ISAG 2017

36th International Society for Animal Genetics Conference

16th - 21st July 2017

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ABSTRACT BOOK

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Contents

ORAL PRESENTATIONS 1
- Animal Forensic Genetics Workshop 1
- Applied Genetics and Genomics in Other Species of Economic Importance 3
- Domestic Animal Sequencing and Annotation 5
- Genome Edited Animals 8
- Horse Genetics and Genomics 9
- Avian Genetics and Genomics 12
- Comparative MHC Genetics: Populations and Polymorphism 16
- Equine Genetics and Thoroughbred Parentage Testing Workshop 19
- Genetics of Immune Response and Disease Resistance 20
- ISAG-FAO Genetic Diversity 24
- Ruminant Genetics and Genomics 28
- Animal Epigenetics 31
- Cattle Molecular Markers and Parentage Testing 33
- Companion Animal Genetics and Genomics 34
- Microbiomes 37
- Pig Genetics and Genomics 40
- Novel, Groundbreaking Research/Methodology Presentation 44
- Applied Genetics of Companion Animals 44
- Applied Sheep and Goat Genetics 45
- Comparative and Functional Genomics 47
- Genetics and Genomics of Aquaculture Species 50
- Livestock Genomics for Developing Countries 53

POSTER PRESENTATIONS 56
- Animal Epigenetics 56
- Animal Forensic Genetics 61
- Applied Genetics and Genomics in Other Species of Economic Importance 61
- Avian Genetics and Genomics 64
- Cattle Molecular Markers and Parentage Testing 75
- Companion Animal Genetics and Genomics 81
- Comparative MHC Genetics: Populations and Polymorphism 87
- Domestic Animal Sequencing and Annotation 90
- Equine Genetics and Thoroughbred Parentage Testing: 94
- Genetics of Immune Response and Disease Resistance 95
- Genome Edited Animals 107
- Horse Genetics and Genomics 108
- ISAG-FAO Genetic Diversity 121
- Microbiomes 125
- Pig Genetics and Genomics 127
- Ruminant Genetics and Genomics 143
- Applied Genetics of Companion Animals 167
- Applied Sheep and Goat Genetics 169
- Comparative and Functional Genomics 177
- Gene Function 186
- Genetics and Genomics of Aquaculture Species 189
- Livestock Genomics for Developing Countries 193

Author Index 199

Key Word Index 212

ORAL PRESENTATIONS

Animal Forensic Genetics Workshop

1 *De novo* genome assembly of *Agapornis roseicollis* and SNP discovery for parentage verification. H. van der Zwan*[1], R. van der Sluis[1], and C. Visser[2], [1]*North-West University, Potchefstroom, North-West, South Africa;* [2]*University of Pretoria, Pretoria, Gauteng, South Africa.*

The African parakeet *Agapornis*, or lovebirds, are globally bred as pets. The main breeding selection criterion is plumage coloration. Birds with rare coloration and their heterozygous offspring (with wildtype coloration) are sold at a premium. Currently, there is no genetic test inferring parentage of the heterozygous offspring, nor has the genes or mutations linked to colour variation been identified. The aim of this study was to discover SNPs to develop a SNP-based parentage verification test for *A. roseicollis* by sequencing, assembling and annotating its *de novo* genome. One young male was selected and its genome sequenced at 100x coverage on the Illumina HiSeq platform. The size of the genome was 1.1Gbp and 15 045 genes were identified. This is comparable to other bird species such as the budgerigar (*Melopsittacus undulates)*. The genomes of the male's parents were sequenced at 30x coverage on the same platform. The parents' reads were mapped to the offspring's reference genome using the Burrow-Wheeler aligner. Making use of the command line Linux-based program Genome Analysis Toolkit (GATK), variants were discovered using the HaplotypeCaller module. Approximately 1.2 million raw SNPs were discovered for the mother and 800 000 for the father. Since it is a non-model organism, hard filtering parameters had to be applied to first extract SNPs and then indels from the raw SNP set. SNPs discovered in indels were discarded. These SNPs were further filtered to include only variants where both parents were heterozygous at that locus indicating heterozygosity from all grandparents. This resulted in a total of 614 700 SNPs after all the filters were applied. SNPs not complying with Mendelian inheritance patterns between the parents and offspring were rejected. Where SNPs was located less than 100bp apart only the SNP with the highest quality score was included. A panel of the top 400 SNPs was selected based on the quality scores obtained during the GATK analyses. This panel will form the foundation for the development of a commercial parentage verification test for lovebirds.

Key Words: lovebird, avian genomics, bioinformatics, *de novo* genome assembly, genomic selection

3 Effectiveness of SNPs genotyping assay as a tool for genetic traceability of cattle production chain. A. Pozzi*[1], C. Previtali[1], R. Capoferri[1], S. Arabi[1], A. Galli[2], and G. Bongioni[1], [1]*Istituto Sperimentale Italiano L. Spallanzani, Rivolta d'Adda, Cremona, Italy;* [2]*Centro di ricerca per le produzioni foraggere e lattiero-casearie CREA, Lodi, Italy.*

In the last years the concern of consumer about food safety is increased, so in the European Union great value is placed on accurate and safe animal identification; breeding plan, disease monitoring, and food safety depend heavily on conventional animal identification. For this reason, the protection of the integrity of these programs from fraud or accidental errors is necessary. Genetic traceability is presented here as a way to further enhance conventional traceability; it represents a powerful tool to verify the identity of animal products during every step of the cattle production chain. From the molecular point of view, Single Nucleotide Polymorphism (SNP) markers have gradually replaced microsatellites (STRs), mostly due to their abundance, cost efficiency, and potential for automation. In this work, the amount of information provided by a 32 SNP panel, selected in a previous study was evaluated on different biological matrices. The main sources of genomic DNA are peripheral blood leukocytes, semen, hair follicles and tissues but sampling requires specialised staff and the animals may be subject to stress, influencing negatively welfare, health and productivity. A valid and cheaper alternative of genomic source can be the milk somatic cells (from 2×10^4 to 2×10^5 cells per millilitre of milk). In details, DNA from 50 semen, 30 meat, 30 hair and 30 milk samples were collected. DNA from 140 samples was processed by TaqMan PCR and scanning array on the Open Array platform, the genotypes were generated by SNP Genotyping and TaqMan Genotyper software. Allele frequencies for each SNP were determined by direct counting. A software developed 'ad hoc' (PAF), was used to estimate the levels of genetic variability: expected heterozygosity (He) values ranging from 0.47 to 0.50 with medium value of 0.46, observed heterozygosity (Ho) ranging from 0,38 to 0,60 and medium value of 0,48. Probability of identity (PI) was calculated for the 32 SNPs and it was equal to 8.40×10^{-14} The 32 SNPs assay described in this study represents a valid and useful tool for DNA-based traceability employed in different applied research projects and in the major commercial cattle products.

Key Words: cattle, single nucleotide polymorphisms, product traceability

4 The population and landscape genetics of the European badger (*Meles meles)* in Ireland. A. Allen*[1], J. Guerrero[2], A. Byrne[1,3], J. Lavery[1], E. Presho[1], G. Kelly[1], E. Courcier[4], J. O'Keefe[5], U. Fogarty[6], D. O'Meara[7], G. Wilson[8], D. Ensing[1], C. McCormick[1], R. Biek[9], R. Skuce[1,3], [1]*Agri Food and Biosciences Institute, Belfast, Northern Ireland;* [2]*CEFE-CNRS, Centre D'Ecologie Fonctionelle et Evolutie, Montpelier, France;* [3]*School of Biological Sciences, Queen's University Belfast, Belfast, Northern Ireland;* [4]*Department of Agriculture, Environment and Rural Affairs, Belfast, Northern Ireland;* [5]*Department of Agriculture Food and the Marine, Dublin, Ireland;* [6]*Irish Equine Centre, Johnstown, Republic of Ireland;* [7]*Waterford Institute of Technology, Waterford, Ireland;* [8]*Animal and Plant Health Agency (APHA), Stonehouse, Gloucestershire, England;* [9]*University of Glasgow, Glasgow, Scotland.*

The European badger (*Meles meles*) is an important member of the fauna of Britain and Ireland, not least because it acts as a wildlife reservoir for bovine tuberculosis. Genetic structure of the species is expected to have been influenced by anthropogenic activities and also landscape-level effects. The relative contribution of both factors is debated, but will conceivably have implications for both wildlife and disease management. Recent Europe-wide surveys of genetic diversity have suggested human-aided introduction of badgers into Ireland. These studies have not, however, indexed island-wide diversity of the species, nor comprehensively attempted to detail demographic and geographic factors which shaped the extant population. Herein, we detail the most comprehensive population and landscape genetic study of the badger in Ireland to date. Our data demonstrate that north-eastern and south-eastern counties of Ireland contain a badger sub-population genetically similar to its British contemporaries. Approximate Bayesian computation suggests this sub-population arose in Ireland ~250–3500 years ago through likely import of a small number of badgers from Britain, which then admixed with an already resident Irish badger population. Landscape genetic analyses determined that geographic distance and elevation were the primary drivers of genetic differentia-

tion, in keeping with the philopatric nature of the species elsewhere in Europe. Other factors such as land cover type, earthworm habitat suitability and the River Shannon, had no detectable effect on gene flow. These data are likely to be useful in future efforts to better understand bovine tuberculosis epidemiology and spatial distribution in the Irish badger population.

Key Words: badger, Ireland, colonisation, landscape genetics

5 Comparison of the effectiveness of 19 STR and 22 STR panels for forensic DNA analysis of canine in Poland. A. Radko*, A. Podbielska, and M. Miszczak, *Department of Animal Genomics and Molecular Biology, National Research Institute of Animal Production, Balice, Poland.*

Dog DNA-profiling is of high importance for the investigation of accident and crime, especially for dogs that are strongly integrated into human society. Twenty-one STR *loci* and one sex determination *locus*, recommended for parentage verification by ISAG were tested in the Polish dog population providing allele frequencies necessary for the application of STRs to forensic genetic casework. Two panels STRs, one containing 19 *loci* and second containing three additional *loci* were tested on 452 randomly selected individuals of five dog breed groups (Herding, Greyhound, Terrier, Non-Sporting and Toy Group). To compare the efficiency of the panels for individual identification and paternity testing, we estimated for each panels the cumulative probabilities of parentage exclusion, when one parent is known (CPE_1) and two parents is known (CPE_2), the combined power of discrimination - CPD, the combined probability of identity - $CP_{ID(theoretical)}$ and random match probability - RMP. The cumulative probabilities of parentage exclusion CPE_1 and CPE_2 for 18 loci were 0.9998806 and 0.99999976, respectively and for 21 *loci* were 0.9999829 and 0.9999999901, respectively. The power of discrimination for each marker showed high values above 0.85, CPD values were near 1.0 for both of panels. The theoretical estimates of CP_{ID} for 18 markers were 4.02×10^{-20} and for 21 markers increased to 6.42×10^{-24}. The probability that a dog selected at random from the population (RMP) will have the same profile as the evidence sample, estimated for 19 STRs and 22 STRs ware 2.85×10^{-22} and 1.73×10^{-26}, respectively.

Key Words: canine, STR profiling, forensic parameters

6 Can-ID: The genetic identification system for canine samples based on SNPs. O. Ramírez*[1], A. Cuscó[1], A. Sánchez[2], O. Francino[2], and L. Altet[1], [1]*Vetgenomics, Barcelona, Spain;* [2]*Molecular Genetics Veterinary Service (SVGM), Barcelona, Spain.*

Genetic identification establishes a secure and permanent DNA profile that is very useful in cases of lost or stolen dogs or to prove parentage. In this study, we present a Canine Identification method (Can-ID) based on SNPs genotyping using a TaqMan OpenArray platform. Can-ID is the genetic identification system for canine samples, developed for the purpose of reducing the problem of unhygienic and unpleasant canine excrement in public places, one of the biggest and most widespread antisocial issue that is very hard to eliminate or even curb. Can-ID panel contains three types of markers. First, contains 100 highly polymorphic SNPs to obtain a unique DNA profile for each dog. These highly polymorphic SNPs were selected from two different datasets: (i) the whole genome sequence (5–22 of final coverage) of 22 dogs of 13 breeds and (ii) the massive sequence after enrichment and capture of 0.4 Mb from 335 dogs (7 breeds) and 100 wolves (2 populations). Second, 15 mitochondrial highly polymorphic SNPs that allow Can-ID guarantees that the biological sample comes from a single animal, and has not been contaminated with exogenous DNA (e.g. faeces contaminated with the urine of another dog), and so avoid false positives. And third, 13 SNPs associated to phenotype traits (as body size, sex, head shape, colour and type of fur) allow obtaining for a 'composite picture' of the dog to be formed, in those cases where the genetic profile found in the excrement is not included in the database. Can-ID have been validated in more than a thousand of dogs and it is the identification method used in the first village from Catalonia that established the obligatory dog DNA testing for all dog owners within the community.

Key Words: dogs and related species, genetic identification, genotyping, genetic marker, parentage

7 Application of multiplex microsatellite panel in *Felidae* family. A. Podbielska*[1], A. Radko[1], W. Nizanski[2], J. Kochan[3], A. Nowak[3], and M. Bugno-Poniewierska[1], [1]*Department of Animal Genomics and Molecular Biology, National Research Institute of Animal Production, Balice, Cracow, Poland;* [2]*Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland;* [3]*Faculty of Animal Sciences, Institute of Veterinary Science, University of Agriculture in Krakow, Cracow, Poland.*

The aim of the study was a preliminary assessment of polymorphism domestic cats and free-living cats by using multiplex microsatellite panel recommended by ISAG for cat identification and parentage control. Ten microsatellite markers: FCA310, FCA220, FCA069, FCA441, FCA075, FCA229, FCA678, FCA149, FCA105 and AMEL were used in Laboratory of Molecular Genetics. Forty-four individuals were tested in the family *Felidae* of species: different breeds a domestic cat (*Felis catus*), five tiger (*Panthera tigri*), two lynxes (*Lynx lynx*) and one of animal, which represent the species wildcat (*Felis silvestris*), manul *(Otocolobus manul*), clouded leopard (*Neofelis nebulosa*), and snow leopard (*Panthera uncia*). Genomic DNA was extracted from hairs and buccal swabs. DNA isolates were amplified by one multiplex PCR. Amplification of genes was amplified using the QIAGEN Multiplex PCR Kit, the amplified products were separated on 3100xl Genetic Analyzer and genotyped using GeneMapper Software (Applied Biosystems). PCR products for all markers were obtained for a domestic cat. Studied wild cats, except for a wildcat and snow leopard were different than domestic cats. PCR products were not observed for FCA441 marker in clouded leopard and lynx. The same results obtained for FCA149 marker for manul, clouded leopard and tigers. Identified alleles of the lynx were out of range in FCA220 marker. The same situation was with manul alleles in FCA441 marker. Additionally, in manul we observed different variant in AMEL marker, which produced a 216-bp X allele, while others species had 214-bp X allele. We conclude, based on the presented studies that we should apply other known markers to the assessment of biodiversity free-living cats. Our preliminary studies allow stating that manul is the most different feline but we need further study on much larger population. Financed by: NCBiR PBS3/B8/16/2015.

Key Words: feline, identification, microsatellite

8 Plains bison triallelic SNPs for determining parentage, estimating cattle introgression, and inbreeding. T. Kalbfleisch*[1], J. Tait[2], V. Basnayake[2], B. Simpson[2], T. Smith[3], and M. Heaton[3], [1]*University of Louisville, Louisville, KY, USA;* [2]*GeneSeek, Lincoln, NE, USA;* [3]*USMARC, USDA, Clay Center, NE, USA.*

Bison producers are interested in availing themselves of genomic technologies to make well-informed decisions for breeding and herd management. Two pressing issues for them are parentage, and a measure of bovine introgression in their seed stock. Fortunately, whole genome next-generation sequencing data generated from bison lends itself well to mapping to the bovine reference genome for comparative analysis. In this work, we have mapped the whole genome sequence data from two plains bison. From this data, we have identified a panel of markers which are heterozygous in both bison where one allele is unique to bison by virtue of not appearing

in the USMARC Bovine Diversity panel at 5% minor allele frequency or greater. The second allele does not appear in the Bovine Diversity Panel, and is consistent with the ancestral allele measured in 2 gaur, 2 banteng and 2 yak. A MALDI-TOF multiplex is being developed for this panel of markers that will measure all three alleles, the two found in the bison, as well as the cattle specific allele. This marker panel enables bison producers to inexpensively measure a unique genetic fingerprint for their animals that can be used in parentage, as well as the ability to quantify the amount of bovine introgression in the animal.

Key Words: bison, parentage, introgression

9 Discrimination of native chicken breeds using SNP marker combination. S. Jin[1], N.-R. Choi[1], D. Seo[1], P. Manjula[1], H.-Y. Kim[2], S. H. Lee[1], and J. H. Lee*[1], [1]*Chungnam National University, Daejeon, Republic of Korea;* [2]*Insilicogen, Inc., Yongin, Republic of Korea.*

The consumption of chicken meat is steadily increasing in the world and the rate of increase is much higher in Korea because chicken meat has been recognised as healthy food. Recently, Korean government launched Golden Seed Project (GSP) for developing new chicken breeding stocks using native chickens. In this study, 600K high-density SNP array was used for the genetic verification of new chicken breeding stocks. Total, 192 native chickens were investigated for selection of SNP markers for breed discrimination. As the results, 128 SNPs were initially selected. Based on the validation study, highly significant 48 SNP markers were finally selected with 96% sensitivity and 98.4% specificity for identifying target population. These results can provide useful information for developing of new chicken breeding stocks with further verifications.

Key Words: SNP, new chicken breeding stock, native chicken, breed discrimination

Applied Genetics and Genomics in Other Species of Economic Importance

10 An evaluation of the ISAG recommended parentage and identification panel for the domestic pigeon (*Columba livia domestica*). M. de Groot*, *VHLGenetics, Wageningen, the Netherlands.*

In this study, the ISAG recommended panel for the identification of pigeon is characterised based on commonly used statistical parameters. The assessed marker panel for the domestic pigeon (*Columba livia*) genotyping is based on 16 short tandem repeat (STR) loci (PIGN15, PIGN10, PIGN57, PIGN26, CliμD16, CliμD19, PIGN12, CliμD17, CliμT17, PIGN04, CliμD01, CliμD11, CliμD35, CliμT02, CliμT13, CliμT43). The alleles of the 16 loci consist of a mixture of tri-, tetra- penta- and hexameric repeat patterns. A sex determination marker was included in the multiplex for quality control. The repeat sequence of the PIGN markers was previously unpublished and therefore sequenced to reveal the sequence pattern. In total, 1,421 pigeons were genotyped on all 16 STR loci to generate allele frequency data for each locus. All 16 markers combined a PE1 (combined non-exclusion probability, first parent) of 0.9986 and PE2 (combined non-exclusion probability, second parent) of >0.9999 was observed. Comparing both the alleged father and mother, a PE value of >0.9999 was observed. Two of the markers, CliμD19 and PIGN12, were found to have relatively high HWE and F(Null) values. Therefore it can be considered to replace these markers by other STRs. Another point of discussion can be to add a gender identification marker to the recommended ISAG panel. Not only can this serve as an extra identification marker, but also because it is very hard to determine the sex of an animal based on phenotypical characteristics, especially for chicks. The set of 16 STR markers can be used in routine parentage verification work as well as identification of individuals.

Key Words: STR, genotyping, *Columba livia domestica*, pigeon

11 Selection of SNP markers for a dromedary camel genotyping array. M. Al Abri*[1], H. M. Holl[2], D. Miller[3], S. Abdalla[4], B. Shykind[4], J. Malek[4], Y. Mohamoud[4], K. Pasha[5], A. Khalili[5], D. F. Antczak[3], and S. Brooks[1], [1]*Sultan Qaboos University, Department of Animal and Veterinary Sciences, Muscat, Sultanate of Oman;* [2]*University of Florida, Department of Animal Sciences, Gainesville, Florida, USA;* [3]*Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA;* [4]*Weill Cornell Medical College in Qatar, Cornell University, Doha, Qatar;* [5]*Tharb Veterinary Hospital, Doha, Qatar.*

Domesticated over 3,000 years ago, dromedary camels play a vital role in livestock production systems, especially in arid and semi-arid regions. As problems of global warming and shortages of potable water worsen, camels could be the livestock of choice as they are better adapted than other species to high ambient temperatures and limited resources. However, to take advantage of the agricultural advantages of the camel, improved understanding of the genetics underlying their unique biology is needed. To this day, there are relatively few published studies in the area of camel genetics and genomics. This is due, in large part, to a lack of genomics tools to conduct such studies. Additionally, while genotyping arrays are commercially available for most livestock, such arrays have not yet been developed for camels. In this work, we describe the development of a set of ~80,000 single nucleotide polymorphisms (SNPs) for the dromedary camel. These SNPs are selected from whole genome sequencing (WGS) of 9 camels and Genotyping by sequencing (GBS) data of 244 dromedary camels. We also discuss the development and evaluation of a reduced set of 100–200 SNP markers for parentage testing, a service in high demand for the camel racing industry. The genotyping panels will assist in estimating diversity parameters and genomic relationships between camels. They will also enable estimation of heritabilities for economic quantitative traits, GWA studies, and searches for selection signatures of important production and health traits. Finally, since no pedigree records or registries exist for most camels, genomic improvement/selection through such a SNP panel may be the only feasible method to boost its contribution to meat and milk production and therefore, food security in the future.

Key Words: Old World camelids, breed/population identification, genetic improvement, parentage, single nucleotide polymorphism (SNP)

12 Characterisation of a family of alpacas exhibiting disproportionate dwarfism. K. A. Munyard* and T. Y. K. Tan, *School of Biomedical Sciences, Curtin Health Innovation Research Institute, Curtin University, Perth, Western Australia, Australia.*

An alpaca breeder noted that one of his herdsires (Sire X) produced cria which were noticeably smaller than normal ~50% of the time. Both male and female cria were affected, and there was also perceived mild dysmorphia. We report here the investigation of this

trait, including phenotypic characterisation, inheritance pattern, and a preliminary genomic association study. The animals in the study were Sire X, cria out of normal females that were sired by Sire X, and cria out of these same dams by normal sires. Phenotype was characterised by measuring weight over time, and body proportions at age >1yo. Mode of inheritance was hypothesised from pedigree records. A genome wide association study was performed on seven of these animals. PLINK was used to analyse SNPs generated via double digest reduced representation Ion Proton sequencing, mapped to the vicpac2.0 genome. The affected alpacas conformed to a disproportionate dwarfism phenotype, namely a significant reduction in the length of the spine (P = 0.002), the length of elbow to withers (P = 0.02), spine to hind knee (P = 0.03), and hock to hind fetlock (P = 0.01). Weights were significantly lower at birth (P = 0.01) and approached significance at 1yo (P = 0.056). Pedigree analysis supported an autosomal dominant mode of inheritance. Between 142,831 and 396,069 SNPs were called for each animal, although only 41,261 SNPs were called at >20x coverage in all 7 animals. One SNP (scaff_19: 12429719) had a P-value of 0.000532, and 15 others also had high p-values. However, none of these reached genome-wide significance after correction for multiple testing. A candidate gene near the top SNP encodes for an enzyme that cleaves Amyloid Precursor Proteins (APP). Abnormal accumulation of APP has been linked to a disease which includes deformity of the long bones as a symptom (Kimonis *et al*., 2007). Additional candidate genes will be examined. The genotyping by sequencing protocol proved to be successful, but still needs further optimization to maximise the common regions sequenced, and thus the number of SNPs able to be called across the whole cohort.

Key Words: New World camelids, genome-wide association, genotyping, genetic disorder, animal health

14 Genomic selection for performance and reproduction traits in American mink. K. Karimi*[1], Y. Miar[1], and M. Sargolzaei[2,3], [1]*Department of Animal Science and Aquaculture, Dalhousie University, Nova Scotia, Canada;* [2]*Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada;* [3]*Semex Alliance, 5653 Highway 6 North, Ontario, Canada.*

Phenotypic relationships between performance and reproduction traits were explored using bivariate models on a dataset from a genomic study aimed at improving economically important traits in American mink. Performance and reproduction traits of 21,939 mink during the period of 2002–2016 were collected by Canadian Centre for Fur Animal Research at Dalhousie University. A study to implement genomic selection for these traits on 2,000 mink using genotyping-by-sequencing has been also initiated. Phenotypic variations and correlations between bodyweight at different ages, litter size, the number of weaned kits and mortality rate until weaning were estimated using SAS 9.4 program. The average number of kits born was equal to 6.12 ± 2.58 and varied between 0 and 17 kits per female. On average, 0.83 kit per female was dead at the first 24 h post whelping. Our results indicated that 84.19% of alive kits survived until weaning (6 weeks). The average number of weaned kits was 4.92 ± 2.54 per female and ranged from 0 to 13. The average birthweight was 11.20 ± 2.95 gr per kit and ranged from 5 to 23.8 gr. Furthermore, the average weight at 3 weeks and weaning weight were equal to 134.1 ± 17.4 gr and 389.8 ± 62.2 gr, respectively. Negative phenotypic correlations were found between birthweight with litter size (-0.47 ± 0.17), the number of kits born alive (-0.39 ± 0.14) and the number of weaned kits (-0.20 ± 0.07). Weaning weight was negatively correlated with litter size (-0.31 on average), the number of kits born alive (-0.32 on average) and the number of weaned kits (-0.36 on average). Additionally, the phenotypic correlation between pairing weights and the number of weaned kits was low (-0.10 ± 0.04). Relatively high phenotypic variation and moderate correlation between the studied traits in American mink warrants further investigation to estimate genetic parameters and to investigate the possibility of genetic improvement through implementation of multi-trait genomic selection.

Key Words: Mustelids, animal breeding, performance traits, phenotypic correlation, genomic selection

15 SNP genotyping of reindeer (*Rangifer tarandus)* using BovineHD BeadChips. V. Kharzinova*[1], A. Dotsev[1], V. Fedorov[2], G. Brem[1,3], K. Wimmers[4], H. Reyer[4], and N. Zinovieva[1], [1]*L.K. Ernst Institute of Animal Husbandry, Moscow, Russia;* [2]*Yakut Scientific Research Institute of the Agriculture Federal Agency Scientific Institutions, Yakutsk, Russia;* [3]*Institute of Animal Breeding and Genetics, VMU, Vienna, Austria;* [4]*Institute of Genome Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany.*

For most model species, SNP genotyping assays became a highly effective molecular genetic tool for biodiversity studies. In regards to non-model species (such as reindeer), these targets are currently limited by the lack of SNP arrays. Some recent research demonstrated the successful application of the medium density DNA chips for survey of genetic diversity and population structure of the reindeer populations, but high-density SNP arrays have not been applied for this purpose yet. In our study, the Illumina BovineHD BeadChip genotyping array was tested as a new tool for describing the biodiversity and estimating individual level of inbreeding within reindeer populations. Genomic DNA was isolated from tissue samples of 22 individuals belonging to three semi-domestic reindeer populations (POP1, n = 7; POP2, n = 12; POP3, n = 3) using a Nexttec column (Nexttec Biotechnologie GmbH, Germany). We used PLINK1.07, Admixture 1.3. Software and R packages ('VennDiagram', 'inbreedR' and 'diveRsity') were used for statistical analysis. After QC (GENO 0.1, MAF = 0.01) 9623 SNPs were taken for the further analyses. All populations had approximately equal polymorphism level, while POP1 was characterised by the highest level of genetic diversity (Ho = 0.227 ± 0.002, He = 0.202 ± 0.002 and Ar = 1.499 ± 0.003) and POP3 the lowest (Ho = 0.223 ± 0.003, He = 0.155 ± 0.002 and Ar = 1.392 ± 0.005). The F_{IS} values ranged from -0.055 in POP2 to -0.386 in POP3. The g2 estimator of identity disequilibrium (ID) significantly differed from zero (g2 = 0.00055, se = 0.00022, $P < 0.001$, based on 999 permutations) and the expected r2 between inbreeding level and heterozygosity was 0.64. Multi-dimensional scaling (MDS) components one (C1) and two (C2) explained ~10.5% and 9.0% of the variation, respectively. Admixture analysis revealed a clear differentiation of the studied reindeer populations, which indicates their unique genetic profiles. We demonstrated that SNP genotyping using Bovine 770K SNP BeadChips provides sufficient information on reindeer genetic profiles which might be used for creation of specific SNP panels for this species. This research was funded by Russian scientific foundation project number 14-36-00039.

Key Words: SNP, BovineHD BeadChips, reindeer

16 Combining transcriptome and epigenetic analysis of H3K36me3 and H3K4me3 marks to explore mechanisms of liver-specific gene expression in pigs. J. Huang, M. Schroyen, N. Gabler, J. Dekkers, and C. Tuggle*, *Department of Animal Science, Iowa State University, Ames, IA, USA.*

Post-translational covalent modifications on the histones in nucleosomes play important roles in regulating gene expression at the chromatin level. To study the interplay between RNA expression and histone modification in pig tissues, we selected putative tissue-specific genes using RNAseq analysis of liver, muscle, spleen and?ileum collected from 6 Yorkshire pigs. We found 148, 108, 17 and 109 genes were specifically or highly differentially expressed in liver, muscle, spleen and ileum, respectively. Using Q-RT–PCR, we validated these predictions for 11 liver, 10 muscle, 9 spleen, and 6 ileum genes. Trimethylation of lysine 36 of histone H3 (H3K36me3) and trimethylation of lysine 4 of histone H3 (H3K4me3) are associated with specific transcriptional states, and we predicted that H3K4me3 marks would be enriched in liver chromatin at liver-specific gene promoters, but not at muscle-specific gene promoters. Likewise, we predicted H3K36me3 would be enriched in the 3′UTR only for liver-specific genes. Liver chromatin preparations from two replicates were immunoprecipitated (ChIP) using antibodies to these two marks. We developed Q-PCR tests (at the promoter or the 3′UTR) for 17 expression-validated genes (9 liver-specific and 8 muscle-specific) to test whether H3K36me3 (gene body/3′UTR) and H3K4me3 (active promoter) marks were associated with RNA-seq predictions. Results showed that the H3K36me3 levels measured at the 3′UTR were high for all liver-specific genes but low for muscle-specific genes. Conversely, H3K4me3 levels measured at the promoter were high for only liver-specific genes. Q-PCR assays for gene deserts were negative for both marks, while a broadly expressed gene (RPL30) was positive for both marks. These data indicated that H3K36me3 and H3K4me3 are active marks that correlate well in pigs with their known connections to functional gene components in human and thus are likely to play a vital role in epigenetic control of porcine gene expression. Therefore, these marks should be highly valuable for functional annotation of the porcine genome. Funding acknowledgment: NIFA-AFRI-2011–68004–30336.

Key Words: pig, Functional Annotation of Animal Genomes (FAANG), gene expression, epigenetics, liver

17 Genome-wide analysis of H3K4me3 and H3K27me3 in three tissues in pigs. C. Kern*[1], Y. Wang[1], P. Saelao[1], K. Chanthavixay[1], I. Korf[1], C. K. Tuggle[2], C. Ernst[3], P. Ross[1], and H. Zhou[1], [1]*University of California, Davis, Davis, CA, USA;* [2]*Iowa State University, Ames, IA, USA;* [3]*Michigan State University, East Lansing, MI, USA.*

Epigenetics is an important factor in understanding the link between an organism's genome and phenome. Such knowledge is especially important in the food production industry where it can be applied to improve production efficiency, animal welfare, and food safety. As a part of the International FAANG effort, we have made progress towards generating a catalogue of functional elements for the pig genome using ChIP-seq assays for the H3K4me3 and H3K27me3 histone modifications in liver, lung, and spleen from two biological replicates with more than 20 million mapped reads for H3K4me3 assays and 40 million mapped reads for H3K27me3 per animal (FAANG Consortium criteria). We identified 29,365 H3K4me3 peaks in liver, 25,558 in lung, and 23,979 in spleen using Macs 2 (q-value 0.01). For H3K27me3, we used SICER (q-value 0.01) and identified 123,392 broad peaks in liver, 122,656 in lung, and 152,269 in spleen. From a set of 22,861 promoter regions from Ensembl, in liver 7,461 contained H3K4me3 peaks, 4,095 contained H3K27me3 peaks, and 2,139 contained peaks from both. In lung, these numbers were 6,853, 3,476, and 2,494, respectively, and in spleen 6,711, 4,097, and 2,551, respectively. Liver showed the highest specificity of the H3K4me3 modification, with 821 promoters containing a peak only in liver, compared with 261 in lung and 340 in spleen. For the H3K27me3 mark, the modification was observed only in liver for 873 promoter regions, 471 in lung, and 972 in spleen. Using RNA-seq data generated from the same tissue samples as our ChIP-seq assays, we confirmed that genes with the H3K4me3 modification were expressed at significantly higher levels than those with the H3K27me3 modification. Five additional tissues and three more ChIP-seq marks will enable an integrative analysis to predict chromatin state across the pig genome.

Key Words: pigs and related species, Functional Annotation of Animal Genomes (FAANG), epigenomics, ChIP-seq, bioinformatics

18 Effects of maternal nutrition on the transcriptome and epigenome of the offspring. H. Namous[1], F. Peñagaricano[2], M. Del Corvo[3], E. Capra[4], A. Stella[4], J. Williams[5], P. A. Marsan[3], and H. Khatib*[1], [1]*University of Wisconsin, Madison, Wisconsin, USA;* [2]*University of Florida, Gainesville, Florida, USA;* [3]*Università Cattolica del S. Cuore, Piacenza, Italy;* [4]*Istituto di Biologia e Biotecnologia Agraria, Lodi, Italy;* [5]*University of Adelaide, Roseworthy, Australia.*

The objective of this study was to evaluate the impact of maternal nutrition of pregnant ewes on the epigenome and transcriptome of their fetuses. Ewes were naturally bred to a single sire, and from days 67 ± 3 of gestation until necropsy (day 130 ± 1) they were individually fed alfalfa haylage (HY; fibre) or corn (CN; starch). A total of 26 fetuses were removed from 15 dams and longissimus dorsi muscle, perirenal adipose depot, and subcutaneous adipose depot tissues were collected. Total RNA and genomic DNA were extracted from fetal tissues for transcriptomic and DNA methylation analyses. To assess the effects of maternal diets on the transcriptome of the fetal tissues, a total of 36 pooled samples (12 pooled samples per tissue with 4 biological replicates per diet) were analysed using RNA-sequencing. From 18,393 genes tested for differential expression in fetal longissimus dorsi muscle tissue, 823 genes showed differential expression between CN and HY maternal diets. Many of these genes are directly involved in embryonic and fetal development, skeletal muscle cell and tissue differentiation, and muscle myosin complex and sarcomere organisation. To assess the effects of maternal diet on the epigenome of the fetus, whole genome DNA methylation analysis was performed in 16 fetal longissimus dorsi muscle tissues using MethylMiner, a methyl binding-based method. A total of 61 differentially methylated regions (DMRs) between HY and CN diets were found in which 39 DMRs showed higher methylation levels in HY compared to CN and 22 DMRs showed higher methylation levels in CN compared to HY. Several differentially methylated genes were validated using bisulfite sequencing. In addition, the correlation between gene expression and DNA methylation was validated for differentially-expressed and differentially methylated gene. Overall, these findings provide evidence that maternal diet supplementation during pregnancy can modulate gene expression and epigenetic changes in the offspring.

Key Words: maternal nutrition, transcriptome, epigenome, sheep

19 Profiling the landscape of transcription, chromatin accessibility and chromosome conformation of cattle, pig, chicken and goat genomes [FAANG pilot project "FR-AgENCODE"]. S. Foissac[1], S. Djebali*[1], H. Acloque[1], FR-AgENCODE Consor-

tium[2], M. H. Pinard-Van der Laan[2], S. Lagarrigue[3], and E. Giuffra[2], [1]*GenPhySE, INPT, ENVT, INRA, Université de Toulouse, Toulouse, France;* [2]*GABI, AgroParisTech, INRA, Université Paris Saclay, Paris, France;* [3]*PEGASE, INRA, Agrocampus Ouest, Rennes, France.*

Functional annotation of livestock genomes is a critical and obvious next step to derive maximum benefit for agriculture, animal science, animal welfare and human health. The aim of the Fr-AgENCODE project is to generate multi-species functional genome annotations by applying high-throughput molecular assays on three target tissues/cells relevant to the study of immune and metabolic traits. An extensive collection of stored samples from other tissues is available for further use (FAANG Biosamples 'FR-AGENCODE'). From each of two males and two females per species (pig, cattle, goat, chicken), strand-oriented RNA-seq and chromatin accessibility ATAC-seq assays were performed on liver tissue and on two T-cell types (CD3+CD4+ & CD3+CD8+) sorted from blood (mammals) or spleen (chicken). Chromosome Conformation Capture (*in situ* Hi-C) was also carried out on liver. Sequencing reads from the 3 assays were processed using standard processing pipelines. While most (50–70%) RNA-seq reads mapped to annotated exons, thousands of novel transcripts and genes were found, including extensions of annotated protein-coding genes and new lncRNAs (see abstract #69857). Consistency of ATAC-seq results was confirmed by the significant proportion of called peaks in promoter regions (36–66%) and by the specific accumulation pattern of peaks around gene starts (TSS) *v.* gene ends (TTS). Principal Component Analyses for RNA-seq (based on quantified gene expression) and ATAC-seq (based on quantified chromatin accessibility) highlighted clusters characterised by cell type and sex in all species. From Hi-C data, we generated 40kb-resolution interaction maps, profiled a genome-wide Directionality Index and identified from 4,100 (chicken) to 12,100 (pig) topologically-associating domains (TADs). Correlations were reported between RNA-seq and ATAC-seq results (see abstract #71581). In summary, we present here an overview of the first multi-species and -tissue annotations of chromatin accessibility and genome architecture related to gene expression for farm animals.

Key Words: multispecies, Functional Annotation of Animal Genomes (FAANG), ATAC-seq, RNA-seq, Hi-C

20 Long-non coding RNAs repertoires in liver and two T lymphocyte cell types in four livestock species [FAANG pilot project "FR-AgENCODE"]. K. Muret*[1], S. Djebali[2], T. Derrien[3], C. Cabau[2], C. Klopp[4], D. Esquerré[2,5], K. Munyard[6], G. Tosser-Klopp[2], H. Acloque[2], E. Giuffra[7], S. Foissac[2], and S. Lagarrigue[1], [1]*UMR PEGASE INRA, Agrocampus Ouest UMR PEGASE, Rennes, France;* [2]*UMR GenPhySE, INRA, INPT, ENVT, Université de Toulouse, Castanet-Tolosan, France;* [3]*IGDR, CNRS-University Rennes 1, Rennes, France;* [4]*SIGENAE, INRA, Castanet-Tolosan, France;* [5]*Plateforme GENOTOUL, INRA, Castanet-Tolosan, France;* [6]*School of Biomedical Sciences, Curtin Health Innovation Research Institute, Western Australian Biomedical Research Institute, Curtin University, Perth, Western Australia, Australia;* [7]*UMR GABI, INRA, AgroParisTech, Université Paris Saclay, Jouy-en-Josas, France.*

Understanding genome-to-phenome relationships requires deep and cross-disciplinary genetic analyses among which functional annotation provides crucial insights. The development of High Throughput Sequencing and RNA-seq now help us to find a large number of heterogeneous and low-expressed transcripts known to be long non-coding RNAs (lncRNAs). One of the aims of the FAANG pilot project 'FR-AgENCODE' is to identify and characterise the long non-coding RNAs of multiple tissues and cell lines in 4 farm animals (chicken, bovine, pig and goat) of both sexes. Here, we focus our analysis on the liver tissue and two blood T-cell types (CD3+CD4+, CD3+CD8+) where samples were collected through 4 biological replicates (2 males and 2 females). It allows us to compare lncRNA repertoires between tissues, sex and species in relation with fundamental biological functions like energy storage and immunity. High depth strand-specific RNA-seq produced ~200M paired-end reads for each of the 16 RNA-seq datasets. After transcriptome reconstruction, we used the recently published FEELnc program (Wucher *et al*., 2017, Nucleic Acid Research) to identify lncRNAs longer than 200 bp and without protein-coding capabilities. FEELnc also classifies lncRNAs based on their genomic localizations with respect to the ENSEMBL protein-coding annotation: intergenic lncRNAs are categorized depending on the distance and orientation with respect to the closest mRNAs and the intragenic lncRNAs are extracted based on their overlap with mRNAs exons and introns. We will report these lncRNA repertoires in terms of intergenic/intragenic lncRNA class, structure and expression and comparing these features between livestock species, tissues and sexes. By profiling the transcriptional landscape of lncRNAs in these 4 species, this data will further contribute to the global action for annotating functional elements of livestock genomes.

Key Words: long non-coding RNAs, multispecies, Functional Annotation of Animal Genomes (FAANG), comparative genomics, RNA-seq

21 Genome-wide CRISPR knockout screening for host factors involved in bovine herpes virus type 1 infection. W. Tan*[1], I. Dry[1], S. Lillico[2], C. B. A. Whitelaw[2], and B. Dalziel[1], [1]*Division of Infection and Immunity, the Roslin Institute, University of Edinburgh, Edinburgh, Scotland;* [2]*Division of Developmental Biology, the Roslin Institute, University of Edinburgh, Edinburgh, Scotland.*

Bovine herpes virus type 1(BHV-1) causes infectious bovine rhinotracheitis, fatalities in calves and pregnancy abortions in cows, leading to huge economy loss in Ireland and the UK. Unfortunately, little is known about how host factors interact with this virus, and our lack of knowledge is impeding vaccine and drug developments. CRISPR/Cas9 are novel molecular scissors that cut DNA in a site-specific manner and have been used to edit many genes in livestock. It relies on base pairing between the small CRISPR guide RNA(gRNA) and target DNA, an activity that leads the gRNA-bound Cas9 protein to exert DNA cutting, often resulting in gene inactivation or conversion changes. Since the specificity of CRISPR/Cas9 is largely determined by the base pairing, it is straightforward to introduce many guides together to achieve knockout of multiple genes in parallel. Various groups have fully utilised this strategy and generated CRISPR libraries that target every gene in given organisms. Cells treated with such libraries lead to dissections of host factors involved in infections such as those from flaviviruses and HIV. To enable whole genome screening in cattle, we are creating a CRISPR library with ~120,000 guides targeting every gene in the bovine genome. We will then use it to screen for host factors involved in BHV-1 infection. To achieve this, MDBK cells that stably express Cas9 from the *rosa26* locus will be transduced by a lentivirus library that expresses all guides at low titre, ensuring single knockout events in the majority of the cells. The resulting cell pool encompassing knockout events in all genes will then be challenged by recombinant BHV-1 virus with mCherry. Cell populations with early and late mCherry signals and cells devoid of mCherry post infection will be obtained by FACS sorting. Integrated gRNA in these populations will be amplified and sequenced by Illumina Hiseq which will reveal copy numbers of guides; enriched guides indicate inhibitory roles of corresponding genes whereas depleted guides imply facilitator roles of targeted genes. These candidates will then be validated by transfecting individual guides into MDBK cells which will also be challenged by the virus.

Key Words: BHV-1, CRISPR-Cas9, whole genome screening, gene discovery

22 Beyond sequencing: Assigning function to novel ncRNAs. F. McCarthy*[1], A. Cooksey[1], C. Gresham[2], and B. Nanduri[2], [1]*University of Arizona, Tucson, AZ, USA;* [2]*Mississippi State University, Starkville, MS, USA.*

New genomic technologies are accumulating information about functional elements within genomic sequence at a faster rate than ever before, and the FAANG Project is expected to increase this rate of acquisition. Understanding how these elements contribute to traits and phenotypes requires a concomitant effort to understand the function of these regulatory genes and regions. However, functional annotation relies on curation of published data and computational prediction pipelines; genomic technologies routinely identify novel functional elements (with no existing body of literature) and there are no existing computational pipelines for functional annotation of ncRNAs. We describe here our approach to develop functional annotation workflows for miRNAs and lncRNAs, two classes of ncRNAs that are being annotated in species that are studied as part of the FAANG Project. We pair miRNA target prediction with proteomics data and GO enrichment analysis to (1) better identify true miRNA targets and (2) predict the function of novel miRNAs. For predicting function of novel lncRNAs we use a similar approach, identifying lncRNA-mRNA pairs expressed in the same tissues, identifying interaction networks for these pairs and investigating GO term enrichment to predict function. By linking experimental expression data with computational approaches, we expect to generate preliminary, high-throughput functional information about commonly identified classes on ncRNAs. This in turn will allow us to provide information that can then be incorporated into functional analyses of typical gene expression datasets (e.g. transcriptome data).

Key Words: noncoding RNAs, functional annotation

23 Haplotype resolution of leukocyte receptor complex in cattle through targeted enrichment and SMRT sequencing. D. Heimeier*[1], J. Schwartz[1], D. Bickhart[2], T. Smith[3], and J. Hammond[1], [1]*The Pirbright Institute, Woking, Surrey, England;* [2]*Cell Wall Biology and Utilization Research, USDA-ARS, Madison, WI, USA;* [3]*Meat Animal Research Center, USDA-ARS, Clay Center, NE, USA.*

The highly repetitive nature of cattle leukocyte receptor complex (LRC) has made it difficult to assemble and fully characterise this region with short reads used by second generation sequencing. Previously, we reported the first two cattle killer immunoglobulin-like receptors (KIR) haplotypes; one complete and framed by leukocyte immunoglobulin-like receptor (LILR) and Immunoglobulin α Fc receptor (FCAR) genes (263kb), the other shorter and incomplete, that were resolved from combined Sanger and 454-pyrosequencing sequencing of BAC clones. Through subsequent targeted genome enrichment with Roche Nimblegen probes and Illumina sequencing of different cattle breeds and related species the haplotype variability and gene polymorphism of further haplotypes has been predicted to be gene variable with additional KIR genes. This data has now been combined and validated with another complete and larger KIR haplotype (350kb) that has been assembled using long-read single molecule real time (SMRT) sequencing with Pacific Bioscience technology. More recently, we have developed this targeted sequencing approach for use with SMRT sequencing to resolve the KIR region in more detail from further two individuals. Initial results show between 87 and 91% average base coverage when mapped to the shorter complete KIR haplotype at a maximum divergence of 10%. Average coverage was more than 20x with certain regions reaching more than 100x, which could be indicating additional or missing genes in the investigated individuals. This preliminary data is clearly indicating towards significant structural variation as well as polymorphisms. We are currently developing a bioinformatics pipeline to *de novo* assemble and phase each haplotype, so we can process a large number of individuals and identify gene variable haplotypes and polymorphic variants with confidence.

Key Words: cattle, immunogenetics, targeted enrichment sequencing, leukocyte receptor complex, haplotype

24 The ruminant biology and evolution revealed by a flock of ruminant *de novo* genomes. W. Wang[1], Q. Q. Qiu[1], G. Zhang[3,4], R. Heller[3], H. R. Siegismund[3], and Y. Jiang*[2], [1]*Northwestern Polytechnical University, Xi'an, Shaanxi, China;* [2]*Northwest A&F University, Yangling, Shaanxi, China;* [3]*University of Copenhagen, Copenhagen, Denmark;* [4]*Beijing Genomics Institute at Shenzhen, Shenzheng, Guangdong, China.*

The ruminants form one of the most ecologically important hehbivorous animal groups on Earth, with two major families, the bovids (Bovidae) and the deer (Cervidae). Furthermore, the ruminants include five of the most important livestock species: the cow, water buffalo, yak, sheep and goat. Despite the remarkable diversity and evolutionary success of the ruminants relatively little is known about the evolutionary genomics of the group, let alone how did they evolve. In an international consortium including Danish and Chinese research groups, we are *de novo* assembling 45 ruminant genomes, covering all of the six exist families and most of the 82 exist genera of ruminant suborder. With this big dataset we plan to bring our knowledge about the evolutionary genomics in this important animal group to a whole new level. We will look at several specific ecological and physiological adaptations, such as the evolutionary innovation of the rumen and the horn, the ruminantia population dynamics correlated with the climate change in past millions of years. Combined with comparative genomics and ENCODE genomic features, the ruminant specific conventional regions would be also revealed and used for the cattle/sheep/goat functional genome annotation.

Key Words: ruminant biology, de novo genome, comparative genomics, rumen, horn

25 SheepGenomesDB: Towards 1000 Genomes. S. McWilliam*[1], R. Brauning[2], S. Clarke[2], A. McCulloch[2], N. Cockett[3], G. Saunders[4], M. Naval Sanchez[1], H. Daetwyler[5,6], and J. Kijas[1], [1]*CSIRO, St Lucia, Queensland, Australia;* [2]*AgResearch Ltd, Invermay, Mosgiel, New Zealand;* [3]*Utah State University, Logan, UT, USA;* [4]*EMBL-EBI, Cambridge, United Kingdom;* [5]*Department of Economic Development, Jobs, Transport and Resources, Bundoora, Victoria, Australia;* [6]*La Trobe University, Bundoora, Victoria, Australia.*

The SheepGenomesDB is a repository of genome wide variants produced from publically available sheep genomes. The project applied a harmonised pipeline for raw read filtering, mapping and variant detection. Run 1 captured nearly 500 sheep genomes, and generated nearly 100 million unfiltered variants which were pruned to define a high confidence set. Run 2 increases the genome count to almost 1000 across a wider range of sheep breeds. Both variant collections (raw and high quality) are available via the European Variation Archive (EVA), with the high quality variants annotated against OARv3.1 using the Ensembl Variant Effect Predictor (VEP). We report on the data diversity, variation across breeds and geographical location and the applications to imputation, analysis of domestication, disease mutation identification and linkages with the emerging FAANG project.

Key Words: sheep, genome, variants

26 A novel approach for mapping of animal genome assemblies to a chromosomal level applied to avian genomes. J. Damas[1], R. E. O'Connor[2], M. Farre[1], H. Martell[2], E. A. Slack[1], E. Allanson[1], L. Kiazim[2], R. Jennings[2], A. Mandawala[3], S. Joseph[2],

K. E. Fowler[3], D. K. Griffin[2], and D. M. Larkin*[1], [1]*Department of Comparative Biomedical Sciences, Royal Veterinary College, University of London, London, UK;* [2]*School of Biosciences, University of Kent, Canterbury, UK;* [3]*Canterbury Christchurch University, Canterbury, UK.*

The ultimate aim of a genome assembly is to create a contiguous length of sequence from p- to q- terminus of each chromosome. Most assemblies are however, highly fragmented, limiting their use in studies of trait linkage, phylogenomics and genomic organisation. To overcome these limitations, we developed a novel scaffold-to-chromosome anchoring method combining Reference-Assisted Chromosome Assembly (RACA) and fluorescence *in* situ hybridisation (FISH) to position scaffolds on chromosomes. Scaffolds generated from *de novo* sequenced genomes were ordered and orientated using RACA against a reference and outgroup genome into 'predicted chromosome fragments' (PCFs) for 18 previously published avian genomes. PCFs were verified using PCR for 6 genomes and the second round of RACA was run for all genomes using settings derived from the PCR results. An universal set of FISH probes developed through the selection of conserved regions in 21 avian genomes were then used to map PCFs of the peregrine falcon (*Falco peregrinus*) and rock pigeon (*Columba livia*) genomes. We were able to physically map 87% of the peregrine and 84% of the pigeon genome, improving the N50 of both 7-fold, as well as identify a series of intra and interchromosomal rearrangements. Additional genomes mapped using this method include the ostrich (*Struthio camelus*), the saker falcon (*Falco cherrug*) and the budgerigar (*Melopsittacus undulatus*). Comparative analysis of evolutionary breakpoint regions identified in the upgraded genome assemblies demonstrated that positions of interchromosomal breaks in avian genomes are limited to the genome intervals with unusually low level of sequence conservation. This likely to shed light on why most avian species have very stable karyotypes. Our combined FISH and bioinformatics approach represents a step-change in the mapping of genome assemblies. This makes verification and upgrade of any avian genome assembly with long scaffolds to chromosomes an easy task allowing comparative genomic research at a higher resolution than previously possible.

Key Words: genome assembly, comparative genomes, fluorescent in situ hybridization, avian genomes, chicken

Genome Edited Animals

27 Pest-off: Could gene drive help drive out Australia's invasive pest animals? M. Tizard*[1], T. Strive[2], P. Brown[3], S. Henry[2], A. Sunarto[1], K. McColl[1], C. Cooper[1], D. Moro[4], M. Byrne[4], and T. Doran[1], [1]*CSIRO Health & Biosecurity, Australian Animal Health Laboratory, Geelong, Victoria, Australia;* [2]*CSIRO Health & Biosecurity, Black Mountain Laboratory, Canberra, ACT, Australia;* [3]*CSIRO Agriculture & Food, Black Mountain Laboratory, Canberra, ACT, Australia;* [4]*Department of Parks and Wildlife, Kensington, Western Australia, Australia.*

The CRISPR/Cas9 tool has had a dramatic impact across biomedical research and is opening new opportunities for biotechnology in animal agriculture. Its adaptation as the driving force of synthetic, RNA guided, gene drives has initiated innovative thinking about approaches to dealing with invasive pest animals. Rabbits and cane toads are probably the best known of Australia's problem animals but the list is long – for example feral cats and common carp (*Cyprinus carpio*) are less well known but arguably of similar importance. Current control measures are often insufficient to mitigate the impacts of these and other pest animals on Australia's native wildlife, landscape and agricultural systems. New approaches and new tools are needed and genetic technologies, particularly gene drive, present a significant opportunity, worthy of investigation. There has been a great deal of international debate involving, scientist, ethicists, biosafety experts, government regulators and non-governmental organisations about a range of concerns emerging from the potential power of the technology. As a result there are new international frameworks being adopted for regulation of gene drive research from funding through to execution of research projects, with a particular focus on the biocontainment of any animals that might be created. It is critical that balanced community engagement and appropriate research be undertaken to provide the substrate for informed public, scientific and regulatory debate. It is against this background that the pests of greatest consequence to Australia are being evaluated with particular reference to the practicality and desirability of the solutions that might be proposed. Social licence to operate is perhaps the most critical issue in the development and future of this technology. Dialogue, regarding the safety and efficacy of any proposed gene drive solution to a pest problem, with the public, with policy makers and with regulators will be as important as the development of the technology itself.

Key Words: CRISPR/Cas9

28 Editing the future of the domestic pig. S. Lillico*[1], C. Proudfoot[1], C. Burkard[1], F. Urnov[2], J. Oatley[3], B. Telugu[4], A. Mileham[5], and B. Whitelaw[1], [1]*The Roslin Institute, Roslin, Midlothian, Scotland;* [2]*Sangamo Biosciences, Richmond, CA, USA;* [3]*Washington State University, Pullman, WA, USA;* [4]*University of Maryland, Beltsville, MD, USA;* [5]*Genus Plc, DeForest, WI, USA.*

Selective breeding and more recently genomic selection have had huge impacts on the productivity of domestic swine. However, these approaches are limited to variation that can be readily identified in the breeding population. The genome editing revolution allows facile manipulation of mammalian genomes. Using these tools we have created multiple lines of modified domestic pig, with focus on the creation of novel traits of utility to the swine industry. Our current work encompassing traits such as germ cell ablation or resistance to viral diseases will be presented.

Key Words: pigs and related species, genome editing, CRISPR-Cas9, genetic improvement

29 The role of leptin in nonalcoholic obesity, diabetes and hepatic fibrosis. T. Tan*[1,2], Z. Song[1], Y. Xing[1,2], X. Hu[1,2], and N. Li[1,2], [1]*College of Biological Sciences, China Agricultural University, Beijing, China;* [2]*State Key Laboratory for Agro-Biotechnology, Beijing, China.*

The studies of human obesity disease and related metabolic complications mainly based on rodents models. However, there are significant differences in physiology between human and rodents. In contrast, pig shows many similarities not only in the physiological structure and function, but also in the metabolic characteristics and genetic background. In this research, we generated a pig model of obesity via ZFN-mediated knockout system. The ZFN mutation rate reached 8.3%. Off-target effects have not been detected in Leptin$^{-/-}$ individuals. Leptin$^{-/-}$ pigs showed largely increased obesity related phenotypes corresponding to human. It has significantly improved appetite and body index especially weight. MSCT examination showed a significant increase of body fat rate in mutant pigs, which reflected in the subcutaneous fat and visceral fat. The Leptin$^{-/-}$ pigs showed significant increase in blood glucose concentration in the age of 12 months, and developed severe insulin resistance. By IVGTT assay, the blood glucose regulatory function is disordered in Leptin$^{-/-}$ pigs. Moreover, by HE and IHC analysis, we found that the Leptin$^{-/-}$ pigs show serious liver lesions, such as

fatty liver and hepatitis particularly hepatic fibrosis, which is different from the results previously reported based on mouse models. This may imply that leptin plays different roles in the development of liver fibrosis in pigs. We were surprised at finding the expression of cytochrome oxidase was up-regulated in Leptin$^{-/-}$ pigs. A series of markers also confirmed the enhanced oxidative stress in the Leptin$^{-/-}$ pigs' liver contrary to the results in ob/ob mice. We have confirmed that leptin deficiency enhanced the βoxidation and triglyceride accumulation via JAK-STAT pathway and inhibited the expression of sirt1 via AMPK pathway. So parkin-mediated mitochondrial autophagy which activated by down-regulated sirt1 and enhanced βoxidation played common role in making liver injury secondary hit significantly enhanced. It resulted in deterioration from adipose degeneration to fibrosis in liver. There are some similarities between the pig and human in hepatic fibrosis caused by obesity, so our research provided a new model and a new approach to treat hepatic fibrosis disease.

Key Words: leptin knockout, pig, obesity model, fibrosis, oxidative stress

30 Developing and exploiting new technologies to advance understanding of the avian immune system. A. Balic*, H. Sang, and M. McGrew, *The Roslin Insitute, University of Edinburgh, Edinburgh, Scotland.*

Global poultry production is increasing rapidly, especially in developing countries where poultry are a major source of animal protein. While we have a good understanding of how the mammalian immune system develops, this is not the case for birds. Indeed while birds face similar pathogen challenges to mammals, they have a different repertoire of organs, cells, molecules and genes of the immune system. To address this deficit in our understanding we have generated several transgenic cell reporter lines of chickens which allow the avian immune system to be visualised and manipulated, including transgenic chickens in which either all haematopoietic cells (RUNX1-eGFP line) or macrophages (CSF1R-mApple) can be visualised in embryos and post-hatch birds. In addition, we have produced several additional lines of chickens which utilise the CreloxP system to allow single- or multicolour cell lineage tracing. Using these novel bird lines lines have identified different populations of antigen-presenting cells (APC), such as macrophages and classical dendritic cells and are currently using these lines of birds to further elucidate the development and function of the avian immune system. More recently we have developed gene editing technologies for modifying chicken primordial germ cells (PGCs), as a route to production of genome-edited birds, utilising either transcription activator-linked nucleases or the CRISPR-Cas9 system. In our hands CRISPR/Cas9 gene editing technology has proven to be a relatively simple, highly efficient and precise method for genetic modification of chickens which opens up many potential applications and benefits for avian researchers.We have developed an efficient method for the long-term culture and cryopreservation of PGCs, demonstrated that these PGCs can used to efficiently produce site-directed genome knockout chickens and crucially, has used these technologies to produce a gene knockout chicken in which homozygous mutant chickens are sterile due to the absence of PGCs. We will discuss further applications of these genetic engineering and genome editing approaches to further the reduction of production losses and enhance the selective breeding of more robust, healthy chickens

Key Words: chicken, transgenic, immune, CRISPR-Cas9, PGC

Horse Genetics and Genomics

31 Genetic diversity, evolution and selection in the major histocompatibility complex *DRB* and *DQB* genes in the family Equidae. M. Klumplerova[1], P. Splichalova[1], J. Oppelt[2], P. Musilova[3], S. Kubickova[3], R. Vodicka[4], J. Vahala[5], L. Orlando[6], and P. Horin*[1], [1]*Ceitec VFU, University of Veterinary and Pharmaceutical Sciences, Dept. of Animal Genetics, Brno, Czech Republic;* [2]*Ceitec MU, Masaryk University, National Centre for Biomolecular Research, Faculty of Science, Brno, Czech Republic;* [3]*Veterinary Research Institute, Dept. of Reproduction and Genetics, Brno, Czech Republic;* [4]*Zoo Prague, Prague, Czech Republic;* [5]*Zoo Dvur Kralove nad Labem, Dvur Kralove nad Labem, Czech Republic;* [6]*Centre for GeoGenetics Natural History Museum of Denmark University of Copenhagen, Copenhagen, Denmark.*

The objectives of this study were to study the genomic diversity, evolution and selection of the major histocompatibility complex (MHC) class II *DRB* and *DQB* genes in the family *Equidae*. Two individuals of *Equus caballus, Equus przewalskii, Equus asinus asinus, Equus africanus somaliensis, Equus kiang, Equus hemionus kulan, Equus quagga burchellii, Equus quagga boehmi, Equus quagga chapmanni, Equus quagga borensis, Equus grevyi* and *Equus zebra hartmannae* were used for this purpose. All currently available genomic resources were used for phylogenetic and selection analyses. Due to their functional importance, exon 2 sequences were analysed. Locus specific horse primers were designed and used for amplifying genomic exon 2 sequences in all species studied. Next-generation and Sanger sequencing combined with cloning were used for assessing the genomic sequence variation. Maximum likelihood phylogenetic trees were constructed and site-specific selection analyses were carried out using standard bioinformatic tools. Three *DRB* and two *DQB* genes were identified in the genomes of all equids. A third DQB locus was found in all members of the family except the asses *Equus hemionus kulan* and *E. h. onager.* The loci *DRB2*, *DRB3* and *DQB3* showed little differences, while *DRB1* and *DQB1* seemed to be less similar across all species analysed. The *DQB2* locus showed large differences among individual genomes. Some of the data obtained could be explained by within-species copy number variation contributing to the MHC diversity. Evidence for recombination was found for the *DQB1*, *DQB2*, *DRB1* and *DRB2* loci. It thus seems that with their at least three *DQB* genes, equids are an example of mammals characterised by a complex *DQB* MHC sub-region. Allele sharing was identified in all loci with the exception of *DRB1*. Site-specific selection analysis predicted loci under positive selection both in *DRB* and *DQB* loci. No selected amino acid sites were identified in *DRB2* and in *DQB3*. These data, along with phylogenetic trees clearly deviating from neutrality support the assumption that important pathogen-driven positive selection formatted the MHC class II *DRB/DQB* sub-regions in the *Equidae.*

Key Words: horses and related species, comparative genomics, DNA sequencing, MHC, biomedical model

32 The potential of Y-chromosomal markers for individual lineage tracking in horses. S. Felkel[1], D. Rigler[1], C. Vogl[1], M. Neuditschko[2], S. Rieder[2], V. Jagannathan[3], T. Leeb[3], T. Rattei[4], C. Schlötterer[5], G. Brem[1], and B. Wallner*[1], [1]*Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Vienna, Austria;* [2]*Agroscope, Swiss National Stud Farm, Avenches, Switzerland;* [3]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland;* [4]*Department of Microbiology and Ecosystems Science, Division of Computational Systems Biology,*

University of Vienna, Vienna, Austria; [5]Institute of Population Genetics, University of Veterinary Medicine Vienna, Vienna, Austria.

Like mitochondrial DNA, the Y chromosome is a powerful marker for studying the sex-specific history of populations. They enable to investigate the history of male and female foundation lines in a pedigree independent way. Stallion lines have an enormous role in horse breeding, but the low sequence diversity of the horse Y-chromosome has so far hindered the genetic tracing of male genealogies in a fine-grained way. We overcame this limitation by generating an extensive horse Y-chromosomal reference genome for horses. This reference enables calling of variants in a region covering 1.46 Mb of the male-specific region of the Y-chromosome using NGS data. Here we show two examples for elucidating the ancestry of founder studs within a breed. Based on a backbone Y-chromosomal phylogeny consisting of 24 haplotypes ascertained from 52 horses of 21 modern breeds, we first assign the two founder lineages of the American Saddlebred (Harrison Chief and Gaines' Denmark) to their respective English Thoroughbred ancestors by simply genotyping present Saddlebred stallions for already defined polymorphisms. In the second example we ascertained markers specific for four founder lineages in the Franches-Montagnes breed using NGS and pedigree data from 21 stallions. In addition to specific haplotypes ascertained for each lineage, we also detect three de-novo mutations that occurred during the time frame of written records. This de-novo mutations result in derived haplotypes and therefore distinguish even sub-branches within a lineage. Our setup can be performed in any breed. Combined with a target-enriched sequencing approach it has potential for high-throughput development of lineage characteristic Y-chromosomal markers.

Key Words: horses and related species, evolutionary genomics, genotyping, breed/population identification, Y-chromosome

33 Zooming in on chronic progressive lymphedema using a high-density array in the Belgian draught horse. L. François*[1], A. Schurink[2], B. Velie[3], A. Stinckens[1], S. Blott[4], B. Ducro[2], C. Lamberigts[5], S. Tinel[1], K. De Keyser[1], M. Oosterlinck[6], G. Lindgren[3], S. Janssens[1], and N. Buys[1], [1]*Research Group Livestock Genetics, Department of Biosystems, KU Leuven, Leuven, Belgium;* [2]*Animal Breeding and Genomics Centre, Wageningen University & Research, Wageningen, the Netherlands;* [3]*Department of Animal Breeding & Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [4]*School of Veterinary Medicine & Science, University of Nottingham, Leicestershire, United Kingdom;* [5]*Research Group Livestock Physiology, Department of Biosystems, KU Leuven, Leuven, Belgium;* [6]*Department of Surgery and Anesthesiology of Domestic Animals, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*

Historically, the Belgian draught horse has been globally indispensable in its role as a working horse. However, due the increasing mechanization of agricultural production, the demand for a such a powerful horse has declined rapidly and the breed is now a recognised living heritage breed. One of the issues threatening the future of this breed is the occurrence of chronic progressive lymphedema (CPL) which leads to progressive lower limb swelling and deformation of the soft tissue. While the underlying cause of this condition is still under debate, two diverging hypotheses have been formed. The first hypothesis considers alteration in the skin elastic system as the initial cause, yet the second hypothesis regards CPL as an inflammatory dermatitis. The current study used 301 Belgian draught horses genotyped on the Affymetrix 670K array to assess the genetic background of this disease by performing genome-wide and homozygosity association analyses. The genome-wide association study reached genome-wide significance on ECA10 as well as nominal significance on several additional chromosomes. Several candidate genes have been proposed previously using a candidate-gene approach or whole-genome scan based on microsatellites. None of these can be found in the vicinity of ECA10; however, three are located in the proximity of regions reaching nominal significance: *FOXC2* (ECA3), *HET/MET* (ECA4), *ubiquitin protein ligase E3A* (ECA1), and *CD109* (ECA20). These candidate genes cannot exclude either hypothesis. All regions passing nominal significance in the genome-wide and homozygosity association analyses were subsequently analysed using PANTHER which ultimately suggested the involvement of several processes of the immune response. This seems implies an inflammatory dermatitis as the primary cause of CPL although the presence of alterations in the skin elastic system as cause cannot be excluded. These results provide the first insight into the genetic background of CPL using a dense marker set. Additional studies are necessary for confirmation and look deeper into the genetic mechanisms underlying CPL.

Key Words: horses and related species, genome-wide association, complex trait

34 Genetic contributions to measured speed in Thoroughbred racehorses during early training. G. Farries*, B. A. McGivney, K. F. Gough, L. M. Katz, and E. W. Hill, *University College Dublin, Belfield, Dublin, Ireland.*

Higher speed during sprint bouts in two year old horses has been associated with improved racing career outcomes in racing Thoroughbreds (Santschi *et al*., 2017). We hypothesised that variation in early measures of speed is heritable. Using GPS and heart rate monitoring during high intensity sprint bouts (work days, WD) we derived speed indices (V_{peak}, Acc, aveSpr, Dist6a, Dist6b, Dist6) to be used as phenotypes for genome-wide association studies using n = 131 horses (69 male, 72 female) genotyped across 49,720 SNPs. All horses were less than three years of age (mean = 2.12 years; range = 1.67–2.96) and had completed less than four WDs before measurement. Sex, jockey and track condition were used as covariates. The speed phenotypes were refined by performing principal component analysis—principal component 1 (PC1) explained 67.8% of the variance across the speed indices. PC1 was largely determined by: V_{peak} (0.41), aveSpr (0.32), Dist6a (0.47), Dist6b (0.46) and Dist6 (0.49). Using PC1 as a phenotype, no SNP reached genome-wide significance ($P_{UC} < 3.2 \times 10^{-5}$). However a candidate 19kb region on chromosome 14 was identified, containing three genes: *PCDHGC5 (Protocadherin Gamma-C5)*, *PCDHGB5 (Protocadherin Gamma-B5)*, and *SLC25A2 (Solute Carrier Family 25 Member 2)*. Protocadhedrins are involved in calcium ion binding and the *PCDHB15* (*Protocadherin Beta 15)* gene, 25kb downstream from the candidate region, was found to be differentially expressed ($P < 0.05$) in equine skeletal muscle in response to acute exercise (RNA-seq, n = 27). Several other protocadhedrins were differentially expressed ($P < 0.05$) in equine skeletal muscle in response to both acute exercise and training. Incorporating field exercise measurements along with genomic and transcriptomic data from racing Thoroughbreds will provide insight into the genetic regulation of exercise responses in a highlyadapted athletic animal model.

Key Words: horses and related species, genome-wide association, functional genomics, athletic performance

35 Whole-genome sequencing reveals two Shetland pony specific variants affecting body size and shape. J. Metzger*, F. Naccache, A. Christmann, and O. Distl, *Institute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover Foundation, Hannover, Niedersachsen, Germany.*

The Shetland pony represents a particularly small horse breed with a characteristically large head and strong built often accompanied by thick mane and tail hair and a general robustness. This study aimed at investigating whole genome sequencing data from Shetland ponies for potential signatures of selection and deleterious

variants in these highly selected regions, which might be responsible for the expression of a reduced body size and a Shetland pony specific shape. Runs of homozygosity (ROH) detection performed in three Shetland ponies, revealed 460 shared ROH regions harboring 1494 genes and 5826 variants with predicted high or moderate effects. Filtering for variants in these ROH regions with homozygous mutant genotypes exclusively found in Shetland ponies revealed two missense variants located in genes involved in growth regulation and bone development. The variant effects were predicted to be tolerated (0.07) for the missense/splice region variant as well as deleterious (0.00) for the missense variant. Further validation of these variants in ponies and horses of different breeds confirmed that both variants could be exclusively found in the Shetland pony and in the closely related Classic pony. In one of these variants the homozygous mutant genotype was only present in Shetland ponies smaller than 87.9 cm height at the withers and could therefore be proposed to be fixed in the Miniature Shetland pony population. None of the other investigated pony and horse breeds, Przewalski horses and donkeys harbored the mutant allele. In conclusion, our study revealed two Shetland pony specific variants of which one variant was private for Miniature Shetland ponies and potentially fixed by targeted selection of this specific pony type during domestication. Thus, these investigations add further two candidate genes to the genetically complex development of body size and shape in horses.

Key Words: horses and related species, homozygosity, DNA sequencing, candidate gene

36 Epigenetic characterization of centromeric chromatin in equids. S. G. Nergadze[1], R. Gamba[1], F. M. Piras[1], E. Cappelletti[1], M. Corbo[1], F. Gozzo[1], D. Miller[2], D. Antczak[2], E. Raimondi[1], K. Sullivan[3], and E. Giulotto*[1], [1]*University of Pavia, Department of Biology and Biotechnology, Pavia, Italy;* [2]*Cornell University, College of Veterinary Medicine, Ithaca, NY, USA;* [3]*National University of Ireland, Centre for Chromosome Biology, Galway, Ireland.*

Mammalian centromeres are typically associated with highly repetitive DNA (satellite DNA), which has so far hindered a detailed molecular analysis of this chromatin domain. A large body of evidence indicates that centromeres are epigenetically specified and that binding of the CENP-A protein is their main determinant. Previously (Wade *et al*. Science 2009; Piras *et al*. PLoS Genetics 2010) we showed that, during the evolution of the genus *Equus*, several centromeres moved to new sites lacking satellite DNA. In this system the epigenetic marks related to the centromere can be studied by comparing the centromeric domain of a species with the noncentromeric orthologous locus in other species. We also demonstrated (Purgato *et al*. Chromosoma 2015) that the location of the CENP-A binding domain can vary in different individuals giving rise to *epialleles*, proving that centromeres are autonomous relative to the DNA sequence and are characterised by positional instability. Here we present the results of ChIP-seq experiments with anti-CENP-A antibodies in horse, donkey and zebra cell lines, which led to the precise localization of several satellite-less centromeric domains in the genome of these species. Thanks to this powerful model system we were able to evaluate the possible role of DNA composition, sequence amplification and DNA breakage in centromere specification. In addition, we characterised the association of the centromeric function with several epigenetic features such as DNA methylation and histone modifications. The transcription of satellite-less centromeric domains was also tested. The possible implications of these findings will be discussed. Taking advantage of hybrid families of horse, donkey and mule individuals, the inheritance of centromeric domains through generations was studied. The transmission of centromeric domains in clonal cell populations was also evaluated. Taken together, our findings demonstrate that, in the genus *Equus*, centromeres are extraordinarily plastic and represent an important driving force in genome evolution.

Key Words: horse genome, genus *Equus*, centromere, epigenetic marks, genome evolution

37 Unraveling gene function using co-expression networks in the domestic horse. R. Schaefer*[1], E. Norton[1], J. Mickelson[2], and M. McCue[1], [1]*Department of Veterinary Population Medicine, University of Minnesota, St Paul, MN, USA;* [2]*Department of Veterinary and Biomedical Science, University of Minnesota, St Paul, MN, USA.*

Genome-wide studies in the horse have successfully been used to describe domestication and selection, clinical disease, athletic performance, and population structure and dynamics. Despite their success, the causal genes and functional mechanism underlying many of these studies remain unknown. For example, our recent GWA study of Equine Metabolic Syndrome (EMS) identified >2,000 SNPs representing 183 regions of the genome that were associated with 11 different monomorphic, biochemical and hormonal traits defining EMS. Identifying genes and alleles within the ROIs these contributing to EMS pathophysiology is an arduous task as: 1) these ROIs span ~98.5M base pairs and contain >3,000 genes; 2) little is known about EMS cellular and molecular pathophysiology, thus prioritization is biased towards genes characterised in human/model organisms; and 3) many genes have entirely unknown or unanticipated functions. To mitigate these issues, here, we systematically integrate whole genome SNP data, tissue specific RNAseq, and serum metabolomic data in order to better describe and inter-relate putative genomic loci associated with EMS. Independently, tissue specific gene co-expression networks were built from skeletal muscle and tail-head adipose depot in 28 horses from four breeds that displayed varying signs of EMS. Differential expression analysis between extreme phenotype scores quantifying EMS in these 28 horses identifies 529 genes among muscle and adipose tissues indicating these tissues are biologically active for our trait. In muscle/adipose networks, there were 6.5X and 8.3X enrichment for co-expression among genes within AgriGO terms demonstrating these networks capture biologically relevant information. Functional analysis for EMS was performed by directly extracting co-expression interactions among genes that were within breed specific haplotype windows containing associated GWAS SNPs. From our starting set of 2,375 associated GWAS SNPs we discover 259 genes with co-expression evidence related to EMS. Future work will focus on building disease specific, differential co-expression networks as well as corroborating metabolite abundances from Welsh Ponies affected by EMS.

Key Words: horses and related species, functional genomics, network analysis, bioinformatics tools, genome wide association

38 Mapping transcriptional regulation at multiple layers using ChRO-seq. T. Chu[1,2], L. Choate[1,2], Z. Wang[1,2], E. Rice[1,2], and C. Danko*[1,2], [1]*Baker Institute for Animal Health, Ithaca, NY, USA;* [2]*Cornell University, Ithaca, NY, USA.*

The annotation of functional non-coding regions in our genomes has proven challenging, requiring large numbers of time-consuming molecular assays to exhaustively identify a pantheon of distinct varieties of functional elements. Here we show that genome-wide maps of nascent transcription can recognise multiple varieties of activating functional elements in mammalian genomes. In addition to providing robust measurements of gene and lincRNA expression levels, transcription can be used to accurately impute the levels of activating histone modifications, DNase-I hypersensitivity, and transcription factor binding when used in combination with sensitive machine learning tools. To facilitate the use of this technology across a wide variety of sample types we developed chromatin run-on and sequencing (ChRO-seq), a novel molecular tool that maps

the location of RNA polymerase starting with virtually any cell or tissue sample. Notably, because ChRO-seq measures transcription through intact protein-DNA interactions, our strategy can be used to measure gene expression in tissue samples even after RNA degradation. To illustrate the applications of these tools for understanding the molecular basis of complex disease, we used ChRO-seq to analyse dozens of primary human brain tumours. Our integrative analysis revealed that whereas malignant brain tissue largely retained enhancers that were DNase-I hypersensitive in the tissue of origin, a rare population of ectopic enhancers resembled fetal tissues isolated from the nervous system, consistent with the cancer stem-cell hypothesis. We used these maps to identify transcription factors driving gene regulatory changes in the tumour. Our new technologies have implications for efficiently annotating mammalian genomes as well as for understanding the molecular basis of disease.

Key Words: functional genomics, genome annotation, epigenomics, machine learning, gene expression

39 EquCab3. T. Kalbfleisch*[1], M. DePriest[1], L. Orlando[2], and J. MacLeod[3], [1]*University of Louisville, Louisville, KY, USA;* [2]*University of Copenhagen, Copenhagen, Denmark;* [3]*University of Kentucky, Lexington, KY, USA.*

EquCab3 is now complete and has been released to NCBI and ENSEMBL for annotation in their respective pipelines. This is the culmination of 3 years work on the project, yielding significant improvements in both contiguity and composition. Additional sequence data for Twilight (the Thoroughbred mare on which the reference is based) has been incorporated to the previous assembly comprised of Sanger data. New datasets were produced using Illumina, PacBio, 10X Genomics, Chicago (Dovetail), and HiC platforms/libraries. We will report on the repeat structure of the genome, as well as the amount of structural variation found within Twilight's genome both of which challenged our efforts to produce a haploid representation of her genome.

Key Words: equine, horse, reference genome

40 Protein-coding gene and transcript sequences quantify progress toward the new equine reference genome assembly. M. S. DePriest*[1,2], J. N. MacLeod[2], and T. S. Kalbfleisch[1], [1]*University of Louisville, Louisville, KY, USA;* [2]*University of Kentucky, Lexington, KY, USA.*

The current version of the equine reference genome (EquCab2) was assembled entirely from Sanger sequence reads and published in 2009. Due to limited sequence read coverage and technological issues with Sanger data, some functional genes that are highly conserved in other mammals are not present in EquCab2. The new reference assembly, EquCab3, incorporates newer sequencing technologies that offset the weaknesses of Sanger data. Based on a comparison of mammalian protein-coding gene lists, we identified 1,430 protein-coding genes present in other mammals but not listed in EquCab2. We used tblastn to search for these genes in EquCab3 and found that the new reference assembly incorporates 758 of these genes. When we mapped RNA-Seq data to both EquCab2 and EquCab3, the mapping rate was ~2.5 percentage points higher for EquCab3 than for EquCab2. Also, 7,426 transcripts generated from the EquCab3 RNA-Seq mapping had truncated alignments in EquCab2, indicating that they had been extended in EquCab3. Finally, the distances between these transcripts and their nearest 5′ gaps tended to be longer in EquCab3 than in EquCab2, suggesting that missing 5′ regulatory regions of these genes had been captured in the new reference. These comparisons of functional loci demonstrate that EquCab3 is more complete than EquCab2.

Key Words: horses and related species, genome annotation, comparative genomics, genome sequencing, RNA-Seq

41 Progress toward functional annotation of the equine genome. J. Petersen*[1], E. Burns[2], M. Bordbari[2], E. Scott[2], B. Ming-Whitfield[2], V. Affolter[2], C. Ramirez Alanis[2], M. Barro[2], M. Mack[2], G. Gianino[2], F. Gianino[2], E. Giulotto[3], K. Hilburger[2], T. Kalbfleisch[4], J. MacLeod[5], M. Mienaltowski[2], S. Katzman[2], T. Leeb[6], T. Raudsep[7], P. Saelao[2], S. Vig[2], H. Zhou[2], R. Bellone[2], and C. Finno[2] [1]*University of Nebraska-Lincoln, Lincoln, NE USA;* [2]*University of California-Davis, Davis, CA USA;* [3]*University of Pavia, Pavia, Italy;* [4]*University of Louisville, Louisville, KY USA;* [5]*University of Kentucky, Lexington, KY USA;* [6]*University of Bern, Bern, Switzerland;* [7]*Texas A&M, College Station, TX USA.*

High-quality reference genomes have accelerated the discovery of variants functioning to alter phenotype. However, significant phenotypic variation of traits associated with animal health and performance still cannot be explained. It is hypothesised that unexplained variation is due, in part, to alterations in genome regulation. As part of the international Functional Annotation of Animal Genomes (FAANG) initiative and with the overarching goal of understanding genome regulation in the horse, a biobank of tissue was generated from two adult Thoroughbred mares for tissue-specific assays to elucidate regulatory elements of the genome. As the objective of FAANG is to 'accelerate genome to phenome,' emphasis was placed on extensive phenotyping to allow for downstream data analyses with full knowledge of any pathology. Antemortem phenotyping included full physical examinations, lameness, ophthalmologic and neurologic evaluations, complete blood counts and serum biochemistries. At postmortem, all tissues were grossly and histologically evaluated to identify any subclinical pathology and characterise the cellular makeup of each tissue. Multiple aliquots of 86 tissues, 3 cell types, serum, whole blood, plasma, cerebrospinal fluid, synovial fluid, gastrointestinal contents, and mucosal samples were collected. Nuclear extraction was performed on 16 tissues for future DNase-I hypersensitivity assays. Initial funding, providing for RNA- and ChIP-sequencing of 8 tissues has been supplemented by the equine research community to allow for RNA-seq of 20 additional tissues in the first phase of this project. The involvement of the equine community has also led to additional data collection including: cell culture, centromere mapping, SNP genotyping, reduced representation bisulfite sequencing, karyotyping, and microbiome sequencing. Data generated to date includes whole-genome sequence (gDNA) and RNA-seq (small and mRNA). Datasets are made publically available as they are generated. ChIP-seq assays to investigate 4 histone marks are currently being optimized. These data represent a valuable advancement for connecting genome to phenome in the horse and will also provide for cross-species analyses of genome regulation.

Key Words: horses and related species, functional genomics, RNA-seq, ChIP-seq, genome regulation

Avian Genetics and Genomics

42 The evolving chicken genome reference. W. Warren*[1], L. Hillier[1], C. Tomlinson[1], P. Minx[1], M. Kremitski[1], T. Graves[1], S. Sullivan[2], I. Liachko[2], M. Delaney[3], J. Fulton[4], M. Abrahamsen[5], R. Hawken[5], M. Miller[6], and H. Cheng[7], [1]*Washington University School of Medicine, St Louis, MO, USA;* [2]*Phase Genomics Inc., Seattle, WA, USA;* [3]*University of California-Davis, Davis, CA, USA;* [4]*Hy-Line International, Dallas Center, IA, USA;* [5]*Cobb-*

Vantress Inc., Siloam Springs, AR, USA; [6]Beckman Research Institute, Duarte, CA, USA; [7]USDA-ARS, East Lansing, MI, USA.

Given the continued interest in further improvements to the chicken genome reference (Gallus_gallus-5.0) we have built a version Gallus_gallu-6.0 from longer single molecule read technology (SMRT) reads. Most assembled contigs were ordered and oriented (i.e. scaffolded) using a proximity-based map into 39 scaffolds at 94% of the assembly size; 865 contigs remained as unplaced. The scaffolds and contig sequences were aligned to the Gallus_gallus-5.0 reference or the published chicken genetic linkage map in order to break chimeric scaffolds and contigs. We found 87 scaffolds and 28 contigs required manual breaks due to *de novo* assembly or scaffolding error. At this phase Gallus_gallus-6.0 assembled bases were 1.05 Gb, with an N50 contig and scaffold lengths of 18 and 35 Mb, respectively. Of the 1.05 Gb genome, ~94% of the assembled bases have been anchored to chromosomes. Next steps will be to assign large unplaced contigs to the remaining unknown autosomes after validating no genetic linkage exists to any known autosomes. In addition, we plan to add novel reference sequence to clusters of immune system genes (MHC) that are challenging to *de novo* assemble and genotype. Furthermore, copy number variation within MHC genes are likely to play an important role in the host immune responses, thus, we will present local assemblies of phased haplotypes from commercial birds chosen for the homozygous state of MHC-B and MHC-Y regions. The Gallus_gallus-6.0 reference is a substantial improvement in base contiguity and autosomal assignment. However, the use of more targeted approaches will be required to elevate the chicken genome to levels comparable to human.

Key Words: chicken, genome, MHC

43 Low number of mitochondrial DNA sequences inserted into the turkey (*Meleagris gallopavo*) nuclear genome: Implications for evolutionary inferences. G. Schiavo[1], M. G. Strillacci[2], S. Bovo[1,3], A. Ribani[1], S. I. Roman-Ponce[4], S. Cerolini[2], F. Bertolini[1,5], A. Bagnato[2], and L. Fontanesi*[1], [1]*Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy;* [2]*Department of Veterinary Medicine, University of Milan, Milano. Italy;* [3]*Biocomputing Group, Department of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy;* [4]*Centro Nacional de Investigación en Fisiología y Mejoramiento Animal, Instituto Nacional de Investigaciones Forestales, Agricola y Pecuarias (INIFAP), Col. Centro Veracruz, Mexico;* [5]*Department of Animal Science, Iowa State University, Ames, IA, USA.*

Mitochondrial DNA (mtDNA) insertions have been detected in the nuclear genome of many eukaryotes. These sequences are pseudogenes that derives from the horizontal transfer of mtDNA fragments (both from coding and non-coding regions) into the nuclear genome, producing nuclear DNA sequences of mitochondrial origin, called *numts.* Some of these regions, that derives from recent insertion events in terms of evolutionary time, have high homology with the original mtDNA genome and may affect the interpretation of population genetic and phylogenetic studies based on mtDNA sequences. A few studies have shown that, in general, bird genomes contain a lower number of *numts* than mammalian genomes. At present in the turkey (*Meleagris gallopavo*) genome the frequency of these mtDNA originated pseudogenes is not known. In this study, we filled this gap providing a first picture of *numt* distribution in the genome of this avian species. The turkey reference genome (Turkey_2.01, GCA_000146605.1) was aligned with the reference linearized mtDNA sequence using LAST and BLASTN software. A total of at least 16 *numts* were identified using both software. A few of them were validated by amplifying and then sequencing the corresponding regions in a population of wild turkeys and in commercial and local lines or breeds. Identities between *numts* and the corresponding mtDNA sequences ranged from ~70% to 100%, spanning from ~60 to 500 bp and representing in total only 0.0003% of the whole nuclear genome. These fewer *numts* do not cover the whole mtDNA genome. The largest *numt* was located on chromosome 2 in an intronic region of the *UFL1* gene and was 100% identical to part of the NADH dehydrogenase subunit 5 mtDNA gene (*ND5*). These results confirm that the low number of *numts* in bird genomes probably derives by an evolutionary selection towards compact genomes and reduced repetitive DNA regions where these mitochondrial pseudogenes are preferentially integrated.

Key Words: avian, turkey, numt, mtDNA, evolution

44 *In situ* and *in silico* improvement of the Japanese quail genome assembly. S. Galkina*[1], M. Kulak[1], A. Saifitdinova[1], A. Komissarov[1], A. Dyomin[1], V. Volodkina[1], J. Damas[2], M. Farre[2], D. Griffin[3], D. Larkin[2], and E. Gaginskaya[1], [1]*Saint-Petersburg State University, Saint-Petersburg, Russia;* [2]*Royal Veterinary College, University of London, London, UK;* [3]*University of Kent, Kent, UK.*

The Japanese quail *Coturnix coturnix japonica* is a biomedical model species and one of the highest-producing poultry species. It has a relatively small genome (≈1.41 Gb) packed into 39 chromosome pairs. The first draft of its genome assembly was released in 2013. Since 2016 the quail genome assembly comprising of 32 (of 38+Z and W) linkage groups is available (https://www.ncbi.nlm.nih.gov/genome/?term = japanese+quail). However, it contains a significant number of sequence gaps due to the presence of repetitive coding and non-coding DNA elements that complicate contiguous assembly. Importantly, the repetitive DNA elements play a crucial role in evolution of chromosome structure and regulation of gene expression. Here, (1) we identified new highly repeated sequences of Japanese quail genome within unassembled short raw reads and mapped them *in silico* and *in situ*. (2) We assembled the Japanese quail rRNA gene cluster on the basis of raw read library using Geneious 9.0.5 software package. Repeat searching and annotation were done by Repeatmasker 4.0.5. (3) To verify the quail draft genome assembly we performed systematic ZOO-FISH experiments on Japanese quail chromosomes with chicken BAC clone probes from CHORI-261 chicken BAC library. BAC clones were selected according to their chromosomal positions and assigned for specific sequence markers and high cross-species hybridization efficiency. All BACs used were positioned in the quail genome assembly. In some cases we have found discrepancies between positions of the markers in the quail genome assembly and physical maps, caused by the newly identified interspecific rearrangements. Support: 'Chromas' Research Resource Center and Theodosius Dobzhansky Center for Genome Bioinformatics (SPbSU), RFBR #15–04–05684 & #16–04–01823.

Key Words: *Coturnix coturnix japonica*, repeat, rRNA cluster, rearrangement

45 The broiler chicken transcriptome. C. Schmidt*[1] and S. Lamont[2], [1]*University of Delaware, Newark, DE, USA,* [2]*Iowa State University, Ames, IA, USA.*

Next-generation RNA-seq transcriptome analysis provides a deep description of the genes expressed in tissues. We have sequenced over 10 billion Illumina reads from multiple tissues isolated from the Ross 708 broiler line of chickens. Tissues sampled include the pituitary, hypothalamus, retina, pineal, cerebellum, heart, breast muscle, liver, spleen, intestine and adipose tissue. Relative tissue analysis has been used to compare between tissues and identify known and novel sequences that are enriched in each examined tissue. In addition, we have analysed the impact of chronic heat stress on gene expression patterns in these broiler chickens. These studies have identified genes up or down-regulated by heat stress and the pattern of responsive genes varies across organs. Finally,

a combination of transcriptomic and metabolomic approaches has provided an integrated description of the liver's response to chronic heat stress in the Ross 708 birds.

Key Words: chicken, transcriptome, metabolome

46 The not-so-missing genes in birds. T. Hron*, H. Farkasova, P. Pajer, J. Paces, P. Bartunek, and D. Elleder, *Institute of Molecular Genetics of the AV CR, v.v.i., Vídenská, Prague, Czech Republic.*

Thanks to the advances in high throughput sequencing methods and data analysis, genome of the huge number of species has been recently read and assembled. Despite of this, subset of essential and well-described mammalian genes, such as erythropoetin, leptin or TNF α, have not been identified in birds and their existence has been controversial for a long time. After thorough analysis of combined sequencing data with high coverage, we were able to identify some of these genes and thus prove their existence. Interestingly, all these 'missing' genes are characterised by exceptionally high GC content and long G/C stretches. Such characteristics cause difficulties in PCR amplification preventing efficient sequencing and can, therefore, lead to the absence of these sequences in databases and genome assemblies. This observation is probably general and seems to apply for a significant portion of avian genes thought to be missing. In all genes we analysed, the GC richness was observed exclusively in the avian orthologs and not in the orthologs of other species. This arises the question what is the cause of such an extreme evolutionary driven accumulation of GC nucleotides in this subset of avian genes. We hypothesise that these regions are important for formation of G-quadruplex structures involved in the pairing of homologous chromosomes during meiosis. However, this possibility has to be further investigated. Our work also demonstrates that sequences biased in their nucleotide content are often underrepresented in sequencing data. Thus, genome assemblies should be treated with caution.

49 Evaluation of semen characteristic of the high and low sperm motility groups in two different strains of chicken. M. Farahi, A. A. Masoudi*, and A. Ehsani, *Tarbiat Modares University, Tehran, Tehran, Iran.*

Successful fertility in males is very important in the poultry industry. One of the key factors affecting the fertility of males during the lifetime is semen quality. The aim of this study was to understand the trends of the semen parameters quality in two different groups of high and low sperm motility in two different strains (commercial and indigenous). An experiment was started by sperm motility evaluation of Arian dame line and Urmia native roosters at the age of 27 weeks. Then the samples were allocated to two High Motility level (HML) and Low Motility Level (LML) for next analyses. In the following sperm parameters, sperm viability and sperm integrity was performed individually for each bird at regular intervals every other week for the eight-month course. The results indicated that although the trend of sperm motility was diverse among the groups, the sperm motility in HML was greater than in LML in both strains throughout the time of the semen production. In addition the trend of sperm motility in high and or low groups of commercial and native strains was almost the same. The results of the sperm motility showed an acceptable quality of sperm motility (0.58 to 0.87) for all the groups during the period of semen production. The sperm viability, however, showed a little reduction but generally the tendency of sperm viability was extremely constant across the production course in HML and LML of both strains. Sperm membrane integrity was significantly lower ($P < 0.05$) in LML of the native strain. Results of this research indicated that the birds ranking based on the semen characteristics at the early breeding of the chicks will be fixed across of the course of the bird rearing and therefore it could be a useful indicator of male selection in the poultry industry.

Key Words: chicken, fertility, pedigree line, sperm motility, native

51 Goose transcriptome provides insights into novel mechanisms of adipogenesis. G. Wang*[2,1], Y. Liu[1], L. Jin[1], D. Shang[1], C. Gill[2], M. Li[1], and J. Wang[1], [1]*Sichuan Agricultural University, Chengdu, Sichuan, China;* [2]*Texas A&M University, College Station, TX, USA.*

Goose is a widespread poultry species with large markets in several countries including China and France. Because geese are migratory birds, their metabolism has adapted to acquire a highly tolerable balance between the rapid intake of massive amounts of glucose before flight and energy consumption during flight. An understanding of the molecular basis of metabolism in geese will strengthen development of the consumer market worldwide, and could provide insights into human obesity-related metabolic diseases. The objective of this study was to investigate the network of genes affecting lipogenesis in geese. Our approach was to use RNA-seq to compare the transcriptomes of 3 tissues involved in adipogenesis (liver, abdominal adipose and subcutaneous adipose) from 3 geese fed a control diet and 3 geese fed a high-energy diet. RNA-seq reads were aligned to the goose genome sequence with Tophat. Between the case and control groups, we identified ~1,900 differentially expressed coding genes for liver, ~800 and ~500 differentially expressed coding genes for abdominal adipose and subcutaneous adipose, respectively. We found there was significant enrichment for metabolic-related pathways among the up-regulated genes. Interestingly, several cancer pathways, including the PI3K-AKT signalling pathway were significantly enriched among the down-regulated genes. Using cuffcompare, CPC, and cmscan, we also identified ~2,000 long non-coding RNAs among the 3 tissues. Several of these lncRNAs were located near key genes in the enriched pathways, indicating they are potentially cis-acting regulatory elements. These results suggest there may be a self-protection mechanism involved in metabolic adaptation in the goose.

Key Words: goose, transcriptome, adipogenesis

52 Integrating genome and transcriptome profiling for dissection of the mechanism of muscle growth and lipid deposition in ducks. L. Wang*, X. Li, J. Ma, Y. Zhang, and H. Zhang, *Lab of Animal Genetic Resource and Molecular Breeding, China Agricultural University, Beijing, China.*

The objectives here were to detect candidate genes on muscle growth and lipid deposition in Pekin ducks through comprehensive analysis combining whole genome sequencing (WGS) and RNA-seq. Pekin duck is famous for fast growth and high intramuscular fat (IMF). Cherry Valley Pekin duck (CD) had higher growth rate, whereas native Pekin duck (BD) deposited more lipids. Combing of WGS and RNA-seq has been widely used to explain genetic basis of different phenotypes. 80 individuals (40 BD and 40 CD) were slaughtered at 3- and 6-weeks of age. Phenotypes of water and IMF content, density and diameter of breast fibre were measured (n = 20). Genomic DNA was resequenced on Illumina HisEqn 4000 platform with PE-150bp. Transcriptomic data of breast were sequenced in HisEqn 2000 platform. BD ducks displayed higher IMF content than CDs at 6-weeks of age, while CD ducks displayed significant differences in both density and diameter of muscle fibres than BDs at 3-weeks. After screening for genomic variations, 206 positively selected genes (PSGs) from 224 windows were detected. Analysis of differentially expressed genes (DEGs) between periods detected 195 and 243 DEGs in BD and CD, respectively. At 3-week, the 52 DEGs between breeds were mainly involved in muscle tissue development. At 6-week, the 206 DEGs were mainly involved in PPAR and adipocytokine signalling pathways. After integrated analysis, 7 genes, both PSGs and DEGs, including PODXL, KY, FABP5, FLT1, ENPP3, NGEF, and NEU2 were confirmed. Another seven PSGs (TTN, TNNI2, CDH2, MEF2A, INSIG1, PCSK1, and PLPPR1) and five DEGs (FoxO3, MAT1A, TNNT2, ADORA1, and ACSL1) were also detected. Finally, we screened 19 candidate genes, of which, 11 (PODXL, KY, FLT1, NEU2, TTN, TNNI2,

CDH2, MEF2A, FoxO3, MAT1A, and TNNT2) were identified to regulate muscle growth involving in pathways like muscle organ development and muscle organ maintain. Eight genes (FABP5, ENPP3, NGEF, INSIG1, PCSK1, PLPPR1, ADORA1, and ACSL1) involving pathways like PPAR signalling were identified to regulate lipid deposition.

Key Words: ducks, integrative genomics, candidate gene

53 Liver and whole blood transcriptome response to chronic heat exposure in laying hens. F. Jehl[1], A. Rau[1], C. Désert[2,3], M. Boutin[2,3], K. Muret[2,3], S. Leroux[4], D. Esquerré[5], C. Klopp[6], D. Gourichon[7], F. Pitel[4], A. Collin[8], S. Lagarrigue[2,3], and T. Zerjal*[1], [1]*GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [2]*INRA, UMR1348 Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'élevage, Saint-Gilles, France;* [3]*Agrocampus-Ouest, UMR1348, Rennes, France;* [4]*UMR INRA/INPT ENSAT/INPT ENVT-GenPhySE, Castanet Tolosan, France;* [5]*INRA, Plateforme GENOTOUL, Castanet-Tolosan, France;* [6]*INRA, SIGENAE, Castanet-Tolosan, France;* [7]*INRA-PEAT, Nouzilly, France;* [8]*URA, INRA, Nouzilly, France.*

Adaptation to heat exposure is required to maintain animal welfare and productivity under high ambient temperature (AT) conditions. In this study we investigate the effects of chronic heat exposure (5 weeks at a constant temperature of 32°C) on the liver and whole blood transcriptome of brown egg layers from 2 divergent lines selected for low (R-) and high (R+) residual feed intake. The R+ and R- hens were equally distributed among 2 temperature-controlled chambers and reared under thermo-neutrality (22°C). At 28 wk of age the AT of one chamber was increased to 32°C until 33 wk of age, when 32 animals (8 per line and treatment) were slaughtered. Total RNA was obtained from the liver and blood and was sequenced using the Illumina HiSEqn 3000, yielding an average per sample of 90 million paired-end reads. The reads were mapped to the *Gallus gallus*-5 reference genome by STAR software and counted by RSEM software using the Ensembl V87 GTF annotation. Comparisons between the two AT groups were made using the *edgeR-robust* R/Bioconductor package. Patterns of AT-specific differential expression were largely shared by the two lines, and no evidence of temperature × line interactions were observed. In liver, a total of 229 differentially expressed genes (DEG) were identified (adjusted *P*-values < 0.05) with respectively 104 and 125 over and under expressed in the heat-exposed compared to the control group. In blood, 960 DEG were identified between the two AT groups with 479 and 481 over and under expressed. Most DEG were tissue specific, and only 18 genes were DE in both liver and blood. Ingenuity Pathway Analysis revealed that many of the DEG in liver were associated with amino acid and lipid metabolisms and energy production. Key genes involved in *fatty acid β-oxidation, ketogenesis, cholesterol biosynthesis were under-expressed in the heat-exposed animals.* In blood, many of the DEG were associated with cell related functions. Based on the DEG expression profile, down-regulation was observed for the PI3K/AKT, the VEGF and the PDGF signalling pathways involved in cell survival and growth, vasculogenesis and angiogenesis. Taken together, these results indicate a tissue-specific response to heat exposure.

Key Words: heat exposure, liver, blood, RNA-seq, laying hens

54 Mapping QTLs affecting Marek's disease by selective DNA pooling in eight lines across 15 generations. E. Lipkin*[1], J. Smith[2], D. Burt[2], M. Soller[1], and J. Fulton[3], [1]*Dept. of Genetics, Silberman Life Sciences Institute, The Hebrew University of Jerusalem, Jerusalem, Israel;* [2]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Midlothian, UK;* [3]*Hy-Line International, Dallas Center, IA, USA.*

Marek's disease (MD) is a major disease affecting the poultry industry. The aim of the present study was to map QTLs, genes and mutations affecting chicken MD mortality. Selective DNA pools were made of 9,391 sires of 8 lines and 15 generations per line, that had progeny test results for MD mortality. The pools were genotyped using an Affymetrix 600 K chicken SNP array. Allele frequencies were obtained by raw intensities of alleles A and B, B% = B/(A+B). The significance of allele frequency differences between sires with low or high progeny mortality was obtained using empirical SE. Moving average of -LogP and Log drop were used to define QTL regions (QTLRs). A total of 42 QTLRs were found among the eight lines, averaging 32 QTLs per line and ranging from 0 to 11. Overlap and proximity were used to condense the QTLRs to 28 QTLRs shared across families. The shared QTLRs averaged 1.2 Mb in length and covered a total of 33.3 Mb (3.1% of the entire genome). RNASeq and bioinformatics analyses of the QTLRs identified differentially expressed and functional candidate genes. Markers from the candidate genes were used to individually genotype 9,391 individuals from the same lines. Association analysis of the individual genotyping confirmed most of the QTLs found by the pools. Putative quantitative trait genes (QTG) and candidate causative nucleotides (QTN) were identified. This study confirmed most of the QTLRs found by the F6 study reported elsewhere at this meeting.

Key Words: poultry, genome-wide association, quantitative genetics, animal health, complex trait

55 Genome-wide association study of complex traits in response to Newcastle disease virus in chickens. K. Rowland*[1], H. Zhou[2], R. Gallardo[3], T. Kelly[2,3], A. Wolc[1,4], and S. J. Lamont[1], [1]*Iowa State University, Department of Animal Science, Ames, IA, USA;* [2]*University of California-Davis, Department of Animal Science, Davis, CA, USA;* [3]*University of California-Davis, School of Veterinary Medicine, Davis, CA, USA;* [4]*Hy-Line International, Dallas Center, IA, USA.*

Newcastle disease (ND) causes up to 80% mortality in chickens in developing countries where velogenic NDV strains are endemic. Genetic improvement of disease resistance, complementary to vaccination, has the potential to be an important tool to reduce the impact of NDV. We hypothesise that many genes regulate NDV response in chickens. Our specific objective was to identify genetic markers associated with NDV resistance (reduced viral load, high antibody titer) so these markers can be applied in a genetic selection program benefiting areas of endemic NDV challenge. The experiment was replicated across three hatches from 150 dams of a commercial egg-laying line, Hy-Line Brown. We inoculated with NDV, La Sota strain, on day 21 of age by an ocular-nasal route. Virus load was estimated from viral mRNA level in lachrymal fluid by qRT–PCR. Systemic antibody response to NDV in serum was measured by ELISA. Genomic DNA was genotyped via Affymetrix 600K chicken SNP array. Analyses of viral RNA and antibody levels confirmed response of challenge groups to the virus and lack of response in control groups. ASReml estimated heritabilities of 0.34, 0.34, 0.105, 0.117, 0.19, and 0.05 for hatch weight, day 31 body weight, 0 and 10 days post-infection (dpi) antibody levels, and 2dpi and 6dpi viral load, respectively. Genome-wide associations with 250 kb windows were tested using the Gensel program. BayesC was used to estimate variance components, subsequently, BayesB (pi=0.999) tested associations. Several QTL regions were found to confirm previously reported QTL for these traits. Identification of novel SNPs and regions will provide insights into genetic control of response to NDV infection in chickens as well as the genetic archi-

tecture of these complex traits. Targeted genotyping and single SNP association tests may further elucidate genetic mechanisms that influence host response to NDV infection. Support: USAID Feed the Future Innovation Lab for Genomics to Improve Poultry, Hy-Line International, and Hatch project #5357.

Key Words: poultry and related species, genome-wide association, genotyping, disease resilience, genetic improvement

Comparative MHC Genetics: Populations and Polymorphism

56 IPD-MHC 2.0: An improved interspecies database for the study of the major histocompatibility complex. G. Maccari*[1,2], J. Robinson[2,3], K. Ballingal[4], L. Guethlein[5], U. Grimholt[6], J. Kaufman[7], C. Ho[8], N. de Groot[9], R. Bontrop[9], P. Flicek[10], J. Hammond[1], and S. Marsh[2,3], [1]*The Pirbright Institute, Pirbright, Woking, Surrey, UK;* [2]*Anthony Nolan Research Institute, Royal Free Hospital, London, UK;* [3]*UCL Cancer Institute, Royal Free Campus, London, UK;* [4]*Moredun Research Institute, Pentlands Science Park, Scotland, UK;* [5]*Stanford University, Stanford, CA, USA;* [6]*Norwegian Veterinary Institute, Oslo, Norway;* [7]*University of Cambridge, Cambridge, UK;* [8]*Gift of Life Michigan, Michigan, USA;* [9]*Biomedical Primate Research Centre, Rijswijk, Netherlands;* [10]*European Molecular Biology Laboratory, Wellcome Genome Campus, Hinxton, UK.*

The IPD-MHC Database (www.ebi.ac.uk/ipd/mhc/) is a key resource for the collection, study and comparison of the major histocompatibility complex sequences from nonhuman species, providing the infrastructure and tools to enable accurate analysis. Since the first release in 2003, IPD-MHC has grown and currently hosts several specific sections, with more than 8,000 alleles from 76 species. Data is expertly curated and made publicly available through an open access website, recording an average of 1,500 unique visitors and more than 5,000 page views per month. As the database has grown in size and complexity, it has created several challenges in maintaining and organizing information, particularly the need to standardize nomenclature, while incorporating new allele submissions. To address these challenges a new version of the IPD-MHC Database was released in 2016, with the key aims of developing a universal cross-species data submission and display tool, aligned with the streamlining and standardisation of curator workflows. A new data submission system has been implemented to facilitate the inclusion of extensive metadata regarding MHC sequence origin and features. In the first eight weeks following release more than 500 new sequences were successfully submitted and curated. The advent of high-throughput sequencing technologies has allowed the generation of large amount of high quality data from highly polymorphic genes, providing the potential for extending the database coverage to include genomic sequences, rather than individual exons. For this reason the IPD-MHC Database has recently introduced the ability to analyse and annotate genomic data. These developments also incorporate a new internal sequence analysis tool that allows the automatic annotation of genomic data, giving users the ability to compare and analyse sequences at different levels of complexity. Furthermore, new tools are presented, enhancing database queries of high quality MHC data from an increasing number of species disseminated to the wider scientific community.

Key Words: bioinformatics, MHC, databases/repositories, polymorphism, sequence variation

57 Major update to the Swine Leukocyte Antigen (SLA) Nomenclature System of the International Society for Animal Genetics (ISAG) and the International Union of Immunological Societies (IUIS). S. Ho*[1], J. Lunney[2], A. Ando[3], C. Rogel-Gaillard[4], J.-H. Lee[5], L. Schook[6], and S. Hammer[7], [1]*Gift of Life Michigan, Ann Arbor, MI, USA;* [2]*USDA, Beltsville, MD, USA;* [3]*Tokai University School of Medicine, Isehara, Kanagawa, Japan;* [4]*INRA, Jouy-en-Josas, France;* [5]*Chungnam National University, Daejeon, Korea;* [6]*University of Illinois, Urbana, IL, USA;* [7]*University of Veterinary Medicine Vienna, Vienna, Austria.*

The SLA system is among the most well characterised major histocompatibility complex (MHC) systems in nonhuman animal species. The ISAG/IUIS SLA Nomenclature Committee was established 15 years ago with primary objectives to: 1) validate newly identified SLA sequences according to the guidelines established for maintaining high quality standards of the accepted sequences; 2) assign appropriate nomenclatures for new alleles as they are validated; and 3) serve as a curator of the IPD-MHC SLA Sequence Database (www.ebi.ac.uk/ipd/mhc/group/SLA), which is the repository for all recognised SLA genes, their allelic sequences and haplotypes. The newly released and improved IPD-MHC Database version 2.0 has incorporated the latest sequence updates, provide new tools that enhance database queries and improve the submission process. The SLA Nomenclature Committee met at last year's ISAG and made some major revisions to the allele naming system. The Committee decided to retire the provisional alphanumerical naming system for unconfirmed alleles and re-designate each allele an official number, adopting the HLA Nomenclature System with colons as field separators (e.g. SLA-1*01rh28 → SLA-1*01:03). Phylogeny will remain the primary approach for assigning SLA-1, -2, -3, DRA, DRB1, DQA and DQB1 alleles into allele groups with similar sequence motifs, while alleles of SLA-5, -6, -7, -8, -12, DMA, DMB, DOA, DOB1, DRB2, DRB3, DRB4, DRB5 are designated sequentially as they are discovered. Naming convention for alleles of other loci (SLA-4, -9, -11, DQB2, DOB2, DYB, MIC1, MIC2, TAP1, TAP2) is to be determined as sequences accumulate. There are currently 223 class I, 214 class II, 2 SLA-related and 2 non-SLA alleles officially designated. There are also 61 class I (SLA-1–3-2) and 49 class II (DRB1-DQB1) haplotypes designated at allele level resolution. This systematic nomenclature for SLA genes is critical to the understanding of the architecture and polymorphism of the SLA system and their role in swine diseases, vaccine development and allo- or xeno-responses in transplantation research.

Key Words: MHC, SLA, immunogenetics, polymorphism, nomenclature

58 Studies of MHC class II content in three common Arabian horse haplotypes. D. Miller*[1], A. Case[1], L. Younger[1], J. Tseng[1], H. Holl[2], Y. A. Mohamoud[3], A. Ahmed[3], J. Malek[3], S. Brooks[2], and D. Antczak[1], [1]*Cornell University, Ithaca, NY, USA;* [2]*University of Florida, Gainesville, FL, USA;* [3]*Weill Cornell Medicine, Doha, Qatar.*

Previous studies from our laboratory have identified microsatellite-defined haplotypes within the Major Histocompatibility Complex (MHC) of the horse, also known as the Equine Leukocyte Antigen (ELA) complex. We have developed a panel of 11 polymorphic microsatellite markers in the MHC region that span from 28.9Mb to 33.5Mb on horse chromosome 20, for a total coverage of 5.6 Mbp. This panel includes two markers in the MHC class I region, two in MHC class III, and seven in MHC class II. In identifying new microsatellite-defined haplotypes our strategy has been to test family groups (sire, dam, and offspring, or paternal half-siblings

and sires). Different horse breeds have very distinct complements of MHC haplotypes defined in this way. In a large sample of over 550 Arabian horses, we identified over 60 new haplotypes not observed previously in other breeds. We also found a small number of MHC homozygous Arabian horses that carried common Arabian haplotypes. In this study we examined ELA Class II gene and variant content in three microsatellite defined haplotypes commonly found in Arabian horses, namely COR007, COR008, and COR026. This study utilised Illumina sequencing of genomic DNA, SNP content from a commercial 670K genotyping array, and sequencing of full length cDNA transcripts from multiple Class II loci. Furthermore, we examined both homozygotes, and heterozygotes who shared a combination of these common haplotypes in both domestic (US) populations, and populations based in Qatar. We will report the sequences identified and compare them with MHC class II alleles that we described previously. The new full-length sequences will be deposited into the equine section of the IPD database. These data will increase our knowledge of ELA Class II gene polymorphism and structural variation with the Class II region. This study was made possible in part by NPRP Grant 6-1303-4-023 from the Qatar National Research Fund (a member of Qatar Foundation). The findings achieved herein are solely the responsibility of the authors.

Key Words: horses, immunogenomics, microsatellite, MHC

59 A rapid, direct sequencing–based MHC genotyping system for populations with insufficient information on allelic variation. J. Buitkamp* and J. Semmer, *Bavarian State Research Center for Agriculture Institute of Animal Breeding, Grub, Bavaria, Germany.*

The individual repertoire of MHC molecules determines which antigenic peptides are presented to the immune system. MHC coding genes belong to the most polymorphic genes in vertebrates and show a high degree of heterozygosity. Usually some main alleles with moderate frequencies occur aside with many rare alleles. Multiple polymorphic positions and different number of genes per haplotype hamper the correct definition of alleles. Haplotyping of heterozygous is particularly complicated when information on allelic variation is missing. We developed an efficient, sequence based MHC-genotyping system for poorly studied populations. It combines the initial definition of main alleles with a reference library that allows the rapid identification of alleles from heterozygous animals. The animals were Merino sheep from Bavaria. All lambs were genotyped for the microsatellite upstream from *DRB1* exon 2. Selected rams were genotyped for exon 2 of *DRB1* and *DQB* using multiple primer pairs that ensure amplification of a complete set of genes. From 500 lambs 53 were homozygote at the *DRB1* microsatellite. From these, 11 lambs representing all main alleles were sequenced at *DRB1* and *DQB* genes. In addition *DRB1* and *DQB* genes were sequenced from heterozygote animals carrying a rare allele in combination with a main allele allowing the determination of a large number of alleles occurring in the population using comparatively few animals. We identified 16 (including 3 new) *DRB1* alleles, and 19 (including 2 new) *DQB1* alleles. Using the sequences from homozygote lambs as a starting point, 11 MHC class 2 haplotypes could be defined. A reference database was build using these alleles in combination with all published alleles. To derive the correct alleles from heterozygote sequences a pipeline based on the blast algorithm was used that allows the identification of published and population specific MHC alleles. This system provides the basis for fast genotyping of MHC haplotypes and the identification of new rare alleles for small to medium scale projects in populations with limited knowledge of MHC diversity.

Key Words: sheep, immunogenomics, genotyping, MHC, animal health

60 Molecular characterisation of Ovar-MHC class II region reveals novel alleles in the Djallonke and Sahelian sheep breeds of Ghana. M. Yaro*[1], K. Munyard[1], E. Morgan[1], M. Stear[2], and D. Groth[1], [1]*Curtin University, Perth, WA, Australia;* [2]*La Trobe University, Melbourne, Victoria, Australia.*

The Djallonke is the most ancient sheep breed in Africa. It is more resistant to many livestock diseases including haemonchosis and trypanosomosis than the Sahelian sheep from the same region. Both sheep breeds are of immense socio-economic relevance in more than 14 countries within the sub Saharan region. Resistance to nematode infection in sheep has been associated with variations within genes in the MHC class II region. These genes encode glycoproteins that present antigen to circulating CD4+ T-cells, and the most polymorphic ones are reported to be the DRB1, DQA1 AND DQA2. In this study we conducted sequencing-based genotyping and analysis of the MHC class II DRB1, DQA1 and DQA2 loci in a population of 200 sheep from the Djallonke and Sahelian breeds from Ghana. Genomic DNA was genotyped with standard MHC primers and sequenced. New alleles and unresolved sequences were cloned into pGem-T easy plasmid vector, which then were used to transform *E. coli* competent cells and were sequenced in both directions using M13 primers. Preliminary results for both breeds show animals harbouring multiple amino acid substitutions (greater than 4) within the coding region of the second exon of DRB1 in reference to closest matched allele accessions at the Immuno-polymorphism database (IPD), suggesting novelty. Interestingly, there is a high frequency of variants of the FN543119.1 (ovar-DRB1*0901) allele only in the Djallonke population, and of EU176819.1 allele in the Sahelian population. Of particular interest is the presence of the FR751085.1 (ovar-DRB1*1303) allele in both sheep populations which was previously identified in the red Maasai sheep breed from Kenya. This suggests an ancestral relationship between these three African sheep breeds. To the best of our knowledge, this the first analysis of MHC architecture and allelic diversity in both breeds. Our ongoing studies aim to provide information that will enhance the sustainable management of these important sheep breeds.

Key Words: Djallonke, Sahelian, sheep, MHC, sub-Saharan Africa

63 Large-scale analysis of the specificities of livestock MHC class I and II molecules. D. B. Steen-Jensen[1], T. Østerbye[1], M. Rasmussen[1], M. Nielsen[2], A. Stryhn[1], and S. Buus*[1], [1]*Laboratory of Experimental Immunology, University of Copenhagen, Copenhagen, Denmark;* [2]*Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark.*

It is well established that T-cells recognise peptide epitopes presented in the context of MHC molecules, however, the identification and validation of T-cell epitopes is still a challenge; something that in real life situations is complicated by the size of pathogen 'peptidomes' and the extreme polymorphic/polygenic nature of the MHC of outbred host populations. Peptide binding to MHC is the most selective event involved in processing and presentation of antigens to T-cells and is therefore essential for our understanding of T-cell immunogenicity. We have proposed that the peptide binding specificity of all human MHC molecules should be mapped. To this end, we have developed recombinant human MHC class I and II molecules and generated the corresponding peptide-binding assays and large-scale affinity binding data. We have also confirmed that peptide-MHC stability is a better correlate of immunogenicity than affinity is, and begun to generate large-scale stability data. We have used the resulting data to develop a series of accurate bioinformatics predictors (e.g. NetMHCpan, NetMHCIIpan and NetMHCStab) and made these predictors publicly available. An efficient T-cell epitope discovery approach has transpired: a) use overlapping peptides representing proteins (or smaller proteomes) of interest to search for T-cell stimulatory peptides in outbred populations, then b) use

the above bioinformatics resources to identify T-cell epitopes and their restrictions elements, and finally c) generate the corresponding MHC class I or II tetramers to validate the proposed T-cell epitopes. To extend these efforts to other species, we have generated unbiased and generally applicable methods, which only depends on the sequence of the MHC in question being available, and successfully begun to address the specificity of pig, cattle, and avian MHC class I and II molecules.

64 The ligands and polymorphic residues of chicken MHC YF class I-like molecules. R. M. Goto[1], G. Gugiu[1], B. M. Stadtmueller[2], P. J. Bjorkman[2], and M. M. Miller*[1], [1]*Beckman Research Institute, City of Hope, Duarte, CA, USA;* [2]*California Institute of Technology, Pasadena, CA USA.*

Polymorphic amino acid residues in the binding groove of classical polymorphic MHC I molecules confer specificity for the types of peptides that bind to MHC class I and shape adaptive immune responses of conventional T lymphocytes. Non-classical MHC class I molecules direct responses of other lymphocyte populations by other means. Of particular interest among non-classical molecules are those encoded by polymorphic *YF* genes within MHC-*Y*, the second region of MHC genes in the chicken. It may be that MHC-*Y* genetics influences the incidence of infectious disease in chickens. Previous structural studies of YF revealed a binding groove too narrow to accommodate peptide ligands, but ligands have not been identified and how the polymorphic amino acid residues of different YF isoforms might affect ligand binding is not known. To define YF ligands, chicken gene sequences for YF1*7.1 heavy chain and β2 microglobulin (β2m) were expressed in *E. coli* using a recombinant expression vector to produce inclusion bodies. Inclusion bodies were purified, then YF1*7.1/β2m renatured first without added candidate ligands and then, in later experiments, renatured with added lipid extract from *E. coli* or with selected candidate ligands. To screen for bound ligand, renatured YF1*7.1/β2m was FPLC purified then analysed by ultra high performance liquid chromatography. Candidate ligands were identified in a Thermo Orbitrap Fusion mass spectrometer. YF1*7.1 ligands were found to be *lyso*-phospholipids including 17:1 *Lyso*-PE, 18:1 *Lyso*-PE and 17:1 *Lyso*-PG. The optimum fatty acid chain-length for ligand binding was 17 but *lyso*-phospholipids with 16 to 19 carbons were also bound. A second YF isoform also bound *lyso*-phospholipids even though differing from YF1*7.1 by 25 residues across the domains forming the ligand binding groove. Mapping the polymorphic residues for this second YF isoform and additional isoforms onto the original YF1*7.1 structure revealed that the side chains of polymorphic residues mostly point away from the ligand binding groove, suggesting that the polymorphic residues may be more important in recognition of different YF isoforms than in selection of ligand bound within the groove.

Key Words: chicken, major histocompatibility complex class I-like molecules, lyso-phospholipids, polymorphism

65 Evolution by gene duplication of the horse major histocompatibility complex class II structure. A. Viluma*, S. Mikko, G. Andersson, and T. F. Bergström, *Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences (SLU), Sweden.*

The major histocompatibility complex (MHC) is a gene dense genomic region that harbors multigene families encoding for class I and class II antigen presenting molecules. The MHC structure has been widely studied in primate species and it is known for variable number of paralogous loci and evolution by gene duplication. Recently, a high quality assembly and annotation of the horse MHC class II region was generated. In comparison to six other mammals (human, mouse, cattle, pig, dog and cat), the corresponding region in horse showed increased number of the *Eqca-DRB*, *-DQA*, *-DQB* and *-DOB* loci, as well as different relative location and directionality of five *Eqca-DRB* loci. The aim of this study was to better understand the molecular mechanisms that have shaped the horse MHC class II region by defining size and order of the duplication events and by investigating break point sequences of the duplicated blocks. The duplication blocks were defined by comparing the horse MHC class II sequence to itself using PipMaker. Furthermore, the intronic sequences of the individual gene families (*Eqca-DRB*, *-DQA*, *-DQB* and *-DOB*) were aligned and the genetic distances were calculated using Jukes-Cantor model. The results of the horse MHC class II sequence self-comparison showed a block-wise duplications of tail-to-tail oriented *Eqca-DQA* and *-DQB* gene pairs. However, the *Eqca-DRB* and *-DOB* paralogous loci were more likely to be individual duplication events of a single gene. The genetic distances of all six *Eqca-DRB* loci suggested that the inversion of *Eqca-DRB* locus predates the rest of the *Eqca-DRB* duplication events. The comparison of genetic distances of all the multigene family members indicated that expansion of the horse MHC class II region occurred in a relatively short evolutionary time period following the inversion of *Eqca-DRB* locus.

Key Words: MHC, horse, evolution, duplication

66 Chicken MHC-*B* diversity detected by a high-density SNP panel. J. E. Fulton*[1], B. Bed'Hom[2], and M. M. Miller[3], [1]*Hy-Line International, Dallas Center, IA, USA;* [2]*GABI, INRA, AgroParisTech, Jouy-en-Josas, France;* [3]*3Department of Molecular and Cellular Biology, Duarte, CA, USA.*

The chicken MHC was initially identified as the B blood group locus with variation detected serologically. Most early MHC-*B* studies were done with inbred lines or limited breeds due to the difficulties of using alloantisera to define MHC-*B* in heterogeneous outbred lines. The tandem repeat LEI0258 marker located within MHC-*B* has been particularly useful in identifying MHC-*B* haplotypes in multiple sources, including outbred populations. However, the LEI0258 marker has limitations in that the same allele size can occur in serologically different haplotypes and mutations occasionally arise within lines that change LEI0258 allele size. To provide a better typing method a single nucleotide polymorphism (SNP) typing panel encompassing 210K of the chicken MHC-*B* region was developed. The MHC-*B* SNP typing methodology is based in allele-specific PCR, with fluorescence detection of endpoint reads. The panel comprises 101 SNP with average spacing of 2,300 bp. The SNP are found in exons, introns or intragenic regions. For inclusion in the panel, SNP had to detect both alleles, be reliable in revealing genotypes, and consistently reveal the haplotypes in parent and offspring among animals with serologically known MHC-*B* haplotypes. This SNP panel allows a large number of samples to be rapidly and inexpensively genotyped for variation within the MHC-*B* region. Genotypes were generated for over 7,500 samples sampled from diverse sources, including serologically-defined MHC recombinants, MHC-defined inbred lines, in populations held at universities within North America, heritage broiler lines, and elite layer lines used for commercial egg production. Genotyping with the SNP panel revealed 78 unique haplotypes and 44 additional recombinant haplotypes with this diverse sample set. The panel revealed hotspots of recombination as well as regions of gene duplication and deletion. Associations are often observed between MHC-*B* variation and disease resistance in the chicken. To date such studies have been done mostly with the small number of haplotypes defined originally by serology. The new haplotypes revealed by MHC-*B* SNP typing provides new opportunities for enhancing the understanding of MHC-*B* and disease resistance in chickens.

Key Words: chicken MHC, SNP, haplotypes, diversity

67 Genetic diagnosis of sex chromosome aberrations in horses based on analysis of microsatellite and X- and Y-linked markers. J. A. Bouzada*, J. M. Lozano, M. R. Maya, A. Trigo, I. Bonet, F. Castillo, J. Fernández-León, T. Mayoral, E. Anadón, and L. B. Pitarch, *Laboratorio de Genética y Control, Algete, Madrid, Spain.*

Equine sex chromosome aberrations are often associated with clinical signs affecting health and reproduction. However, abnormal manifestation with sex chromosome aberration usually appears at maturity and potential disorders may be suspected infrequently. A reliable survey at an early stage is therefore required because detect and characterise sex chromosome aberrations in newborn has important economic effects. Through the routine DNA genotyping of animals, it is possible to identify profiles that are indicative of chromosome abnormalities. Including additional DNA markers in usual panels for pedigree and parentage verification can be useful identifying animals possessing chromosomal abnormalities. Abnormal profiles of genetic markers located on sex chromosomes can help identify animals with chromosomal defects. Markers panel used for horse DNA testing by Laboratorio Central de Veterinaria of Algete (Madrid) consisting of seventeen autosomal microsatellite markers (AHT4, AHT5, ASB2, ASB17, ASB23, CA425, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, LEX33 and VHL20), two microsatellite markers linked to sex chromosomes (LEX3 and LEX27) and the Amelogenin marker, a gene with distinct X and Y alleles, has proved be very useful for genealogical control and detection of chromosomal abnormalities. Additional sex-linked markers (LEX22, LEX24, LEX28 and TKY598) are also available at the laboratory for extended analyses of suspect cases. Detection at an early age and understanding of the prevalence of sex chromosome aberrations should assist in the diagnosis and management of horses kept for breeding. Further, the parental origin of the X chromosome of each disorder could be proved by the results of genetic analysis, thereby contributing to cytogenetic characterisation.

68 Rate of sex reversal cases in horses of Argentina. M. Martinez*, M. Costa, B. Elguero, and C. Ratti, *Laboratorio de Genética Aplicada, Sociedad Rural Argentina, Buenos Aires, Argentina.*

This work describes the sex reversal cases found on 168,000 horses tested for different breeds in Argentina. Sex reversal syndromes describe sexual and development disorders in mammals, including horses. Affected animals show a disagreement between sex chromosome constitution, gonadal and phenotypic/behavioural sex. According to XX/XY constitution and the Sex-Determining Region Y gene (SRY) presence, the sex reversal cases fall in 4 categories: female XY SRY-negative or SRY-positive genotypes and male XX SRY-negative or SRY-positive genotypes. Our laboratory genotypes horses for parentage verification using ISAG STR panel and the sexing marker amelogenin (AME, X and Y linked). When the gender reported by the owner disagrees with the inferred by AME, then SRY is tested. Doing so, 38 females XY SRY-negative, 6 females XY SRY-positive and 5 male XX SRY-negative genotypes were detected. Even when there are not males XX SRY-positive horses reported in the literature, one case was found out at the laboratory. Percentage rates of 0.019 and 0.038 were estimated in Thoroughbred and Polo breeds, respectively, for females XY SRY-negative genotypes. The higher rate in Polo may be linked to artificial assisted reproductive technologies used in this breed. It also may be explained by the preferential use of some sire lines with higher tendency to produce XY female offspring. Some of these animals have offspring, an unexpected outcome related to the extreme heterogeneity of this condition. Female XY SRY-positive genotypes are very unusual and some of them have been linked to autosomal/X chromosome gene mutations. In those reports, the animals were related. Not relationship was found for our cases. Three from the 5 male XX SRY-negative cases were horses showing chimeric genotype profiles, suggesting fusion of fraternal twins during the embryo development. Similar findings were previously showed in horses with no hereditary brindle coat colour patterns. In all the cases, except one, described as hermaphrodite by the breeder, not phenotype abnormalities were reported by the owners. Therefore, all the findings were done during routine parentage testing. Gender discrepancy was then informed to the owners for further research on affected animals.

Key Words: sex reversal, X chromosome, Y chromosome

69 Characterization of equine STR panel "15 TKY system" by imputation from dense SNP genotypes in a Thoroughbred population. M. Kikuchi*, H. Kakoi, T. Tozaki, K. Hirota, and S. Nagata, *Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan.*

Single nucleotide polymorphisms (SNPs) are an informative resource for genetic studies and recently, SNP genotyping is being considered an alternative to short tandem repeat (STR) genotyping, as a tool for parentage testing in many animals. Imputation of STR alleles from SNP genotypes is well developed for some species; however, there is little information, based on SNPs, on equine STR parentage panels. Our group developed a panel with 15 STRs, namely 15 TKY system. The ISAG has sanctioned it as a secondary parentage panel in horses, and some laboratories routinely employ several TKY markers in parentage testing. Therefore, as a model for imputation of STR markers in horses, we characterised the 15 TKY system by identifying SNP haplotypes corresponding to STR alleles in a Thoroughbred population. For the STR analysis, 94 randomly selected Japanese Thoroughbred horses (48 males and 46 females) were genotyped, and 90 alleles were observed on the 15 TKY STRs. This covered 88% of the previously reported alleles. Subsequently, all horses were subjected to SNP genotyping using 670K Axiom Equine Genotyping Array (Affymetrix). We extracted 200–737 (average 278) SNPs within 500 kb on either side of each STR (1 Mb) and selected SNPs that were completely genotyped for all horses and exceeded minor allele frequency of 5%. Then, the SNP haplotypes with STR alleles were phased by SNPAlyze Ver. 9, using 1,000 iterations. The total number of SNPs required to impute STR alleles was minimized by referring to a previously reported process. The number of SNPs corresponding to each STR was 3–6, and the SNPs were located within 34–469 kb (average 170 kb). Finally, 90 alleles of the 15 TKY STRs were theoretically explained by 105 haplotypes derived from 69 SNPs in the analysed population. With this model for imputation from a high-density SNP platform within a Thoroughbred population, it was concluded that the 15 TKY system could be mostly imputed by using the 69 SNPs in the Thoroughbred population. Further analysis for larger population size and simulation for parentage verification using both 15 TKY system and SNP haplotypes are in progress.

Key Words: SNP, STR, haplotype, Thoroughbred, parentage verification

70 Preliminary results of genetic monitoring of the occurrence of three genetic diseases (CA, SCID, LFS) in Arabian horses from Poland. M. Bugno-Poniewierska*[1], M. Stefaniuk-Szmukier[2], A. Piestrzynska-Kajtoch[1], A. Fornal[1], and K. Ropka-Molik[1], [1]*National Research Institute of Animal Production, Department of Animal Genomics and Molecular Biology, Balice n.*

Krakow, Poland; [2]*University of Agriculture, Department of Horse Breeding, Kraków, Poland.*

Polish horse breeding industry is well known from Arabian horse breeding tradition. The origin of the horse lines traces back more than 200 years which can be confirmed by historical sources. Polish Arabians are commonly known from their desirable beauty and performance ability. Balanced selection is based on preserving traits combining unquestionable appearance and utility. Therefore, the Polish population of Arabian horses are undoubtedly one of the most influential in the world. Genetic screening for heritable disorders in Arabians is an extremely important step for population management to avoid the production of affected offspring, reducing the incidence of carriers and preventing economic losses. There are three common genetic disorders tested in Arabian horses: Cerebellar Abiotrophy (CA); Severe Combined Immunodeficiency Disorder (SCID) and Lavender Foal Syndrome (LFS). The genetic background of causative mutations responsible for each conditions has been previously described (Brault *et al*. 2011, Shin *et al*. 1997, Brooks *et al*. 2010). The aim of the presented study is to determine the occurrence of mutant alleles of the three diseases in a sample population of Arabians from Poland using DNA-based test. Analysis included 448 healthy horses that were introduced into Polish Arabian Stood Book. The scan against mutant alleles reveled an absence of SCID and LFS carriers among investigated individuals. However, the investigation for CA alleles showed 11,4% frequency and all of them were in heterozygous state. Although the carriers of the mutant alleles do not show clinical signs and there is a lack of reports describing negative consequences for health and athletic performance in heterozygous individuals, further monitoring of active population is essential. The absence of SCID carriers in Polish Arabians has been reported previously (Terry *et al*. 199) which resulted in an increased efforts of breeders to maintain the population clear. The LFS is most common linked to Egyptian origin lines, even though that polish breeding program introduce stallions of Egyptian origin, the mutation causing LFS is not determined. Financed: BIOSTRATEG2/297267/14/NCBR/2016.

Key Words: horse, genetic diseases, CA, SCID, LFS

Genetics of Immune Response and Disease Resistance

71 Enhanced genetic disease control with selection for low susceptibility and infectivity. S. Tsairidou*, O. Anacleto, J. A. Woolliams, and A. Doeschl-Wilson, *Roslin Institute, Edinburgh, UK.*

Genetic heterogeneity in host infectivity and susceptibility to infectious diseases has a great impact on spread and severity of livestock epidemics. Current genetic selection schemes exploit heritable variation in susceptibility to reduce disease prevalence. However, increasing evidence suggests that there is also variation in infectivity and super-spreaders have been documented in disease outbreaks. This study examined if combined selection for both low infectivity and susceptibility can be more efficient in reducing epidemic severity and risk compared to selection on susceptibility alone. Infectivity depends on genetics and disease epidemiology. Thus, a stochastic SIR (Susceptible, Infected, Recovered) epidemiological model was used to model disease dynamics accounting for polygenic genetic heterogeneity in infectivity and susceptibility. A population of 5,000 animals was generated and divided in groups of 100. Epidemics were simulated within each group and different scenarios for genetic variance and reproductive ratios R_0 were tested. Selection on infectivity and susceptibility was simulated over 20 generations following standard quantitative genetics theory and alternative selection schemes were tested by varying selection accuracy and intensity. There was a greater reduction of epidemic severity and risk by combined selection for susceptibility and infectivity compared to selection only for susceptibility. After 4 generations of combined selection with accuracy of 0.7 for both traits the proportion of infected animals was 44% lower compared to selection only on susceptibility. After 12 generations of combined selection the percentage of groups producing an epidemic was reduced by 50% for diseases with R_0 of 1.5, and by 20% for severe epidemics with R_0 of 6, while selection on susceptibility alone required more than 20 generations. Finally, even for an accuracy for infectivity of 0.2, there was substantial improvement from combined selection. In conclusion, combined selection for susceptibility and infectivity was more efficient than selection only on susceptibility. Further studies are needed to estimate genetic effects for both traits.

Key Words: animal breeding, infectious disease

73 Genetic basis for resistance to avian influenza in commercial egg layer chicken lines. W. Drobik-Czwarno*[1], A. Wolc[2,3], J. Fulton[3], T. Jankowski[4], J. Arango[3], P. Settar[3], N. O'Sullivan[3], and J. Dekkers[2], [1]*Department of Animal Genetics and Breeding, Faculty of Animal Science, Warsaw University of Life Sciences, Warsaw, Poland;* [2]*Department of Animal Science, Iowa State University, Ames, IA, USA;* [3]*Hy-Line International, West Des Moines, IA, USA;* [4]*Nutribiogen, Poznan, Poland.*

A 2015 outbreak of H5N2 Highly Pathogenic Avian Influenza (HPAI), resulting in mandatory euthanization of millions of chickens, was the most fatal in US history. The aim of this study was to identify genomic regions associated with survival following natural infection with HPAI. Blood samples were obtained from 274 individuals from three commercial White Leghorn varieties. Survivors and age and genetics matched non-infected controls from each variety were included in the comparison. All individuals were genotyped on the 600k Affymetrix SNP array. A genome-wide association study was performed within the varieties with standard frequency test in PLINK, while logistic regression with the first three multi-dimensional scaling (MDS) components of SNP genotypes as covariates was used for all varieties together. Several SNPs located within three regions in two varieties were significant at a 5% Bonferroni genome-wide threshold ($P < 3.87E-06$): on chromosomes 5 and 18 for variety 1 and on chromosome 11 for variety 2. Genome wide scan with F_{ST} was also performed as an alternative method of analysis, using windows of 40, 100 and 500kb. The regions with highest F_{ST} values between cases and controls were located on chromosomes 1 and Z and overlapped several genes with immunological function and previously identified quantitative trait loci for health. Only few regions were consistent between the GWAS analysis (approaching significance) and at the same significance level in the F_{ST} genome wide scan. This study confirms that resistance to HPAI is a complex, polygenic trait and that mechanisms of resistance can be population specific. This study was supported by the Iowa Egg Industry Center.

Key Words: chicken, highly pathogenic avian influenza, resistance, GWAS

74 Integrated network analysis for mRNAs and miRNAs expressed in PRRSV vaccinated peripheral blood mononuclear cells of pigs. M. A. Islam[1], C. Neuhoff*[1], S. Rony[1], C. Große-Brinkhaus[1], M. J. Uddin[2], M. Hölker[1], D. Tesfaye[1], E. Tholen[1], M. J. Pröll[1], and K. Schellander[1], [1]*Institute of Animal Science, Animal Breeding and Husbandry group, University of Bonn, Endenicher*

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MicroRNAs, posttranscriptional regulators of gene expression, have been emerged as potential tools for evaluating host immune response to infection or vaccination. The current study aimed to investigate the expression profiles of mRNA and miRNA in peripheral mononuclear cells (PBMCs) to uncover the miRNA-mRNA regulated host immune response to porcine reproductive and respiratory syndrome virus (PRRSV) vaccines. For this we analysed the global miRNA profiles of PBMCs collected at before (0 h), and 6, 24 and 72 h post PRRSV vaccination in German Landrace (DL) and Pietrain (Pi) pigs. Expression analysis identified 12, 259 and 14 differentially expressed (DE) miRNAs in PBMCs of DL and 0, 222 and 13 DE miRNAs in PBMCs of Pi at 6, 24 and 72 h post vaccination, respectively. The mRNA expression analysis of PBMCs of pigs was performed from the same sample pools at 0, 6, 24 and 72 h post vaccination. The list of predicted target genes of DE miRNAs were overlaid onto the list of vaccine induced differentially expressed gene (2,920) and a total of 1,397 matched mRNAs were found as true differentially expressed target genes (TDETGs) in PBMCs after PRRSV vaccination. The TDETGs are involved with regulation of biological processes including response to signal transduction, innate immune response regulation of MAPK kinase activity and apoptosis. The miRNA-mRNA co-regulatory network was drawn between down-regulated miRNA and their up-regulated true mRNA targets. The miRNA and gene co-regulatory network revealed that miR-6762, miR-23a-5p, miR-181b-5p, miR-4454 and miR-125–5p are the putative regulators of PRRSV vaccine induced genes including SIRT1, FOS, ARNTL, PKM, CD9, WNT1, CDKN1A, ABCG2, VEGFA and TNFAIP3 in PBMCs. In conclusion, the results of this study evidenced the immune response during PRRSV vaccination is associated with co-regulatory miRNA-mRNA networks in PBMCs.

Key Words: PRRSV, miRNA, PBMCs, vaccination, pig

75 Mammary epithelial cells, rather than professional immune cells dictate the pathogen species-specific immune reaction of the udder. J. Hehl*[1], M. Koy[2], A. Berthold[1], H.-J. Schuberth[2], M. Weinert[3], S. Engelmann[3], C. Kühn[1], H.-M. Seyfert[1], and J. Günther[1], [1]*Leibniz Institute for Farm Animal Biology (FBN), Institute for Genome Biology, Dummerstorf, Germany;* [2]*Immunology Unit, University of Veterinary Medicine Foundation, Hannover, Germany;* [3]*Institute of Microbiology, Technical University Braunschweig, Braunschweig, Germany.*

The aetiology determines extent and quality of the immune response after an udder infection (mastitis). Gram-negative bacteria (e.g. *Escherichia coli*) will quickly elicit strong inflammation of the udder, fully activate its immune defence and often eradicate the pathogen. In contrast, Gram-positive bacteria (e.g. *Staphylococcus aureus*) will slowly elicit a much weaker inflammation and immune response frequently resulting in chronic infections. It was unclear which of the different cell types residing in the udder determines the pathogen-species specific norm of immune reaction of that organ. Therefore, we diagnosed the pathogen-specific immune response of different relevant cell types from udder and blood. We challenged primary cultures of bovine mammary epithelial cells (pbMEC), fibroblasts from udder, bovine monocyte-derived macrophages (boMdM) and the established MEC line MAC-T with heat-killed *E. coli*, *S. aureus* and *S. uberis* and analysed their immune responses. *E. coli,* but not the other pathogens fully activated the immune response in pbMEC, fibroblasts and MAC-T-cells, including increased cytokine and chemokine expression and NF-kB activation. *S. aureus* and *S. uberis* induced weak or no immune reactions respectively. Yet, the macrophage models (boMdM and murine RAW 264.7 cells) responded strongly to all three pathogens including activation of IkB/NF-kB signalling. The models for MEC and fibroblasts responded with distinctly graded immune reactions to each of the three pathogens. *E. coli* induced a strong, transient cytokine storm in the models but neither of the Gram-positive bacteria did. This distinction was caused by the failure of MEC to activate TLR-mediated signalling upon challenges with *S. aureus* or *S. uberis*. Hence, the pathogen-species dependent immune reaction norm of MEC complies best with - and by inference - dominates *in vivo* that of the udder. We are now scrutinizing on humoral and genetic factors modulating the immune responsiveness of the MEC. Therefore, we isolated pbMEC and factor secreting boMdMs from cows have been selected for an inherited divergent susceptibility against mastitis causing pathogens.

Key Words: bovine mastitis, pbMEC, *E. coli*, *S. aureus*, immune response

76 Integrative network genomics of the bovine host response to infection with *Mycobacterium bovis*. T. J. Hall*[1], K. E. Killick[1,2], M. P. Mullen[3], K. E. McLoughlin[1], N. C. Nalpas[4], I. W. Richardson[5], D. A. Magee[1], C. N. Correia[1], J. A. Browne[1], H. M. Vordermeier[6], B. Villarreal-Ramos[6], D. P. Berry[7], E. Gormley[8], S. V. Gordon[2,8], D. E. MacHugh[1,2], [1]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland;* [2]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland;* [3]*Department of Life and Physical Sciences, Athlone Institute of Technology, Athlone, Ireland;* [4]*Quantitative Proteomics and Proteome Centre Tübingen, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany;* [5]*IdentiGEN Ltd., Blackrock Business Park, Blackrock, Dublin, Ireland;* [6]*Animal and Plant Health Agency (APHA), Weybridge, Addlestone, United Kingdom.;* [7]*Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Cork, Ireland;* [8]*UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland.*

Bovine TB (BTB), caused by infection with *Mycobacterium bovis*, is a major endemic disease affecting global cattle production, particularly in many developing countries. In the current study we used correlation- and interaction-based network approaches to analyse the host response to infection with *M. bovis* at the level of the transcriptome to identify core disease response pathways. These networks were then integrated with genome-wide association study (GWAS) datasets to enhance detection of genomic variants for susceptibility/resistance to *M. bovis* infection. The host gene expression data consisted of bovine RNA-seq data generated using peripheral blood samples from cattle infected with *M. bovis* across a 14-week infection time course. These data were then used for weighted gene correlation network analysis (WGCNA) to construct a correlation-based network to identify gene modules associated with disease response. A base gene interaction network of the mammalian host response to mycobacterial infection was also generated using 213 genes identified from a GeneCards (www.genecards.org) search for relevant keywords and the network was constructed using InnateDB (www.innateDB.com). Differential gene expression data were superimposed on this base network and the JActiveModules Cytoscape (www.cytoscape.org) plugin was used to extract functional modules. Bovine GWAS data was obtained from a published BTB susceptibility/resistance study. SNPs from genes within the top functional modules (5 kb up- and downstream of each gene) were used with single-SNP regression and Bayesian approaches to analyse GWAS data from 841 Holstein-Friesian bulls with composite multi-relative BTB susceptibility/resistance phenotypes. These analyses identified new genomic variants associated with susceptibility and resistance to BTB, demonstrating that integration of transcriptomics and GWAS data is a useful method for studying the host response to mycobacterial infection.

Key Words: integrative genomics, cattle, systems biology, bioinformatics

77 Identification of putative key transcription factors in canine macrophages after infection with *Leishmania infantum* and stimulation with a toll-like receptor-2 agonist. L. Solano-Gallego[2], S. Montserrat[2], F. Mayer[1], A. Castello[1], L. Alborch[2], S. Heath[3], A. Esteve-Codina[3], J. Gomez-Garrido[3], R. A. Cigliano[4], and A. Clop*[1], [1]*Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Cerdanyola del Valles, Catalonia, Spain;* [2]*Universitat Autonoma de Barcelona, Cerdanyola del Valles, Catalonia, Spain;* [3]*Centre Nacional d'Anàlisi Genómica CNAG-CRG, Barcelona, Catalonia, Spain;* [4]*Sequentia Biotech, Barcelona, Catalonia, Spain.*

Leishmaniosis is a common zoonotic disease in dogs and humans caused by the parasite *Leishmania infantum*. PAM3CSK4, a TLR2 agonist, promotes inflammation and reduces the parasite load in macrophages. In order to understand the molecular changes occurring in relation to *L. infantum* infection and after PAM3CSK4 stimulation, we compared the RNA-seq profiles of a canine macrophage cell line (DH82) (i) before infection, (ii) after infection with *L. infantum* promastigotes, (iii) after stimulation with the TLR2 agonist and (iv) after infection and TLR2 stimulation. We also performed ChIP-seq to map two histone modifications: H3K4me3 and H3K27ac, which mark promoters and genomic activity, respectively. We used an improved dog genome annotation built in-house with RNA-seq data from this project and from NCBI. In total, 530 million 75bp × 2 paired-end reads were generated in an Illumina HiSEqn 2000 system. RNA-seq profiles were determined with STAR and RSEM and differential expression was assessed with DESEqn 2. These analyses show that TLR2 stimulation but not infection has an impact (FDR ≤ 0.05) on gene expression. Taking into account any of the comparisons involving TLR2 stimulation, 281 genes were differentially expressed (DE). 257 of these genes were DE when (i) and (iv) were compared. There was an enrichment of immunoinflammatory genes. Of note, 19 of the 257 genes are transcription factors (TF). ChIP-seq reads were mapped to the dog genome with BWA and peaks were called with MACS2. The peaks occurring between 4Kb upstream and 2Kb downstream of the transcription start site (putative regulatory regions) of the 257 DE genes were further investigated to identify potential binding sites for the 10 DE TFs with annotated motifs using Transfac/Jaspar. Cognate binding sites were present in 198 DE genes. RelA, Rel and Foxj1, three key immune-related TFs, displayed the largest number of binding sites. Further analysis will help determining whether these TFs orchestrate the gene dysregulation caused after stimulating the cells with a TLR2 agonist.

Key Words: leishmaniasis, TLR2 stimulation, RNA-seq, ChIP-seq, differential expression

78 Analysis of the genomic regions associated to response to coccidiosis caused by *Eimeria maxima* in broiler chickens. B. Bed'Hom*[1], M. H. Pinard-Van der Laan[1], R. Hawken[2], and H. Edin[1], [1]*INRA, Jouy-en-Josas, France;* [2]*Cobb-Vantress Inc., Siloam Springs, AR, USA.*

Coccidiosis is an intestinal parasitic disease caused by species from *Eimeria* genus, widespread in poultry farming, leading to important losses by its direct impact on production and the associated control measures (such as vaccines or cocciostats). The integrative management against this disease should lead to include new parameters of animal's response to coccidiosis in selection schemes. Our goal was to study the variability of broilers' response to coccidiosis caused by *Eimeria maxima*, to identify associated genomic regions and putative candidate genes and to understand underlying biological mechanisms. For this aim, we performed an experimental infection with 2 024 animals (Cobb500 broilers) and measured phenotypes of animals' response (such as bodyweight, temperature, lesion scores and many blood parameters). Animals have been genotyped using the Affymetrix 580K SNP panel, and the genome-wide association study has revealed genomic regions highly significantly associated to some traits of interest (bodyweight gain, plasma colour and plasma β2-globuline concentration). However some genetic markers have contrasted effects on phenotypes between control or infected animals, indicating putative trade-offs between production and health. The analysis of biological functions of genes from regions associated to phenotypes has also shown the major role played by biological pathways of metabolism and some innate immune parameters. Moreover, the inference of interaction networks between genes highlighted very significant networks centred on gut repair or cardiovascular functions. The genetic markers identified during this study can be used in selection programs.

Key Words: chicken, genome-wide association, disease resilience, coccidiosis

79 Genetic individual variability of vaccine responses in pigs. F. Blanc*[1], G. Lemonnier[1], J. Leplat[1,2], E. Bouguyon[3], Y. Billon[4], J. Estelle[1], and C. Rogel-Gaillard[1], [1]*GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [2]*CEA, DRF/IRCM/SREIT/LREG, Jouy-en-Josas, France;* [3]*VIM-INRA-Université Paris-Saclay, Jouy-en-Josas, France;* [4]*GenESI, INRA, Surgères, France.*

Impact of host genetic variation in shaping innate and adaptive immune responses is an emerging lever to be included in new vaccination strategies. Our aim was to analyse the genetic control of individual vaccine responses in pigs and we addressed this question by studying the variation of antibody responses induced by vaccination against *Mycoplasma hyopneumoniae* (*M. hyo*) or Influenza A Virus (IAV). Large White pigs housed in a conventional facility were vaccinated at weaning (around 28 days of age) with a booster vaccination 3 weeks later. Forty eight families were produced in five batches. 190 and 192 piglets were vaccinated against *M.hyo* or IAV, respectively, and 64 non vaccinated piglets were included as controls for the two vaccine experiments. The humoral vaccine response was measured by following the dynamics of seric *M. hyo*- or IAV-specific IgGs, prior vaccination on the vaccine day, every week during five weeks post-vaccination, and before slaughtering. In addition, haemagglutination inihibition (HAI) assays were performed for IAV-vaccinated pigs. The individual variability of responses to vaccination was revealed by the differences in the levels of *M. hyo*- or IAV-specific IgGs in the sera of vaccinated animals. One week after the booster vaccination, *M. hyo* Ab levels (S/P values) ranged from 0.012 to 2.151 (mean = 1.322, s.d. = 0.337) and IAV-specific IgGs levels ranged from 0.59 to 128.5 µg/mL (mean = 33.39 µg/mL, s.d. = 2.475). For IAV-vaccinated animals, HAI titres ranged from 10 to 2560 (geometric mean = 332.2). Interestingly, females exhibited a higher humoral immune response to vaccination against *M. hyo* compared to males, with significant differences (unpaired *t*-test) two weeks post booster vaccination ($P = 0.024$) and also before slaughtering ($P = 0.0002$). This experimental design and the wide range of individual variabilities obtained for both vaccines will allow us to estimate the heritability of the phenotypes and to perform genome wide association studies (GWAS) to identify the genomic regions and candidate genetic markers associated with individual variability of vaccine responses.

Key Words: pigs and related species, immunogenomics, immune system, animal health

80 QTLs associated with resistance to MAP infection in Holstein-Friesian cattle. S. Mallikarjunappa*[1,3], M. Sargolzaei[4], K. Meade[2], N. Karrow[3], and S. Pant[1], [1]*Graham Centre for Agricultural Innovation, Charles Sturt University, Wagga Wagga, NSW, Australia;* [2]*Teagasc Animal and Bioscience Research Department, Grange, Co. Meath, Ireland;* [3]*Department of Animal Biosciences,*

University of Guelph, Guelph, ON, Canada; [4]*The Semex Alliance, Guelph, ON, Canada.*

The objective of this study was to perform genome-wide association (GWA) analysis using high-density (HD) imputed genotype data in order to identify putative Quantitative Trait Loci (QTLs) associated with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection that causes Johne's disease in cattle. The study was based on the hypothesis that the use of imputed HD SNP genotypes leads to identification of additional associations especially in the regions with low coverage on 50k panel used in the previous study. Therefore, previously analysed 50k genotypes of 232 Holstein-Friesian cows, comprised of a MAP-positive (n = 90) and MAP-negative cohort (n = 142), were imputed to a 777k SNP chip panel using the software package FImpute. Subsequently, principal component regression analysis was used for GWA of imputed genotypes, which revealed 15 putative QTLs (P = 1.99E-6) associated with the MAP infection phenotype on 8 different chromosomes (BTA 5, 7, 10, 14, 15, 16, 20 and 21) of which the QTLs identified on BTA 15, 16, 20 and 21 constituted the new additional QTLs identified in this study. Post-GWAS bioinformatic analysis identified several novel candidate genes underlying these QTLs including *NLRP3*, *IFi47*, *TRIM41, TNFRSF18, TNFRSF4*. Several of these genes have pro-inflammatory properties, which could be indicative of the role of phagocytic cells in eliciting an anti-MAP response during early stages of Johne's disease pathogenesis. To our knowledge, this is the first study to carry out Johne's disease GWA analysis using high density SNP genotypes. Our analysis revealed potential QTLs that were associated with resistance to MAP infection, which will now be functionally investigated. Once validated, associated QTLs could be exploited via marker-assisted selection to breed for Johne's disease resistance in cattle.

Key Words: cattle, genome-wide association, imputation, genetic marker, genetic improvement

81 The association of copy number variations with tick count in South African Nguni cattle. L. Pickering*[1], K. Dzama[1], F. Muchadeyi[2], and M. Wang[2], [1]*Animal Science Department, University of Stellenbosch, Stellenbosch, Western Cape, South Africa;* [2]*Agriculture Research Council Biotechnology Platform, Pretoria, Gauteng, South Africa.*

Ticks and tick-borne diseases pose a major threat to livestock industries worldwide. The South African Nguni is a locally adapted cattle breed that is known for its resilience to ticks and tick borne diseases. Copy number variations regions (CNVR) comprise insertions, duplication and deletions within the genome that are larger than 1kb and are reported to play a possible role in adaptation. A preliminary investigation to determine the association between CNVR and tick resistance in South African Nguni cattle was performed. Tick count data was collected from 347 randomly selected Nguni cattle from three different locations within South Africa over a period of two years. Data was split per location and summary statistics were used to determine quartile and interquartile ranges of tick counts. If an animal had a tick count average lower than or equal to the first quartile it was classified as resistant (1), animals with a tick count average greater than or equal to the third quartile were classified as susceptible (0) and those with values in between were grouped as unclassified (2). DNA extracted from hair and blood samples was genotyped using the Illumina BovineSNP50 assay. Quality control and sample pruning was performed using Plink (Version 1.07) leaving 41193 high quality SNPs. LogR ratios and B allele frequency data of filtered SNPs was extracted and PennCNV software was utilised to identify 1831 unique CNVR. A data file containing respective copy number states (0, 1, 2, 3) of CNVR loci for each animal was generated. A general linear model testing the hypothesis that tick resistance is associated with CNVR was run using R Studio. Sixty-six CNVR located on chromosomes 4, 5, 6, 13 and 14 demonstrated a significant (P < 0.05) association with tick count. Associated CNVR covered 16 genes that played a role in multiple molecular functions and biological processes including catalytic activity, binding functions and immune, metabolic, cellular, reproduction and developmental processes respectively. This study is the first of its kind to demonstrate a significant association between tick count and copy number variations in South African Nguni cattle.

Key Words: cattle, tick resistance, copy number variation region, bovine 50K BeadChip

82 Investigating genetic control of resistance to avian pathogenic *Escherichia coli* colonization in chickens. M. Monson*[1], M. Kaiser[1], A. Wolc[1,2], and S. Lamont[1], [1]*Iowa State University, Ames, IA, USA;* [2]*Hy-Line International, Dallas Center, IA, USA.*

Colibacillosis in poultry is caused by extraintestinal infections with avian pathogenic *Escherichia coli* (APEC) and impacts commercial production worldwide. Initial exposure to APEC occurs primarily through the respiratory tract, after which the bacteria can spread rapidly throughout the host. Poultry infected with APEC exhibit decreased growth and egg production and increased mortality and carcass condemnation. Improving host resistance to APEC colonization would maintain better poultry health and reduce the economic losses for the industry. This study aims to identify quantitative trait loci (QTLs) in chickens (*Gallus gallus*) linked to variation in the bacterial load after APEC challenge. These QTL regions could provide a first step towards developing markers for genomic selection and could point to novel target genes for resistance. In this study, 324 birds from the F_{24} generation of an advanced intercross line (AIL) and 46 from the parental lines (a closed broiler line and an inbred Fayoumi line) were challenged at 14 days of age with 10^7 CFU of APEC O1:K1:H7 via right-side intra-airsac injection. Blood and tissue (right lung, left lung, spleen, and liver) samples were collected after 1 day of exposure. Bacterial loads [$\log_{10}$(CFU/g)] in each sample were determined by plating 10-fold serial dilutions of tissue homogenates. The bacterial load differed significantly between tissues (right lung > spleen > left lung and liver > blood). For each AIL individual, genotypes were collected on the Affymetrix Axiom 600K Genome-wide Chicken Genotyping array, providing ~200,000 usable SNPs (call rate ≥ 95%; minor allele frequency ≥ 2%). Tissue colonization levels were determined to be lowly heritable traits (h^2 < 0.3) and used as quantitative phenotypes for Bayesian genome wide association analysis (GWAS). Association of specific regions of the genome with resistance to APEC colonization could inform future efforts to reduce colibacillosis in commercial chickens. Support: USDA-NIFA-AFRI US-UK Collaborative grant, Hatch project #5424.

Key Words: poultry, genome-wide association, genotyping, infectious disease, animal health

84 Genome-wide association study for monocyte count at day 7 post-challenge with bovine viral diarrhea virus in F_2 and F_3 Nellore-Angus halfblood steers. K. M. S. Davila*[1], A. D. Herring[1], J. E. Sawyer[1], J. F. Ridpath[2,3], and C. A. Gill[1], [1]*Texas A&M University, College Station, TX, USA;* [2]*National Animal Disease Center, Ames, IA, USA;* [3]*Ridpath Consulting, Ames, IA, USA.*

Bovine viral diarrhoea viruses (BVDV) are prevalent worldwide, and outbreaks in USA beef herds are estimated to cost between $50 and $100 per animal. BVDV infections are associated with varying degrees of immunosuppression. Monocyte counts have been shown to drop at day 7 or 8 of infection. The objective of this study was to identify genetic variants associated with monocyte counts following BVDV challenge in crossbred cattle. A population of 372 F_2 and F_3 Nellore (*Bos indicus*)-Angus (*Bos taurus*) halfblood steers were balanced across sires regarding modified-live, killed and non-vaccinated experimental treatments and subsequent-

ly intra-nasally challenged with a BVDV type 1b noncytopathic field strain. . Monocyte count at day 7 post-challenge was evaluated and adjusted for vaccine type, calf type and weaning temperament score. Genotypes were imputed within family to high density and after quality control filtering there were 555,670 SNP available for a genome-wide association study for bovine monocyte count applying the univariate procedures of GEMMA that fitted the genomic relationship matrix to account for genetic covariance among animals. To correct for multiple tests, the Benjamini and Hochberg false discovery rate was constrained to 0.05 and there were 37 SNP associated with bovine monocyte count on bovine chromosomes (BTA) 2, 3, 14, 17, 19 and 29. There were 11 significant SNP within a 100kb region on BTA 17:72,729,721 - 73,483,993, which explained 7.5% of the variation in monocyte count at day 7. This region contains 2 genes, SLC5A1 and SLC5A4, which code for glucose co-transporter family proteins. In human studies the expression of glucose transporter genes in monocytes and other leukocytes has been shown to be vital to providing the necessary cellular fuel to mount an immune response. The genomic region identified may be important to immune response to viral challenge or vaccination.The identification of genetic variants associated with reduced impact of BVDV infection in *Bos indicus* influenced cattle would be of great economic importance globally to cattle producers in tropical and sub-tropical regions.

Key Words: *Bos indicus*, BVDV, monocytes

85 Polymorphism in TLR2 in different dairy cattle breeds suggests immune functional modulation. M. Bartens*, K. Tombacz, A. Gibson, and D. Werling, *Department of Pathobiology and Population Sciences, Royal Veterinary College, University of London, Hatfield, UK.*

There is strong evidence that high yielding cows are highly susceptible to infectious diseases. As the innate immune system is the first barrier for pathogens entering an organism, its activation by pattern recognition receptors (PRRs) is crucial for an adequate immune response. Within these PRRs, toll-like receptors are the most important and polymorphisms within TLRs are suggested to be associated with disease resistance traits in farm animals. TLR2 plays a major role in recognising multiple pathogens, therefore, we compared TLR2 sequences of two popular dairy breeds by cloning and sequencing their coding TLR2 sequences from PBMC-derived macrophages. Assembled TLR2 contigs were translated to amino acid sequences and aligned. A total of 18 SNPs within the coding sequence of TLR2 were detected across the breeds. Of note, two were found in the crucial ligand-binding domain of the extracellular domain of the TLR2 receptor, H326Q and T63G. A functional role of the detected SNPs, was investigated using a cellular reporter assay for TLR2 receptor activation. Human embryonic kidney (HEK) 293 T-cells were stably transfected with the NF-kB-inducible reporter gene secreted embryonic alkaline phosphatase (SEAP) and our selected TLR2 receptor constructs. The transfectants were stimulated with TLR2-specific ligands such as Pam_3CSK_4 and FSL-1 as well as with heat-killed *E. coli*, *S. dublin* and *M. bovis BCG* as these are common pathogens in the farm environment. Significant increases in TLR2 activation were measured in TLR2 constructs containing H326Q, (SNP rs68343167). This variation was also previously found in an indigenous, resistant, Bos taurus Anatolian breed, thus we hypothesise the presence of this SNP to confer Brown Swiss (and Anatolian Black) to be less susceptible to infections than the Holstein Friesian breed. We suggest that immune function is compromised in those breeds under strong selective pressure for high production traits. Our results suggest that the variations seen may be of functional relevance leading to a stronger immune response within the Brown Swiss breed.

Key Words: bovine TLR2, cattle breeds, innate immunity, disease resistance, reporter assay

86 Delineating Indian native cattle–specific allelic variants and haplotypes in lactoferrin gene: A potential candidate for disease resistance. A. Sharma*[1,2], M. Sodhi[2], P. Jain[1], M. Kumar[2], and M. Mukesh[2], [1]*University Institute of Engineering & Technology, Kurukshetra, Haryana, India;* [2]*National Bureau of Animal Genetics Resources, Karnal, Haryana, India.*

Lactoferrin, a bioactive glycoprotein is member of transferrin family and plays an important role in immune defence, iron homeostasis, antioxidant and regulation of cell growth. The present investigation was undertaken to establish polymorphism data for Indian native cattle (INC) breeds in lactoferrin gene. Sequence data was generated for 2.3 kb comprising of 5′ flanking and untranslated, coding (17 exons) and 3′-untranslated regions across 72 animals representing 12 cattle breeds from different agro-climatic regions of India. Comparative data analysis across INC and taurine animals revealed a total of 19 SNPs with distribution of 3 in 5′-flanking region, 2 in 5′-untranslated region, 13 in CDS and one novel SNP in 3′-untranslated region. Out of 13 CDS SNPs, 6 were identified as non-synonymous - I145V, S538T, T546N, T596S, K627E, and H632R. Among these, I145V showed complete fixation with frequency of (1.0) and was found to be specific for INC. SNPs in 5 UTR occurred within transcription factor binding sites (AP-2α, SP1) could affect the transcription rate and novel SNPs in 3′UTR might alter the stability of gene. These SNPs in UTRs might be responsible for differential expression of lactoferrin gene in Indian cattle. The haplotype and LD analysis revealed 12 haplotypes and 3 haploblocks specific to INC breeds. The highest frequency (0.546) was observed for haplotype-1 (ACT) and LD occurred with Lodsq >2 indicated lower recombination rate for observed haploblocks. In addition, different physiochemical parameters of 6 nsSNPs were predicted by ProtPram Server and further anaysed by SIFT, PROVEAN, I Mutant and PloyPhen-2 tools revealed their non-deleterious nature. The study is first to report on nucleotide substitutions in lactoferrin gene among various Indian native cattle breeds. The data presented here provides baseline information to carry out the functional aspect of these identified variants and depicts the evolutionary differences from taurine cattle that could correlate the higher disease tolerance ability of Indian cattle breeds.

Key Words: lactoferrin, cattle, DNA sequencing, haplotypes, allelic variants

ISAG-FAO Genetic Diversity

87 An ancient genomic perspective on the horse domestication process. P. Librado[1], A. Fages[1,2], C. Gaunitz[1], N. Khan[1], K. Hanghøj[1,2], C. Gamba[1], C. Der Sarkissian[1], M. Leonardi[1], M. Schubert[1], and L. Orlando*[1,2], [1]*University of Copenhagen, Centre for GeoGenetics, Natural History Museum of Denmark, Copenhagen, Denmark;* [2]*Université de Toulouse, University Paul Sabatier (UPS), Laboratoire AMIS, CNRS UMR 5288, Toulouse, France.*

The domestication of the horse in the Pontic-Caspian steppes some 6,000 years ago represents one major turning point in human history. With horses, humans could travel for the first time well above their own speed and carry their germs, culture and genes across vast geographic areas. The development of horse-drawn

chariots and cavalry also radically changed the history of warfare and was instrumental to the emergence of transcontinental empires. Additionally, beyond the battlefield, farm horses have massively impacted agricultural productivity. The biological changes that accompanied the process of horse domestication are, however, difficult to reconstruct from current patterns of genetic diversity both due to the development of intensively selected and extremely influential breeds during the last two centuries, and the almost extinction of wild horses. Recent developments in ancient DNA research have opened for the characterisation of complete genomes, epigenomes and microbiota over long time series. We have applied such approaches to a large panel of horse remains spread across Eurasia and dated to 44,000–200 years ago. This started revealing the genetic structure of horse populations before and during early domestication stages as well as the history of genetic changes that accompanied their further transformation in a range of cultural contexts. I will present our latest progress made on an extensive dataset of ancient horse genomes spanning the whole domestication temporal and geographical range.

Key Words: ancient DNA, horse, domestication

89 When *taurus* met *indicus*. Exploring admixture events in ancient cattle. M. Verdugo*, *Trinity College Dublin, Dublin, Ireland.*

The Bronze Age is an important period in prehistory that comprised many socio-economic changes including the rise and fall of city states in Mesopotamia. In this period the establishment of trade routes allowed for the exploitation of raw materials and animals. From archaeology, there is evidence of zebu cattle moving into in the Near East from the Indus Valley from the end of the Late Bronze Age. Ancient DNA provides a direct view into the past of these early domestic animals. We present a dataset of ~50 high and low coverage ancient Near Eastern cattle genomes through time and space. From comparison using both 700K Bovine SNP chip data and whole genomes we observe at least one main admixture event in the Bronze Age. This contrasts with mitochondrial data in which such admixture is invisible. We also show the power of low coverage data from ancient domestic cattle to confidently detect admixture between taurine and indicine animals, increasing the utility of sequencing of archaeological samples from hot regions with poor preservation.

Key Words: cattle and related species, evolutionary genomics, ancient DNA, admixture

90 Genetic continuity of maternal lineages in Iberian cattle populations since Roman times. L. Simões[1], A. E. Pires[2,3], C. Detry[4], I. Ureña[2], E. Svensson[1], J. Matos[5], C. Rodriguez-Fernández[6], A. M. Arruda[4], I. Fernandes[7], S. Davis[3], A. Götherström[8], and C. Ginja*[2], [1]*Department of Organismal Biology, Uppsala University, Uppsala, Sweden;* [2]*CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, Vairão, Portugal;* [3]*Laboratório de Arqueociências-InBIO, DGCP, Lisboa, Portugal;* [4]*UNIARQ, Centro de Arqueologia da Universidade de Lisboa, Faculdade de Letras, Universidade de Lisboa, Lisboa, Portugal;* [5]*Grupo de Biologia Molecular, Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal;* [6]*Departamento de Historia, Facultad de Filosofía y Letras, Universidad de León, León, Spain;* [7]*Câmara Municipal de Palmela, Palmela, Portugal;* [8]*Archaeological Research Laboratory, Department of Archaeology and Ancient History, Stockholm University, Stockholm, Sweden.*

Cattle mitochondrial DNA is geographically structured. This enables the association of maternal lineages to specific regions. For example, the T1 and T3 haplogroups predominate in Africa and Europe, respectively. Extant cattle from the Iberian Peninsula show high genetic diversity despite their considerable geographical distance from their presumed Near-Eastern centre of domestication. However, it is not clear if this pattern is recent or ancient. Our aim is to use a zooarchaeogenetics approach to characterise the genetic diversity and investigate phylogenetic relationships of past domestic cattle populations from the Iberian Peninsula. Sixty-two specimens dating between the 1st century BC and the 15th century AD were selected for ancient DNA analysis. Targeted high-throughput 454-JS Junior sequencing technology (Roche) was used to examine ~220 bp of the mitochondrial D-loop region. Consensus sequences were aligned against NCBI reference data from extant cattle representing major haplogroups and phylogenetic relationships were inferred using median-joining networks. We successfully extracted and analysed DNA sequences from cattle remains preserved under suboptimal environmental conditions, i.e. a temperate climate. High-coverage allowed sequence validation and authentication. Overall, T3-haplotypes predominate in the Iberian Peninsula (~80% of the total) at least since the Roman occupation, but T1-lineages of putative African origin were also detected (~15%). The distinct Q-lineage, which is found in low frequency in extant Iberian cattle (~5%), was observed for the first time in one specimen from Roman Monte Molião (southern Portugal), but also in one specimen from Moslem Alcáçova de Santarém (near Lisbon, Portugal) and in one specimen from post-medieval Christian Beja (southern Portugal). The T2-lineage, which is predominant in Asia, was only found in one specimen from Beja. Our results corroborate observations from other studies of both ancient and extant domestic cattle that indicate a genetic continuity of maternal lineages over time between cattle populations from the same location, and suggest post-Medieval cattle were improved locally.

Key Words: Iberian cattle, ancient DNA, mitochondrial sequencing, biodiversity

91 Comparative genome-wide characterisation of five rare British Isles cattle breeds. P. Flynn*[1,2], J. Carlsson[2], and D. Berry[3], [1]*Weatherbys DNA Laboratory, Johnstown, Naas, Co. Kildare, Ireland;* [2]*University College Dublin, School of Biology & Environmental Science, UCD, Belfield, Dublin, Ireland;* [3]*Teagasc, Moorepark, Fermoy, Co. Cork, Ireland.*

Several cattle breeds within the British Isles have been subjected to reduction in population numbers over recent centuries. Five such breeds are Kerry (KY), Dexter (DX), DroimFhionn (DF), Irish Moiled (IM) and White Park (WP). Comparative genome wide characterisation studies contribute towards conservation strategies, aiming to ensure future survival and progression of such bovine genetic resources. Using a 4,345 SNP subset from the International Dairy and Beef (IDB) SNP chip, this study established genetic parameters such as diversity, differentiation and population structure for these five breeds. Samples were collected for each breed (total $n = 225$), with selection based on pedigree knowledge to maximise within breed representation. Available datasets for Angus (AN) and Holstein Friesian (HF) breeds were also included for comparative purposes (total $n = 100$). Reduced within breed genetic diversity, relative genetic isolation and strong population structure (@ K = 7) was observed for both WP (He 0.36502 ± 0.14465) and IM (He 0.36712 ± 0.14396). Greatest genetic distance was also observed between WP and IM (Fst 0.21624, $P < 0.01$). KY (He 0.41602 ± 0.10763) and DX (He 0.43498 ± 0.08935) displayed comparable within breed genetic diversity, however Principle Component and Structure analysis (@ K = 7) generated distinct clusters for both breeds. The DF breed displayed genetic diversity (He 0.41854 ± 0.10712) similar to the overall mean (He 0.41214 ± 0.10882) with comparative genetic distances ranging from closest - HF (Fst 0.09263, $P < 0.01$) to furthest - WP (Fst 0.17342, $P < 0.01$). Key findings within this dataset include – reduced genetic diversity and

genetic isolation for both IM and WP, along with evidence of distinct differentiation between KY and DX. Novel insights into DF genetic diversity and distinctiveness were revealed within this breed's first ever comparative genome wide analysis. Results provide a genome wide snapshot of current genetic status for each rare breed and a benchmark to monitor future breeding strategies or genetic shifts.

Key Words: cattle and related species, comparative genomics, single-nucleotide polymorphism (SNP), breed diversity, conservation

92 Diversity of sheep breeds in Russia based on SNP analysis. T. Deniskova*[1], A. Dotsev[1], M. Selionova[2], K. Wimmers[3], H. Reyer[3], V. Kharzinova[1], E. Gladyr[1], G. Brem[1,4], and N. Zinovieva[1], [1]*L.K. Ernst Institute of Animal Husbandry, Podolsk, Moscow region, Russia;* [2]*All-Russian Research Institute of Sheep and Goat, Stavropol, Stavropol region, Russia;* [3]*Institute of Genome Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany;* [4]*Institute of Animal Breeding and Genetics, VMU, Vienna, Austria.*

Due to a huge territory, Russia is characterised by great variety of climate and relief conditions. The combination of these features led to creation of wide range of sheep breeds with high adaptive capacity to local environmental conditions and variety of productivity qualities. High-throughput SNP arrays allowed to investigate genetic traits of animals at the genome level and to understand more fully the character of relationships between breeds. Nevertheless, such a detailed study has not been performed up to now on Russian sheep breeds. In this regard, the aim of our work was to evaluate the genetic diversity and to study genetic relationships between the most widespread sheep breeds in Russia using whole-genome analysis. We have genotyped 411 individuals from the most popular sheep breeds in Russia, including 11 coarse wool (CW, n = 205), 5 semi-fine wool (SFW, n = 78) and 9 fine wool breeds (FW, n = 128) by using Illumina OvineSNP50 BeadChip. Statistical indicators were calculated in PLINK 1.07, GENETIX 4.05, HP-Rare 1.1. The lowest level of genetic diversity was found for CW group (Ho = 0.374; Ar = 1.89), whereas the FW and SFW groups showed similar levels of observed heterozygosity (Ho = 0.383…0.385) and allelic richness (Ar = 1.92). Among all the breeds the maximum measures of diversity were detected for Baikal fine wool (Ho = 0.394; Ar = 1.93), whereas minimum values were estimated in Romanovs (Ho = 0.350; Ar = 1.86). The breeds were characterised by insignificant heterozygote excess from 1.1% in the SFW group to 1.6% in the CW and the FW groups. Phylogenetic tree showed that breed`s distribution corresponded to their wool type. Thus, the CW breeds formed distant cluster from FW and SFW and had more branchy structure. The most CW originated from unique local sheep whereas the SFW were created with using English Long Wool rams, and Australian Merino improved the FW. The whole genome SNP data on sheep breeds is the first step to design effective selection and conservation programs for local breeds. Besides introduction of new methods, this will lead to prosperity of Russian sheep breeding. The research was funded by Russian Scientific Foundation (14–36–00039).

Key Words: sheep, biodiversity, single nucleotide polymorphism

93 Ancient whole mitochondrial genomes and insights into the prehistory of goats. K. Daly*, *Smurfit Institute of Genetics, Trinity College, Dublin, Ireland.*

The domestication of goats (*Capra hircus*) from bezoar (*Capra aegagrus*) is thought to have occurred in the Near East ~10,000 years ago. As one of the earliest domesticated animals, elucidating the patterns, pace and major events of the process is of great interest. However, such analyses using genomic data from modern goats are hampered by 10,000 years of human-mediated movement of goat. Ancient DNA allows populations before this be directly sampled. We present an initial analysis of whole mitochondria data from goat sampled from a range of time depths. We observed a high degree of mitochondrial diversity at earlier periods followed by a significant restriction which has shaped modern goat mitochondrial diversity. We also report a 14 thousand year old caprid mitochondrial lineage most similar to the Caucasian Tur (*Capra caucasica*), having diverged from it over 100,000 years ago.

Key Words: goats and related species, ancient DNA, animal domestication

94 Genome-wide analysis for signature of selection in domestic chicken and red jungle fowl. R. A. Lawal*[1] and O. Hanotte[1,2], [1]*The University of Nottingham, Nottingham, Nottinghamshire, UK;* [2]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia.*

The red jungle fowl *Gallus gallus* is the main maternal ancestor of modern chicken having been domesticated from multiple subspecies of *Gallus gallus* more than 5400 years ago. Following domestication, environmental and human selection pressures have shaped the domestic chicken making them one of the most diverse livestock species with population of more than 50 billion across the world. Here, we report the analysis of indigenous chicken population from Ethiopia, Saudi Arabia and Sri Lanka, alongside the red jungle fowl, for signature of positive selection across the autosomes. Using the pool heterozygosity and composite likelihood ratio methods, we identified several candidate genomic regions that may correspond to local adaptation along with five candidate domestic regions shared and unique to all domestic populations. Only two candidate regions located on chromosomes 7 and 23 out of the 191 found in the red jungle fowl were shared with all domestic chicken populations. Gene ontology show that most of the seven genes located within the sweep region on chromosome 23 are associated with development with particular emphasis on central nervous system, memory, emotion and learning traits. Finally, this study identify several regions unique to each chicken ecotypes and several regions linked to production and growth traits in Saudi Arabia chicken

Key Words: genome sequencing, genomic selection, candidate gene, gene ontology, animal domestication

95 Genome-wide assessment of genetic diversity in the Synbreed Chicken Diversity Panel. S. Weigend*[1], A. Weigend[1], D. Malomane[2], and H. Simianer[2], [1]*Friedrich-Loeffler-Institut, Institute of Farm Animal Genetics, Neustadt-Mariensee, Höltystraße 10, Germany;* [2]*University of Göttingen, Animal Breeding and Genetics Group, Göttingen, Albrecht-Thaer-Weg 3, Germany.*

Genetic diversity within a given farm animal species refers to the variety of genetic variants accumulated during domestication. High density SNP genotyping arrays allow genome-wide assessment of structural variation between genomes of individuals, families and populations. Within the framework of the SYNBREED project a wide range of chicken breeds were sampled in 34 countries across 4 continents. Sampling was supported by a world-wide collaborative effort including 21 partners from 17 countries (Argentina, Australia, Bangladesh, Chile, Egypt, Ethopia, Finland, Germany, Hungary, Pakistan, Saudi Arabia, Sudan, Switzerland, Tanzania, Turkey, United Kingdom, and Vietnam). It was augmented by samples of two Red Junglefowl populations (*Gallus gallus gallus* and *Gallus gallus spadiceus*) as well as 12 commercial purebred chicken lines (brown layers, white layers, broilers) and 10 local chicken breeds taken from the previous EU project AVIANDIV. This 'Synbreed Chicken Diversity Panel (SCDP)', which encompasses more than 3200 individuals of 175 populations, was genotyped with the 580K SNP Affymetrix Chicken Genotyping array. First cluster analyses showed a continuous transition from Asian type breeds to European breeds as well as a separation of breeds according to body size, i.e. normal sized breeds and bantam breeds. Wild populations as well as

commercial broiler lines cluster within this spectrum of diversity, whereas commercial white and brown egg layer lines formed distinct and rather opposite edges of it. Chicken populations from Africa and Asia showed a lower proportion of genomic regions in Runs of Homozygosity (ROH), while chicken populations sampled in Europe displayed a wide variation ranging from 4 to ~70 percent of the genome being included in ROH. Regarding the commercial lines, the genome of white layer lines was least polymorphic, while broiler lines clustered at the polymorphic end of the SCDP spectrum. Brown egg layers showed a medium degree of variability. Detailed analyses will evaluate the extent and distribution of variation, the extent of linkage disequilibrium and the distribution of ROH across chromosomes. The SCDP is an excellent resource to get insight into mechanisms underlying the diversification within the species.

Key Words: chickens, biodiversity, SNP markers

97 Detection of selection signals between Merino and Churra sheep breeds. B. Gutierrez-Gil*[1], P. K. Chitneedi[1], A. Suarez-Vega[1], P. Wiener[2], C. Esteban-Blanco[1], and J. J. Arranz[1], [1]*Department of Animal Production, Faculty of Veterinary Sciences, University of León, León, Spain;* [2]*Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush Campus, Midlothian, United Kingdom.*

Selection affecting desired phenotypes has left detectable signatures of selection within the genomes of modern sheep. The aim of this study was the identification of selection signals related to wool production. To achieve this, we contrasted the OvineSNP50 BeadChip genotypes of 332 samples from three Australian Merino sheep populations, characterised by the production of extremely fine wool, and 378 samples of Spanish Churra sheep, a coarse wool sheep breed phylogenetically close to Merino. With the aim of identifying genomic regions harboring selection signatures in the two breeds, we performed four different analyses. In addition to genetic differentiation (F_{ST}) and reduced heterozygosity (*ObsHtz*) analyses, we also performed two complementary analyses based on haplotype structure using the *hapFLK* and *reHH* (*XPEHH* test) programs. We defined selection signature candidate regions (CR) by grouping the positions showing extreme values from the four approaches into discrete regions, based on the extent of linkage disequilibrium in the two breeds. Subsequently, convergence candidate regions (CCRs) were identified as those CRs that overlapped between the different approaches, i.e. to qualify as a CCR, overlap was required between a region identified by at least one of the methods based on allele/genotype frequencies (F_{ST}/*ObsHtz*) and at least one of the methods based on haplotype analysis (*hapFLK/XPEHH)*. The F_{ST} analysis identified a total of 49 CRs, whereas the *ObsHtz* analysis identified 96 and 72 regions in Merino and Churra respectively. The *hapFLK* and the XPEHH analyses identified seven and 98 significant (P-value < 0.001) selection sweeps regions, respectively. Overlap between these regions defined a total of 18 CCRs, on chromosomes 2, 3, 6, 8, 10, 11, 15 and 25. Five of the CCRs were related to positive selection in Merino while the rest were related to positive selection in Churra. Further study of genetic variation within these regions may help to identify candidate mutations underlying the selection signals reported here, some of which are expected to be related to the specialization of the Australian Merino for wool quality traits.

Key Words: sheep and related species, single-nucleotide polymorphism (SNP), selection scan, wool production

98 Distribution of polymorphisms in major and candidate genes for productive and domestication-related traits in European local pig breeds. A. Fernández[1], M. Muñoz[1], F. García[1], Y. Núñez[1], C. Geraci[2], A. Crovetti[3], J. García-Casco[1], E. Alves[1], M. Skrlep[4], J. Riquet[5], M. Mercat[6], R. Bozzi[3], M. Candek-Potokar[4], L. Fontanesi[2], C. Óvilo*[1], [1]*INIA, Madrid, Spain;* [2]*UNIBO, Bologna, Italy;* [3]*UNIFI, Firenze, Italy;* [4]*KIS, Ljubljana, Slovenia;* [5]*INRA, Toulouse, France;* [6]*IFIP, Paris, France.*

TREASURE is a multidisciplinary European project focused on the development of activities for the benefit of sustainable pork chains based on European local pigs. One of its main objectives is the genetic characterisation of local pig breeds participating in the project, by using genetic and genomic tools. The most relevant genes and mutations associated with pig productive, meat quality, reproductive and disease resistance traits were prioritized and analysed in order to identify useful markers for authentication, traceability, conservation and breeding programmes. A panel of 32 SNPs were selected and a genotyping chip was designed and employed to genotype 48 animals from each one of 20 breeds included in the project (Schwäbisch Hällisches, Iberian, Black Majorcan, Basque, Gascon, Black Slavonian, Turopolje, Apulo Calabrese, Casertana, Cinta Senese, Mora Romagnola, Black Sicilian, Sarda, Lithuanian indigenous wattle, Old Lithuanian White, Alentejano, Bisaro, Mangalitsa, Moravka, Krskopolje). Twenty seven SNPs located in 24 genes were succesfully genotyped (*MC1R, TYRP1, NR6A, PCK1, RYR1, IGF2, MC4R, PHKG1, SCD, GBP5, TAS2R39, TAS2R4, MUC4, ESR1, CYP2E1, LEP, CAST, MTTP, CYB5A, FTO, PPARGC1A, CAPN1, PPARD, CTSL*). Results show very interesting findings, as lack or scarce segregation of markers in genes involved in coat colour or productive and reproductive traits, such as *MC1R*, *ESR1* or *CTSL* in all the analysed breeds, with useful implications for traceability. On the other hand, major gene alleles with contrasted effects on production and fatness (such as *RYR1*, *IGF2*, *MC4R*, *LEP*), meat quality (*SCD*, *CAST*, *MTTP*) or disease resistance (*MUC4*, *GBP5*) segregate in most breeds, in some cases with intermediate frequencies, opening selection possibilities. These results joint with ongoing genomic, transcriptomic and metagenomic assays, will provide essential information regarding genetic diversity, structure, selective signatures and population-specific biological processes responsible for specific production and quality traits. TREASURE project is funded under European Union's Horizon 2020 research and innovation programme, grant no. 634476

Key Words: pig, local breed, SNP, major gene, allele frequency

188 Genetic diversity among domestic goats (*Capra hircus*) and wild goats (*Capra aegagrus*) in Turkey. I. S. Yildirim[1], M. Nizamlioglu[1], M. D. Oncu[2], E. K. Bastanlar[2], and Z. Bulut*[1], [1]*Selcuk University, Faculty of Veterinary Medicine, Departments of Biochemistry, Konya, Turkey;* [2]*TUBITAK-MAM, Genetic Engineering and Biotechnology Institute, Gebze, Kocaeli, Turkey.*

Characterisation of populations at the molecular level makes it possible to define genetic distances among and between populations. For this purpose, microsatellites are frequently preferred for that they generally are not subject to natural selection and that they show variation among and between populations directly according to time and mutation rate. In this study, the genetic similarities and differences between Kilis, Shami, Honamli, Saanen, Hair, Angora and wild goats were investigated through the use of 320 individual goat samples. DNA isolation was conducted using a standard phenol/chloroform method. Twenty different microsatellite loci were identified and PCR-amplified. The Beckman Coulter CEQ-8000 Genetic Analysis System was used to fractionate and genotype individual microsatellite markers. The total number of alleles, observed heterozygosity and expected heterozygosity values under Hardy–Weinberg Equilibrium were estimated. It was observed that the number of total alleles varied between four and 25 for different loci. While observed mean heterozygosity values differed between 0.499 and 0.632, it was seen that expected mean heterozygosity ranged between 0.609 and 0.705. Using the Structure program and FCA graphs, it was observed that wild goats are more genetically homogenous than domestic goats and that they could be classified separately. Interestingly, wild goat heterozygosity was lower than domestic goats. Furthermore, it was found that there were no ge-

netic differences between the Ankara goat Eskisehir and Lalahan populations. This study was supported by Selcuk University BAP (Project Number: 15202004) and TUBITAK-KAMAG (Project Number: 109 G 103).

Key Words: genetic diversity, wild goat, domestic goat

Ruminant Genetics and Genomics

99 Evidence from the bovine of major differences between individuals in the rate of *de novo* single nucleotide mutation and transposon mobilization in the germline. C. Harland[1,2], K. Durkin[1], M. Artesi[1], L. Karim[1,3], N. Cambisano[1,3], M. Deckers[1,3], N. Tamma[1,3], E. Mullaart[4], W. Coppieters[1,3], M. Georges[1], and C. Charlier*[1], [1]*Unit of Animal Genomics, GIGA-R, University of Liège, Liège, Belgium;* [2]*Livestock Improvement Corporation, Research & Development, Hamilton, New Zealand;* [3]*GIGA-Genomics Platform, University of Liège, Liège, Belgium;* [4]*CRV, Research & Development, Arnhem, Netherlands.*

To study the process of *de novo* mutations in the bovine germline, we have sequenced the whole genome of >750 individuals constituting 130 sire-dam-offspring trios with at least five grand-offspring each. A first study using four pedigrees revealed the common occurrence of somatic and germline mosaicism for *de novo* mutations pointing towards mutation-prone early cleavage cell divisions (http://biorxiv.org/content/early/2016/10/09/079863). We herein characterise *de novo* mutations in the remaining 126 pedigrees. Two observations point towards major inter-individual differences in the rate of *de novo* mutations. We first identify one sire characterised by a mutation rate that is ~3-fold larger than the population average. We show that this remarkable increase is due to a ~7-fold excess of mutations occurring at the very early stages of development (on the basis of observed mosaicism). The corresponding mutations are characterised by a ~8-fold excess in C to T transitions outside the CpG context. The corresponding animal was shown to be the only individual of the pedigree to be homozygous for a rare disruptive mutation in components of the DNA repair or replication machinery: a *P* > L substitution in the REV1 DNA Directed Polymerase. The causality of this mutation is presently being examined. We further developed a pipeline to detect *de novo* transposition and pseudogene mobilization events. We identified a family of LTR elements that are still active in the bovine genome. We detected five corresponding *de novo* transposition events, of which three occurred in the same individual including two in the same gamete. Latest results of both studies will be presented.

Key Words: de novo mutations, mosaicism, transposable elements, pseudogenes, whole genome sequences

100 Pinpointing causal mutations among imputed sequence variant genotypes in three cattle breeds. H. Pausch*[1,2], I. MacLeod[1], P. Bowman[1,3], R. Emmerling[4], R. Fries[5], B. Gredler-Grandl[6], H. Daetwyler[1,3], and M. Goddard[1,7], [1]*Agriculture Victoria, AgriBio, Centre for AgriBioscience, Bundoora, VIC, Australia;* [2]*Animal Genomics, Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland;* [3]*School of Applied Systems Biology, La Trobe University, Bundoora, VIC, Australia;* [4]*Institute of Animal Breeding, Bavarian State Research Centre for Agriculture, Poing-Grub, Germany;* [5]*Chair of Animal Breeding, Technische Universitaet Muenchen, Freising, Germany;* [6]*Qualitas AG, Zug, Switzerland;* [7]*Faculty of Veterinary and Agricultural Science, University of Melbourne, Melbourne, VIC, Australia.*

The whole-genome sequencing of key ancestors of many cattle breeds yielded genotypes at millions of polymorphic sites. These data can be used as a reference population to impute sequence variant genotypes for tens of thousands of animals that have dense array-derived genotypes. Accurately imputed sequence variant genotypes may improve genomic predictions and facilitate pinpointing causal mutations in genome-wide association studies because the polymorphisms that underlie phenotypic variation are included in the data. We assessed the accuracy of imputing sequence variant genotypes in 249 Fleckvieh and 450 Holstein bulls using 39,728,987 sequence variants of 1577 animals from the fifth run of the 1000 bull genomes project. Imputation was performed using either *Minimac* or *FImpute* considering either within- or multi-breed reference populations. Regardless of the composition of the reference population and imputation software tested, the overall accuracy of imputation was high in both breeds (0.898 – 0.952). However, several segments with poor imputation quality were detected particularly at regions where the bovine genome contains large structural variants. The highest accuracy of imputation was obtained when *Minimac* was used to infer sequence variant genotypes and when allele dosages rather than best guess genotypes were considered at the imputed sequence variants. Using a multi-breed reference population increased the accuracy of imputation particularly at low-frequency variants. Next, we inferred genotypes for more than 23 million sequence variants in 6778 Fleckvieh, 5204 Holstein and 1646 Brown Swiss bulls using 1577 sequenced animals from various breeds as a reference population. Association tests between the predicted allele dosages at imputed sequence variants and daughter-derived phenotypes for protein and fat percentages in milk revealed several QTL and candidate causal mutations that were mostly detected in more than one breed. Two causal mutations in the *DGAT1* and *GHR* genes were the most significantly associated variants at two QTL on chromosomes 14 and 20 demonstrating that highly precise QTL mapping is possible with imputed sequence variant genotypes.

Key Words: cattle and related species, genome sequencing, imputation, genome-wide association, complex trait

101 Genotyping by sequencing for genomic selection in dairy goats (*Capra hircus*). S. Clarke*[1], K. Dodds[1], R. Brauning[1], T. Van Stijn[1], R. Anderson[1], M. Wheeler[2], B. Foote[3], A. Cameron[4], and J. McEwan[1], [1]*AgResearch, Mosgiel, Dunedin, New Zealand;* [2]*AgResearch, Ruakura, Hamilton, New Zealand;* [3]*Foote Dairy, Hikurangi, Northland, New Zealand;* [4]*Meredith Dairy, Meredith, Victoria, Australia.*

High-throughput genotyping by sequencing (GBS) methodology produces SNP genotypes that are supported by varying depth of sequence reads, dependent on the number of samples and proportion of the genome assayed within a lane of sequencing. Although additional samples per lane is more cost effective, a balance is needed to achieve the required sequence read depth to support the SNP genotype for downstream applications such as genetic diversity and differentiation, parentage assignment, inbreeding estimation, genomic selection and genome wide association studies. Advances in statistical methods tailored to GBS data have enabled the use of GBS-derived genomic relationship matrices to implement genomic selection via GBLUP for the dairy goat industry. GBS is a cost effect alternative to chip arrays that are further compromised by large and inflexible setup costs. Although GBS has suffered from a variety of technical challenges (increased complexity of data processing, a high proportion of missing genotypes, and low accuracy of genotype calls), these have been overcome enabling this technology to be implemented in the dairy goat herd. Here we present an example in both a New Zealand and Australian dairy goat herd utilising

a methodology that comprises ~60,000 SNPs for less than US$20/sample. The use of GBS has considerable implications for future goat genomic research especially when utilising this technology for high density genotyping (>200k SNPs), due to the lack of a suitable HD chip.

Key Words: genomic selection, GBLUP, dairy goats, genotyping by sequencing, GBS

102 Identification of polymorphisms modifying gene expression regulation in cattle. G. Guillocheau* and D. Rocha, *GABI, INRA, AgroParisTech, Université Paris Saclay, Jouy-en-Josas, France.*

Thanks to the advent of novel sequencing technologies, an increasing number of polymorphisms have been identified in genic regions. These polymorphisms can play an important role in gene expression regulation. Allele-specific expression (ASE) analysis is a robust approach to detect *cis*-regulatory variations of gene expression. Because of its economic importance, cattle were one of the first mammals to have its genome sequenced. During this sequencing more than 2.2 million putative Single Nucleotide Polymorphisms (SNPs) have been detected. Since many bovine genomes have been sequenced and there is currently more than 99 Million SNPs. Polymorphisms showing allele-specific expression could be linked to economic important traits and therefore could help to improve genetic selection. The aim of our project is to develop a pipeline to predict polymorphisms that modify the regulation of gene expression. Association studies between these predicted polymorphisms and important phenotypes will later be performed. We used RNA-seq data from muscle samples of 19 Limousin bull calves (77 Million reads in average per samples) and from eight different tissue samples (heart, kidney, liver, lung, muscle, ovary, spleen and uterus) of six Holstein cows (65 Million reads in average by samples). We had also the whole-genome DNA sequences for these 25 animals (an average coverage of 15 Limousin samples and 5 Holstein samples). The RNA-seq data was aligned with STAR, an ultrafast RNA-seq aligner and we predicted polymorphisms (SNPs and small insertions/deletions) with GATK for DNA and RNA sequence data. The ASE detection was performed using ASEReadCounter and binomial test. We detected more than 150,000 SNPs showing ASE in all samples with this method. Currently, our pipeline can detect SNPs in genes with an allelic imbalance for species with a reference genome sequence available. We select interesting polymorphisms to perform a experimental validation using pyrosequencing.

Key Words: bioinformatics, polymorphisms, genome regulation, sequencing, transcriptomics

103 GWAS for response to vaccination in Angus calves. L. Kramer*[1], M. Mayes[1], J. Williams[1], E. Fritz-Waters[1], E. Downey[2], R. Tait Jr.[3], A. Woolums[4], C. Chase[5], J. Ridpath[6], and J. Reecy[1], [1]*Iowa State University, Ames, IA, USA;* [2]*Elanco Animal Health, Larchwood, IA, USA;* [3]*Neogen GeneSeek Operations, Lincoln, NE, USA;* [4]*Mississippi State University, Mississippi State, MS, USA;* [5]*South Dakota State University, Brookings, SD, USA;* [6]*Ridpath Consulting, Gilbert, IA, USA.*

Bovine Respiratory Disease Complex (BRDC) is an economically important disease and an animal welfare issue. While vaccines have been shown to be efficacious, morbidity and mortality still persists. To examine the genetics of response to vaccination, Iowa State University Angus calves (>2000 head) were vaccinated for Bovine Viral Diarrhoea Virus 1 and 2, Bovine Respiratory Syncytial Virus, and Bovine Herpes Virus 1. Serum neutralization scores for each virus were collected across multiple time points to allow for identification of genomic regions associated with response to vaccination. The response to vaccination traits were initial serum neutralization score (week 0), initial response to vaccination (week 3 – week 1), response to booster vaccination (week 6 – Week 3), overall response to vaccination (week 6 – week 0), and final antibody titer score (week 6). In addition, maternal decay and pre-vaccination titer scores were collected on a subset of individuals for Bovine Viral Diarrhoea Virus 1 and 2 only. A genome wide association study was performed using imputed 770k (BovineHD beadchip) markers and a BayesB statistical model with pi of 0.999 (575 most associated markers). 1-Mb windows with a posterior probability of inclusion (PPI) 0.9 or greater were identified from the BayesB analysis, with each window accounting for a minimal portion of the total genetic variation. Genes within the 1-Mb windows were examined for potential candidacy for causality. These associated windows may give insight into the genetic control of response to vaccination, and indicate avenues of advancement in improving vaccines and vaccination protocols against BRDC.

Key Words: cattle, genome-wide association, antibody response, bovine respiratory disease complex

104 The water buffalo gene expression atlas. R. Young*[1], L. Lefevre[1], S. Bush[1], J. Williams[2], S. Gokhale[3], S. Kumar[4], A. Archibald[1], and D. Hume[1], [1]*The Roslin Institute and Royal (Dick) School of Veterinary Studies (RDSVS), Easter Bush, Midlothian, UK;* [2]*School of Animal and Veterinary Sciences, The University of Adelaide, Adelaide, South Australia, Australia;* [3]*BAIF Development Research Foundation, Central Research Station, Pune, Maharashtra, India;* [4]*Centre for Cellular and Molecular Biology, Hyderabad, Telangana, India.*

The domestic water buffalo (*Bubalus bubalis*) contributes significantly to the global agricultural economy through milk, meat, hides and draught power, and a larger part of the human population depends on domestic water buffalo than on any other livestock species in the world. Despite its agricultural importance, the buffalo genome is not fully annotated. We have generated a fine scale gene expression atlas of 220 tissue and cell types collected from adult riverine water buffalo (Mediterranean, Pandharpuri and Bhadawari breeds). Accompanying whole genome sequence data were also generated for each breed. Gene expression was quantified from RNA-Seq data and visualised using the network analysis tool Miru, allowing the co-expression of genes to be explored across tissues. The atlas data are also being used to analyse alternative splicing, candidate expressed SNPs and allelic expression imbalance and compared to other ruminant species. The sample metadata have been loaded into BioSamples and the sequence data will be deposited in ENA. This data will be a valuable resource for the international Functional Annotation of Animal Genomes (FAANG) initiative. This study is the largest gene expression atlas generated in water buffalo to date. Potential variation identified between domestic breeds will form the basis for the development of predictive marker-assisted selection and breed improvement in the buffalo industry.

Key Words: water buffalo, transcriptome, genome annotation, FAANG, breed improvement

105 Origin and evolutionary history of the European bison unraveled through ancient DNA. T. Grange*, D. Massilani, S. Guimaraes, and E.-M. Giegl, *Institut Jacques Monod, CNRS, University Paris Diderot, Paris, France.*

The European bison or wisent (*Bison bonasus*), one of the last wild European large mammals, narrowly escaped extinction at the onset of the 20th century. It shares a common ancestry with both American bison (*Bison bison*) and modern cattle (*Bos primigenius* f. *taurus*), although its evolutionary history since the divergence of these three lineages during the Early Pleistocene has never been well characterised. Here we describe complete and partial mitog-

enomes from 57 ancient Eurasiatic bison specimens dating from 50 kiloyears ago (kya) to the early 20th century, just preceding the last major bottleneck, and covering the area from Western Europe to the Caucasus and to Siberia. Our results reveal several waves of population expansion, contraction and extinction. Three bison populations successively occupied Western Europe during this time frame and their presence can be correlated with major climatic and environmental fluctuations. First, an ancestral, now extinct, *Bison bonasus* clade was dominant during most of the temperate Marine Isotope Stage (MIS) 3 (ca. 57 – 29 kya). Second, a steppe bison (*Bison priscus*) population originating from North-East Eurasia recolonized Western Europe during the following cold period of the last glaciation (MIS2 ca. 29 – 14 kya). We hypothesise that the population overlap we observed in southern France during this transition period (39–34 kya) is reflected in the contemporaneous rock paintings of the Chauvet cave in the same area. Third, the steppe bison was replaced after the last glacial maximum at the beginning of the Holocene (MIS1) by a separate *Bison bonasus* population that previously occupied a refuge encompassing the southern Caucasus. This last population survived up to the Middle Ages in France, while a related population survived into the 20th century in Eastern Europe, where it can still be found today. The present-day pattern of reduced genetic diversity of the wisent preceded the last major bottleneck of World War I. Climatic oscillations, environmental changes and, recently, anthropogenic pressure have both shaped the evolutionary history of this emblematic species in different ways.

Key Words: bison, ancient DNA, Europe

106 Signals of adaptive introgression between European taurine and indicine cattle revealed by local ancestry inference. M. Barbato*[1], M. Del Corvo[1], T. Sonstegard[2], and P. Ajmone-Marsan[1], [1]*Istituto di Zootecnica, Universitá Cattolica del Sacro Cuore, Piacenza, Italy;* [2]*Recombinetics Inc., St. Paul, MN, USA.*

European taurine cattle (*Bos taurus*) was domesticated in the Fertile Crescent around 8,000 year BC and later colonised Europe, Asia and Africa following the agriculture wave. A second domestication involving the ancestors of the modern humped zebu cattle (*Bos indicus*) occurred 2,000 years later in the Indus valley, in Central Asia. Admixture between taurine and indicine species occurred extensively in the past, the indicine species sometimes contributing to taurine's genetic pool with a better adaptation to tropical climate and diseases, and an improved ability to thrive with very poor fodder. Interestingly, a small percentage of indicine ancestry can be detected in several modern taurine breeds. With the aim to identify the putative adaptive nature of such indicine × taurine introgression, we analysed Illumina BovineHD SNP genotypes of 16 Chianina cattle sampled in central Italy, along with 187 individuals from six reference breeds of taurine (3) and indicine (3) origin. Local ancestry investigations involving nine reference population combinations were performed and smoothed using the CIWI (Consistently Introgressed Windows of Interest) analytical framework, able to identify concordant and reference-independent genomic regions of a given ancestry. Among the CIWIs of indicine ancestry identified in Chianina, the strongest signal was recorded in chromosome 18. Haplotype homozygosity-based selection sweep analysis evidenced signatures of selection occurring within the same genomic region. Here, we infer the putative adaptive nature of this ancestral indicine genome portion, and suggest its association to indicine cattle's superior ability to efficiently use poor quality fodder.

Key Words: cattle, local ancestry, adaptive introgression, SNP array, selection signature

107 Cattle on the Western Atlantic edge of Europe: A time series of ancient cattle genomes through Ireland and Britain. V. Mullin*, *Trinity College Dublin, Dublin, Ireland.*

The domestication of cattle marks a significant period of time in human prehistory. The initial neolithic movement of domestic cattle across western Europe concluded with the movement of animals to the Western Atlantic edge; the islands of Ireland and Britain. One approach to understanding this past is the study of variation in modern cattle genomes to model past demography, admixture and selection. However, an alternative, more challenging and promising approach is the the direct study of archaeological genomes. Ancient genomes provide a snapshot of the genetic diversity present in the past, allowing for the exploration of the timing of population events such as an admixture, migration and turnover. The combination of technological advancements in next-generation sequencing and improved sampling techniques of archaeological samples enables the sequencing of many more ancient individual animals than previously possible, and has allowed us to compare the genomes of multiple animals across space and time. We have sequenced a time series of ~20 ancient Irish and British cattle genomes with genome coverage ranging from 0.2× to 17× sampled from the Neolithic (3500BC) to the Middle Ages (1200AD). The application of population genomics techniques to these data provide new insights into the demography of the prehistoric livestock of these islands.

Key Words: cattle and related species, palaeogenomics, ancient DNA, animal domestication

108 Whole genome structural analysis of Caribbean hair sheep reveals quantitative link to West African ancestry. G. Spangler*[1], B. Rosen[1], O. Hanotte[2], T. Sonstegard[3], and C. Van Tassell[1], [1]*USDA/ARS/AGIL, Beltsville, MD, USA;* [2]*U of Nottingham/School of Life Sciences, Nottingham, UK;* [3]*Acceligen of Recombinetics, St Paul, MN.*

Hair sheep of Caribbean origin have become an important part of the USA sheep industry. Lack of wool eliminates several health concerns and drastically reduces the cost of production. More importantly, Caribbean hair sheep demonstrate robust performance even in the presence of drug resistant gastrointestinal nematodes, a rising concern to the industry. Despite the growing importance of hair sheep in the Americas their genetic origins have remained speculative. Prior to this report no genetic studies were able to identify a unique geographical origin of hair sheep in the New World. Our study clarifies the African and European ancestry of Caribbean hair sheep. Whole genome structural analysis was conducted on four established breeds of hair sheep from the Caribbean region. Using breeds representing Africa and Europe we establish an objective measure indicating Caribbean hair sheep are derived from Iberian and West African origins. Caribbean hair sheep result from West African introgression into established ecotypes of Iberian descent. Genotypes from 47,750 autosomal single nucleotide polymorphism markers scored in 290 animals were used to characterise the population structure of the St Croix, Barbados Blackbelly, Morada Nova, and Santa Ines. Principal components, admixture, and phylogenetic analyses results correlate with historical patterns of colonization and trade. These results present an important basis for further investigation into desirable traits attributed to hair sheep of the region such as heat tolerance and nematode resistance.

Key Words: sheep, genome, genotyping, admixture, breed identification

109 A worldwide investigation of the effects of climate selection on goat genomes. F. Bertolini*[1], E. Rochat[2], S. Joost[2], B. Servin[3], P. Crepaldi[4], A. Stella[5], and M. F. Rothschild[1], [1]*Department of Animal Science, Iowa State University, Ames, IA, USA;* [2]*LASIG, EPFL, Lausanne, Switzerland;* [3]*INRA, Casta-*

net-Tolosan, France; [4]*DIMEVET, University of Milan, Milan, Italy;* [5]*PTP, Lodi, Italy.*

Climate factors can cause genomic selection pressures that can affect several traits in livestock. To investigate these effects on the goat genome, the ADAPTMAP consortium compiled a dataset of high-throughput genotyped animals collected from more than 30 countries. For the purpose of this project, each animal was classified according to its GPS location, discarding animals with no GPS coordinates, and an individual Köppen climate group was assigned. Then the following filters were applied: 1) For each group only breeds with at least 10 animals were considered and 2) If two breeds were located in different climate groups only the animals that belonged to the groups of known breed origin were considered. The filtered groups were as follows: 160 animals/7 breeds for group A (Tropical), 1,020 animals/30 breeds for group B (Arid), 744 animals/33 breeds for group C (Temperate) and 136/4 breeds for group D (Cold). The single SNP Fst analyses were performed comparing one group against the others merged together. The top 20 SNPs of each analysis were compared with the results provided by the landscape genomics approach to the same dataset filtered with independent criteria and performed using the Sambada software. At least 20 SNPs detected with the Fst were concordant for Fst and landscape genomics results, and were analysed to find genes nearby (± 100Kb). Among these 20 SNPs, 7 SNPs were found to differentiate the A groups from the others. Particularly, two SNPs on chromosome 5 were close to members of the HOX gene family that controls body plan of an embryo along the craniocaudal axis, has been linked to reproductive behaviour and is subject to selective pressure in several species. A total of six SNPs were detected for the B and C groups, separately. The allele frequency analyses of these SNPs revealed that the two groups have opposite major alleles that confirmed the different selection that may occur in temperate and arid environments. The regions nearby these SNPs contain genes that are linked to many functions, such as feed intake, growth phenotypes and pubertal development in cattle.

Key Words: goat, climate, adaptation, ADAPTmap

Animal Epigenetics

110 DNA methylation and microRNA modifications in scrapie. J. Toivonen[1], A. Sanz[1], O. López-Pérez[1,2], D. Sanz-Rubio[1], M. Salinas-Pena[1], J. Alejo[1], R. Bolea[2], J. Espinosa[3], J. Badiola[2], P. Zaragoza[1], J. Torres[3], and I. Martín-Burriel*[1,2], [1]*Laboratorio de Genética Bioquímica, IIS Aragón, IA2, Universidad de Zaragoza, Zaragoza, Spain;* [2]*Centro de Investigación en Encefalopatías y Enfermedades Transmisibles Emergentes, IIS Aragón, IA2, Universidad de Zaragoza, Zaragoza, Spain;* [3]*Centro de Investigación en Sanidad Animal, CISA-INIA, Valdeolmos, Madrid, Spain.*

Scrapie is a transmissible spongiform encephalopathy (TSE) of sheep and goats. Recent evidence suggests an important role for epigenetic mechanisms, such as DNA methylation or regulation of gene expression by microRNAs (miRNAs), in the pathogeny of neurodegenerative diseases. Studies on epigenetics may help in clarifying some of the molecular mechanisms of the scrapie associated pathology and could allow identification of molecules for diagnostics (biomarkers) and for therapeutic targets. We present here an analysis of global DNA methylation levels and miRNA profiling in the central nervous system of transgenic (Tg) murine models of scrapie in early (preclinical) and late (clinical) stages of the disease and a verification of these changes in sheep naturally infected with scrapie. Scrapie-induced alterations in small RNAs, including miRNAs, were determined by RNA sequencing in cervical spinal cord (SC) of Tg501 animals (Tg mice expressing the wild type ARQ goat *PRNP* allele) infected with scrapie in preclinical and clinical stages, and in their age-matched controls. After multiple correction, one and six significant miRNA alterations were found in preclinical and clinical stage, respectively. These miRNA alterations are currently being validated by quantitative PCR (qPCR) in the same Tg501 model, in Tg338 mice (Tg mice homozygous for the sheep VRQ allele) infected with ovine scrapie, and in sheep naturally infected with scrapie. On the other hand, global DNA methylation and hydroxymethylation was quantified using a colourimetric ELISA assay in SC from the three models. DNA methylation was significantly higher ($P < 0.05$) in SC from clinical Tg338 mice than in their age-matched controls. On the contrary, global DNA hydroxymethylation decreased in the early phases of the disease. Epigenetic changes are being validated in the remaining models, and the expression of genes encoding epigenetic enzymes (DNA methylases, histone deacetylases and ten-eleven translocation enzymes) is also being quantified by qPCR. This is the first time that DNA methylation changes are described in any model of prion diseases and we are currently performing genome-wide studies to investigate the true depth of epigenetic changes in these diseases.

Key Words: sheep, epigenomics, microRNA, RNAseq, infectious disease

111 Evaluating the role of epigenomic modifications in host-pathogen interaction for bovine alveolar macrophages infected with *Mycobacterium bovis*. A. O'Doherty*[1], K. Rue-Albrecht[2], J. Browne[1], T. Hall[1], N. Nalpas[3], D. Magee[1], S. Gordon[1,4], D. Vernimmen[5], and D. MacHugh[1,6], [1]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland;* [2]*NDM Research Building, University of Oxford, Oxford, UK;* [3]*Quantitative Proteomics and Proteome Centre Tübingen, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany;* [4]*UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland;* [5]*The Roslin Institute, University of Edinburgh, Easter Bush Campus, Midlothian, UK;* [6]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland.*

Epigenetic modifications, such as DNA methylation and chromatin modifications, are pivotal in orchestrating various biological processes, representing an important mechanism for conveying cellular response to external environmental stimuli. The impact of *Mycobacterium bovis* infection, the cause of bovine tuberculosis, on the transcriptome of bovine alveolar macrophages (bAM) has been well documented; however, possible effects on the macrophage epigenome are currently not well understood. In the current study, whole genome bisulfite sequencing (WGBS), chromatin immunoprecipitation sequencing (ChIP-seq) and RNA-seq were used to examine the effect of *M. bovis* infection on the epigenome of bAM. *M. bovis*-infected bAM were compared to non-infected control bAM 24 h post infection (WGBS $n = 8$, ChIP-seq $n = 3$, RNA-seq $n = 3$). DNA methylation was assessed across the genome, with particular emphasis on the following genomic features: intergenic sequences, intragenic sequences and promoters with- or without CpG islands. This analysis revealed that the bAM DNA methylome is resistant to perturbations induced by *M. bovis*. Gene ontology analysis, focusing on the degree of methylation at proximal promoters (hyper-, hemi- or hypomethylated), revealed that genes with hemimethylated promoters were enriched for immune-related categories; this enrichment was not observed for genes with hyper- or hypomethylated promoters. Dual ChIP-seq and RNA-seq was also performed on

bAM 24 h post-infection with *M. bovis*. Results from these experiments can be used to elucidate the role of chromatin reconfiguration in the host macrophage response to *M. bovis* infection. This is one of the first studies to examine macrophage epigenomic perturbations induced by *M. bovis* infection. It provides novel information for a more complete understanding of host-pathogen interaction in mycobacterial infections and has relevance to human tuberculosis caused by *Mycobacterium tuberculosis*, which has 99.95% genome sequence identity to *M. bovis*.

Key Words: cattle and related species, epigenomics, Functional Annotation of Animal Genomes (FAANG), genome regulation, animal health

112 Maternal nutrition during the first 50 days of gestation alters expression of histone and histone modifying genes in bovine fetal liver. M. S. Crouse*[1], J. S. Caton[1], R. A. Cushman[3], K. J. McLean[2], C. R. Dahlen[1], P. P. Borowicz[1], L. P. Reynolds[1], and A. K. Ward[1], [1]*Department of Animal Sciences, North Dakota State University, Fargo, ND, USA;* [2]*Department of Animal and Food Sciences, University of Kentucky, Lexington, KY, USA;* [3]*USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA.*

During the first 50 days of gestation, organogenesis is taking place. Nutritional influences during this time may alter the mammalian phenotype through affecting gene regulatory mechanisms, thus 'programming' potential susceptibilities to chronic disease and metabolic issues into the animal's genome. We tested the hypothesis that maternal nutrition during the first 50 days of gestation would alter the transcriptome of histones and histone related genes in the developing fetal liver. Fourteen beef heifers were oestrus synchronized and assigned to 2 dietary treatments at breeding (CON-100% of nutrient requirements to gain 0.45kg/d; RES-60% of CON). Heifers were ovariohysterectomized on d 50 of gestation and fetal livers were dissected, flash frozen, and RNA extracted. RNA-seq analysis was conducted on the Illumina HiSEqn 2500 platform using 50-bp paired-end reads at a depth of 2 × 10.4M reads/sample. Transcriptome analysis was performed in collaboration with USDA-ARS-MARC using the Tuxedo Suite, and KEGG pathways were analysed with DAVID 6.8. A total of 548 genes ($P < 0.01$) were used for ontological analysis, of which 201 were false discovery rate protected ($q < 0.10$). We found 9 histones that were up-regulated in RES *v.* CON including members of the histone H1, H2A, H2B, and H4 families. The 13 differentially expressed histone modifying transcripts included genes associated with acetylation and de-acetylation, methylation, phosphorylation, and ubiquitination. Of particular note, *HDAC10* was 2.67- fold greater ($q < 0.05$) in liver of RES fetuses. Additionally, the histone deacetylase complex gene, *CIR1* was 2.22-fold greater ($q < 0.05$) in RES. Only one gene associated with histone modifications, *SET* was 1.77- fold lower ($P = 0.006$; $q = 0.16$) in RES. The *SET* gene is involved in preventing H4 lysine acetylation. Thus, a moderate nutrient restriction during the first 50 days of gestation alters the expression of histone and histone modifying genes in the bovine fetal liver. This implies that early maternal nutrition initiates developmental programming through epigenetic remodelling.

Key Words: cattle and related species, development, epigenomics, pregnancy, RNA-seq

113 Transgenerational phenotypic and epigenetic inheritance across three generations in layers induced by Poly(I:C). L. Liu*, D. Wang, Z. Y. Duan, S. Yang, G. Y. Xu, N. Yang, and Y. Yu, *China Agricultural University, Beijing, China.*

Transgenerational epigenetic inheritance is evoked by environmental factors and could transmit the changed information from one generation to their offspring without genetic variations. Polyriboinosinic–polyribocytidylic acid (Poly(I:C)) is a synthetic mimic of viral dsRNA polymer, which can improve cancer immunotherapy outcome and also used as vaccine adjuvant to cure avian plague in husbandry. The aims of the study were to investigate the effects and molecular mechanisms of Poly(I:C) on three generations of a layer model. First, Poly(I:C) (group P) or saline were intravenous injection in 66 Rhode Island White hens at 53 weeks of age in only F0 generation with family in consideration. Compared to the saline treated controls (group C), egg-laying rate and concentration of plasmatic cytokines (IL-6, TNF-α) of group P was significantly increased ($P < 0.05$), while the egg weight was significantly decreased ($P < 0.01$) in both F0 and F1 generations. Bodyweight of group P was lower than controls in F1 and F2 generations ($P < 0.01$). Moreover, we also found Poly(I:C) impeded the embryonic development of F2 chickens. Next, peripheral blood lymphocytes of F1 chickens at 48 weeks of age were used to conduct RNA-seq and whole-genome bisulfite sequencing (WGBS) analysis. We found some pivotal pathways involved in immune response, development and reproduction (MAPK signalling pathway, ErbB signalling pathway, Progesterone-mediated oocyte maturation) in methylation data by KOBAS. Combined the methylome with transcriptome data, some important overlapped genes were detected. Of which, six up-regulated genes (*KCNQ1, MLLT4, MYOF, NOTCH1, SGCD, SREBF2*) and two down-regulated genes (*PDK4, TGM3*) were associated with immune responses and diseases, while two down-regulated genes, *SEMA3D* and *SOX6*, were related with chick embryonic development and cartilage formation. In conclusion, although Poly(I:C) can improve egg-laying rates of hens, it also decreases the immunity and growth performance as a trade-off for three generations.

Key Words: transgenerational phenotypic and epigenetic inheritance, Poly(I:C), chicken, immune, reproduction

114 Update on DNA methylation datasets of FAANG reference samples for the chicken and pig. N. Trakooljul*[1], H. Zhou[2], P. Ross[2], I. Korf[3], M. E. Delany[2], H. H. Cheng[4], C. Ernst[5], C. Kern[2], F. Hadlich[1], S. Ponsuksili[1], and K. Wimmers[1], [1]*Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany;* [2]*Department of Animal Science, University of California, Davis, CA, USA;* [3]*Genome Center, University of California, Davis, CA, USA;* [4]*USDA-ARS, Avian Disease and Oncology Laboratory, East Lansing, MI, USA;* [5]*Department of Animal Science, Michigan State University, East Lansing, MI, USA.*

As part of the FAANG initiative, this study aims at cataloging the DNA methylome of 8 FAANG-reference tissues (liver, spleen, lung, muscle, adipose, hypothalamus, cerebral cortex and cerebellum) for the pig and chicken (two adults) at a single-base resolution using Reduced Representation Bisulfite Sequencing (RRBS) with double-enzyme digestion (MspI & Taq$^{\alpha}$I) and increased selected-fragment size (40 - 350bp) to enhance the genome-wide CpG coverage. A total of 30 RRBS libraries were constructed, multiplexed and deep-sequenced for single-end reads and 114 cycles on a HiSEqn 2500. We obtained a total of 2.1 and 2.2 billion high-quality reads (395Gb in total and 145 ± 1.5 million reads per sample) for the chicken and pig, respectively. The data was pre-processed and aligned to the reference genomes with mapping abilities of 64.34 ± 0.87 (chickens) and 62.44 ± 0.62% (pigs) using BS-Seeker2. Interestingly, the chicken showed a lower percentage of methylated CpGs (24.01 ± 1.47/ 1.21 ± 0.28/ 1.04 ± 0.35 for CG/CHG/CHH) compared to the pig (43.42 ± 1.51/ 1.14 ± 0.16/ 1.01 ± 0.23). Despite the distinct DNA methylation levels, both chickens and pigs showed a similar pattern of hypo-methylation around the transcription start site (TSS) and hyper-methylation in the gene body. Analysis of tissue specific patterns of DNA methylation and correlation analysis between DNA methylation and gene expression (RNA-Seq) should help us gain more functional information of the DNA methylation. Together with other epigenetic assays associated with the FAANG

reference samples will tremendously facilitate the functional annotation of the animal genomes.

Key Words: DNA methylation, gene regulation, pigs, chickens, FAANG

115 Genome-wide identification of imprinted genes and its methylation statuses in various tissues of Hanwoo. K.-T. Lee*, K.-S. Lim, B.-H. Choi, H.-H. Chai, J.-E. Park, E.-W. Park, G.-W. Jang, and D. Lim, *National Institute of Animal Science, RDA, Wanju, Jeonbuk, South Korea.*

Genomic imprinting is a epigenetic phenomenon in which only one of two alleles is expressed, resulting in a functional hemizygosity. Numerous genes showed species-specific and tissue-specific imprint patterns. Here, we aimed to identify imprinted genes transcriptome-widely and to find clues for relation between imprinting and methylation in Hanwoo. For three Hanwoo family trios, the transcriptome data of 17 kinds of tissues were generated, and methylation levels were estimated from methylome data in three offspring. A total of 62 imprinted genes expressed monoallelically in at least one tissue. Comparing genotypes among each family trio, the preference alleles of eighteen genes were identified (maternal expression, $n = 9$; paternal expression, $n = 9$), and the imprinting of random selected three genes were validated by direct sequencing. Imprinted genes were involved in gene regulation, metabolic process and immune response, and in particular six genes encode transcription factors (FOXD2, FOXM1, HTATSF1, SCRT1, NKX6–2 and UBN1) with tissue-specific expressions. Methylated reads were obtained in 2kb upstream region of all imprinted genes, and IFITM2 showed highest methylation level. This is the first study to identify the imprinted genes in aspect of various adults tissues in cattle, and this results could contribute to elucidation of epigenetic effects for phenotype variations.

Key Words: genomic imprinting, cattle, adult tissues, Re-seq, RNA-seq

Cattle Molecular Markers and Parentage Testing

116 Next-generation targeted sequencing panel for verification of bovine parentage. A. Burrell*[1], P. Siddavatam[1], A. Allred[1], C. Willis[1], R. Ferretti[2], and A. Raeber[1], [1]*Thermo Fisher Scientific;* [2]*Neogen GeneSeek Operations.*

Bovine parentage verification is a critical aspect of successful herd management. Due to its highly accurate and reproducible results, SNP genotyping is becoming an increasingly favoured tool for parentage verification. With the utilisation of high-throughput next-generation sequencing platforms like the Ion S5 sequencing system, laboratories can test hundreds of samples and thousands of SNPs simultaneously. We developed a targeted sequencing panel based on 200 bovine SNP markers selected by the International Society of Animal Genetics (ISAG) for the purpose of verifying bovine parentage. Utilising the AgriSeq™ sequencing workflow, a high-throughput targeted amplification and re-sequencing workflow, panel performance was tested on 115 diverse bovine DNA samples. Samples included a panel of 96 samples obtained from the USDA (MARC Beef Cattle Diversity Panel v2.9) as well as samples contained within the 2015 ISAG/ICAR 3rd SNP Typing Bovine Comparison Test panel. Samples originated from 20 different bovine breeds. Libraries were prepared using the high-throughput AgriSeq workflow. The resulting amplicons were ligated to unique barcodes and sequenced on a single run on the Ion S5 sequencing system using an Ion 540 chip. Utilising this system, up to 768 samples can be barcoded and run on a single sequencing run allowing for up to ~1500 samples to be tested a day (2 runs/day). Data was analysed using the Torrent Variant Caller (TVC) plugin as part of the Torrent Suite software package to determine the genotype call for each marker and sample. The mean call rate (the percent of markers generating a genotyping call) was >98%. As a comparison, the USDA gDNA was also hybridized to six replicate arrays to generate a consensus array genotype call. Genotype call concordance was >99% between the array and AgriSeq workflow calls. Accurate parentage determinations were made for the samples within the 2015 ISAG/ICAR 3rd SNP Typing Bovine Comparison Test panel as compared to provided reference genotypes. The data demonstrates that the bovine parentage panel tested with the AgriSeq workflow provides accurate and reproducible results for SNP-based parentage verification.

Key Words: NGS, GBS, bovine parentage, AgriSeq, SNP genotyping

117 SNP data quality control in a national beef and dairy cattle system and highly accurate SNP-based parentage verification and identification. M. McClure*[1], J. McCarthy[1], R. Weld[2], P. Flynn[2], M. Kean[1], K. O'Connell[1], and J. Kearney[1], [1]*Irish Cattle Breeding Federation, Bandon, Cork, Ireland;* [2]*Weatherbys Ireland, Johnstown, Kildare, Ireland.*

As single nucleotide polymorphism (SNP) genotyping costs decrease the level of genotyping in a country's national herd increases. A major use of this genetic data is parentage verification and identification as inaccurate pedigrees result in decreased genetic gain. Since 2012 the international standard for SNP-based verification in cattle has been the ISAG100 or ISAG200 SNP set for *Bos taurus* breeds. While these SNP sets have provided an increased level of parentage accuracy over microsatellite markers (MS), they can validate the wrong parent for an animal and can predict >1 sire or dam at < 1% misconcordance rate levels, indicating that more SNP are needed if a more accurate pedigree is required. With rapidly increasing numbers of cattle being genotyped in Ireland representing 80 *Bos taurus* breeds from a wide range of farm types: beef/dairy, multiple breeds, pedigree/commercial, purebred/crossbred, AI/stock bulls, and large to small herd size the Irish Cattle Breeding Federation (ICBF) analysed different SNP densities to determine that at a minimum >500 SNP are needed to consistently predict only one set of parents at a < 1% misconcordance rate. ICBF currently uses 800 SNP for parentage validation and prediction selected according to SNP clustering quality, ISAG200 inclusion, call rate (CR), and minor allele frequency (MAF) in the Irish cattle population. Microsatellite imputation and genetic relationship matrixes are also used to assist parentage when the true parent is not SNP genotyped. When dealing with large datasets sample and SNP quality control (QC) is paramount. Most publications only deal with SNP QC via CR, MAF, parent-progeny conflicts, and Hardy–Weinberg deviation, but not much on the sample QC. Through trial and error ICBF has developed our own sample QC pipeline to deal with the unique challenges of genotypes from a national herd. We share this pipeline for the benefit of others so they can be proactive with their datasets in dealing with SNP genotype errors from mis-tagging of animals, laboratory errors, farm errors, and multiple other issues that can arise.

Key Words: SNP parentage, quality control, parentage prediction

118 High cross-platform genotyping concordance of Axiom high-density microarrays and Eureka low-density targeted

NGS assays. M. A. Patil* and A. Pirani, *Thermo Fisher Scientific, Santa Clara, CA, USA.*

Microarrays ranging from mid- to high-plex have been developed on the Axiom Genotyping Solution to interrogate single-nucleotide polymorphisms (SNPs) and insertion/deletions (indels) for over 55 agrigenomic organisms. This technology has the flexibility to genotype populations exhibiting diploid to various levels of allopolyploid genetics. It also incorporates methods for accurately genotyping samples originating from normal and inbred populations. The high variant density possible on microarrays has the ability to facilitate multi-breed genomic selection, fine mapping of quantitative trait loci, and detection of copy number variation. The Eureka Genotyping Solution is an affordable, low- to mid-plex, high-throughput genotyping assay that uses common next-generation sequencing (NGS) platforms for signal readout. It enables the detection of tens to thousands of genetic markers which are increasingly in demand for routine animal agrigenomics testing. This routine testing can include parentage and sex validation and genomic evaluation, after imputation. High reliability and concordance across the Axiom and Eureka technologies is essential to allow researchers to migrate between the platforms seamlessly. Thermo Fisher Scientific has accomplished high genotype concordance and high genotype call rate (low missing data rate) by adapting the same genotype calling and SNP QC framework across both platforms. The developed genotype calling algorithm has been shown to work on both microarray intensity and counts of allele × locus barcodes of next-generation sequencing (NGS) reads. Various overlapping animal datasets have been evaluated across these microarray and targeted NGS technologies. A high level of genotype concordance is demonstrated, allowing for easy comparison across and migration between platforms.

Key Words: genotyping, next-generation sequencing, microarray, genomic selection

Companion Animal Genetics and Genomics

119 Genetic variants in *ATP1B2* and *KCNJ10* in Belgian Shepherd dogs with ataxia. N. Mauri[1], M. Kleiter[2], M. Leschnik[2], S. Högler[3], E. Dietschi[1], C. Monney[4], A. Oevermann[4], D. Henke[5], M. Wiedmer[1], J. Dietrich[1], F. Steffen[6], S. Schuller[7], C. Gurtner[8], N. Stokar-Regenscheit[8], D. O'Toole[9], T. Bilzer[10], C. Herden[11], V. Jagannathan[1], and T. Leeb*[1] [1]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland;* [2]*University Clinic for Small Animals, Department for Companion Animals and Horses, University of Veterinary Medicine Vienna, Vienna, Austria;* [3]*Institute of Pathology and Forensic Veterinary Medicine, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria;* [4]*Division of Neurological Sciences, Department of Clinical Research and Veterinary Public Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland;* [5]*Division of Clinical Neurology, Department of Clinical Veterinary Medicine, Vetsuisse Faculty University of Bern, Bern, Switzerland;* [6]*Section of Neurology, Department of Small Animals, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland;* [7]*Division of Small Animal Internal Medicine, Department of Clinical Veterinary Medicine, Vetsuisse Faculty University of Bern, Bern, Switzerland;* [8]*Institute of Animal Pathology, Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland;* [9]*Wyoming State Veterinary Laboratory, University of Wyoming, Laramie, WY, USA;* [10]*Institute of Neuropathology, University Hospital Düsseldorf, Düsseldorf, Germany;* [11]*Institute of Veterinary Pathology, Justus-Liebig-University, Gießen, Germany.*

Ataxia is characterised by uncoordinated movements and may be caused by cerebellar, vestibular or proprioceptive dysfunction. In the Malinois variety of Belgian Shepherd dogs, puppies that showed a severe ataxia with an age of onset at 4–8 weeks had previously been reported. Neuropathologically, ataxic puppies suffered from spongy degeneration targeting the cerebellum, brainstem, and spinal cord in some cases. We performed a genetic investigation in six closely related families with ataxic puppies and seven additional isolated cases. Linkage analysis revealed an unexpected genetic heterogeneity within the families, which was correlated with variation of the neuropathological lesions. The affected dogs from four families and one isolated case shared a ~1.4 Mb common homozygous haplotype segment on chromosome 38. Whole genome sequence analysis revealed a missense variant in the *KCNJ10* gene encoding a potassium channel (c.986T>C; p.Leu329Pro) that was homozygous in the cases from four of the six families and one isolated case. Pathogenic variants in *KCNJ10* were reported previously in humans, mice, and dogs with neurological phenotypes. Therefore, we consider *KCNJ10*:c.986T>C the most likely candidate causative variant for one subtype of ataxia, which we propose to term spongy degeneration with cerebellar ataxia 1 (SDCA1). In all four affected puppies from another family and one of the isolated cases we detected a structural variant in the *ATP1B2* gene encoding a subunit of the Na^+/K^+-ATPase. *Atp1b2* deficient mice show early onset motor incoordination and severe progressive neurodegeneration. Thus, the detected *ATP1B2* variant is a compelling candidate causative variant for a second ataxia form in Malinois dogs. So far, no human patients with *ATP1B2* defects have been described. Therefore, we propose that this gene should be considered a functional candidate gene for unexplained human cases of ataxia. The investigation of the last of the six families is still ongoing and we will present an updated analysis during the conference.

Key Words: dog, molecular genetics, disease, nervous system, high-throughput sequencing (HTS)

120 Phylogenetic analysis of Angora, Van, and stray cats of Anatolia. N. Bilgen*, B. C. Kul, M. Akkurt, O. Cildir, O. Ozmen, and O. Ertugrul, *Ankara University Faculty of Veterinary Medicine Department of Genetics, Ankara, Turkey.*

Unlike agricultural animals (cattle, sheep, pig etc.) or carrying animals (horse and donkey) the cat domesticated due to its feeding habit on rodents, which invade grain storage of farmers showing cats as 'commensal'. Earliest archeological evidence was found in Cyprus pointing that cat's domestication period was determined as between 9500–4000 years ago. Anatolia is considered as the cradle of domestication for many animal species. As an important species of Anatolia, there is not enough molecular study to reveal history of the Angora and Van cats. To shed light on domestic cat breeds of Anatolia, Angora (n = 28), Van (n = 49) and stray cats (n = 51) were sampled. The Cytochrome *b* gene (CYTb) and control region (CR or D loop) on mtDNA were aimed to investigate by Polymerase Chain Reaction (PCR) and sequencing methods, and data analysed by bioinformatics tools. Phylogenetic analysis revealed that the *F. silvestris lybica* was major maternal origin whereas Van, Angora and stray cats also shared branch with *F. silvestris ornata.* Network analysis and frequency calculations showed ~70% of the cats were represented by two major haplotypes, A and D for CR; Haplotype10 and Haplotype15 for CYTb. Unique sequences were found in %9.3 of the population (Van n = 1; Angora n = 3; stray cats n = 8). Haplotype diversity of CYTb and CR region were determined 0.71 and 0.77, respectively. Shared haplotypes were high, thus FST statistics revealed low genetic differentiation between groups. Obtained data

and new mtDNA haplotypes provided in completion of the information about the genetic structure of the domestic Anatolian cat breeds. This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) grant number 114O768

Key Words: Angora cat, control region, cytochrome b gene, mtDNA, Stray cat

121 Canine brachycephaly is associated with a retrotransposon-induced missplicing of *SMOC2*. T. Marchant[1], E. Johnson[1], R. Harrington[1], L. McTier[1], C. Johnson[1], A. Gow[1], T. Liuti[1], D. Kuehn[2], K. Svenson[3], M. Bermingham[4], M. Drögemüller[5], M. Nussbaumer[6], M. Davey[1], D. Argyle[1], R. Powell[7], S. Guilherme[8], J. Lang[9], G. Ter Haar[1], T. Leeb[5], T. Schwarz[1], R. Mellanby[1], D. Clements[1], and J. Schoenebeck[1]* [1]*Royal (Dick) School of Veterinary Studies and Roslin Institute, The University of Edinburgh, Midlothian, UK;* [2]*Friendship Hospital for Animals, Washington, DC, USA;* [3]*The Jackson Laboratory Bar Harbor, Bar Harbor, ME, USA;* [4]*Institute of Genetics and Molecular Medicine, The University of Edinburgh, Edinburgh, UK;* [5]*Institute of Genetics, University of Bern, Bern, Switzerland;* [6]*Naturhistorisches Museum, Bern, Switzerland;* [7]*Powell Torrance Diagnostic Services, Hertfordshire, UK;* [8]*Davies Veterinary Specialists, Hertfordshire, UK;* [9]*Department of Clinical Veterinary Medicine, University of Bern, Bern, Switzerland;* [10]*Department of Clinical Sciences and Services, The Royal Veterinary College, Hertfordshire, UK.*

The accentuated morphological features of the domestic dog have leant themselves to making this species a powerful model for identifying developmental programmes of bone formation and growth. Here, we have used computer tomography scans of 374 pedigree and mixed-breed dogs to generate high-resolution three-dimensional reconstructions of the canine skull. Geometric morphometric analyses of the neurocranium and rostrum enabled us to separate craniofacial size from shape in dogs that were genotyped by high-density SNP arrays. Using haplotype mapping, we identified a 187-kb critical interval on canine chromosome 1 present among the brachycephalic dogs used in our study. Leveraging whole genome sequencing, we identified candidate mutations that included a large structural variant and several other SNPs. All variants are found within introns of the SPARC-related modular calcium binding 2 (*SMOC2*) gene. The structural variant is a fragment of a long interspersed nuclear element (LINE-1), a family of retrotransposons. RNAseq analysis of *SMOC2* mRNA has revealed three alternative *SMOC2* transcripts present among individuals that carry the LINE-1 element. All alternative transcripts incorporate the LINE-1 element and a portion of preceding intron. This is predicted to introduce premature stop codons following exon eight of *SMOC2*'s canonical 13-exon transcript. RT-qPCR has revealed an 80% reduction in *SMOC2* transcript levels for individuals that are homozygous carriers of the LINE-1 which may be the result of nonsense mediated decay of the alternative transcripts. No differential expression was detected among transcripts of genes neighbouring *SMOC2*. Our models of phenotypic effect predict that the LINE-1 insertion explains up to 34% of the overall face length variation captured by our study. Endogenously expressed (mouse) *Smoc2* is observed in the pharyngeal arches during development and the viscerocrania of *Smoc2* null mice are dysmorphic. Our data provide a compelling picture that implicates *SMOC2* in mammalian craniofacial development. Our results are predicted to have health implications to both human and veterinary medicine.

Key Words: dog, craniofacial anomaly, genetics, brachycephaly, retrotransposon

122 Revealing the genetic basis of diabetes mellitus in Burmese cats. G. Samaha[1], J. Beatty[1], L. Lyons[2], C. Wade[3], and B. Haase*[1], [1]*School of Veterinary Science, Faculty of Science, University of Sydney, Sydney, NSW, Australia;* [2]*College of Veterinary Medicine, University of Missouri, Columbia, MO, USA;* [3]*School of Life and Environmental Sciences, Faculty of Science, University of Sydney, Sydney, NSW, Australia.*

This study aims to identify the underlying genetic factors causing feline diabetes mellitus (FDM) in the Australian Burmese cat population. Feline diabetes mellitus (FDM) is a common feline endocrinopathy, characterised by insulin resistance, defective insulin secretion and β-cell loss. Among established breeds, the incidence of FDM is highest among Burmese cats [O'Neill et al. (2016) J. Vet. Intern. Med. 30:964–972]. Variation in the prevalence of this disease among breeding populations is well established. Australian, European, British and New Zealand-bred Burmese are more likely than US-bred Burmese to suffer from FDM [McCann et al. (2007) J. Feline Med. Surg. 9:289–299; Lederer et al. (2009) Vet. J. 179:254–258]. We performed a genome-wide association analysis using 76 samples, including 10 cases and 66 controls. All animals were genotyped on the Illumina Feline 63K DNA array (Illumina, San Diego). Affected cats were diagnosed by a veterinarian based on persistent fasting hyperglycemia, glycosuria with clinical signs; weight loss, polydipsia and polyphagia. Unaffected cats displayed no clinical signs of FDM at the time of sample collection. Association analysis and permutation testing was performed using PLINK [Purcell et al. (2007) Am. J. Hum. Gen. 81:559–575]. HAPLOVIEW [Barrett et al. (2005) Bioinformatics 21:263–265] was used to assess linkage disequilibrium of significant SNPs and to construct haplotype blocks. The GWAS revealed strong associations of FDM to loci on FCA4 ($P_{raw} = 3.72x10^{-13}$) and FCA7 ($P_{raw} = 7.08x10^{-12}$). A high degree of linkage disequilibrium was observed among significant SNPs within the associated regions on FCA4 and FCA7 ($r^2 = 0.77–1.0$ and $r^2 = 0.8$, respectively). The distribution of alleles on FCA4 and FCA7 among cases and controls suggests both regions play a role in the pathogenesis of FDM. Our findings provide new insights into the genetic basis of FDM in domestic cats and may have provided a basis for the identification of functional variants associated with the condition within the Australian Burmese breeding population.

Key Words: diabetes mellitus, feline, Burmese cat, GWAS

123 Localizing the regions of causative mutations in feline amyloidosis: a next-generation genomic approach. F. Genova*[1], B. Gandolfi[2], A. Thomas[3], E. Creighton[2], L. Lyons[2], and M. Longeri[1], [1]*Department of Veterinary Medicine, Università degli Studi di Milano, Milan, Italy;* [2]*Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA;* [3]*Antagene, La tour de Salvagny, France.*

The term "amyloidosis" refers to a heterogeneous group of metabolic diseases that arise as a consequence of the improper folding of some autologous proteins. Protein aggregation and deposition is reported in different organs. In the mutated system the amyloid protein precursors acquire a β - sheet secondary structure, forming fibrillary deposits that are insoluble and resistant to proteolysis. The amyloid fibrils can be deposited locally in one single organ, or they can be distributed systemically. In felids, amyloidosis was observed in fancy pedigreed breeds and random bred cats and cases were also reported in the black-footed cat, the Siberian tiger and the cheetah. In young Abyssinian/Somali and Siamese/Oriental breeds, a familial inheritance in developing the Inflammatory amyloidosis (AA) was described. The pathogenic pathway of this disease is still unknown in felids and the final diagnosis is only *postmortem*. In this study, the whole genome sequences (WGS) of two affected Abyssinians, two affected Siamese and one affected Black-footed cat, were used to identify mutations potentially related to amyloidosis, using additional 127 whole genome cat sequences in the 99 Lives Project context as controls for variant exclusion. Identified variants were prioritized based on a previous GWAS analysis carried out on affected/healthy cohorts of Abyssinians, which showed associations with cat chromosomes B4 and C2. Among all the genes within

the two regions, one above all, on chromosome B4, is considered a good candidate as it is involved in controlling the levels of b-amyloid deposits, through the interaction with a zinc finger protein. Indeed, two mutations within the candidate gene were found, one unique to the affected cats and an additional variant unique to the affected black-footed cat. Other interesting variants were found in two zinc finger proteins as well as in several genes on cat chromosome C2. To confirm the causality link to the disorder, the identified variants will be genotyped on a larger population of pedigreed bred cats, as well as on random bred cats and four black-footed cats.

Key Words: cats and related species, genome sequencing, genome-wide association, candidate gene, animal health

124 Insights into 115 domestic cat and 17 wild felid genomes. L. Lyons*, *University of Missouri, Columbia, MO, USA.*

The 99 Lives Cat Genome Sequencing Consortium was initiated in 2014 and has now reached its goal of over 100 high-quality genomes from domestic cats. Over 90% of genomes are 30x coverage, with a minimum of 20x coverage, and produced using only 100 – 150 bp paired-end reads from PCR-free libraries using Illumina HiSeq sequencing technology. The genome dataset comprises 115 domestic cats, including at least 22 breeds, a representative from the ten racial populations of cats, four parent-offspring trios, and a few affected sib-pairs. Over 20 investigators have contributed sequences to the consortium, as well as three industry partners. Funding has been provided by various contributors for each investigator, the Winn Feline Foundation, the National Geographic Foundation, and industry. Nearly 50% of wild felid species are represented including cats from six of eight lineages within Felidae, including domestic cat, domestic cat, leopard cat, puma, lynx, caracal, and panthera. The 99 Lives dataset has led to the discover of DNA variants likely causal for congenita Myasthenic syndrome, progressive retinal atrophy, bobtailed tail, and a novel form of Niemann-Pick Type C in domestic cats, and a retinal degeneration in Black-footed cats and as well as several other traits that are yet unpublished. The discovery of the Niemann-Pick Type C mutation is an example of whole genome sequencing of a clinical case and discovery of the likely causal variant during the course of the cat's disease. This success demonstrates thay Precision Medicine can be used in feline health care. The dataset has allowed the identification of a vast array of variants in the cat genome that can support the development of a high-density DNA array. Goals are to include at least one representative from each cat breed and species, and to continue variant discovery for diseases and traits in the felids. The success and the limitations of the 99 Lives project will be presented, including the Niemann-Pick Type C Precision Medicine effort.

Key Words: domestic cat, felids, whole-genome sequencing, disease, phenotypes

125 Early events of cat domestication uncovered through ancient mitochondrial DNA analysis. E.-M. Geigl*[1], C. Ottoni[1,2], and T. Grange[1], [1]*Institut Jacques Monod, CNRS, University Paris Diderot, Paris, France;* [2]*KU Leuven, University of Leuven, Department of Imaging and Pathology, Center for Archaeological Sciences; University Hospitals Leuven, Laboratory of Forensic Genetics and Molecular Archaeology, Leuven, Belgium.*

The analysis of mitochondrial DNA in domestic, feral and wildcats showed 10 years ago that domestic cats are the descendants of the wildcat from Northern Africa (NA) and South-west Asia (SWA), *Felis silvestris lybica*. Little is known, however, about their domestication centre(s), process and spread owing to both hybridization between domestic and wild cats blurring the tractability of the genetic signature of wild cats as well as the scarcity of archaeological remains. Solely two archeological finds, a cat skeleton found in a 9,000-year-old child burial in Cyprus and some skeletons excavated from a 5,700-year-old Egyptian Elite cemetery, suggest a cat-human relationship evocative of a taming process. The richest source documenting this relationship is the iconography of ancient Egypt showing an evolution of the cat-human relationship towards an incorporation of the cat into the domestic context. To shed light on the domestication process of cats, data on the predomestication situation are required. In particular, knowledge of the phylogeography in the past and its evolution over time is a prerequisite to disclose the 'where, when and how' of this process. Analysing mitochondrial DNA from ancient specimens covering ~9,000 years, from the Mesolithic to the 19th century CE, and a large geographic area including Europe, SWA and NA, we were able to decipher crucial steps of the spread of wildcats in the ancient world. Our data indicate that *F. s. lybica* was tamed twice at different times, first in SWA and later in ancient Egypt, and that these tamed cats conquered the world as companions of merchants and soldiers on their voyages throughout the ancient world. These translocated ship's cats subsequently reshaped the diversity of wildcats in the corresponding areas.

Key Words: cat, ancient DNA, DNA analysis

126 Characterization of Plakophilin-2 expression in canine skin and identification of differential gene expression in non-lesional skin from dogs affected by atopic dermatitis. G. Andersson*[1], K. Tengvall[2,3], B. Ardesjö-Lundgren[1,2], S. Kozyrev[2], M. Kierczak[2], M. Olsson[2,3], F. F. Farias[2], Å. Hedhammar[4], K. Bergvall[4], and K. Lindblad-Toh[2,5], [1]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [2]*Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, BMC, Uppsala University, Uppsala, Sweden;* [3]*Center for Molecular Medicine, Department of Medicine, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden;* [4]*Department of Clinical Sciences, Swedish University of Agricultural Sciences, Sweden;* [5]*Broad Institute of MIT and Harvard, Cambridge, MA, USA.*

Canine atopic dermatitis (CAD) is an immune-mediated disease caused by interactions between genetic and environmental factors. We previously identified genetic risk factors for CAD located on CFA27 in German shepherd dogs (GSDs). Targeted re-sequencing and fine-mapping identified transcriptional regulatory variants within the *Plakophilin-2 (PKP2)* locus as likely major risk factors (Tengvall et al. PLoS Genet. 2013). Subsequent functional studies of these regulatory variants confirmed their differential transcriptional regulatory activities in epithelial- and immune-derived cells implicating a role for these risk variants in the development of CAD (Tengvall et al. BMC Genet. 2016). We performed immunofluorescence, electron microscopy immuno-labelling, and morphology analyses of PKP2, which is a crucial desmosome component, in non-lesional axillary skin biopsies collected from CAD-affected and healthy control GSDs to define the cellular expression pattern of PKP2 in epidermal cells and its intracellular localization. PKP2 was evenly expressed in keratinocytes and strong expression was also observed in Langerhans cells (LCs) and T-cells. PKP2 protein was located in nuclei and on keratin filaments attached to desmosomes (Ardesjö-Lundgren et al. Vet. Dermatol. 2017). Since LCs and T-cells are known to be involved in atopic disease, altered expression of PKP2 in these cell types may correlate to CAD pathogenesis. To gain further insights into gene expression patterns and pathways influenced in CAD, we performed mRNA sequencing of the transcriptome of non-lesional atopic axillary skin tissue and axillary skin from healthy dogs. Differential gene expression was identified and gene ontology analyses identified involvement of inflammatory as well as structural pathways. Our results will be used for improved annotation of the canine skin transcriptome. The implications of the observed differential gene expression for the development of CAD will be discussed. Funding was provided by the Swedish Research

Council, the Swedish Research Council FORMAS and the European Research Council (ERC).

Key Words: dogs, functional genomics, RNA sequencing, disease genetics, allergy

127 An analysis of canine caudal fossa morphology and its genetics. R. Harrington*, T. Marchant, D. Argyle, D. Clements, T. Liuti, T. Schwarz, K. Marioni-Henry, and J. Schoenebeck, *Royal (Dick) School of Veterinary Studies and Roslin Institute, The University of Edinburgh, Easter Bush, Midlothian, UK.*

The caudal fossa is the concavity of the occipital bone that surrounds the cerebellum. In dogs, abnormalities in caudal fossa (CF) morphology are suspected of contributing to the development of neurological conditions such as syringohydromyelia. This painful condition is a major animal welfare concern, particularly to small breed dogs such as the Brussels Griffon and Cavalier King Charles Spaniel. Although the exact causes of syringohydromyelia are unknown, its correlation with Chiari malformations of the occipital bone have led to speculation about the developmental interplay between the skull and its underlying soft tissues. To date, analyses of the canine CF have not determined the types and breadth of CF shapes observed both within and across dog breeds. We present our methodology to study CF morphology. Using 3D reconstructions of skulls derived from computed tomography (CT) imaging, we have landmarked 500+ skulls from a wide range of dog breeds of varying sizes and head shapes, including the CF morphology. Each patient used in our study was also genotyped on the Illumina Canine HD 170K SNP array. Our quantification of CF morphologies enabled us to conduct genome-wide association studies (GWAS) to investigate the basis of CF shape in a manner that is controlled for individual size. The morphological analysis and GWAS methodology presented can directly improve canine welfare through the identification of CF morphologies which are at higher risk for syringohydromyelia and their genomic associations.

Key Words: dogs and related species, genome-wide association, diagnostic imaging, quantitative trait locus (QTL), morphometrics

128 Detection and characterisation of a genetic association with Norwich terrier upper airway syndrome. T. Marchant*[1], E. Dietschi[2], R. Harrington[1], M. Drögemüller[2], U. Rytz[3], T. Leeb[2], and J. Schoenebeck[1], [1]*The Royal (Dick) School for Veterinary Studies and Roslin Institute, The University of Edinburgh, Edinburgh, Midlothian, UK;* [2]*The Institute of Genetics, Vetsuisse Faculty, The University of Bern, Bern, Switzerland;* [3]*Department of Clinical Veterinary Medicine, Division of Small Animal Surgery, Vetsuisse Faculty, The University of Bern, Bern, Switzerland.*

In domestic dogs, the 'flat-faced' brachycephalic head shape is a risk factor for developing the respiratory defect, Brachycephalic Obstructive Airway Syndrome (BOAS). As the popularity of breeds such as the French bulldog continues to increase in the UK, so too are the expected incidences of BOAS. For this reason, we became interested in the Norwich terrier, a non-brachycephalic breed which presents with Upper Airway Syndrome (UAS), a condition highly reminiscent of BOAS. Here, we have studied this single breed to identify genetic association(s) with UAS. Pathological assessments and grading from laryngoscopic examinations held at the Vetsuisse Faculty of the University of Bern, were used as phenotypes in conjunction with microarray genotypes to perform GWAS. In total, 233 Norwich terriers were examined. We identified the same QTL on canine chromosome (CFA) 13 to be associated with the abnormal positioning of laryngeal cartilage and everted saccules in the dogs most severely affected by UAS. We phased genotypes at the CFA13 QTL to conduct haplotype mapping, which led us to define a 413 kb critical interval which encompasses a single positional candidate gene. The derived haplotype within this interval is overrepresented: it is found to be homozygous in 61 of 81 (74%) severely affected cases. In contrast, this homozygous haplotype was identified among 7 of 86 (8.1%) mild/unaffected controls. We have resequenced four dogs representing phenotypic extremes to 16-fold depth to identify putatively causal variants. We will provide an update to this ongoing project, which is expected to guide Norwich terrier breeding and inspire additional exploration of the CFA13 locus to improve animal welfare.

Key Words: dog, animal health, genome-wide association, quantitative trait locus (QTL), candidate gene

129 Canine diversity and disease: Genome analysis in Australian dogs. S.-A. Mortlock, J. Marin-Cely, R. Booth, P. Soh, M.-S. Khatkar, and P. Williamson*, *The University of Sydney, Sydney, NSW, Australia.*

Dogs have tremendous natural diversity in form and stature, and are recognised as excellent models for genetic studies. There are also many documented diseases in dogs that represent comparative models of human disease and which are found within breed sub-populations. We have utilised genome technologies customised for dogs to study disease risk and variation in biological processes in Australian dogs, specifically in the Kelpie, Bullmastiff, Border collie, and German Shepherd dog (GSD) breeds. In this study we analysed natural variation in metabolomic profiles of GSD. Genotyping data was generated using the Illumina Canine HD array, and 52 metabolites were measured in plasma. Amino acid profile measurements measured by liquid-chromatography mass spectrometry were obtained from 82 dogs for 20 amino acids. A mixed linear model association analysis was conducted in GCTA for each of the 20 amino acids to detect any loci associated with these metabolite measurements. A single association was detected for threonine levels, the peak signal observed from a 5.3Mb region on CFA25 (p-value < 0.05, FDR < 0.05). Subsequently, a sliding window analysis identified haplotypes within the region that were associated with threonine levels. The most significantly associated haplotype contained six SNPs and two genes, *MTMR9* and *LOC477365*, and was shown to be predominantly homozygous in dogs with high threonine values. The *LOC477365* gene, which is now annotated as a threonine dehydrogenase, was the top candidate gene owing to its involvement in threonine metabolism and location within the top haplotype. Analysis of expression or structural polymorphisms within the gene are underway.

Key Words: canine, genome-wide association study, metabolomics, amino acids

Microbiomes

130 Advanced bioinformatics and molecular analysis of whole-genome-shotgun metagenomics data from rumen microbiomes reveals remarkable diversity, structure and function. M. Watson*[1], R. Stewart[1], A. Warr[1], T. Snelling[3], M. Auffret[2], A. Walker[3], R. Wallace[3], and R. Roehe[2], [1]*The Roslin Institute, University of Edinburgh, Easter Bush, Scotland;* [2]*SRUC, Easter Bush, Scotland;* [3]*The Rowett Institute, University of Aberdeen, Aberdeen, Scotland.*

Rumens contain highly complex microbial communities, which are crucially important for the growth, development and health of the host animals. Despite huge numbers of microbial

genomes becoming publicly available, they are often from model organisms and are not relevant for the interpretation of rumen microbiomes. As a result, metagenomics software tools based on public data can be misleading or simply do not work when applied to ruminants. We have applied several standard and custom molecular and bioinformatics techniques to help better understand whole-genome-shotgun sequencing data from rumen microbiomes, including (but not limited to) deep Illumina sequencing, long-read nanopore sequencing and rumen-specific classification databases. The results of our work show remarkable diversity, structure and function within rumen microbiomes and demonstrate that we have much to discover from these rich and diverse microbial communities

Key Words: rumen, microbiome, genomics, bioinformatics

131 Bovine genes regulate the rumen microbial composition. O. Gonzalez-Recio*[1], I. Zubiria[2], A. García-Rodriguez[2], A. Hurtado[3], and R. Atxaerandio[2], [1]*Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain;* [2]*Departamento de Producción Animal. NEIKER-Tecnalia, Granja Modelo de Arkaute, Vitoria-Gasteiz, Alava, Spain;* [3]*Departmento de Salud Animal. NEIKER-Tecnalia, Derio, Bilbao, Spain.*

The rumen microbiota plays an important role during feed digestion, and influences relevant traits like feed efficiency and methane yield. The microbiota composition is partially regulated by the host, and is associated to rumen conformotion and phisiology, feed transit speed and eating behaviour. All this characteristics are also regulated at the host genetic level. Hence, we hypothesise that the rumen microbiome is also regulated at the host genetic level. Sequencing technoligies allow to determine the taxonomy of the microbiome with a rather satisfactory depth. This study reveals certain genetic control on the microbiome composition of the rumen in cattle. In total, 13 genera were analysed for bacteria (5), archaea (1), and ciliates (7) in cattle rumen from 16S and 18S rRNA gene-based analyses. All these bacteria and archaea genera showed association to the host genetic background both for breed and SNP markers. *Butyrivibrio* and *Ruminococcus* genus showed association with the SNP markers but not with the breed composition. The breed composition had a significant effect on *Isotricha*, *Ophryoscolex* and *Polyplastron* genus, and the SNP markers on *Entodinium*, *Ophryoscolex* and *Polyplastron*. In total, 77% (10/13) of microbes analysed showed to be associated to the host genetic background. This study also evaluated statistical association between candidate genomic regions and the relative abundance of these microbes. Then, DGAT1, ACSF3, AGPAT3, STC2 genes showed to be associated to the relative abundance of *Prevotella* genus with a false discovery rate lower than 15%. We showed some evidences for a host genetic control of the microbiome in cattle.

Key Words: genomic, breed, microbiome, NGS

132 16SrRNA amplicon sequencing of mock microbial populations to investigate DNA extraction methodology, primer selection and PCR cycles. E. McGovern*[1,2], M. S. McCabe[1], A. K. Kelly[2], D. A. Kenny[1], P. Cormican[1], and S. M. Waters[1], [1]*Teagasc, Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre, Grange, Dunsany, County Meath, Ireland;* [2]*University College Dublin, School of Agriculture and Food Science, Belfield, Dublin, Ireland.*

Elucidating the composition, adaptation, and function of the rumen microbiome is of international interest due to its implications in climatology and applied animal production. Despite extensive use of 16SrRNA amplicon sequencing for rumen microbial phylogenetic analysis, it may not be generating an accurate representation of constituent microbial communities in samples due to inefficiencies/biases in DNA extraction method, primer selection and/or PCR amplification. The objective of this study was to assess these factors in relation to selected currently established methods for 16S phylogenetic community analysis on a microbial community standard (MS) and a DNA standard (DS) (ZymoBIOMICS). DNA was extracted from MS, and rumen solid digesta sample as a positive control (PC), using the repeated bead beating and column (RBB+C) method. 16S rRNA amplicon libraries were generated for MS, PC, DS and a negative control, using both 515F/806R and Pro341F/Pro805R designed by Caparaso *et al*. and Takahashi *et al*. respectively and subjected to both 20 and 28 PCR cycles under identical cycle conditions. Sequencing was conducted using the Illumina MiSeq platform. A high-throughput BLAST search against a NCBI 16S database was performed. Linear regression analysis of the 8 bacterial species present in MS amplified using 515F/806R and Pro341F/Pro805R showed the relative abundance tended towards the theoretical composition of MS ($P > 0.1$), indicating that the protocol is suitable for DNA extraction gram positive bacteria. The relative bacterial abundances from 515F/806R and Pro341F/Pro805R were comparable across each sample type ($r^2 = 0.9$). The relative abundance of bacteria amplified from DS with 515F/806R at 20 PCR cycles, showed a correlation with its theoretical composition ($P > 0.01$), indicating that 515F/806R are sufficient for accurate determination of microbial communities. Communities amplified with 20 PCR cycles resulted in a higher correlation to expected mock community composition than samples generated with 28 PCR cycles. In conclusion, using the RBB+C method for DNA extraction, 515F/806R primers to target the 16SrRNA gene using 20 PCR cycles was sufficient for amplicon sequencing to generate relatively accurate depiction of the bacterial communities present in rumen samples.

Key Words: next-generation sequencing, mock communities, 16SrRNA, DNA extraction, microbiota

134 Host genetics influences gut microbiota composition in pigs. J. Estellé*[1], N. Mach[1,2], Y. Ramayo-Caldas[1], F. Levenez[2], G. Lemonnier[1], C. Denis[1], M. Berri[3], M.-J. Mercat[4], Y. Billon[5], J. Doré[2], C. Larzul[1,6], P. Lepage[2], and C. Rogel-Gaillard[1], [1]*GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [2]*MICALIS, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [3]*ISP, INRA, Université de Tours, Nouzilly, France;* [4]*IFIP-BIOPORC, Pôle génétique, Le Rheu, France;* [5]*GENESI, INRA, Surgères, France;* [6]*GenPhySe, INRA, INP, ENSAT, Université de Toulouse, Castanet-Tolosan, France.*

Microbiomes and their effects on hosts have emerged as outstanding factors to take into account in livestock production. Despite the well-acknowledged impact of maternal colonization and environmental factors for driving the gut microbiota composition, the genetics of the host is also likely to play a role. In this study, we aimed at studying the interplay between host genetics and variations of gut microbiota composition in pigs. A cohort of 518French Large White 60-day-old piglets was scored for faecal microbiota composition by sequencing the 16S rRNA bacterial gene, and genotyped with the Illumina PorcineSNP60 DNA chip. The relative abundances of operational taxonomic units (OTUs) and bacterial genera were obtained by using the Qiime package. Genetic parameters were estimated for a set of 63 bacterial genera present in the gut microbiota of pigs included in the study. Results showed that heritability was low ($0.1 < h^2 < 0.2$) for seven genera, medium ($0.2 < h^2 < 0.4$) for 15 genera, and high ($h^2 > 0.4$) for eight genera. Positive and negative genetic correlations were found between the relative abundances of various bacterial genera, with *Prevotella*, *Oribacterium*, *Selenomonas*, *Dialister* and *Megasphaera* genera being positively correlated. Genome-wide association studies (GWAS) revealed significant associations between genomic regions and relative abundances of *Flexispira*, *Megasphaera*, *Mitsuokella* or *Streptococcus* genera. GWAS uncovered also additional genomic regions associated with variations in OTU abundances. In conclusion, our results provide

new evidences that the gut microbiota composition is influenced by host genetics. We anticipate that genetic approaches will provide complementary strategies to nutrition solutions able to modulate gut microbiota composition for shaping production and health phenotypes relevant for promoting sustainable farm systems.

Key Words: pigs and related species, microbiomics, heritability, genome-wide association

135 The MetaPig project: Leveraging potentials in pig genomics and metagenomics to boost feed efficiency and gut health in modern pig production. P. Karlskov-Mortensen*[1], A. Ø. Pedersen[1], N. Canibe[2], P. Kiilerich[3], K. Kristiansen[3], and M. Fredholm[1], [1]*Department of Veterinary and Animal Science, Faculty of Health & Medical Sciences, University of Copenhagen, Frederiksberg, Denmark;* [2]*Department of Animal Science, Aarhus University, Tjele, Denmark;* [3]*Department of Biology, Faculty of Science, University of Copenhagen, Copenhagen, Denmark.*

Feed efficiency is a trait of great importance for profitability and environmental sustainability in modern pig production. Hence, it is of utmost importance to elucidate and understand all the complex factors and mechanisms involved in nutrient absorption and growth. In this project we take a holistic approach with the aim to characterise individual and combined effects of the host genome, metagenome, foodstuff composition and feed additives on feed efficiency and gut health. The project is divided into a discovery and a verification phase. In the first phase, the direct effect of feed formulation, feed additives and host genome on microbiota composition and subsequent effect on feed efficiency and gut health will be characterised in a total of 900 pigs. This phase includes genotyping of 700K SNP in 400 pigs and collection of gut epithelium for RNA sequencing and/or Fluidigm qPCR. Additionally, individual gut microbiota composition will be characterised using metagenome sequencing and/or 16S RNA gene sequencing. Together, the assembled data allow for an integrated analysis of the interplay between host genome, host transcriptome, microbiome, feed formulations and additives on gut health and feed efficiency. The analyses include QTL and eQTL mapping, transcriptome profiling and gene co-expression network analyses to identify major signalling pathways and host genes of key importance for microbiota composition, feed efficiency and gut health. Based on discoveries in phase one, up to four different feeding regimes including pre- and/or probiotic feed additives will be designed for the verification phase. In this phase, each of four diets will be tested in 1000 pigs to evaluate and verify host response and confirm positive effects on feed efficiency and gut health. We here present some preliminary results and outline perspectives for this line of investigations regarding feed efficiency and gut health for future pig production.

Key Words: pig, GWAS, RNAseq, metagenomics, systems biology

136 Characterization of the gut microbiome along the digestive tract of Iberian pigs. D. Crespo-Piazuelo*[1,2], J. Estellé[3], M. Revilla[1,2], L. Criado-Mesas[1,2], Y. Ramayo-Caldas[3], C. Óvilo[4], A. I. Fernández[4], M. Ballester[5], and J. M. Folch[1,2], [1]*Plant and Animal Genomics, Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB Consortium, Bellaterra, Barcelona, Spain;* [2]*Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain;* [3]*Génétique Animale et Biologie Intégrative (GABI), Institut National de la Recherche Agronomique (INRA), AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [4]*Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain;* [5]*Departament de Genètica i Millora Animal, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Caldes de Montbui, Barcelona, Spain.*

The Iberian pig is a rustic animal with high intramuscular fat content which is relevant for the production of cured products like ham. Recent studies have found that pig lipid metabolism may be modified by the gut microbiome through gene expression regulation. Therefore, the aim of this study was to describe the differences of the microbiome found along the Iberian pig gut and evaluate their possible role in the whole-body energetic homeostasis. DNA was extracted from luminal content of five gut sections (duodenum, jejunum, ileum, and proximal and distal colon) of thirteen 120-day-old Iberian pigs with PowerFecal (MoBio) kit and the region V3-V4 of the 16S rRNA gene was sequenced in a MiSeq (Illumina) instrument. With QIIME pipeline, a total of 1,669 operational taxonomic units (OTUs) distributed in 179 genera were found, from which 643 were new regarding the GreenGenes 13.8 database. *Lactobacillus* and *Clostridium* spp. were the two most abundant genera in the small intestine, while *Prevotella* spp. was predominant in colon. Diversity studies were made with *vegan* R package showing that the α diversity was increasing whereas the β diversity was decreasing while advancing through the gut. The OTUs presence/absence analysis was carried out with *metagenomeSeq* R package using a model where the animal was included as co-factor and with a FDR ≤ 0.01 cut-off. From the total 1,669 OTUs, 946 were absent in the small intestine sections while 325 were not present in the large intestine. Metagenome KEGG Orthologies (KOs) were predicted with *PICRUSt* software. The differences in abundance of these KOs were pointed out by *DESEqn 2* R package. Due to the abundance of the previous genera, one of the most relevant pathways found in the small intestine was the phosphotransferase system while the dicarboxylate/4-hydroxybutyrate was most important in the large intestine. In summary, this study confirms that the energy pathways of the gut microbiome are different along its sections, and besides, these results represent, to our knowledge, the first description of the gut microbiota composition along the intestine in Iberian pigs.

Key Words: Iberian pig, gut microbiome, 16S rRNA, metagenome prediction, OTUs

137 A metagenomics study on a non-metagenomics experiment: Mining next-generation sequencing datasets from porcine DNA identified unexpected viral sequences. S. Bovo[1,2], G. Mazzoni[1,3], A. Ribani[1], V. J. Utzeri[1], F. Bertolini[1,4], G. Schiavo[1], and L. Fontanesi*[1], [1]*Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy;* [2]*Biocomputing Group, Department of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy;* [3]*Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Copenhagen, Denmark;* [4]*Department of Animal Science, Iowa State University, Ames, IA, USA.*

Shot-gun next-generation sequencing (NGS) on whole DNA extracted from specimens collected from mammals often produces reads that are not mapped (i.e. unmapped reads) on the host reference genome and that are usually discarded as by-products of the experiments. In this study, we mined Ion Torrent reads obtained by sequencing DNA isolated from archived blood samples collected from 100 performance tested Italian Large White pigs. Two reduced representation libraries were prepared from two DNA pools constructed each from 50 equimolar DNA samples. A specific bioinformatics pipeline was designed to mine unmapped reads on the reference pig genome that were obtained from the two NGS datasets. *In silico* analyses included read mapping and sequence assembly approaches for a viral metagenomics analysis using the NCBI Viral Genome Resource. Our approach identified sequences matching several viruses of the *Parvoviridae* family: porcine *Parvovirus* 2 (PPV2), PPV4, PPV5 and PPV6 and porcine *Bocavirus* 1-H18 isolate (PBoV1-H18). The presence of these viruses was confirmed

by PCR and Sanger sequencing of individual DNA samples. PPV2, PPV4, PPV5, PPV6 and PBoV1-H18 were all identified in samples collected in 1998–2007, 1998–2000, 1997–2000, 1998–2004 and 2003, respectively. Our study provided a retrospective evaluation of apparently asymptomatic parvovirus infected pigs providing information that could be important to define occurrence and prevalence of different parvoviruses in South Europe and to evaluate resistance of these animals to viral infections. This study demonstrated the potential of mining NGS datasets non-originally derived by metagenomics experiments for viral metagenomics analyses in a livestock species.

Key Words: metagenomics, unmapped reads, parvovirus, disease resistance, NGS

Pig Genetics and Genomics

138 Expression of identical genetic mutations across Oncopig cell types results in distinct expression profiles recapitulating transcriptional hallmarks of human tumors. K. M. Schachtschneider*[1], R. M. Schwind[1], K. A. Darfour-Oduro[2], Y. Liu[2,3], S. Mäkeläinen[4,5], A. K. De[2], L. A. Rund[2], O. Madsen[4], M. A. M. Groenen[4], R. C. Gaba[1], and L. B. Schook[1,2], [1]*Department of Radiology, University of Illinois at Chicago, Chicago, IL, USA;* [2]*Department of Animal Sciences, University of Illinois, Champaign-Urbana, IL, USA;* [3]*Department of Animal Genetics and Breeding, Sichuan Agricultural University, Chengdu, China;* [4]*Wageningen University & Research, Animal Breeding and Genomics, Wageningen, The Netherlands;* [5]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Difficult questions confront clinicians attempting to improve patient outcomes for a wide range of cancer types. A large animal model with genetic, anatomic, and physiologic similarities to humans is required for transitioning between preclinical mouse models and human clinical trials in order to address unmet clinical needs. We previously reported the production of an inducible porcine cancer model (Oncopig) encoding Cre recombinase inducible porcine transgenes encoding $KRAS^{G12D}$ and $TP53^{R167H}$, which represent a commonly mutated oncogene and tumour suppressor in human cancers, respectively. To validate the ability of the Oncopig cancer model to mimic human cancers on a transcriptomic level, gene expression profiles were produced for Oncopig primary and transformed cell lines (fibroblasts and hepatocytes), as well as *in vivo* tumours (leiomyosarcomas and hepatocellular carcinomas (HCC)) using RNA-seq. Results based on the relative expression of 11,041 known genes for which expression information was available for each sample resulted in samples clustering by group. In addition, both sarcoma and HCC cell lines and tumours recapitulated key transcriptional hallmarks observed in their respective human malignancies. These included *TERT* reactivation, apoptosis evasion, angiogenesis activation, altered cell cycle regulation, and Wnt signalling activation in HCC samples, and altered *TP53* signalling, Wnt signalling activation, and epigenetic reprogramming in sarcoma samples. Master regulators of Oncopig gene expression previously implicated in human sarcoma (*FOSL1*, *SPI1*, *MEF2C,* and *ETV4*) and HCC (*HDAC2*, *HNF4A*, *FOXA2*, and *EP300*) development were also identified. Direct comparisons also identified conservation of 8 master regulators across Oncopig and 18 human HCC cell lines. Collectively, these results demonstrate expression of identical genetic mutations ($KRAS^{G12D}$ and $TP53^{R167H}$) across Oncopig cell types results in distinct expression profiles recapitulating transcriptional hallmarks of human tumour types, demonstrating the value of the model across distinct human cancer subtypes.

Key Words: gene expression, biomedical model, pig, sarcoma, hepatocellular carcinoma

139 Genome-scale sgRNA library construction and use for CRISPR/Cas9-based genetic screens in the pig. C. Zhao*[1], G. Yang[1], X. Han[1], Y. Gao[1], X. Li[1,2], S. Xie[1,2], and S. Zhao[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding, and Reproduction of the Ministry of Education & Key Laboratory of Swine Genetics and Breeding of the Ministry of Agriculture, Huazhong Agricultural University, Wuhan, China;* [2]*The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan, China.*

The CRISPR-Cas9 system enables efficient targeting to large numbers of genes through the use of single guide RNA (sgRNA) libraries. The ability to systematically disrupt porcine genes serves as a powerful tool for understanding their function. One key challenge is the design and production of high-quality oligonucleotides. In this study, a Pig Genome-scale CRISPR Knockout library (PigGeCKO) was designed and constructed. First, we designed a CRISPR library (CRISPR-lib) design software, which implements an end-to-end design of custom sgRNA libraries targeting the genomes of many different species. CRISPR-lib automates all tasks for the generation of sgRNA libraries. It can design libraries of variable size ranging from a few hundred genes to genome-scale for any genomes. Then, we used CRISPR-lib to design a PigGeCKO consisting of more than 85,000 sgRNAs, of which these sgRNAs were directed against the whole genome-wide porcine genes, lincRNAs, miRNAs and 1000 scrambled-sequence sgRNAs serving as negative controls. All genes were covered with 3 sgRNAs per gene. For library construction, the PCR- and Gibson assembly based approaches were employed to create large polyclonal pools of lentiviral sgRNA vectors. Subsequently, library coverage was determined by Illumina sequencing of plasmid libraries, the result showed that up to 96.17% designed sgRNAs were detected in the plasmid pool. In addition, a stable Cas9 expression PK-15 cell line was constructed for genetic screening using PigGeCKO. The PigGeCKO plasmid pool was packaged into lentiviral particles and used to generate knockouts in the PK-15-Cas9 cell lines. In summary, CRISPR-lib software is suitable for the design of libraries using CRISPR/cas9 and targeting any species. And the PigGeCKO is also suitable for High-throughput screening of a CRISPR/Cas9 library for functional genomics in the pig. This work was supported by the National High Technology Research and Development Program of China (863 Program, 2013AA102502) and the National Transgenic Project of China (2016ZX08006003–004).

140 Dietary vitamin A differentially affects fat desaturation in porcine stearoyl-CoA desaturase genotypes. J. Estany*[1], S. Gol[1], M. Tor[1], L. Bosch[2], J. Reixach[3], and R. Pena[1], [1]*Departament of Animal Science, University of Lleida Agrotecnio Center, Lleida, Spain;* [2]*Departament of Agriculture Engineering and Food Technology. University of Girona, Girona, Spain;* [3]*Selección Batallé, S.A, Riudarenes, Spain.*

The single nucleotide polymorphism (g.2228T>C) in the promoter region of the porcine stearoyl-coA desaturase (*SCD*) gene is associated to fatty acid desaturation in Duroc pigs (Estany *et al*. 2014 PlosOne 20;9(1):e86177). This mutation is positioned in the core sequence of two putative transcription binding motifs for retinoid and retinoic acid receptor response elements. Thus, it could be hypothesised that the effect of the *SCD* genotypes on fat desaturation may differ according to dietary vitamin A. To test this hypothesis, we conducted an experiment in which two batches of Duroc pigs of the three S*CD* genotypes (n = 142) were subjected to two identical growing-finishing diets differing in the vitamin A content.

The first diet was not supplemented with vitamin A while the second diet was supplemented with retinyl acetate at commercial levels. At slaughter, a sample of m. gluteus medius and m. longissimus thoracis were taken to determine the intramuscular fat content and fatty acid composition. As expected, the *SCD* genotype modified the desaturation ratio, with allele T increasing C16:1/C16:0 and C18:1/C18:0 ratios ($P < 0.01$). The diet had no effect on fatty acid composition, but an interaction between the diet and the *SCD* genotype was revealed for C16:1/C16:0 and C18:1/C18:0, both in m. gluteus medius ($P < 0.01$) and in m. longissimus thoracis ($P < 0.05$). In both muscles, the allele substitution effect of allele T for allele C at *SCD* g.2228T>C for C16:1/C16:0 and C18:1/C18:0 was, respectively, around 3-fold and 2-fold higher when vitamin A was not added to the diet. The results obtained indicate that supplementation of vitamin A at commercial amounts promotes the activity of allele C but depresses that of allele T, thereby giving rise to a good example of how genetic variation influences nutrient response.

Key Words: pig, vitamin A, intramuscular fat, oleic acid, MUFA

141 Effects of diet supplementation with oleic acid or carbohydrates on *Biceps femoris* transcriptome in growing Iberian pigs. R. Benítez*[1], B. Isabel[2], A. Fernández[1], Y. Núñez[1], E. De Mercado[3], E. Gómez Izquierdo[3], J. García-Casco[1], C. López-Bote[2], and C. Óvilo[1], [1]*INIA, Madrid, Spain;* [2]*UCM, Madrid, Spain;* [3]*Centro Pruebas Procino Itacyl, Hontalbilla, Segovia, Spain.*

Tissue composition largely determines the quality of meat and meat products and is influenced by factors as diet, genetic type, age or sex. Diet influences animal body and tissue composition due to direct deposition and to the nutrients effects on metabolism. In this study we evaluated the effects of a diet supplemented with 6% high oleic sunflower oil or carbohydrates as energy source on muscle composition and quality traits of growing Iberian pigs. A comparative study of the *Biceps femoris* transcriptome with RNA-seq between animals fed with both types of diet was carried out. A total of twenty nine Iberian males started the dietary treatment at 19.9 kg of average live weight (LW) and were kept under identical management conditions and fed with two different isocaloric and isoproteic diets (3.3 Kcal of digestible energy and 15.6% of crude protein) provided *ad libitum*: HO diet enriched with 6% high-oleic sunflower oil and CH diet with carbohydrates as energy source. All animals were slaughtered after seven days of treatment, with 24.1 kg of average LW. Fatty acid composition of animal tissues reflected the diet composition and indicates higher lipogenesis in CH group, as expected. We detected 25 differentially expressed (DE) genes, 17 were overexpressed in HO (FC = 2.3 to 23.5; $q < 0.1$) (i.e. *ALB* and *APOC3*) and 8 in CH diet (FC = 2 to 11.2; $q < 0.1$) (i.e. *microRNA143, INSIG* and *TBXAS1*). We performed a functional analysis (metabolic pathways and GO enrichment) of the DE genes, which showed the enrichment of functions related to lipid metabolism (synthesis and accumulation of fatty acids), cellular differentiation and proliferation, inflammatory response and metabolic processes related to oxidative stability. The bioinformatic analysis also allowed to predict potential regulators (*ATF4, PPARGC1A* and *TNF*) for the expression differences observed. The results indicate the direct deposition of nutrients and a small effect of the diet on gene expression, affecting relevant biological pathways, in agreement with previous results.

Key Words: Iberian pig, RNA-Seq, tissue composition, diet, transcriptome

142 Porcine bloodomics: Identification of porcine neutrophil-specific genes through gene expression correlations to neutrophil abundance and comparative expression data. G. Vella[1,2], M. Schroyen[2], H. Beiki[2], C. L. Loving[3], and C. K. Tuggle*[2], [1]*College of Veterinary Medicine, Iowa State University, Ames, IA, USA;* [2]*Department of Animal Science, Iowa State University, Ames, IA, USA;* [3]*Food Safety and Enteric Pathogens Research Unit, USDA-ARS-NADC, Ames, IA, USA.*

Identification and expression profiling of porcine immune cell-specific genes will provide researchers with a more vivid portrait of healthy and diseased states in individual pigs, permit more accurate biomarker screening, and will improve porcine genome annotation. Cell-specific gene profiling has been conducted in human and mouse cells under both resting and inflammatory models. Evidence of conservation of lymphoid and myeloid signature genes in mice and humans has been published, but there is a lack of pig cell-specific data. As an initial study to begin cataloging porcine cell-specific patterns, we used existing human granulocyte-specific expression data in combination with correlation of gene expression to neutrophil proportion in porcine whole blood RNA-seq data to predict neutrophil-specific pig genes. The RNA-seq datasets included collections from healthy as well as lipopolysaccharide-treated pigs. Correlation network comparisons were also used to visualise neutrophil-correlated modules. Pig neutrophil-correlated genes were significantly enriched ($P < 0.01$, Fisher's exact test) in a published list of human granulocyte-specific genes. A subset of these genes ($n = 26$) were tested using Fluidigm q-RT–PCR with RNA from neutrophils versus peripheral blood mononuclear cells (PBMC, monocytes and lymphocytes only), as well as the original blood samples to verify RNA-seq data. Quantitative PCR experiments verified 80–100% of these genes to be 2 fold higher in expression in neutrophils compared to PBMC, depending on the porcine whole blood RNA-seq dataset used for correlation, while 60–80% of tested genes were 10-fold higher in neutrophils over PBMC. To extend this work, we are currently analysing neutrophils by using RNA-seq and comparing human and porcine neutrophil-specific networks to expand our understanding of porcine immunogenomics and increase functional annotation of the porcine genome.

Key Words: pig, FAANG, comparative genomics, immunogenomics, biomarker

143 Is there genetic variation in both resistance and tolerance of pigs to porcine reproductive and respiratory syndrome virus? G. Lough[1], A. Hess[4], H. Rashidi[2], H. Mulder[2], J. Dekkers[4], I. Kyriazakis[3], M. Hess[4], N. Deeb[5], A. Kause[6], B. Rowland[7], J. Lunney[8], and A. Doeschl-Wilson*[1], [1]*The Roslin Institute, University of Edinburgh, Edinburgh, Midlothian, UK;* [2]*Animal Breeding and Genomics, Wageningen University and Research, Wageningen, the Netherlands;* [3]*School of Agriculture Food and Rural Development, Newcastle University, Newcastle upon Tyne, UK;* [4]*Iowa State University, Ames, IA, USA;* [5]*Genus plc, Hendersonville, TN, USA;* [6]*Natural Resources Institute Finland, Jokioinen, Finland;* [7]*Kansas State University, Manhattan, KS, USA;* [8]*USDA, Beltsville, MD, USA.*

A host can adopt two response strategies to combat infection: resistance (reduce pathogen load) and tolerance (minimize impact of infection on performance). Both strategies may be under genetic control and could thus be amenable to genetic improvement. Although there is evidence in support of a genetic basis for resistance to Porcine Reproductive and Respiratory Syndrome (PRRS), it is not known whether pigs also differ genetically in tolerance. The aim of this study was to determine the extent to which pigs that have been shown to vary genetically in resistance to PRRS also exhibit genetic variation in tolerance. Random regression sire models were fitted to PRRS Host Genetics Consortium data from 1320 weaned pigs (offspring of 54 sires) experimentally infected with a virulent strain of PRRS virus to obtain genetic parameter estimates for resistance and tolerance. Random regression models using individual average growth rates and cumulative serum virus load measures and a tolerance slope fitted for each sire was not indicative of genetic variation in tolerance. However, strong evidence for genetic variation in tolerance was obtained when the 42-day infection period

was partitioned into different stages of infection based on individual serum virus load profile characteristics, and by either assessing tolerance genetics at each particular stage, or by utilising the information from longitudinal data in a repeated-measurement model. Multi-variate mixed models applied to the diverse individual infection stages gave highly variable estimates for genetic correlations between resistance and tolerance, indicating that the relationship between both traits varied throughout the time course of infection. In contrast, when considering the whole 42 day infection period, pigs with greater genetic resistance to PRRSV tended to also be genetically more tolerant (r_g = 0.79; se. 0.31). In conclusion, pigs previously found to vary genetically in resistance to PRRS also vary genetically in tolerance. The results imply that both traits could be targeted for genetic selection to improve resilience of pigs to PRRS.

Key Words: tolerance, resistance, resilience, pigs, PRRS

144 Integrative and differential analysis of transcriptomes and chromatin accessibility regions reveals regulatory mechanisms involved in pig immune and metabolic functions [FAANG pilot project "FR-AgENCODE"]. S. Djebali[1], K. Munyard[2], N. Villa-Vialaneix[3], C. Cabau[1], A. Rau[4], E. Crisci[4], T. Derrien[5], C. Klopp[3], M. Zytnicki[3], S. Lagarrigue[6,7], H. Acloque[1], S. Foissac*[1], and E. Giuffra[4], [1]*GenPhySE, INPT, ENVT, INRA, Université de Toulouse, Castanet-Tolosan, France;* [2]*Curtin University, School of Biomedical Sciences, Perth, Australia;* [3]*MIAT, Université de Toulouse, INRA, Castanet-Tolosan, France;* [4]*GABI, AgroParisTech, INRA, Université Paris Saclay, Jouy-en-Josas, France;* [5]*UMR6290 IGDR, CNRS, Université Rennes 1, Rennes, France;* [6]*UMR PEGASE, INRA, Rennes, France;* [7]*UMR PEGASE, Agrocampus Ouest, Rennes, France.*

In the context of the FAANG pilot project 'FR-AgENCODE' to improve the functional annotation of livestock genomes, we characterised the transcriptome and the chromatin accessibility of pig hepatocytes and two types of lymphocytes. More specifically, CD3+CD4+ ('CD4') and CD3+CD8+ ('CD8') T-cells were sorted from the blood of two male and two female Large White adult pigs. These samples, along with liver samples from the same animals, were processed by strand-oriented RNA-seq and ATAC-seq experiments. Principal Component Analyses on log-transformed TMM-normalized read counts in genes (from RNA-seq) and regions of chromatin accessibility (from ATAC-seq) consistently highlighted the variability between liver and the T-cells, and to a lesser extent within T-cells (CD4 *v.* CD8), as well as between the male and female samples. Comparative analyses identified differentially expressed genes between cell types as well as potential regulatory sites from differentially accessible chromatin regions. As expected, ontology annotations of differentially expressed genes were enriched for either immunity- or metabolism-related terms. Interestingly, correlations between gene expression and promoter accessibility across samples were enriched for both extreme positive and negative values, which suggests that ATAC-seq can efficiently capture distinct regulatory mechanisms of gene expression. Candidate enhancers and repressors were identified by comparing ATAC-seq regions with predicted binding sites of 500+ transcription factors. By integrating these results with those from Hi-C chromosome conformation capture on the liver samples, we further characterised the differences between 'active' and 'repressed' topological domains in terms of functional features, including gene density and general chromatin accessibility. Altogether, these results lead to a better understanding of the molecular mechanisms involved in pig immune and metabolic functions, and illustrate a useful contribution to the functional annotation effort of the FAANG initiative.

Key Words: Functional Annotation of Animal Genomes (FAANG), pigs and related species, ATAC-seq, RNA-seq, Hi-C

145 Genome-wide scanning of the *cis*-effects of sequence variations on enhancer activity in the F6 swine heterogeneous stock. Z. Zhang*, Y. Zhu, Z. Zhou, W. Li, and L. Huang, *State Key Laboratory of Pig Genetic Improvement and Production Technology, Jiangxi Agricultural University, NanChang, JiangXi Province, China.*

Genome-wide association studies (GWAS) have highlighted thousands of variants associated with numerous traits in human and farm animals. And the majority of these variants located in non-coding regions, leading to difficulty in functional annotation. To meet the significant challenges in post-genomic era, working together with FANNG, a two phase research project has been carried out to identify and annotate the functional elements of the pig genome at different developmental stages, different tissues in both male and female individuals of Asian and western pig breeds (Phase I) and then to investigate the sequence variations of these genomic elements with the corresponding transcriptome, proteome, metaboliome and phenome change of the swine complex traits for the benefit of effective meat production as well as disease models for human beings (Phase II). Here, we report our effort to systematically archive enhancers in liver tissues in an experimental F6 heterogeneous pig population (multigenerational outbred comprising of 8 pig breeds) and decipher the *cis*-effects of genome-wide sequence variants on the activity of these enhancers and expression of related genes. We established a stable protocol to define active enhancers by ChIP-seq with H3K27Ac antibody in liver, and ~89% enhancer peaks we detected are nicely overlapped with that from previous report (Villar et al., 2015). We will characterize the active enhancers in livers of ~150 F6 mosaic pigs. Integrating of DNA re-sequencing, liver transcriptome as well as detailed phenotyping of these pigs with ~220 traits, we will create a resource to understand how sequencing variants perturb enhancers and further affect gene expression patterns in liver in pigs. This resource will be valuable not only for functional interpretation of the genome wide active enhancer variants but also their potential effects on the related complex traits in this important farm animal.

Key Words: swine heterogeneous stock, ChIP-seq, enhancer, integrative genomics, complex trait

146 The effect of histone acetyl-transferase inhibitor (trichostatin A) administration on porcine mesenchymal stem cells transcriptome. A. Gurgul*, J. Opiela, K. Pawlina, T. Szmatola, and M. Bugno-Poniewierska, *National Research Institute of Animal Production, Balice, Poland.*

The use of histone acetyl-transferase inhibitors such as Trichostatin A (TSA) for epigenetic modulation of mesenchymal stem cells (MSCs) is an interesting approach in research involving somatic cell cloning of pigs and other mammalian species. Despite the effectiveness of TSA in cloning applications was already confirmed, the detailed mechanisms underlying this effect are not yet fully recognised, especially for pig MSCs. To add to this knowledge, in this study we performed a comprehensive transcriptome analysis using high-throughput RNA sequencing of pig bone-morrow derived MSCs, treated and untreated with TSA, and evaluated the effect of TSA administration on their transcription profile after 24 h of *in vitro* culture. Subsequently, the stability of introduced epigenetic modifications was evaluated after another 50–72 h of culture without TSA. The results showed a wide stimulating effect of TSA on MSCs transcription, affecting genes across the whole genome with some minor signs of site-specific acting in regions located on SSC2 and SSC6. TSA had stronger impact on already expressed genes with only minor influence on silenced genes. Genes with expression altered by TSA were related to a wide range of biological processes, however, we found some evidence for specific stimulation of genes associated with development, differentiation, neurogenesis or generation of muscles. The analysis of cell

transcriptome after prolonged culture following the TSA removal, showed that the expression level of majority of genes affected by TSA is restored to the initial level. Nonetheless, the set of about six hundred genes was altered even after 50–72h of culture without TSA. TSA also enhanced expression of some of pluripotency marker genes (*FGF2, LIF, TERT*) but their expression was stabilised during further culture without TSA. The detailed analysis of factors connected with neuron-like differentiation allows us to assume that TSA mostly stimulates neurogenic differentiation pathway in the pig MSCs and thus seems to trigger mechanisms conducive of epigenetic reprograming. This research was supported by the Polish National Science Centre resources allocated on the basis of decision number 2014/15/B/NZ9/04288.

Key Words: MSC, pig, transcriptome, trichostatin A

147 Characterization of 3D genomic interactions in fetal pig muscle. M. Marti-Marimon*[1], H. Acloque[1], M. Zytnicki[2], D. Robelin[1], S. Djebali[1], N. Villa-Vialaneix[2], O. Madsen[3], Y. Lahbib-Mansais[1], D. Esquerré[1], F. Mompart[1], L. Liaubet[1], M. Groenen[3], M. Yerle-Bouissou[1], and S. Foissac[1], [1]*GenPhySE, University of Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France;* [2]*MIAT, University of Toulouse, INRA, Castanet Tolosan, France;* [3]*Animal Breeding and Genomics Centre (ABG), Wageningen University, Wageningen, the Netherlands.*

Genome sequence alone is not sufficient to explain the overall coordination of nuclear activity in a particular tissue. The nuclear organisation and genomic long-range intra- and inter-chromosomal interactions play an important role in the regulation of gene expression and the activation of tissue- specific gene networks. Here we present an overview of the pig genome architecture in muscle at two late developmental stages. The muscle maturation process occurs between the 90th day and the end of gestation (114 days), a key period for survival at birth. To characterise this period we profiled chromatin interactions genome-wide with *in situ* Hi-C (High Throughput Chromosome Conformation Capture) in muscle samples collected at 90 and 110 days of gestation, specific moments where a drastic change in gene expression has been reported. About 200 million read pairs per library were generated (3 replicates per condition). This allowed: (a) the design of an experimental Hi-C protocol optimized for frozen fetal tissues, (b) the first Hi-C contact heatmaps in fetal porcine muscle cells, and (c) to profile Topologically Associated Domains (TADs) defined as genomic domains with high levels of chromatin interactions. Using the new assembly version *Sus scrofa* v11, we could map 82% of the Hi-C reads on the reference genome. After filtering, 49% of valid read pairs were used to infer the genomic interactions in both developmental stages. In addition, ChIP-seq experiments were performed to map the binding of the structural protein CTCF, known to regulate genome structure by promoting interactions between genes and distal enhancers. The Hi-C and ChIP-seq data were analysed in combination with the results of a previous transcriptome analysis, focusing on the hundreds of genes that were reported as differentially expressed during muscle maturation. We will report the observed general differences between both developmental stages in terms of transcription and structure.

Key Words: nuclear architecture, fetal development, Hi-C (high-throughput chromosome conformation capture), pig muscle, CTCF

148 Exploiting long read sequencing technologies to establish high quality highly contiguous pig reference genome assemblies. A. Warr[1], R. Hall[2], K. Kim[2], E. Tseng[2], S. Koren[3], A. Phillippy[3], D. Birkhart[4], B. Rosen[4], S. Schroeder[4], D. Hume[1], R. Talbot[5], L. Rund[6], L. Schook[6], W. Chow[7], K. Howe[7], D. J. Nonneman[8], G. A. Rohrer[8], N. Putnam[9], R. E. Green[9], R. O'Connor[10], D. Griffin[10], B. M. Skinner [11], C. A. Sargent[11], N. A. Affara[11], C. Tyler-Smith[7], M. Watson[1], T. P. L. Smith[8], and A. Archibald*[1] [1]*The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, UK;* [2]*Pacific Biosciences, Menlo Park, CA, USA;* [3]*National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA;* [4]*Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD, USA;* [5]*Edinburgh Genomics, University of Edinburgh, Edinburgh, UK;* [6]*University of Illinois, Urbana, IL, USA;* [7]*The Wellcome Trust Sanger Institute, Hinxton UK;* [8]*USDA, ARS, USMARC, Clay Center, NE, USA;* [9]*Dovetail Genomics LLC, Santa Cruz, CA, USA;* [10]*University of Kent, Canterbury, Kent, UK;* [11]*University of Cambridge, Cambridge, UK.*

The current pig reference genome sequence (Sscrofa10.2) was established using Sanger sequencing and following the clone-by-clone hierarchical shotgun sequencing approach used in the public human genome project. However, as sequence coverage was low (4–6x) the resulting assembly was only of draft quality. We have built new *de novo* genome assemblies from whole genome shotgun (WGS) sequence reads generated using Pacific Biosciences (PacBio) long read sequencing technology for two pigs – the original reference animal (Duroc sow 2–14) and a Duroc/Landrace/Yorkshire crossbred barrow. About 60–70x coverage WGS data per animal were assembled with the Falcon assembler and error corrected with Quiver/Arrow and Pilon using high coverage WGS PacBio and Illumina reads, respectively. The estimated accuracy (99.999%) of the Duroc assembly meets the requirement of a Gold standard finished sequence. The Duroc assembly was scaffolded with paired-end reads from isogenic BAC and fosmid clones and assigned to chromosomes based on fluorescent *in situ* hybridisation using probes generated from BAC clones. The crossbred assembly was scaffolded using Dovetail's Hi-Rise. The current statistics for these assemblies are: Duroc 2-14 (Sscrofa11) for SSC1-18, SSCX (2.39 Gbp, 122 contigs; contig N50=58.5 Mbp; scaffold N50=107.6 Mbp); Duroc/Landrace/Yorkshire crossbred for SSC1-18, SSCX, SSCY (2.62 Gbp, 14,924 contigs; contig N50 =6.5 Mbp; scaffold N50=132 Mbp). The Sscrofa11 assembly has been updated recently to Sscrofa11.1 by the addition of the SSCY sequence data from Skinner et al. 2016 (Genome Res 26:130-9). The BAC and fosmid clone resource from Duroc 2-14 will facilitate further targeted sequence closure. These improved genome assemblies will be a key resource for research in pigs and will enable applications in agriculture and biomedicine. The assemblies are being deposited in the public database under the pre-publication data release terms of the Toronto Statement (Nature 461:168-70).

Key Words: pig, reference genome sequence, long read sequencing technology

149 Updated pig genome resources in Ensembl. T. Hourlier*[1], L. Eory[2], K. Billis[1], C. García Girón[1], L. Haggerty[1], O. Izuogu[1], D. N. Murphy[1], R. Nag[1], F. J. Martin[1], A. L. Archibald[2], B. Aken[1], and P. Flicek[1], [1]*European Molecular Biology Laboratory, European Bioinformatics Institute, Hinxton, UK;* [2]*The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, Edinburgh, UK.*

Ensembl provides high-quality, reference annotation resources for publicly available genome assemblies, including domestic pig (*Sus scrofa*). Pig is an important model for cardiovascular disorders, infectious diseases, and xenotransplantation, and also an economically important species for meat production. We have fully updated all pig genome resources in Ensembl based on the recently improved reference assembly Sscrofa11.1 (GCA_000003025.6) produced by the Swine Genome Sequencing Consortium. These updates include a curated gene annotation using new Illumina and PacBio transcriptome data that comply with the FAANG meta data submission guidelines, which we will describe in detail. Also updated to the new assembly are our pig genome variation resources, gene names, gene-trees and orthologues, cross-references to external databases including UniProt and RefSeq, vertebrate multiple whole genome

alignments, and conserved and constrained elements. These updates will be released as part of Ensembl version 90, which is expected in July. All data are freely available through our website at http://www.ensembl.org, REST API (http://rest.ensembl.org) and our public MySQL server (ensembldb.ensembl.org) as well as tools such as the Ensembl Variant Effect Predictor (www.ensembl.org/Tools/VEP) and BioMart (http://www.ensembl.org/biomart). Ensembl also supports the upload and visualisation of data in multiple file formats such as BAM or GFF3. We have developed the Track Hub Registry (http://trackhubregistry.org/) to facilitate data sharing through discovery of publicly accessible track data hubs and viewing them alongside Ensembl data. Additionally, data can now be viewed in Ensembl even if the files are not hosted locally by submitting them in CRAM format to the ENA and registering a track hub with the ENA accession numbers.

Key Words: pigs and related species, genome annotation, Functional Annotation of Animal Genomes (FAANG), bioinformatics tools, databases/repositories

Novel, Groundbreaking Research/Methodology Presentation

150 Sheep parchment as a genetic resource. M. Teasdale*, *Trinity College Dublin, Dublin, Ireland.*

Before the mass production of paper, parchment was the major medium for codices and until the widespread adoption of typewriters, they were a clerk's preferred medium for many formal legal documents and records. Unlike modern parchment, which is typically made from goat or calf, ~1,000 English legal documents that we have analysed via peptide mass fingerprints (MALDI-TOF mass spectrometry), were made of sheepskin. Parchment has been shown to harbour sufficient concentrations of DNA to allow for high-throughput sequencing and therefore offers tremendous scope for documenting the recent genetic history of British sheep breeds. Documents contained within the British archival collections span the transition from a wool- to a meat-based economy and the beginnings of intense artificial selection. We have sequenced a range of parchment samples from the county of Yorkshire (UK) that date from the 14th to the 19th century to low coverage. These animals were then compared to modern breeds from the Sheep HapMap and the Sheep Genomes Database and scanned for evidence of recent positive selection between the time points.

Key Words: genomics, ancient DNA, selection

Applied Genetics of Companion Animals

151 AgriSeq targeted sequencing panel for determination of canine parentage and genetic health. M. Karberg*[1], A. Burrell[1], P. Siddavatam[1], A. Allred[1], M. de Groot[2], and W. van Haeringen[2], [1]*Thermo Fisher Scientific, Austin, TX, USA;* [2]*VHL Genetics, Wageningen, the Netherlands.*

The objective of this presentation is to show the performance of canine parentage and genetic health AgriSeq primer panels, and demonstrate how the panels can be combined, or otherwise modified, without detrimental effects to detection. Ensuring an accurate pedigree is particularly important for purebreds, having both economic and animal health implications. Historically, microsatellites (short tandem repeats or STRs) have been used for genetic identification, traceability and paternity. In recent years, other DNA based tests such as single nucleotide polymorphism (SNPs) detection have become increasingly used for this purpose. High-throughput targeted amplification and re-sequencing, using the Applied Biosystems AgriSeq target enrichment technology and the Ion S5 sequencing system, allows for the simultaneous and accurate genotyping of a large number of SNPs to interrogate the heritage and genetic health of an animal. In addition, the AgriSeq approach is very flexible, allowing panels to be combined or modified easily, thereby helping both breeders and diagnostic laboratories stay relevant with evolving content needs. Here, we describe the development of two AgriSeq panels targeting canine SNP markers: a parentage panel based on 200 ISAG canine targets, and a genetic health panel based on over 140 well characterised genetic markers. To demonstrate the modularity of the AgriSeq approach, the performance of the genetic health and parentage panels were analysed independently and also combined and analysed simultaneously using 192 samples pooled on an Ion S5 540 chip. Variant calling was performed using the Torrent Variant Caller (TVC) plugin as part of the Ion Torrent Suite software package. The data show that the two panels work similarly, regardless of whether they are used separately, or combined together on one sequencing chip. The mean sample call rate was above 95% and the sample concordance between the separate and combined panels was over 99.9%.

Key Words: dogs and related species, DNA sequencing, parentage

152 Pedigree and genomic-based relationships in a dog population. A. Talenti*[1], D. L. Dreger[2], F. Danelli[1], S. Frattini[1], B. Coizet[1], S. P. Marelli[1], G. Pagnacco[1], G. Gandini[1], M. Polli[1], R. Caniglia[3], M. Galaverni[3], E. A. Ostrander[2], and P. Crepaldi[1], [1]*Department of Veterinary Medicine, University of Milan, Milan, Italy;* [2]*National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA;* [3]*Laboratorio di Genetica, Istituto Superiore per la Protezione e la Ricerca Ambientale, Ozzano dell'Emilia, Italy.*

In many dog breeds, small population sizes, associated with intense selection schemes, have led to considerable losses of genetic diversity. This complicates the production of accurate genomic estimates of parentage. The Lupo Italiano, with ~300 living dogs, is an Italian breed created in 1966 by crossing the Apennine grey wolf (*Canis lupus italicus*) with German Shepherd dogs (GSD). The aim of this work was to compare calculated relationships from genomic and pedigree data, using the Lupo Italiano as an example of a small population. The entire pedigree of the Lupo Italiano is known, consisting of up to 12 generations and dating to the founder animals. The pedigrees of 28 Lupo Italiano dogs (provided by AAALI) were used to build an additive relationships matrix (A) (CFC software). These 28 dogs were genotyped on the Illumina CanineHD 170K SNP chip (University of Milan and National Institutes of Health in Bethesda, MD), and the resultant genotypes were used for the estimation of the within-breed Genomic Relationships Matrix (GRMa) (GCTA64 software). The mean parentage values for GRMa (-0.02±0.05) and A (0.80±0.05) were not equivalent, however, they did display a significant positive correlation ($R = 0.75$; $P < 0.001$). Four additional populations, genotyped on the same panel, consisted of 20 Apennine grey wolves (ISPRA), 30 GSDs, 14 grey wolves, and 31 village dogs (publicly available

in Dryad, Shannon et al. 2015). The GRM produced with the combined set (GRMb) led to a higher correlation with A (R = 0.80; $P < 0.001$) and to higher estimates of parentage between Lupo Italiano individuals (0.53±0.05). The calculation was expanded a final time to include an additional 250+ GSDs (GRMc). This matrix showed a decrease in the correlation with A (R = 0.76; $P < 0.001$) balanced by a strong increase in the parentage values (0.82±0.08), making it the closest to the A matrix. These results show that estimation of genomic relationships from populations with greater allelic diversity can improve the correlation and accuracy with pedigree-derived estimates. Consideration of these implementations can allow for better management of mating schemes and conservation of genetic variation in dog breeds with small population sizes. The authors thank AAALI for the kind collaboration.

Key Words: dog, parentage, SNP

Applied Sheep and Goat Genetics

153 Introgression of wool-shedding genes into the Romane breed sheep. L. Drouilhet*[1], B. Pena[1], C. Huau[1], D. Marcon[2], Y. Bourdillon[2], and D. Allain[1], [1]*GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France;* [2]*INRA UE0322, La Sapinière, Bourges, France.*

Wool production in Europe became unprofitable compared to the meat or milk productions. One major reason is that the wool income is lower than the cost of shearing. The Romane breed, a French composite breed between the Berrichon du Cher breed (meat breed) and the Romanov breed (prolific breed), showed little ability to shed. We decided to introgress in the Romane breed the phenotype of wool-shedding of the Martinik Hair breed. Indeed, the Martinik Hair sheep have the ability to annually naturally woolshed. The experimental trial of introgression was realised on 4 successive backcrosses from the Martinik Hair (MH) to the Romane breed (RM): F1 (MH*RM), then BC1 (F1*RM), BC2 (BC1*RM) and BC3 (BC2*RM). Two traits were considered: the ability to shed at least a part of the fleece or not, as a binary trait and the extent of shedding as the ratio of wool shed area to total body area. During those backcrosses, animals were measured and selected on their ability to shed at 7 months of age. The BC3 population represents the first generation (G1) of the introgressed population for the wool-shedding phenotype. This introgressed breeding stock (n = 150 ewes) was then selected using estimated breeding values based on shedding extension. A high heritability estimate (0.50 ± 0.09) and a large genetic gain (2.2 genetic standard deviations) on wool-shedding were observed after 6 generations of selection, without impairing the production fitness on the Romane sheep. We did not observe a bimodal distribution of wool-shedding extension phenotype, suggesting that not only one mutation is segregating in our population, but more probably a few major genes with large effects due to the large genetic gain observed. At the G6, 96 animals (9 family sires, 6 to 11 progeny per sire, 10 dams) with extreme phenotypes (total wool shedding or not) including some full-sibs and their dam were selected and genotyped on 50K SNP chip. This dataset is currently analysed using linkage analysis (LA), linkage disequilibrium (LD) and joint LD-LA mapping using QTLMAP software. The first results showed at least 3 different loci influencing the ability to shed on chromosome OAR3, OAR12 and OAR15. The analysis are in progress to precise those intervals of localization.

Key Words: sheep, genetic introgression, genome wide-association, wool shedding

154 Community-based sheep breeding programs in Ethiopia resulted in substantial genetic gains. A. Haile*[1], T. Mirkena[3], G. Duguma[2], S. Gizaw[2], M. Wurzinger[4], J. Solkner[4], O. Mwai[2], T. Dessie[2], A. Abebe[6], M. Mamiru[8], T. Tadesse[7], R. N. B. Lobo[5], and B. Rischkowsky[1], [1]*International Center for Agricultural Research in the Dry Areas, Addis Ababa, Ethiopia;* [2]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, and Nairobi, Kenya;* [3]*FAO, Addis Ababa, Ethiopia;* [4]*BOKU University, Vienna, Austria;* [5]*EMBRAPA-goat and sheep, Sobral, Brazil;* [6]*Debre Berhan Agricultural Research Center, Debre Berhan, Ethiopia;* [7]*Bako Agricultural Research Center, Bako, Ethiopia;* [8]*Bonga Agricultural Research Center, Bonga, Ethiopia.*

In small ruminants, centralized breeding schemes, entirely managed and controlled by governments – with minimal, if any, participation by farmers – were developed and implemented in many developing countries. Such programs have generally failed to sustainably provide the desired genetic improvements to smallholder livestock keepers. Community-based breeding Programs (CBBPs) have been suggested as an alternative and are being implemented in a few pilot countries. A team of international and national scientists designed and implemented sheep CBBPs in three sites/breeds (Bonga, Horro and Menz) in Ethiopia. The team developed an innovative methodological framework on how to design, implement and sustain CBBPs. Selection traits identified through participatory approaches were six month weights in all the three sites, and in Horro and Bonga, where resources, particularly feed and water, permit larger litter sizes, twinning rate was included. Eight years (2009–2016) performance data from the programs were analysed using WOMBAT (Meyer, 2007). The results indicate that the birthweight of lambs has not improved over the years in Menz and Bonga. In Horro, there is even a slight decrease. Given that we have not selected for birthweight in the community flocks we did not expect genetic change. However, there could have been an effect through correlated responses which was not the case in all the three breeds. Six months weight, the major selection trait in our CBBPs, increased over the years in all breeds. In Horro the average increase was 0.31 ± 0.060 kg per year, followed by average increase of 0.26 ± 0.058 kg per year in Bonga and 0.14 ± 0.006 kg for Menz. This is quite substantial in an on-farm situation. In Horro and Bonga sheep, where twinning rate was one of the selection traits, the litter size of lambs born increased over the years in both breeds: the increase was 12% (from 1.28 to 1.46) in Horro and 8% (from 1.48 to 1.61) in Bonga. This increase combined with the increased bodyweight has made a substantial impact on the incomes of the farmers. Our results show that CBBPs are technically feasible and result in measurable genetic gains in performance traits.

Key Words: sheep and related species, animal breeding, genetic improvement

155 Identification of two major genes affecting prolificacy in the French Noire du Velay sheep. L. Chantepie*, L. Bodin, F. Woloszyn, J. Sarry, and S. Fabre, *GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France.*

Classical selection methods of prolificacy in sheep assume a full polygenic inheritance, each gene having an infinitesimal effect. However, in the last decades, many mutations having a major effect on prolificacy were discovered in four major fecundity genes namely *BMP15 (FecX)*, *BMPR1B (FecB)*, *GDF9 (FecG)* and *B4GALNT2 (FecL)*. When present, these mutations should be taken into account to obtain relevant prolificacy breeding values for the selection process. Based on litter size records (LS), we suspected the segregation of such major gene in the French Noire du Velay (NdV) sheep population. The first approach was to genotype selected highly prolific ewes for the already known mutations at the four major loci. We

evidenced the segregation of the *FecL*^{L} mutation in the *B4GALNT2* gene originally discovered in the Lacaune breed. By a specific genotyping of 1224 NdV ewes, we observed 10% of *FecL*^{L} carriers with a mutated allele effect of +0.4 lambs/lambing. Nevertheless, it still existed extremely prolific NdV ewes non-carrier of the *FecL*^{L} allele. Then in a second approach, we genotyped 150 *FecL*$^{+}$ NdV ewes using the ovine 50k SNP array, followed by an association analysis under a case (n = 100, mean LS 2.2) *v.* control (n = 50, mean LS 1.1) design. A unique significant signal was located on the X chromosome (53.8Mb), near the *BMP15* candidate locus. The whole genome sequencing of 3 ewes heterozygous, homozygous carrier and non-carrier of the supposed prolific allele, identified a novel A>T SNP 290bp upstream of the *BMP15* gene. Using a specific RFLP test, we genotyped 756 *FecL*$^{+}$ NdV ewes and evidenced 125 ewes as carriers of the new polymorphism (16%). We observed that this variant increased significantly the prolificacy by +0.2 lambs/lambing. By real-time PCR analysis, we showed that the A>T nucleotide change was associated to a decrease of the oocyte-specific expression of the *BMP15* gene. According to the nomenclature of *Fec* genes, this new mutation was named *FecX*^{N}. In conclusion, by a combination of candidate gene and whole genome scan approaches, we identified *FecL*^{L} and *FecX*^{N} mutations essential for improving the genetic evaluation and selection of prolificacy in Noire du Velay sheep.

Key Words: sheep, genome-wide association, monogenic trait, reproduction, genetic improvement

156 Genomic regions associated with entropion in Columbia, Polypay, and Rambouillet breeds of sheep. M. R. Mousel*[1,2] and S. N. White[1,3], [1]*Animal Disease Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Pullman, WA, USA;* [2]*Paul G. Allen School of Global Animal Health, Washington State University, Pullman, WA, USA;* [3]*Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.*

Entropion is an inward rolling of the eyelid allowing the eyelashes and cornea to have direct contact, potentially causing abrasions which may lead to infections and blindness if not treated. Typically in domestic sheep entropion only occurs with the lower eyelid and is a congenital defect. Entropion has a wide frequency (0–80%) worldwide in domestic sheep, was found to be heritable (0.08–0.21) and it is speculated to be recessive in inheritance. To eliminate this condition from their flocks, producers must cull afflicted sheep and their parents. Identification of genomic regions or genes associated with entropion could lead to the development of genetic marker(s) to reduce entropion through selective breeding. Therefore, a genome-wide association scan was conducted with 473 Columbia, Polypay, and Rambouillet sheep genotyped using the Illumina OvineSNP600 BeadChip. Entropion status was recorded within 48 h of birth and corrected if present. The overall prevalence was 6.1% in these 473 sheep. Data was analysed using a mixed model with EMMAX that accounted for relatedness, breed, and SNP minor allele. Heritability was estimated to be 0.28. Five genome-wide significant ($P < 1\times10^{-8}$) SNP were identified on chromosomes 2, 3, and 15 as well as nine SNP that were genome-wide suggestive ($P < 1\times10^{-7}$) on chromosomes 2, 3, 7, 14, 15, 20 and 22. Previously, locations on ovine chromosome 2, 3, and 15 were found to be associated with entropion by Mousel *et al*., 2015 and/or Hadfield *et al*., 2016. We are working to narrow the range of these associated regions and identify the underlying casual mutations for the benefit of sheep producers.

Key Words: entropion, genome-wide association, domestic sheep, breeds

157 Genome-wide scan reveals *NF1* locus is associated with fat tail phenotype rather than high-altitude adaptation in Asian sheep. K. Dong[1,2], M. Yang[1], N. Gorkhali[1], Y. Ma[1], and L. Jiang*[1], [1]*Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China;* [2]*USDA, Agricultural Research Service, Avian Disease and Oncology Laboratory, East Lansing, MI, USA.*

The main morphological changes such as tail length and distinct patterns of tail fat deposition are considered to be some of the major changes that followed domestication. However, nowadays, a decrease in the size of sheep fat-tail is desirable for both producers and consumers. To identify genes underlying the ovine fat tail phenotype, we performed a genome-wide scan for the highly differentiated loci between seven thin-tailed sheep breeds from Asia and six fat-tailed breeds from Middle East using Illumina Ovine 50KSNP Beadchip. By combing an independent dataset of fat-tailed and thin-tailed Chinese indigenous sheep breeds, we found a total of 90 common SNPs corresponding to 251 genes. Among those were several genes known to be involved in lipid metabolic process, including *RAB7A*, *ENPP6*, *PLA2GA*, *INSIG2*, *PEX2*, *BMP2*, *VDR* etc. GO analysis showed one of the most significant GO categories was 'lipid glycosylation' (P = 9.33E-4). Intriguingly, we found that the locus of *NF1* (containing *NF1* and three small genes, *EVI2A*, *EVI2B* and *OMG*), previously linked to the high-altitude adaptation, displayed the highest differentiation across the ovine genome. Our study demonstrated that sheep living at different altitudes showed no evidence of divergence at this locus and therefore, *NF1* locus is likely the predominant factor for the fat tail phenotype, rather than high-altitude. This finding suggested caution in drawing a conclusion in genomic studies without excluding the potential confounding factors. Additionally, by combining re-sequencing data for the *NF1* locus and RNA-sequencing (RNA-seq) data of adipose tissues from divergent tail types, we found that the potentially functional genes within the *NF1* locus were *EVI2A* and *OMG*, rather than *NF1*. Furthermore, two missense mutations at the well-conserved positions in *EVI2A* and *OMG* genes were identified in fat-tailed sheep. As the function of *EVI2A* and *OMG* are currently little known, the two missense mutations represent a unique opportunity to study the function of these two genes in an organismal context, especially for the potential novel roles in fat deposition.

Key Words: fat-tailed, genomic selection signature, sheep, NF1

158 Investigating genetic associations with meiotic recombination in rams. K. M. Davenport*, A. M. Rodriguez, R. J. Sawyer, T. M. Badigian, H. K. Jaeger, M. A. Follett, and B. M. Murdoch, *University of Idaho, Moscow, ID, USA.*

Meiotic recombination is an important process during gametogenesis that contributes to genetic variation. Understanding the process of recombination will lead to enhanced genetic predictions that will promote the sustainability of the sheep industry. It is clear from previous studies that recombination is not random, and at least one recombination event or crossover (CO) per chromosome arm is necessary for proper chromosome segregation. In addition, CO experience location preferences termed 'hotspots,' as well as interference in that one CO cannot occur in too close proximity with another. Previous studies in sheep have identified loci associated primarily in females with recombination rate derived from linkage data. Our objective was to investigate genetic associations with CO counts in rams acquired cytogenetically. We quantified the number of CO in Suffolk, Icelandic, and Targhee rams using a cytogenetic approach because it allows us to accurately identify all recombination events during meiosis without a large number of offspring. In total, we examined over 165,000 CO events from ~2,600 spermatocytes. We identified significant differences in CO number between individual rams within Suffolk, Icelandic, and Targhee breeds ($P <$ 0.05), as well as differences between breeds ($P < 0.01$). Using the mean CO counts obtained from the spermatocytes from individual rams as a quantitative phenotype, we performed a genetic association study. The OvineSNP50 BeadChip was used to genotype rams

and an association study was performed with PLINK v1.09. The results of the association study identified genomic regions of interest on chromosomes 1, 4, 6, 9, 14, 22, 23, and 24 after a Bonferroni correction ($P < 1E-06$). This study identifies potentially important genomic regions of interest associated with the number of CO in these rams. These data contribute important information towards the understanding of individual and breed recombination differences. Furthermore, this research will advance breed specific selection strategies that support the sustainability of the sheep industry.

Key Words: sheep, recombination, quantitative trait locus (QTL), genome-wide association, genetic improvement

Comparative and Functional Genomics

159 Identification of regulatory elements in livestock species. H. Zhou*[1], P. Ross[1], C. Kern[1], P. Saelao[1], Y. Wang[1], M. Halstead[1], K. Chanthavixay[1], I. Korf[1], M. Delany[1], H. Cheng[2], J. Medrano[1], A. Van Eenennaam[1], C. Tuggle[3], and C. Ernst[4], [1]*University of California, Davis, Davis, CA, USA;* [2]*USDA-ARS, Avian Disease and Oncology Laboratory, East Lansing, MI, USA;* [3]*Iowa State University, Ames, IA, USA;* [4]*Michigan State University, East Lansing, MI, USA.*

Regulatory elements play an essential role in understanding how an organism's genotype determines its phenotype. The technologies and assays developed in the human and mouse ENCODE projects provide a solid foundation to functionally annotate farm animal genomes. The recent international FAANG (Functional Annotation of ANimal Genomes) initiative has stimulated such efforts on livestock species, which will ultimately be leveraged to improve production efficiency, animal welfare, and food safety. The overall objective of this study is to functionally annotate regulatory elements in three major livestock species: chicken, cattle, and pig. The first key step is to identify regulatory elements in the genomes by integrating RNA-seq, DNase-seq and ChIP-seq data. As one of FAANG pilot projects coordinated by UC Davis, we will present the current progress in generating and analysing data from these three important species. We have collected samples from adipose, cerebellum, cortex, hypothalamus, liver, lung, muscle, and spleen in two male biological replicates from each species, allowing us to identify both universal and tissue-specific functional elements. High depth of RNA-seq has allowed the identification of 9,393 long non-coding RNAs in chicken, 7,235 in cattle, and 14,428 in pig. From DNase-seq sequencing in chickens, we have identified 132,362 open chromatin regions across the genome, many of which are tissue-specific or only present in some tissues. Per tissue, the values range from a high of 57,703 regions in spleen to the lowest of 14,700 in cerebellum. Genes present in these open chromatin regions show generally higher expression in our RNA-seq data. ChIP-seq for the H3K4me3 histone modification from all eight tissues in chicken identified a total of 31,174 peaks, while 35,081 were identified from liver, lung, and spleen in pig. H3K27me3 from the same three tissues in pig identified a total of 104,640 peaks. Preliminary results from H3K27me3 assays in chickens identified a total of 16,247 peaks. In the future, an integrative analysis of five ChIP-seq assays and DNase-seq will produce genome-wide chromatin state predictions and allow the identification of promoters, enhancers, and silencers in all three species.

Key Words: bioinformatics, Functional Annotation of Animal Genomes (FAANG), ChIP-seq, epigenomics

160 Transcription factor binding sites enrichement in ruminant and cetartiodactyl specific conserved non-coding elements. L. Buggiotti*, M. Farrè, and D. Larkin, *Royal Veterinary College, London, UK.*

Gene regulatory sequences (e.g. transcription factor binding sites (TFBSs)) contribute to differences in gene expression patterns within and between species and from *de novo* in clade specific-evolution. To investigate association between lineage-specific conserved non-coding elements (CNEs) and potential TFBSs, we have done identification of CNEs from several mammalian lineages and looked for association between these elements and TFBSs found in the cattle genome. A multiple-species alignment of 28 mammalian genomes, CNEs, and TFBSs detections were done as follows: i) lastZ pairwise alignments of each genome against the cattle genome assembly (bosTau6); ii) mulitZ was used to combine pairwise alignments in multiple alignment files; iii) conserved elements (CEs) were estimated using phastCons; iv) TFBStools with JASPAR CORE vertebrate motifs was utilised to perform the whole-genome scan for TFBS; v) CNEs were defined as those CEs that did not overlap with the coding parts of all known genes. Genomic association test was done to test for enrichment of TFBSs in the ruminant-, cetartiodactyl- and mammalian-specific CNE sets compared to the rest of the cattle genome. Overall, mammalian CNEs (>20bp) cover 1.3% of the cattle genome, while cetartiodactyl and ruminant CNEs cover ~1% and ~1.5%, respectively. *In silico* analysis of TFBS predicted over 25.9 million binding sites in the three groups of CNEs or ~3% of all TFBSs. The enrichment analysis revealed that >200 TFBS motifs were significantly overrepresented (qvalue=0.001) in the ruminant, cetartiodactyl and mammalian CNE sets. The TFBS motif EWSR1-FLI1 had the highest overrepresentation in the ruminant CNEs compared to the mammalian CNE set, while multiple interferon-regulatory factor (IRF) and heat shock factor (HSF) TFBSs were most overrepresented both in the cetartiodactyl and ruminant CNEs compared to the mammalian set.

Key Words: multispecies, comparative genomics, bioinformatics tools/ data mining, regulatory element

161 Gene regulation in sheep alveolar macrophages: Genome-wide identification of active enhancers. A. Massa*[1], M. Mousel[2,1], B. Murdoch[3], and S. White[2,1], [1]*Washington State University, Pullman, WA, USA;* [2]*United States Department of Agriculture-ADRU, Pullman, WA, USA;* [3]*University of Idaho, Moscow, ID, USA.*

Lung macrophages provide a first line of defence for the cell-mediated innate immune system against many inhaled pathogens. Annotation of regulatory elements within these cells aids advanced understanding of gene regulation and genetic priming of the immune system. Acetylation of lysine 27 on histone protein three (H3K27ac) is one of the most dynamic histone modifications, denoting active enhancer regions of the genome. Changes in H3K-27ac correspond to changes in gene expression that control cellular differentiation. In this study, alveolar macrophages were harvested from the lungs of 2 healthy, one-year-old, Suffolk-cross sheep. Chromatin immunoprecipitation with high throughput sequencing (ChIP-seq) was performed for histone modification H3K27ac. Approximately 50,000 peaks were identified in the immunoprecipitation dataset over control input DNA. Peaks with a false discovery rate of less than 5% included 12,984 peaks with an average enrichment of 18-fold. This is comparable with published data from humans and other mammalian species for active enhancer marks. Overall, 51% of active enhancers were either within genes or were less than 500 base pairs upstream of genes. While 13% were located greater than 50,000 base pairs from any gene. Peak length was similar to expected values of ~200–2000 base pairs for mammalian enhancers. However, sequence motif discovery in sheep suggested

many are unique when compared with known enhancer motifs in humans, indicative of sequence divergence between species. These data provide a basis for regulatory landscape comparison among sheep cell types and for cross-species comparative regulome analysis in macrophages and immune cells.

Key Words: ChIP-seq, H3K27ac, sheep, enhancers

163 The reconstruction and evolutionary history of eutherian chromosomes. J. Kim[1], M. Farre[2], L. Auvil[3], B. Capitanu[3], J. Ma[4], H. A. Lewin[5], and D. M. Larkin*[1], [1]*Department of Biomedical Science and Engineering, Konkuk University, Seoul, Korea;* [2]*Royal Veterinary College, University of London, London, UK;* [3]*Illinois Informatics Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA;* [4]*Computational Biology Department, School of Computer Science, Carnegie Mellon University, Pittsburgh, PA, USA;* [5]*Department of Evolution and Ecology, University of California, Davis, CA, USA.*

Whole genome assemblies of 19 placental mammals and two outgroup species were used to reconstruct the order and orientation of synteny blocks in chromosomes of the eutherian ancestor and six other descendent ancestors leading to human. For ancestral chromosome reconstructions, we developed a new algorithm (DESCHRAMBLER) that probabilistically determines the adjacencies of syntenic blocks using chromosome-scale and fragmented genome assemblies. The reconstructed chromosomes of the eutherian, boreoeutherian and euarchontoglires ancestor each included >80% of the entire length of the human genome, while reconstructed chromosomes of the most recent common ancestor of simians, catarrhini, great apes, and humans and chimpanzees included >90% of human genome sequence. These high coverage reconstructions permitted reliable identification of chromosomal rearrangements over ~105 million years (My) of eutherian evolution. Orangutan was found to have eight chromosomes that were completely conserved in homologous sequence order and orientation with the eutherian ancestor, the largest number for any species. Ruminant artiodactyls had the highest frequency of intrachromosomal rearrangements, while interchromosomal rearrangements dominated in murid rodents. A total of 162 chromosomal breakpoints in evolution of the eutherian ancestral genome to the human genome were identified; however, the rate of rearrangements was significantly lower (0.80/My) during the first ~60 million years of eutherian evolution, then increased to greater than 2.0/My along the five primate lineages studied. Our results significantly expand knowledge of eutherian genome evolution and will facilitate greater understanding of the role of chromosome rearrangements in adaptation, speciation, and the aetiology of inherited and spontaneously occurring diseases.

Key Words: ancestral chromosome reconstruction, primates, human, chromosome evolution, algorithm

164 iTRAQ-based proteomic analysis reveals key proteins affecting muscle growth and lipid deposition in pig. Z. Wang*[1], P. Shang[1,2], Q. Li[3], L. Wang[1], H. Zhang[1], and C. Wu[1], [1]*China Agricultural University, Beijing, China;* [2]*Tibet Agriculture and Animal Husbandry University, Linzhi, China;* [3]*Anhui Academy of Agricultural Sciences, Hefei, China.*

Pig growth rate and meat quality that are the main economic traits may be involved in multiple genes and biological pathways. The Tibetan pig (TP) and Diannan Small Ear pig (DSP) are indigenous Chinese breeds; they have significantly lower growth rates, higher lipid deposition ability, and better meat quality than those of introduced pig breeds such as Yorkshire (YY) and Landrace (LL). Nowadays, the proteomic analysis is a powerful method to identify key functional genes for the complex quantitative traits. Parallel reaction monitoring (PRM) is a recent development in targeted mass spectrometry and involves the use of a quadrupole-equipped Orbitrap. In present study, the *longissimus dorsi* muscle tissues were collected from the TP, DSP, YY and LL pig breeds at the age of six month and were performed the iTRAQ-based quantitative proteome analysis. The protein expression obtained using iTRAQ analysis was confirmed by quantifying the expression levels of twelve selected proteins by a PRM-MS analysis. Totally, 4,815 peptides corresponding to 969 proteins were detected. Comparison of expression patterns between TP-DSP and YY-LL revealed 288 differentially expressed proteins (DEPs), of which 169 were up-regulated and 119 were down-regulated. Functional annotation suggested that 28 DEPs were related to muscle growth and 15 to lipid deposition. Protein interaction network predictions indicated that differences in muscle growth and muscle fibre morphology between TP-DSP and YY-LL breeds were regulated by ALDOC, ENO3, PGK1, PGK2, TNNT1, TNNT3, TPM1, TPM2, TPM3, MYL3, MYH4, and TNNC2, while those in lipid deposition ability were regulated by LPL, APOA1, APOC3, ACADM, FABP3, ACADVL, ACAA2, ACAT1, HADH, and PECI. Twelve DEPs (up-regulated: UQCRC1, ACAT1, ACADM, PECI, MYL3, NNT, ACAA2, TTN, and HADH; down-regulated: PRDX4, MYL1, and LDB3) were selected for the PRM to confirm the reliability of the iTRAQ analysis. The fold changes and *P* values for these proteins were significantly different between the TP-DSP and YY-LL groups at $P < 0.10$, which was in agreement with the findings of the iTRAQ analysis. Our expression profiles provide new insights into the key proteins involved in muscle growth and lipid deposition in the pig.

Key Words: iTRAQ, PRM, muscle growth, lipid deposition, pig

165 Hypothalamus transcriptome during the early rise in LH secretion related to puberty age in bull calves. J. Liron[1], M. Fernández*[2], A. Prando[3], A. Baldo[3], and G. Giovambattista[2], [1]*Center of Veterinary Research (CIVETAN, CONICET), Faculty of Veterinary Sciences, UNCPBA, Tandil, Buenos Aires, Argentina.;* [2]*Institute of Veterinary Genetics (IGEVET, CONICET), Faculty of Veterinary Sciences, National University of La Plata, La Plata, Buenos Aires, Argentina;* [3]*Cátedra de Zootecnia Especial (II Parte), Faculty of Veterinary Sciences, National University of La Plata, La Plata, Buenos Aires, Argentina.*

Cattle puberty influences reproduction rates and profitability. In pre-pubertal bull calves there is an early transient rise in gonadotropin secretion between 10 and 20 weeks of age. The early elevation in mean circulating concentrations of LH and FSH most likely causes the proliferation of Sertoli and Leydig cells, respectively. This post-natal gonadotropin rise is considered one of the main factors in determining the age at which bulls reach puberty. In order to enhance our knowledge about genes and regulatory pathways involved in this phenomenon, we characterised the hypothalamus transcriptome from six Angus calves along this early expression pattern of LH (4, 6, and 14 wk of age) using the RNA-Seq technology. Of 37 million RNA-Seq reads per sample generated using the Illumina HiSEqn 2000 sequencer, at least 95% were mapped to the customized reference genome BosTau6. The gene annotation revealed that 13,976 genes were expressed in the hypothalamus. Tophat2, EdgeR, DESEqn 2, Bioconductor and R packages were utilised to performed differential expression (DE) analysis between groups. We detected 915 DE genes (*P* adjusted values < 0.05). The top gene ontology term enrichment of the highest expressed genes in the hypothalamus included cellular synapse, ion channel complex, neuron projection and plasma membrane part (Cellular component category); cell-cell signalling, transmembrane transport, behaviour and organism process (Biological process); metal ion transmembrane transporter activity and neuropeptide hormone activity (Molecular function). Enrichment analysis identified 40 KEGG signicantly enriched pathways. Based on the observation of the lowest *P*-value, calcium, oxytocin, circadian entrainment, cholinergic, glutamatergic, dopaminergic, serotonergic and GABAergic synapse, GnRH, oestrogen, Rap1, MAPK, ErbB, Ras and cancer signalling and several drugs addiction were among the most significant enriched pathways. The list of highest DE genes includes

OTP, AVP, OXT, CRH and TH, known for their physiological roles associated with lactation and mammalian social behaviours.

Key Words: cattle, functional genomics, RNA-seq, puberty, genetic improvement

166 Integrative genomics of human and bovine tuberculosis. K. E. Killick*[1,2], M. P. Mullen[3], T. Hall[1], N. C. Nalpas[4], I. W. Richardson[5], D. A. Magee[1], C. N. Correia[1], J. A. Browne[1], D. P. Berry[6], D. Bradley[7], V. Naranbhai[8], A. Hill[8], E. Gormley[9], S. V. Gordon[2,9], D. E. MacHugh[1,2], [1]*University College Dublin, UCD College of Health and Agricultural Sciences, University College Dublin, Dublin, Ireland;* [2]*University College Dublin, UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland;* [3]*Athlone Institute of Technology, Department of Life and Physical Sciences, Athlone Institute of Technology, Athlone, Ireland;* [4]*University of Tübingen, Quantitative Proteomics and Proteome Centre Tübingen, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany;* [5]*IdentiGEN Ltd, IdentiGEN Ltd., Blackrock Business Park, Blackrock, Dublin, Ireland;* [6]*Teagasc, Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Cork, Ireland;* [7]*Trinity College, Smurfit Institute of Genetics, University of Dublin, Trinity College, Dublin, Ireland;* [8]*University of Oxford, Wellcome Trust Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, Oxford, UK;* [9]*University College Dublin, UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland.*

Human tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent. Bovine TB (BTB), caused by the closely related *Mycobacterium bovis* (99.95% sequence identity), is a major endemic disease affecting global cattle production, particularly in many developing countries. In the current study we used a network-based approach to integrate host gene expression data with high-density single-nucleotide polymorphism (SNP) genome-wide association (GWA) data to enhance detection of genomic variants for susceptibility/resistance to both *M. tuberculosis* and *M. bovis* infection. The host gene expression data used consisted of human and bovine RNA-seq data from macrophages infected with *M. tuberculosis* and *M. bovis*, respectively. A base gene interaction network of the mammalian host response to mycobacterial infection was generated using 213 genes identified from GeneCards (www.genecards.org). Differential gene expression data (FDR P value < 0.001) were superimposed on to this base network and the JActiveModules Cytoscape (www.cytoscape.org) plugin was used to extract functional modules with DE gene sets from macrophage infection experiments. SNP array population data was obtained from large human and bovine TB susceptibility/resistance studies, including the Wellcome Trust Case Control Consortium (WTCCC – www.wtccc.org.uk) resource and a published GWAS study in dairy cattle. SNPs from the top functional modules (5 kb up- and downstream of each gene) were identified for both human and bovine gene expression data. These analyses identified new genomic variants in humans and cattle associated with susceptibility and resistance to tuberculosis disease in both species. Comparison and integration of human and bovine gene expression data with GWAS data for TB and BTB can be used to identify shared and specific mechanisms underlying the mammalian host response to mycobacterial infection. In summary, the integrative genomics approach described here can be used to generate new knowledge by leveraging distinct but complementary omics datasets from a wide range of biological contexts.

Key Words: integrative genomics, bovine tuberculosis, mycobacterium tuberculosis

167 Circulating microRNAs as potential novel biomarkers to diagnose *Mycobacterium avium* ssp. *paratuberculosis* infection in cattle. K. Zhao[1], S. Hendrick[2], and L. Guan*[1], [1]*Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, AB, Canada;* [2]*Coaldale Veterinary Clinic, Lethbridge, Canada.*

Johne's disease (JD) is an infectious disease caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in ruminants. The circulating microRNAs are promising biomarkers for prediction and diagnosis of variety of diseases in humans. Therefore, the purpose of this study is to explore the potential of using the circulating microRNAs as diagnosis markers for early MAP infection detection. The microRNAomes of sera collected from three-year-old cows with clinical symptoms (CC, n = 20) and subclinical carriers (SC, n = 24) were generated using RNA-seq. In total, 116 expressed miRNAs were detected across all samples, and 11 of them were differentially expressed (DE) between CC and SC (fold change >2 and FDR < 0.05). Among them, seven miRNAs (miR-1468, 296–3p, 2284x, 2284y, 181a, 181b, and let-7f) were up-regulated in SC and four miRNAs (miR-192, 22–5p, 24–3p, and 361) were up-regulated in CC. Functional analysis showed that function of highly expressed miRNAs in SC was enriched to 'Metabolic pathway' by inhibiting the expression of genes related to glycolysis, fatty acid metabolism, and ATP synthesis. The function of those DE miRNAs in CC was enriched in 'Phagosome', including the genes regulate phagosome formation and function. Moreover, principal component analysis showed that sera miRNAome profiles of 24 SC cattle segregated into two groups (SC1 and SC2, n = 12, respectively), with profile of SC2 overlapped with those of the CC. This suggests that SC2 cattle may potentially progress to CC, while the SC1 may retain as SC. Moreover, 51 DE miRNAs were identified between SC1 and SC2 with 16 up-regulated in SC1 and 35 up-regulated in SC2. Functional analysis of up-regulated miRNAs in SC1 and SC2 showed similar pattern with those DE miRNAs between SC and CC, including 'Metabolic pathway' and 'Platelet activation'. Our results indicate that the decreased glucose/energy metabolism and innate immune response contributed partially to the molecular mechanism of MAP infection in SC and CC, respectively. The panels of circulating miRNAs (miR-2284x, 2284y, 181a, 181b, 24–3p, and 361) can be potentially used as novel biomarkers for early diagnosis of MAP infection at subclinical stage.

Key Words: biomarker, miRNAome, serum, Johne's disease

168 Generating customized integrated functional annotation datasets with BovineMine. C. Elsik*, D. Unni, A. Tayal, and D. Hagen, *University of Missouri, Columbia, Missouri, USA.*

BovineMine is the data mining resource of the Bovine Genome Database (BGD, http://BovineGenome.org). The objective of this presentation is to show how BovineMine can accelerate genomics research by enabling scientists without scripting skills to create and export customized annotation datasets merged with their own research data for use in downstream analyses. BovineMine allows researchers to leverage the curated gene pathways of model organisms (e.g. human, mouse and rat) based on orthology, and is especially useful for GO and pathway analyses in conjunction with GWAS and QTL studies. BovineMine also includes the reference genomes of sheep and goat so researchers can leverage information across ruminants. BovineMine uses the InterMine platform to integrate data from a variety of sources, including reference genome assemblies, genes (NCBI, Ensembl, Official Gene Set), proteins (UniProt), protein families and domains (InterPro), orthologs and paralogs (EnsemblCompara, Homologene, OrthoDB, TreeFam), pathways (BioCyc, KEGG, Reactome), interactions (BioGRID, IntAct), Gene Ontology (GO), QTL (AnimalQTLdb), variation (dbSNP, dbVar) and publications (PubMed). Pre-computed data from BGD, including variant effects and RNAseq-based gene expression, allow users to query tissue specific gene expression levels together with genomic variation data. BovineMine provides simple and sophisticated data mining tools. Built-in query templates pro-

vide starting points for data exploration, while the QueryBuilder tool supports construction of complex queries. The List Analysis and Genomic Regions search tools execute queries based on uploaded lists of identifiers and genome coordinates, respectively. BovineMine supports meta-analyses by tracking identifiers across gene sets and genome assemblies, which will be particularly valuable with the release of the upgraded bovine reference genome assembly. Future plans include the incorporation of FAANG datasets to enable fine-grained data mining of functional elements in combination with gene annotations and additional biological data.

Key Words: cattle and related species, functional genomics, comparative genomics, bioinformatics tools, data mining

169 The Vertebrate Gene Nomenclature Committee (VGNC). P. Denny*, B. Yates, S. Tweedie, B. Braschi, K. Gray, R. Seal, and E. Bruford, *European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Hinxton, Cambridgeshire, UK.*

Standardised gene nomenclature is essential for effective communication and provides a critical resource for all biomedical researchers. However, an ever-increasing number of vertebrate genomes are being sequenced and the data released into the public domain without systematic annotation or gene naming. There are only six vertebrate model organisms with an established gene nomenclature committee (mouse, rat, chicken, Anolis, *Xenopus* and zebrafish), all of which base their gene names on those approved by the HUGO Gene Nomenclature Committee (HGNC) for human genes. The Vertebrate Gene Nomenclature Committee (VGNC) is a new initiative that extends the remit of the HGNC to approve consistent gene names and symbols across other vertebrates. Our naming strategy for each species starts by identifying a high confidence set of gene annotations with 1:1 human orthologs, using our HCOP tool (http://www.genenames.org/cgi-bin/hcop) that combines orthology predictions from multiple sources. The human gene nomenclature is transferred in a semi-automated way to these 1:1 orthologs. Genes with multiple predicted orthologs, members of complex gene families, pseudogenes and RNA genes require additional manual curation, such as review of phylogeny, synteny, gene structures and encoded proteins. Our pilot species for VGNC naming has been chimpanzee and we have named over 14,000 protein-coding chimp genes with a 1:1 human orthologue. During this process, we have taken the opportunity to simplify and improve the consistency of our human gene names, taking care to minimise transfer of species-specific information, such as susceptibility to pathogens. This naming process will soon be expanded to other species, including dog, horse and cow. We plan to prioritize species based on the quality of genome assembly and annotations, perceived importance as a model for humans and demand from the research community. An online vertebrate gene nomenclature portal has been created that stores, displays and makes this new nomenclature data accessible both to individual researchers and available for dissemination to other key resources including Ensembl and NCBI Gene. Further information and requests for individual gene names and symbols can be made via: http://vertebrate.genenames.org.

Key Words: nomenclature, comparative, bioinformatics, annotation, website

Genetics and Genomics of Aquaculture Species

170 Comparative genomics of disease resistance traits in salmonids. J. M. Yáñez*, *Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile.*

Infectious diseases generate large economic losses in salmon farming. For instance, *Piscericketssia salmonis,* the causal agent of Salmon Rickettsial Syndrome, affects several salmon species and is considered one of the major pathogens of the salmon farming industry. A feasible and sustainable alternative to prevent disease outbreaks is represented by genetic improvement for disease resistance. The information from causative mutations involved in resistance against diseases may be used to accelerate the genetic progress for these traits. Comparative genomics can provide useful information from common functional variants associated with disease resistance traits in salmonids. Here, we perform genome-wide association studies (GWAS) for resistance against *Piscirickettsia salmonis* in three commercial salmonid species: Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*). The analyses were performed using phenotypic data from challenged fish and genotypes obtained from 50K and 57K Affymetrix single nucleotide polymorphisms (SNPs) arrays available for Atlantic salmon and rainbow trout, respectively, and from a Double Digest Restriction Associated DNA (ddRAD) Sequencing experiment for coho salmon. We found loci significantly associated with *P. salmonis* resistance in the three species. There were five SNPs in chromosomes *Ssa*01 and *Ssa*17, one SNP in chromosomes *Omy*25 and one SNP in scaffold *Oki*01025 significantly associated with *P. salmonis* resistance in Atlantic salmon, rainbow trout and coho salmon, respectively. However, the proportion of the phenotypic variance explained by each marker was small (< 5%) for each species. A comparison of the most important genomic regions associated with resistance against *P. salmonis*, representing 1% of the most significant associated SNPs for each species, was carried out in order to identify common genomic regions among species. Biological candidate including genes related with immune response and iron metabolism have been found to be physically linked with these genomic regions and may play an important role in the differential immune response against this pathogen in salmonids.

Key Words: *Piscirickettsia salmonis*, genome-wide association analysis, coho salmon, Atlantic salmon, rainbow trout

171 GWAS reveals the architecture of two maturation traits in Tasmanian Atlantic salmon. J. Kijas*[1], A. Mohamed[1], S. McWilliam[1], B. Evans[2], H. King[3], P. Kube[3], and K. Verbyla[4], [1]*CSIRO, Brisbane, Queensland, Australia;* [2]*SALTAS, Hobart, Tasmania, Australia;* [3]*CSIRO, Hobart, Tasmania, Australia;* [4]*Data61, Canberra, ACT, Australia.*

A key developmental transformation in the life of all vertebrates is the transition to sexual maturity, whereby individuals are capable of reproducing for the first time. In the farming of Atlantic salmon, unwanted maturation that occurs before harvest size has a serious negative impact as it retards growth while severely diminishing flesh quality. Recent findings in European Atlantic salmon report the presence of a gene that exerts a large effect on age of maturation (*VGLL3*). We performed two genome wide association studies in Tasmanian animals, which are derived from North American stock, to map genetic loci that contribute to variation. First, a total of 2721 fish with trait data describing maturation in the marine environment were genotyped using a custom SNP50 array. Second, genotypes were collected from 1846 fish with trait data describing maturation in freshwater. For both experiments, a case-control design lineage regression analysis was performed to identify associated regions. Neither GWAS suggests *VGLL3* plays a major role in the two maturation traits as measured in the Tasmanian population. Further, the two traits have different architecture as few highly associated SNP were common to both experiments. We present findings from a systematic assessment of the gene content of associated ge-

nomic regions. This revealed genes involved with energy metabolism, neuroendocrinology and steroid biosynthesis.

Key Words: salmon, GWAS

172 Optimum-contribution selection increases genetic gain in Atlantic salmon breeding schemes. B. Hillestad*[1] and M. Henryon[2], [1]*SalmoBreed AS, Bergen, Norway;* [2]*Seges, Copenhagen, Denmark.*

We tested the hypothesis that genetic gain in an Atlantic-salmon breeding population will be increased by changing from truncation selection (TS) to optimum-contribution selection (OCS). This was tested by simulating salmon breeding populations under OCS and TS. Selection was performed for a single trait with $h^2 = 0.25$ and $\sigma^2_P = 0.722$. The schemes were run over 10 generations, with a rate of inbreeding (ΔF) equal to 1%. For each following generation 300 new families were produced with a litter size of 40 fish. The TS schemes were setup with a 2x1 factorial design, where each male where allowed to mate with two females. The maximum number of individuals per family that was selected to mate varied from five to 40. There were two different factorial designs set up for the OCS schemes: 3x3 and 300x300, where each selected animals was mated to three or 300 individuals, respectively. The second OCS scheme then allowed for full OCS. For OCS schemes as well, the number of individuals selected to mate per family varied from 5 to 40. Preliminary results showed that ΔG can be increased by at 8–15% when the ΔF is set to approx. 1%. Further results will also give us insight on how the number of males and females allowed to mate per family will affect both ΔG and ΔF, and how restriction on the number of mating sires and dams are allowed to do in OCS. This suggests that genetic gain in Atlantic salmon breeding population would be increased by implementing OCS.

Key Words: fish, animal breeding

173 Exploiting linkage disequilibrium information in turbot selection programs. M. Saura*[1], A. Fernández[1], J. Fernández[1], M. Toro[2], P. Martínez[3], A. Millán[4], M. Hermida[3], A. Blanco[3], S. Cabaleiro[5], A. Doeschl-Wilson[6], and B. Villanueva[1], [1]*Departamento de Mejora Genética Animal, INIA, Madrid, Spain;* [2]*Departamento de Producción Agraria, ETS Ingenieros Agrónomos, Madrid, Spain;* [3]*Departamento de Xenética, Facultade de Veterinaria, Universidade de Santiago de Compostela, Lugo, Spain;* [4]*Geneaqua SL, Lugo, Spain;* [5]*CETGA, Cluster de Acuicultura de Galicia, Aguiño-Ribeira, Spain;* [6]*Division of Genetics and Genomics, The Roslin Institute and Royal (Dick) School of Veterinary Studies, Midlothian, Scotland, UK.*

The turbot aquaculture sector holds a top position in the international market and breeding programs associated to the culture of this fish is a well established process. The recent availability of a high quality genome for this species opens new opportunities to study the genomic architecture of complex traits and to improve selection efficiency through genomic selection. The success of these approaches depends however on the magnitude and extent of the linkage disequilibrium (LD) across the genome. The aim of this study was to characterise genomic LD patterns in a Spanish turbot population challenged with the parasite *Philasterides dicentrarchi,* in order (1) to investigate the potential of within-family genomic selection, and (2) to identify QTL regions for disease resistance using genome-wide association analysis (GWAS). RAD-sequencing was used to identify and genotype 18,824 SNPs in 1,393 fish belonging to 36 families. Linkage disequilibrium was estimated as the squared correlation between SNP pairs (r^2). GWAS for resistance was performed by analysing each SNP independently. LD was moderately high between closely linked markers at the within-family level (r^2 ~0.40 between markers separated by 5 Kb), while it was considerably lower at the population level (r^2 ~0.10). With increasing distance between SNP pairs, r^2 decreased by half over the first 4 - 5 Mb and 1 Mb at within-family and population level, respectively. Results from GWAS revealed 60 associations with the disease trait. Our preliminary results suggest that within-family genomic evaluation for disease resistance could have great potential in turbot aquaculture. The consensus physical map currently under development will allow to identify candidate QTL regions and explore the genetic content within them.

Key Words: aquaculture, disease resilience, genome-wide association, genomic selection, linkage disequilibrium

175 Transcriptomic profile of *Salmo salar* skin in response to the Chilean sea louse *Caligus rogercresseyi* using *de novo* transcriptome assembly. K. Neumann*[1], D. Cichero[2], and V. Martinez[1], [1]*FAVET-INBIOGEN-Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile;* [2]*Blue Genomic SPA, Puerto Varas, Chile.*

The Chilean sea louse *Caligus rogercresseyi* is the most important copepod ectoparasite responsible for significant economic losses in the salmon industry. In order to better understand the biological mechanisms involved in the response of the host to the parasite, the main objective of this study was to characterise the transcriptome profile of *Salmo salar* skin in response to *C. rogercr*esseyi through RNA-Sequencing analysis using a *de novo* transcriptome assembly. A challenge trial was conducted on a pedigree population of *S. salar* from the genetic program of AquaGen Chile SA (Puerto Varas, Chile), which were challenged with *C. rogercresseyi* in its infestive stage. The sea lice were allowed to develop until the chalimus III – IV stage. Chalimus counts were conducted on all fish, and individuals with extreme phenotypes were classified as having low (L) or high (H) lice count. The skin samples were RNA-sequenced on a MiSeq platform (Illumina, USA). A *de novo* assembly using Trinity was annotated using Trinotate. The RNA sequences were then mapped to the *de novo* assembled transcriptome using Salmon software, which also it allows quantifying the expression of transcripts. Differential expression analysis was performed with EdgeR with a false discovery rate p-value < 0.05. The enrichment analysis on Gene Ontology (GO) terms were performed by a Fisher's exact test using TopGo with a p-value < 0.01. Among the down-regulated transcripts in the L group compared to the H group, we found 19 GO terms enriched for biological process (BP), 9 terms for molecular functions (MF) and 4 terms for cellular component (CC). While the up-regulated transcripts in the L group, had 10 GO terms enriched for BP, 4 terms for MF and 2 for CC. Some important terms are related to protein metabolism, immune system processes and cell death which were down-regulated in the skin from L group. These results show the complexity of local host response to the sea louse, in term of processes involved and its relationship with the background of fish. This work contributes to better understanding the response of *S. salar* to sea louse *C. rogercresseyi*.

Key Words: fish, functional genomics, RNA-seq, gene expression, genomic selection

176 Mining the European Sea Bass (*Dicentrarchus labrax*) genome for the characterization of tandem repeat variability. F. Bertolini*[1,2], S. Bovo[2,3], M. F. Rothschild[1], and L. Fontanesi[2], [1]*Department of Animal Science, Iowa State University, Ames, IA, USA;* [2]*Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Bologna, Italy;* [3]*Biocomputing Group, Department of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy.*

Dicentrarchus labrax L., the European Sea Bass (also known as European Seabass), is a teleost fish mainly distributed along the coasts of the northeastern Atlantic Ocean and of the Mediterranean and Black Seas. This species of the family Moronidae is one of the

most relevant marine species for professional and sport fisheries and aquaculture production, raising attention and concerns for conservation and management of natural genetic resources on one hand and for adaptation to aquaculture conditions, domestication and selective breeding on the other hand. Therefore, the development of a large number of microsatellite markers could provide useful tools for these purposes. Microsatellites (with motif length < 10) are considered a subgroup of tandem repeats that traditionally includes minisatellites (with motif length of 10–60) and macrosatellites (with motif length >60). Tandem repeats been have frequently utilised in forensics, population genetics, and low-cost genetic scans in many different species. In this study, we mined the current draft version of the European Sea Bass genome encompassing 25 assembled chromosomes to identify tandem repeat regions and then we complemented this information using next-generation sequence data we produced from DNA pools obtained from 12 fish of this species. The European Sea Bass genome were scanned for tandem repeats with the Tandem Repeats Finder software. A total of 75,805 microsatellites, 12,790 minisatellites and 410 macrosatellites were identified across the genome with at least the 95% of identity. All these regions were profiled using the lobSTR software in two seabass DNA pools composed of six animals each and derived by two different hatcheries, and sequenced with Proton Torrent sequencer. A total of 1,471 regions for the pool 1 and 4,102 regions for the pool 2 had a minimum depth of 3. Among these regions, that on the whole intersected 381 genes, a subgroup of them, i.e. 1,313 for pool 1 and 3,724 for pool 2, showed variation from the profile of the reference genome. This study produced a whole tandem repeat map of the European Sea Bass genome useful for the characterisation of the genetic variability and future breeding programs.

Key Words: European Sea Bass, tandem repeats, NGS

177 Reconstructing the complex structure of the sex determination locus in Atlantic herring using SMRT sequencing. N. Rafati*[1], C.-J. Rubin[1], C. Feng[1], M. Petterson[1], A. B. Martinez[2], S. Lamichhaney[1], I. Bunikis[3], and L. Andersson[1,5], [1]*Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden;* [2]*Science for Life Laboratory, Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden;* [3]*Science for Life Laboratory, National Genomics Infrastructure, Uppsala University, Uppsala, Sweden;* [4]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [5]*Department of Veterinary Integrative Biosciences, Texas A&M University, College Station,, TX, USA.*

The mechanism for sex determination considerably varies among species. Fish (similar to some of reptiles) have established two distinct sex determination systems: environmental sex determination (ESD) and genetic sex determination (GSD), and sometimes both systems work in concert. GSD can be under control of sex chromosomes or master genes on autosomal chromosomes, yet in most fish species genes with predominant roles in sex determination have not been reported. An exception is the *dmrt1* paralogous copy (*dmy*) on medaka's (*Oryzias latipes*) Y-chromosome governing sex determination. It is now clear that a GSD system has evolved independently in several lineages of teleosts. However in the majority of them, including Atlantic herring (*Clupea harengus*), the sex determination system is still unknown. Herring is among the most abundant species with vast economical and ecological importance in Northern Atlantic. Atlantic herring reproduces throughout the Baltic Sea and Atlantic Ocean in different salinities (2–35‰) and seasons. We generated a high-quality draft genome assembly by short read sequencing technology to unravel the genetic basis of ecological adaptation to both salinity and seasonal reproduction. We identified a large region (~100 Kb) for which males and females showed significant differentiation (male specific region). Our study on unmapped reads revealed male unique sequences belonging to a member of the *cation channel sperm-associated protein* (*CATSPER*) gene. But our efforts in linking these two segments by PCR failed. To gain further insight into the herring genome, we generated a new assembly by single-molecule real-time (SMRT) sequencing technology. In this new assembly, we revealed the organisation of the previously identified signals indicative of early stages of sex chromosome evolution. This is the first report on identifying a sex determination locus and proto-Y chromosome in Atlantic herring. This study enhances our understanding of the evolution of sex chromosome in this species and other teleosts.

Key Words: sex determination, evolution, sex chromosome, reproduction, SMRT sequencing

178 Rapid cold shock induces only slight shift in gene expression of rainbow trout *(Oncorhynchus mykiss*). T. Goldammer*[1], A. Borchel[1,2], M. Verleih[1], and A. Rebl[1], [1]*Leibniz Institute for Farm Animal Biology, Inst. for Genome Biology, Dummerstorf, Germany;* [2]*University of Bergen, SLCR-Sea Lice Research Centre, Bergen, Norway.*

A rapid decline in temperature poses a major challenge for poikilothermic fish, as their entire metabolism depends on ambient temperature. We compared the gene expression of rainbow trout (*Oncorhynchus mykiss*) having undergone such a cold shock (0°C) to a control (5°C) using microarrays and quantitative real-time PCR. The number of genes found to be regulated at 0°C was surprisingly low. Instead of classical genes involved in temperature shock, the three genes encoding fibroblast growth factor 1 (fgf1), growth arrest and DNA-damage-inducible, α (gadd45a) and sclerostin domain-containing protein 1 (sostdc1) were up-regulated in the liver upon cold shock in two different rainbow trout strains, suggesting that these genes may be involved in the response to cold shock in rainbow trout.

Key Words: stress response, fgf1, gadd45a, microarray, sostdc1

179 Allele-specific expression analysis related with jaw deformities in Yellowtail kingfish (*Seriola lalandi*) larvae. P. Dettleff*, A. Patel, and V. Martinez, *FAVET-INBIOGEN, Faculty of Veterinary Science, University of Chile, Santiago, Chile.*

Seriola lalandi is a globally distributed species with increasing relevance for Chilean aquaculture. Skeletal deformities represent an important issue on this species. The aim of this study was to identify SNPs that present Allele-specific expression (ASE) exclusively in normal or deform *S. lalandi* larvae using RNA-seq data. We used samples of four normal and four jaw deform larvae of 23 days posthatch for Illumina sequencing. Reads were mapped to a previous *de novo* transcriptome assembly of *S. lalandi* larvae and *de novo* SNPs identification was performed across all samples with CLC Genomics Workbench. To identify ASE, the deviation of the expected ratio 50:50 of the expression of each allele was evaluated using a Chi-squared test (FDR < 0.01), determining those SNPs with ASE in each sample. Subsequently, we identify those SNPs that present ASE exclusively on normal or deform group. A total of 5815 SNPs were identified across all samples. We identify 597 SNPs with ASE exclusively on normal group, corresponding to 190 transcripts. We determined 41 SNPs with ASE exclusively on deforms larvae, corresponding to 41 transcripts. Interestingly, we found differences in *cis*-regulation between deform and normal larvae. These includes genes related to extracellular matrix as *PLOD1* and *COL10A1* (involved in endochondral ossification); protein synthesis initiation factors as *EIF3* and *EIF6*; with muscle structure and function as *MYBPC1K*, *TNNI2, DNM2* (previously associated with morphological abnormalities during development); the calcium ion-binding protein *PVALB* (previously observed in other fish that is affected in skeletal deformities). Finally, we observed *cis* regulation only in deform larvae of the *RARG* gene, a retinoid receptor with role in the

normal embryonic development and previously associated with jaw and vertebral column deformities in other fish species. This study provides relevant information about *cis*-acting factors involved in jaw deformities in *S. lalandi*. Funding: Programa de Diversificación de la acuicultura chilena (PDACH).

Key Words: fish, aquaculture, RNA-seq, allele-specific expression

Livestock Genomics for Developing Countries

180 Development of genomic tools to select for economic traits in tropical adapted cattle breeds. F. F. Cardoso*[1,2], G. S. Campos[2], C. C. Gulias-Gomes[1], B. P. Sollero[1], and A. R. Caetano[3], [1]*Embrapa Pecuária Sul, Bagé, RS, Brazil;* [2]*Universidade Federal de Pelotas, Pelotas, RS, Brazil;* [3]*Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil.*

Tropical areas in developing countries have great potential to help supply increasing demands for livestock products, as cattle and other ruminants have the ability to convert fast-growing tropical grasses into high-quality protein for human consumption. Nevertheless, sustainability of beef production systems requires the use of livestock that are both highly productive and adapted to tropical environments. Work to apply genomic tools to enhance beef cattle productivity and tropical adaptation is well underway in Brazil. Brazilian Braford and Hereford cattle herds have been systematically measured for key phenotypes related to parasite resistance: tick counts (TC); heat tolerance: eye pigmentation (EP), hair coat at weaning (HCW) and at yearling (HCY); growth: birth (BW), weaning (WW) and long-yearling (LYW) weight, and post-weaning gain (PWG); and reproduction: scrotal circumference (SC). Phenotypes and historical pedigree data of 169,839 animals were combined with 3,954 genotypes for 41,011 SNP markers using different methods (single and multi-step) to derive prediction equations for genomic selection. Estimated heritability (h^2) and accuracy (r) of best performing single-step genomic predictions within this population were 0.19 ± 0.03 and 0.56 for TC, 0.46 ± 0.02 and 0.74 for EP, 0.44 ± 0.03 and 0.93 for HCW, 0.41 ± 0.02 and 0.67 for HCY, 0.28 ± 0.01 and 0.78 for BW, 0.22 ± 0.01 and 0.65 for WW, 0.26 ± 0.01 and 0.78 for LYW, 0.10 ± 0.01 and 0.57 for PWG, and 0.48 ± 0.03 and 0.83 for SC, respectively. These moderate to high r estimates were largely proportional to the respective trait h^2 and represented gains of up to 93% when compared to traditional pedigree-based predictions. Moreover, genome-wide association studies detected chromosome segments that explain up to 5% of the trait genetic variability, providing candidate regions for fine mapping. Low-density marker panels with 41 to 159 markers, based on informative tag-SNP identified within these regions, retain ~70% of the full panel r, and represent alternatives for lower cost predictions. Finally, selection indexes have been derived for optimizing selection of animals combining a desirable balance of economically relevant traits.

Key Words: adaptation, cattle, genome-wide association, genomic selection, meat production

181 Genomic selection based on current status in developing countries. R. Mrode*[1], J. Ojango[1], O. Mwai[1], and J. M. Mwacharo[2], [1]*Animal Biosciences, International Livestock Research Institute, Nairobi, Kenya;* [2]*Small Ruminant Genetics and Genomics Group, International Centre for Agricultural Research in the Dry Areas, Addis Ababa, Ethiopia.*

Genomic selection (GS) has opened new opportunities in animal breeding in developed countries resulting in rapid rates of genetic progress and detection of genomic regions associated with QTLs. The current status of GS and use of molecular tools in breeding programs of developing countries, where smallholder systems predominate and the basic components for conventional breeding are lacking excepting for a few established breeds, is examined. Genotypic data in smallholder systems offer quick wins in terms of parent verification, breed composition determination and genetic evaluation using G. The few studies on genomic prediction (GP) in developing countries are mostly in dairy and beef cattle and are characterised by small reference populations (≈1000 to 2000 animals). The small reference populations indicate the need for across regional GP that pull data across countries. The gains in accuracy from molecular information range from 0.33 to 0.45. The G matrix has allowed the estimation of breeding values (BVs) and parameters applying random regression models in populations lacking pedigree. Multi-trait single-step has been used to incorporate information on foreign bulls with deregressed proofs and genotypic data only in developed countries, into the BVs of bulls in developing countries. Thus GS in developing countries would benefit from collaborations with developed countries, especially in the dairy sector as a large number of sires used are from developed countries where they may have been genotyped. The development of next-generation sequencing tools such as SNP panels have allowed determination of breed composition for crossbreds in eastern Africa and parent verification for beef breeds and small ruminants (SR) in South Africa. The tools have been used to investigate genomic diversity and genome-wide selection sweeps. The most prominent selection sweeps found in breeds/populations of cattle and SR from across Africa represent candidate regions spanning genes of known major effects (coat and skin properties, high temperatures, muscle function, feed efficiency, etc.) associated with adaptation. This information is important for incorporation into breeding programs aiming to utilise GS in developing countries.

Key Words: genomic selection, developing country, QTL, accuracy, GWAS

182 Genetic admixture and identity by descent in Senegalese dairy cattle. P. J. N. Ema[1,3], A. Missohou[1], K. Marshal[2], S. F. Tebug[2], J. Juga[4], and M. Tapio*[5], [1]*Interstate School of Veterinary Science and Medicine of Dakar, Dakar, Senegal;* [2]*International Livestock Research Institute, Nairobi, Kenya;* [3]*University of Ngaoundere, Ngaoundere, Cameroon;* [4]*University of Helsinki, Helsinki, Finland;* [5]*Natural Resources Institute Finland, Jokioinen, Finland.*

Cattle keeping is an important livelihood in Senegal. As a part of non-transhumant dairy production analysis to understand factors influencing farmer's net benefit, we analysed the composition of production stock of small and moderate scale farms in two Senegalese study sites. The data included genotypes of 624 Senegalese cows based on BovineSNP50 BeadChip. Published data of 455 genotypes from 26 breeds was used as a reference. This set included several improved taurine and indicine breeds and African cattle populations. The interviews and field visits suggested the presence of several breeds and crossbreeds. Trained Bayesian clustering analysis supported idea of heterogeneity and uneven influence of non-native taurine and indicine cattle was detected. The main types were *Indigenous zebu*, *Indigenous zebu by Guzerat*, *Indigenous zebu by improved taurine*, and *High improved taurine*. Identity by descent analysis revealed that cows that were more crossbred also shared a larger proportion of their genomes with each other. Thus, the crossbred cattle are related to each other. This may reflect the higher relatedness within the improved breeds. While the average IBD sharing was low in *Indigenous zebu* group, the variation across genome regions was more pronounced. Pedigree recording and

managed crossbreeding schemes may combine benefits from local and exotic cattle, while limit the increase in the relatedness.

Key Words: cattle and related species, genome-enabled breeding, population structure, milk production, crossbreeding

183 Towards the unraveling of the genomic basis of milk production traits in African dairy zebu cattle. A. Tijjani*[1,4], J. Kim[3], R. Mrode[2], B. Salim[5], N. Oyekanmi[4], H. Kim[3], and O. Hanotte[1,2], [1]*School of life Sciences, University of Nottingham, Nottingham, UK;* [2]*International Livestock Research institute (ILRI), Nairobi, Kenya;* [3]*C&K genomics, Seoul National University Research Park, Seoul, South Korea;* [4]*National Biotechnology Development Agency, Lugbe, Abuja, Nigeria;* [5]*Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum, Khartoum, Sudan.*

Among the few African *Bos indicus* cattle considered as major milk producers is the Kenana cattle population, which are indigenous to northern Sudan. Together with the Butana cattle breed, they account for ~25 percent of the total cattle population in Sudan. In this study, we carried out full genome sequencing of nine Kenana cattle belonging to a single population in order to provide insight into the genomic basis of their milk production capability in comparison to European dairy cattle (Holstein and Jersey) and African zebu beef breeds (Boran and Ogaden). A total of 26 million autosomal biallelic single nucleotide polymorphisms (SNPs) were identified after comparison to the UMD3.1 bovine reference assembly. Three different signature of positive selection approaches were used (pooled heterozygosity (hp), integrated haplotype score (iHS) and global fixation index (FST)). We identified a total of 109, 61, and 71 significant candidate loci showing the signal of positive selection in Kenana, Holstein, and Jersey respectively, of which 85, 58, and 55 regions have overlap with known cattle quantitative trait loci (QTL) associated with milk traits. Five regions, located on BTA 1 and 7 are shared with Jersey, one region on BTA 20 is shared with Holstein and no candidate region is commonly detected in the three breeds. Several protein coding genes such as LEMD3, WIF1, GPCPD1, ZCCHC11, and CCND2 were found to overlap the Kenana milk traits linked candidate regions, these genes may have roles in milk production and have been documented to be under selection in other cattle breeds and livestock. This study suggests that Kenana and Jersey cattle may have witnessed partially common selection pressure for better milk production traits distinct from the selection mechanisms which have shaped milk production in Holstein.

Key Words: Africa dairy zebu, milk traits, Kenana, positive selection, full genome sequencing

184 Matching breeds to production clusters using high-density SNP arrays: The case of East Africa. F. D. N. Mujibi*[1], E. K. Cheruiyot[2], T. Dusingizimana[3], M. Chagunda[4], J. Ojango[5], and R. Mrode[4,5], [1]*Nelson Mandela Africa Institution for Science and Technology (NMAIST), Arusha, Tanzania;* [2]*University of Nairobi, Nairobi, Kenya;* [3]*University of Rwanda, Kigali, Rwanda;* [4]*Scotland Rural University College, SRUC, Edinburgh, Scotland;* [5]*International Livestock Research Institute, Nairobi, Kenya.*

In East Africa, dairy farming is dominated by smallholder operations, typified by less than ten crossbred dairy cattle on small land holdings. Predominantly, most of the animals are Holstein-Friesian - local indicus breed crosses, reflecting farmer desire for higher milk yield and adaptation to local environments. However, due to lack of pedigree and breed information, performance evaluation is often impossible, and farmers end up with poor yielding cattle. Application of high-density SNP arrays for breed composition determination would allow performance evaluation in different production systems to be undertaken. This study evaluated breed composition and milk yield for ~3,000 crossbred dairy cattle in Kenya, Uganda, Tanzania and Rwanda. These cows were genotyped at 150,000 – 700,000 SNP loci, using high-density (HD) SNP arrays. The proportion of genes from international dairy breeds was estimated using admixture analysis with Holstein, Friesian, Canadian Ayrshire, Norwegian Red, Jersey, Guernsey, Ndama, Gir and Zebu as references. Production clusters were defined based on several factors including supplementary feeding, milk productivity and household wealth status. A fixed regression model using the G matrix was used to analyse milk test day yield accounting for year-month-test-date, parity, age, and breed type. Production clusters were fitted as fixed effects, while animals and permanent environment effects were considered random effects. Admixture results indicated large within-population genetic diversity ranging from less than 30% to 100% exotic dairy percentage. In all but feed intensive commercially oriented production clusters, the best performing animals had higher proportion of Norwegian Red-Friesian genes. In the feed-intensive systems, animals with a Holstein genetic background with at least 75% dairy composition were the best performing. These results indicate that matching breed type to production cluster is central to maximizing productivity and will be critical in shaping breed development. Additionally, SNP arrays will increasingly play a major role in providing information to guide breed improvement and decision making in smallholder production systems.

Key Words: breed/population identification, crossbreeding, single-nucleotide polymorphism (SNP), genotyping

185 Finding optimum levels of admixture in crossbred sheep populations in Ethiopia by use of ancestry informative genetic markers and phenotypes. T. Getachew[1,2], H. J. Huson[3], M. Wurzinger[1], J. Burgstaller[4], S. Gizaw[5], A. Haile[6], B. Rischkowsky[6], G. Brem[4], S. A. Boison[1], G. Mészáros[1], A. O. Mwai[7], and J. Sölkner*[1], [1]*University of Natural Resources and Life Sciences, Vienna, Austria;* [2]*Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia;* [3]*Cornell University, Ithaca, NY, USA;* [4]*University of Veterinary Medicine, Vienna, Austria;* [5]*International Livestock Research Institute, Addis Ababa, Ethiopia;* [6]*International Center for Agricultural Research in the Dry Areas, Addis Ababa, Ethiopia;* [7]*International Livestock Research Institute, International Livestock Research Institute, Nairobi, Kenya.*

Understanding the relationship between genetic admixture and performances is crucial for the success of crossbreeding programs. In this study we selected a small set of ancestry informative makers (AIMs) from Ovine 50KSNP data and validated their ability in estimating the contributions of parental breeds to get information about optimum admixture levels. Awassi × Ethiopian fat-tailed crossbred sheep populations kept in farmer villages in different districts were included in the study. AIMs were chosen based on differentiation (high Fst). We estimated admixture levels using ADMIXTURE software. Reducing the number of AIMs from 74 to 65, 55, 45, 35 and 25 did not substantially change the predicted admixture levels of individuals ($r = 0.996–0.960$). Association of admixture levels with lamb growth showed that Awassi level affected ($P < 0.05$) eight months weight in both farmer locations, lambs with higher Awassi levels were heavier. Lambing interval of ewes was longer as Awassi level increased, but this drawback was outweighed by the increased productivity of ewes in terms of eight months lamb weight per year. The results indicate that linking AIMs with on-farm phenotypic records provides a cheap and powerful tool for decision support for the optimum levels of crossbreeding under farmer conditions. Based on the results presented here, we were able to suggest optimum levels of breed composition for the two farmer environments investigated.

186 Can genomics be used in the smallholder livestock sector? Case studies from South Africa. F. C. Muchadeyi*,

Agriculture Research Council, Biotechnology Platform, Pretoria, South Africa.

High-density SNP genotyping technology has numerous possible applications in livestock improvement programs including breed characterisation, genetic diversity analysis, genome-wide association studies as well as genomic selection. In South Africa, dissection of livestock genome structures particularly in the marginalized smallholder sector is increasingly turning to SNP genotypes. Case studies are presented wherein population genomic tools have been applied in a range of livestock species to determine intra-species diversity and population genetic structures as well as infer on genetic adaptation using signatures of selection. Examples are given where genome-wide SNP data is utilised in estimating effective population sizes and genomic inbreeding levels in populations where pedigree information is unavailable. Determination of causal mutations for livestock genetic disorders through application of genome-wide association analysis is presented. The value of these genomic tools to smallholder livestock populations of diverse non-descript phenotypes, limited breeding records because of random and unmonitored mating systems is demonstrated. SNP array data has been useful in assessing as the genomic response of animals to exposure to extreme and fluctuating environmental conditions.

Key Words: SNP arrays, livestock production systems, diversity, breed improvement

188 Follows abstract 98 on page 27

POSTER PRESENTATIONS

Animal Epigenetics

MT1 DNA methylation profiles in red blood cells of adult hens correlate to their previous rearing conditions. F. Pértille*[1,2], M. Brantsæter[4], J. Nordgreen[4], L. Coutinho[1], A. Janczak[4], P. Jensen[2], and C. Guerrero-Bosagna[2], [1]*Linköping University, Linköping, Östergötland, Sweden;* [2]*University of São Paulo, Piracicaba, São Paulo, Brazil;* [3]*Swedish University of Agricultural Sciences, Uppsala, Sweden;* [4]*Faculty of Veterinary Medicine, Oslo, Norway.*

Stressful conditions are common in the environment where production animals are raised. Stress in animals is usually determined by the levels of stress-related hormones. A big challenge, however, is in determining the history of the exposure of an organism to stress because the release of stress hormones can show a recent but not a sustained exposure to stressful conditions. Epigenetic tools are usefull to investigate long-term stress and detect environmental exposures that affect gene regulation during the lifetime of organisms. It is expected that if animals are constantly subjected to stress and systemic hormonal changes, this exposure will imprint the epigenome of many cells types, including blood cells. Epigenomic effect of stress in red blood cells have been reported in monkeys and humans. Chickens, however, provide a unique model to study stress effects in red blood cells (RBCs), which are nucleated in birds. Moreover, chickens are the most consumed meat source in the world and are therefore subjected to a variety of conditions in their production environment. The fact that chickens contain nucleated red blood cells allows for a straightforward measuring of the epigenome in a cell type of easy access, and in live animals. The present study investigates in chickens whether two different rearing conditions can be identified by looking at DNA methylation patterns in their RBCs later in life. The conditions tested are rearing in a system of open aviaries versus in cages. These rearing conditions are likely to differ regarding the amount of stress to which birds are exposed, as suggested by observations showing long-term differences in fearfulness and cognitive functions. We found 115 differentially methylated regions in RBCs ($P < 0.0005$) between experimental groups. Network analyses of genes associated with these regions showed connections with important biological pathways, mainly related to immune system and signal transduction in opioid signalling. The objective of the present study is to generate a proof-of-concept for future detection of long-term stress in production animals, using epigenetic measurements in cell types of easy accessibility in live animals.

Key Words: stress, epigenetics, chicken, DNA methylation, animal welfare

MT2 DNA methylation of bactericidal/permeability-increasing protein (*BPI*) gene promoter is involved in the regulation of its mRNA expression in pigs. H. Wang*, S. Wu, and W. Bao, *College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China.*

Expression of the *BPI* gene was found associated with the resistance of pigs to *E. coli* infection, while the regulatory mechanisms for *BPI* expression remain largely unknown. The objectives of this study were to analyse the role of promoter DNA methylation in *BPI* expression regulation, and explore the patterns of promoter methylation in different pig breeds and the associations of promoter methylation with *E. coli* susceptibility. We measured the DNA methylation levels of *BPI* promoter CpG island by bisulfite sequencing PCR and quantified *BPI* mRNA expression by qPCR in Meishan, Sutai and Yorkshire pig breeds. The duodenal samples were obtained from 15 individuals ($n = 5$ per breed) raised under the identical conditions. Results showed that the *BPI* promoter CpG island methylation level was higher in Yorkshire pigs than in Sutai and Meishan pigs with significant differences between Yorkshire and Meishan pigs ($P < 0.05$). However, *BPI* mRNA expression in Meishan pigs was significant higher than in Yorkshire pigs ($P < 0.05$). Pearson correlation analysis indicated a significant negative correlation between CpG island methylation and *BPI* mRNA expression ($P < 0.05$). Luciferase assay showed that methylation of *BPI* promoter can result in significant reduction of promoter transcriptional activity *in vitro*. Furthermore, we identified the DNA methylation level of the CpG island and *BPI* expression in three pairs of pigs differing in *E.* coli F18 susceptibility. Results showed that the CpG island methylation level was significantly higher in sensitive individuals than in resistant individuals ($P < 0.05$), while the expression level of *BPI* was significantly lower in sensitive individuals ($P < 0.05$), indicating a potential link between *BPI* promoter methylation, *BPI* expression and *E. coli* susceptibility. Our findings provided strong evidence for the negative relationship between promoter methylation and *BPI* expression, showed distinct *BPI* promoter methylation patterns across different pig breeds, and revealed the potential of *BPI* promoter methylation as an important marker for *E. coli* susceptibility.

Key Words: pig, BPI gene, DNA methylation, gene expression

MT3 Global quantification of DNA hydroxymethylation and DNA methylation in the bovine brain. B. Cantrell*[1], H. Lachance[1], R. Funston[2], R. Weaber[3], and S. McKay[1], [1]*University of Vermont, Burlington, VT, USA;* [2]*University of Nebraska, North Platte, NE, USA;* [3]*Kansas State University, Manhattan, KS, USA.*

Among the lesser characterised epigenetic modification is DNA hydroxymethylation. Levels of 5-hydroxymethylation (5-hmC) appear to be highest in brain tissues which raises questions of the role 5-hmC may have with regard to brain function. DNA hydroxymethylation results from the oxidation of methylated DNA by Ten-Eleven-Translocation enzymes. The objective of this study is to characterise global DNA hydroxymethylation and DNA methylation in blood and nine regions of the limbic system in the bovine brain: amygdala, bed nucleus of the stria terminalis, cingulate gyrus, dorsal raphe, hippocampus, hypothalamus, nucleus accumbens, periaqueductal grey and prefrontal cortex. DNA was extracted from brain and blood samples of two Red Angus × Simmental steers (less than 20 months of age) using the DNA Extraction Kit from Agilent Technologies (Santa Clara, CA) and a phenol chloroform extraction respectively. Percent global DNA hydroxymethylation was determined using the MethylFlash Global DNA Hydroxymethylation (5-hmC) ELISA Easy Kit (Colourimetric) and percent global DNA methylation was determined using the MethylFlash Methylated DNA Quantification Kit (Colourimetric) from Epigentek (Farmingdale, NY). This study is the first to show global quantification of DNA methylation and DNA hydroxymethylation between different tissue types in the limbic system of the bovine brain. Understanding the global differences in epigenetic regulation of DNA methylation and hydroxymethylation within in the bovine brain will facilitate future research to determine the role of epigenetic modifications with regard to economically important traits.

Key Words: hydroxymethylcytosine, methylcytosine, epigenetics, brain, bovine

MT4 Methylation of CpG within *IGF2R* DMR2 are associated with prenatal nutrition and genetic potential for residual feed intake from birth to slaughter in purebred Angus cattle. C. Fitzsimmons*[1,2], J. Devos[1,2], C. Straathof[2], F. Paradis[1,2], C. Li[1,2], H. Block[4], M. Colazo[3], and H. Bruce[2], [1]*Agriculture and Agri-Food Canada, Edmonton, Alberta, Canada;* [2]*University of Alberta, Edmonton, Alberta, Canada;* [3]*Alberta Agicutlure and Forestry, Edmonton, Alberta, Canada;* [4]*Agriculture and AgriFood Canada, Lacombe, Alberta, Canada.*

Methylation of insulin-like growth factor 2 (*IGF2)* DMR2 is sensitive to prenatal maternal nutrition in cattle (Paradis *et al*. submitted), and IGF2 receptor (*IGF2R*), an important mediator IGF2, displays imprinted expression in the bovine fetus (Bebbere *et al*. 2013). Selection for residual feed intake (RFI) has the potential to alter metabolism and energy partitioning in the pregnant cow, which may interact with prenatal nutrition resulting in differences in methylation patterns in the DNA of their calves. Therefore we hypothesised that methylation of the *IGF2R* DMR2 might be sensitive to prenatal nutrition and/or genetic potential for RFI in cattle. To test our hypothesis, *longissimus dorsi* biopsies were collected shortly after birth (n = 49), weaning (n = 42), and slaughter (n = 23, steers only), as well as liver and *semimembranosus* at slaughter (n = 23); from calves born of parents with differential genetic potential for high or low RFI (HRFI, LRFI), and whose dams had either been fed a diet formulated for an average daily gain (ADG) of 0.5 (L-diet), or 0.7 kg/d (H-diet) from 30–150d of gestation. In both neonatal and weaning samples, female calves exhibited higher methylation levels in 5 and 4 CpG groups (respectively) within *IGF2R* DMR2 (11 CpG groups total) as compared to males ($P < 0.05$). In neonatal samples the prenatal L-diet was associated with higher ($P < 0.05$) methylation in 3 CpG (groups) in the combined data from both sexes. At weaning HRFI was associated ($P < 0.05$) with higher methylation levels of 3 CpG (males/females combined). *Longissimus dorsi* sampled at slaughter had higher ($P < 0.05$) CpG methylation in 4 CpG for L-diet treatment. In *semimembranosus* 2 CpG were associated with prenatal diet ($P < 0.05$, L-diet had higher methylation), and in liver 1 CpG had higher ($P < 0.05$) methylation in LRFI steers. However, in *semimembranosus* several CpG were significant for the interaction between prenatal diet and genetic potential for RFI. These results highlight the sensitivity of the bovine genome to differences in the prenatal maternal environment and stages of physiological development.

Key Words: IGF2R, prenatal nutrition, cattle, epigenetics, bovine

MT5 Methylation assessment of selected bovine genes in relation to aging and inflammation in dairy cattle. T. Zabek*[1], E. Semik[1], A. Gurgul[1], T. Szmatola[1], K. Pawlina[1], and E. Bagnicka[2], [1]*National Research Institute of Animal Production, Balice, Poland;* [2]*Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Jastrzebiec, Poland.*

The methylation status of genes important for aging research was evaluated in the bovine blood. DNA prepared from blood leukocytes of two groups of HF cows differing in age (nine 2-month-old heifer calves and nine 10-year-old cows) were bisulfite converted. Amplification and Sanger sequencing were performed for CpG islands resided near the transcription start sites. The subject of study were two genes with documented opposite effect of complete methylation of their regulatory sequences in mammals – the first one in which methylation decreases gene expression (ATG5) resulting in recurrent inflammatory lesions in the old age and the other one where the methylation prompts gene activity (IGF1R) in aged individuals especially in malignant tissues acting as an anti-apoptotic agent. The third locus under study was telomerase reverse transcriptase (TERT) which is responsible for the shortage of telomeres during aging. Investigation included also DGAT1 gene affecting lipid metabolism which polymorphisms are known QTLs for milk fat content. Given the increased susceptibility to fat deposition after reaching adulthood, aging might be a positive factor of DGAT1 activity rising demethylation of its regulatory elements. Bisulfite sequencing revealed that for all samples no methylation of regulatory sequences were detected for ATG5 and IGF1R gene whereas complete methylation was identified for DGAT1. TERT locus was mostly characterised with partially methylated CpG sites and a high variability of methylation percent (PM) was observed within both groups of cows. For majority of CpGs of the TERT CpG island no significant differences in PM were observed between calves and old cows except the highly significant differences detected for two CpG sites. Lack of the more clearly detectable differences of PM between both aging groups of cows in TERT locus might be the effect of heterogeneous mixture of the blood cells. In turn, the effect of aging-related methylation differences for DGAT1 would be potentially visible in the target tissues like adipose or skeletal muscles. This work was supported by the project No. 2015/17/B/NZ9/01561 of the National Science Center of Poland.

Key Words: aging, DNA methylation, dairy cattle

MT6 Analysis of differentially expressed genes and long non-coding RNAs from multiple pig tissues using RRBS and RNA-seq. M.-K. Choi, J. Lee, D. Kim, B. Y. Ahn*, and C. Park, *Department of Animal Biotechnology, Konkuk University, Seoul, South Korea.*

Recent advances in genome sequencing technology have made it possible to analyse diverse aspects of transcribed sequences from diverse species. Long non-coding RNAs (lncRNAs) have been reported as one of potential gene expression regulators in diverse biological processes. However, their detail functions and mechanisms are still not clear. To discover a high-quality set of lncRNAs and a correlation between lncRNAs and gene expression in pigs, we generated over 300 million RNA sequencing reads from four tissues and a PAM cell line including the neocortex, liver, femoral muscle, spleen and 3D4/2 cells. We analysed the expression patterns of genes and lncRNA across tissues and their associated CpG methylation. In detail, clean reads were mapped to current pig genome assembly Sscrofa10.2 with Ensembl gene annotation (release 84) using STAR aligner and then cufflinks2 was used to identify differentially expressed genes (DEGs). Across five tissue comparisons, we identified 3,079 DEGs (p_value < 0.001). Among them, 56.3% of DEGs (1,735 DEGs) were associated with PAM cell comparisons. LncRNAs were discovered and classified using slnck and further analyses are in progress. CpG methylation profiling also performed within promoter region (upstream 2 kb regions from TSS) of all identified differentially expressed transcripts. These results will contribute to constructing the baseline for transcriptome analysis for pigs.

Key Words: pigs, epigenomics, RNA-seq, gene expression, DNA methylation

MT7 Small non-coding RNA from frozen bull sperm cells: biomarkers of male fertility? E. Sellem*[1], S. Marthey[2], H. Kiefer[2], C. Le Danvic[1], A. Allais-Bonnet[1], J. Perrier[2], L. Jouneau[2], A. Rau[2], H. Jammes[2], and L. Schibler[1], [1]*ALLICE, Paris, France;* [2]*INRA, Jouy-en-Josas, France.*

New sperm function in embryo development have emerged recently, relying on their small noncoding RNAs (sncRNAs) content. Indeed, involvement of sperm-borne miRNAs in mouse epigenetic inheritance has been evidenced and paternal sncRNAs have been shown to be dispensable for fertilization but crucial for the development of zygotes and 2 cells-embryos. Our study was conducted to unravel the sncRNA content from bull frozen sperm cells and identify miRNA associated with fertility. Total RNA was extracted from 65 ejaculates originating from Holstein and Montbeliard bulls

with contrasting fertility and morphological abnormalities (Holstein only), using a novel enhanced protocol. The two quality controls were done on RNA to measure RNAs quantities (Qubit technology) and check whether a reference miRNA (miR125) could be detected by RTqPCR. NGS sequencing libraries were prepared using small RNA (< 200 nucleotides) and sequenced at modest depth (40 million 50 bp single reads, Illumina HiSeq; Exiqon). 67% of the reads could be annotated as miRNA (16%), rRNA (13%), tRNA (7.5%), long non-coding RNA (7.2%), mitochondrial RNA (6.5%) and mRNA (17%). The piRNAs have not been identified, due to the lack of a specific database from bos Taurus. But the remaining unknown sequences are consistent in terms of size with piRNA, which are known to be expressed spermatocytes, spermatids and sperm cells. By the use of miRDeep2 software, 3196 miRNA expressed in all samples have been identified, including 583 known and 2613 putative miRNAs. The first statistical analysis carried out using the DESEqn 2 package, emphasised 47 and 34 differentially expressed miRNAs respectively among the fertility groups and between morphological abnormality groups. By taking into account the signature effect of miRNAs, the BCA highlighted the best contributors (168 and 125 miRs, respectively for Holstein and Montbeliard breeds) separating all the bull groups. This study highlights the potential of sperm cells' sncRNA as biomarkers for bull fertility. Supported by ANR and APIS-GENE (Labcom SeQuaMol).

Key Words: microRNA, bull sperm cell, fertility

MT8 Maternal nutrition during the first 50 days of gestation alters expression of histone and histone modifying genes in bovine fetal liver. M. S. Crouse*[1], J. S. Caton[1], R. A. Cushman[3], K. J. McLean[2], C. R. Dahlen[1], P. P. Borowicz[1], L. P. Reynolds[1], and A. K. Ward[1], [1]*Department of Animal Sciences, North Dakota State University, Fargo, ND, USA;* [2]*Department of Animal and Food Sciences, University of Kentucky, Lexington, KY, USA;* [3]*USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA.*

During the first 50 days of gestation, organogenesis is taking place. Nutritional influences during this time may alter the mammalian phenotype through affecting gene regulatory mechanisms, thus 'programming' potential susceptibilities to chronic disease and metabolic issues into the animal's genome. We tested the hypothesis that maternal nutrition during the first 50 days of gestation would alter the transcriptome of histones and histone related genes in the developing fetal liver. Fourteen beef heifers were oestrus synchronized and assigned to 2 dietary treatments at breeding (CON-100% of nutrient requirements to gain 0.45kg/d; RES-60% of CON). Heifers were ovariohysterectomized on d 50 of gestation and fetal livers were dissected, flash frozen, and RNA extracted. RNA-seq analysis was conducted on the Illumina HiSEqn 2500 platform using 50-bp paired-end reads at a depth of 2 × 10.4M reads/sample. Transcriptome analysis was performed in collaboration with USDA-ARS-MARC using the Tuxedo Suite, and KEGG pathways were analysed with DAVID 6.8. A total of 548 genes ($P < 0.01$) were used for ontological analysis, of which 201 were false discovery rate protected ($q < 0.10$). We found 9 histones that were up-regulated in RES *v.* CON including members of the histone H1, H2A, H2B, and H4 families. The 13 differentially expressed histone modifying transcripts included genes associated with acetylation and de-acetylation, methylation, phosphorylation, and ubiquitination. Of particular note, *HDAC10* was 2.67- fold greater ($q < 0.05$) in liver of RES fetuses. Additionally, the histone deacetylase complex gene, *CIR1* was 2.22-fold greater ($q < 0.05$) in RES. Only one gene associated with histone modifications, *SET* was 1.77- fold lower ($P = 0.006$; $q = 0.16$) in RES. The *SET* gene is involved in preventing H4 lysine acetylation. Thus, a moderate nutrient restriction during the first 50 days of gestation alters the expression of histone and histone modifying genes in the bovine fetal liver. This implies that early maternal nutrition initiates developmental programming through epigenetic remodelling. USDA is an equal opportunity provider and employer.

Key Words: cattle and related species, development, epigenomics, pregnancy, RNA-seq

MT9 Evaluating the role of epigenomic modifications in host-pathogen interaction for bovine alveolar macrophages infected with *Mycobacterium bovis*. A. O'Doherty*[1], K. Rue-Albrecht[2], J. Browne[1], T. Hall[1], N. Nalpas[3], D. Magee[1], S. Gordon[1,4], D. Vernimmen[5], and D. MacHugh[1,6], [1]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland;* [2]*NDM Research Building, University of Oxford, Oxford, UK;* [3]*Quantitative Proteomics and Proteome Centre Tübingen, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany;* [4]*UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland;* [5]*The Roslin Institute, University of Edinburgh, Easter Bush Campus, Midlothian, UK;* [6]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland.*

Epigenetic modifications, such as DNA methylation and chromatin modifications, are pivotal in orchestrating various biological processes, representing an important mechanism for conveying cellular response to external environmental stimuli. The impact of *Mycobacterium bovis* infection, the cause of bovine tuberculosis, on the transcriptome of bovine alveolar macrophages (bAM) has been well documented; however, possible effects on the macrophage epigenome are currently not well understood. In the current study, whole genome bisulfite sequencing (WGBS), chromatin immunoprecipitation sequencing (ChIP-seq) and RNA-seq were used to examine the effect of *M. bovis* infection on the epigenome of bAM. *M. bovis*-infected bAM were compared to non-infected control bAM 24 h post infection (WGBS $n = 8$, ChIP-seq $n = 3$, RNA-seq $n = 3$). DNA methylation was assessed across the genome, with particular emphasis on the following genomic features: intergenic sequences, intragenic sequences and promoters with- or without CpG islands. This analysis revealed that the bAM DNA methylome is resistant to perturbations induced by *M. bovis*. Gene ontology analysis, focusing on the degree of methylation at proximal promoters (hyper-, hemi- or hypomethylated), revealed that genes with hemimethylated promoters were enriched for immune-related categories; this enrichment was not observed for genes with hyper- or hypomethylated promoters. Dual ChIP-seq and RNA-seq was also performed on bAM 24 h post-infection with *M. bovis*. Results from these experiments can be used to elucidate the role of chromatin reconfiguration in the host macrophage response to *M. bovis* infection. This is one of the first studies to examine macrophage epigenomic perturbations induced by *M. bovis* infection. It provides novel information for a more complete understanding of host-pathogen interaction in mycobacterial infections and has relevance to human tuberculosis caused by *Mycobacterium tuberculosis*, which has 99.95% genome sequence identity to *M. bovis*.

Key Words: cattle and related species, epigenomics, Functional Annotation of Animal Genomes (FAANG), genome regulation, animal health

MT10 Genome-wide analysis of H3K4me3 and H3K27me3 in three tissues in pigs. C. Kern*[1], Y. Wang[1], P. Saelao[1], K. Chanthavixay[1], I. Korf[1], C. K. Tuggle[2], C. Ernst[3], P. Ross[1], and H. Zhou[1], [1]*University of California-Davis, Davis, CA, USA;* [2]*Iowa State University, Ames, IA, USA;* [3]*Michigan State University, East Lansing, MI, USA.*

Epigenetics is an important factor in understanding the link between an organism's genome and phenome. Such knowledge is especially important in the food production industry where it can be

applied to improve production efficiency, animal welfare, and food safety. As a part of the International FAANG effort, we have made progress towards generating a catalogue of functional elements for the pig genome using ChIP-seq assays for the H3K4me3 and H3K27me3 histone modifications in liver, lung, and spleen from two biological replicates with more than 20 million mapped reads for H3K4me3 assays and 40 million mapped reads for H3K27me3 per animal (FAANG Consortium criteria). We identified 29,365 H3K4me3 peaks in liver, 25,558 in lung, and 23,979 in spleen using Macs 2 (q-value 0.01). For H3K27me3, we used SICER (q-value 0.01) and identified 123,392 broad peaks in liver, 122,656 in lung, and 152,269 in spleen. From a set of 22,861 promoter regions from Ensembl, in liver 7,461 contained H3K4me3 peaks, 4,095 contained H3K27me3 peaks, and 2,139 contained peaks from both. In lung, these numbers were 6,853, 3,476, and 2,494, respectively, and in spleen 6,711, 4,097, and 2,551, respectively. Liver showed the highest specificity of the H3K4me3 modification, with 821 promoters containing a peak only in liver, compared with 261 in lung and 340 in spleen. For the H3K27me3 mark, the modification was observed only in liver for 873 promoter regions, 471 in lung, and 972 in spleen. Using RNA-seq data generated from the same tissue samples as our ChIP-seq assays, we confirmed that genes with the H3K4me3 modification were expressed at significantly higher levels than those with the H3K27me3 modification. Five additional tissues and three more ChIP-seq marks will enable an integrative analysis to predict chromatin state across the pig genome.

Key Words: pigs and related species, Functional Annotation of Animal Genomes (FAANG), epigenomics, ChIP-seq, bioinformatics

MT11 Update on DNA methylation datasets of FAANG reference samples for the chicken and pig. N. Trakooljul*[1], H. Zhou[2], P. Ross[2], I. Korf[3], M. E. Delany[2], H. H. Cheng[4], C. Ernst[5], C. Kern[2], F. Hadlich[1], S. Ponsuksili[1], and K. Wimmers[1], [1]*Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany;* [2]*Department of Animal Science, University of California, Davis, CA, USA;* [3]*Genome Center, University of California, Davis, CA, USA;* [4]*USDA-ARS, Avian Disease and Oncology Laboratory, East Lansing, MI, USA;* [5]*Department of Animal Science, Michigan State University, East Lansing, MI, USA.*

As part of the FAANG initiative, this study aims at cataloging the DNA methylome of 8 FAANG-reference tissues (liver, spleen, lung, muscle, adipose, hypothalamus, cerebral cortex and cerebellum) for the pig and chicken (two adults) at a single-base resolution using Reduced Representation Bisulfite Sequencing (RRBS) with double-enzyme digestion (MspI & Taq$^{\alpha}$I) and increased selected-fragment size (40 - 350bp) to enhance the genome-wide CpG coverage. A total of 30 RRBS libraries were constructed, multiplexed and deep-sequenced for single-end reads and 114 cycles on a HiSEqn 2500. We obtained a total of 2.1 and 2.2 billion high-quality reads (395Gb in total and 145 ± 1.5 million reads per sample) for the chicken and pig, respectively. The data was pre-processed and aligned to the reference genomes with mapping abilities of 64.34 ± 0.87 (chickens) and 62.44 ± 0.62% (pigs) using BS-Seeker2. Interestingly, the chicken showed a lower percentage of methylated CpGs (24.01 ± 1.47/ 1.21 ± 0.28/ 1.04 ± 0.35 for CG/CHG/CHH) compared to the pig (43.42 ± 1.51/ 1.14 ± 0.16/ 1.01 ± 0.23). Despite the distinct DNA methylation levels, both chickens and pigs showed a similar pattern of hypo-methylation around the transcription start site (TSS) and hyper-methylation in the gene body. Analysis of tissue specific patterns of DNA methylation and correlation analysis between DNA methylation and gene expression (RNA-Seq) should help us gain more functional information of the DNA methylation. Together with other epigenetic assays associated with the FAANG reference samples will tremendously facilitate the functional annotation of the animal genomes.

Key Words: DNA methylation, gene regulation, pigs, chickens, FAANG

MT12 Transgenerational phenotypic and epigenetic inheritance across three generations in layers induced by Poly(I:C). L. Liu*, D. Wang, Z. Y. Duan, S. Yang, G. Y. Xu, N. Yang, and Y. Yu, *China Agricultural University, Beijing, China.*

Transgenerational epigenetic inheritance is evoked by environmental factors and could transmit the changed information from one generation to their offspring without genetic variations. Polyriboinosinic–polyribocytidylic acid (Poly(I:C)) is a synthetic mimic of viral dsRNA polymer, which can improve cancer immunotherapy outcome and also used as vaccine adjuvant to cure avian plague in husbandry. The aims of the study were to investigate the effects and molecular mechanisms of Poly(I:C) on three generations of a layer model. First, Poly(I:C) (group P) or saline were intravenous injection in 66 Rhode Island White hens at 53 weeks of age in only F0 generation with family in consideration. Compared to the saline treated controls (group C), egg-laying rate and concentration of plasmatic cytokines (IL-6, TNF-α) of group P was significantly increased ($P < 0.05$), while the egg weight was significantly decreased ($P < 0.01$) in both F0 and F1 generations. Bodyweight of group P was lower than controls in F1 and F2 generations ($P < 0.01$). Moreover, we also found Poly(I:C) impeded the embryonic development of F2 chickens. Next, peripheral blood lymphocytes of F1 chickens at 48 weeks of age were used to conduct RNA-seq and whole-genome bisulfite sequencing (WGBS) analysis. We found some pivotal pathways involved in immune response, development and reproduction (MAPK signalling pathway, ErbB signalling pathway, Progesterone-mediated oocyte maturation) in methylation data by KOBAS. Combined the methylome with transcriptome data, some important overlapped genes were detected. Of which, six up-regulated genes (*KCNQ1, MLLT4, MYOF, NOTCH1, SGCD, SREBF2*) and two down-regulated genes (*PDK4, TGM3*) were associated with immune responses and diseases, while two down-regulated genes, *SEMA3D* and *SOX6*, were related with chick embryonic development and cartilage formation. In conclusion, although Poly(I:C) can improve egg-laying rates of hens, it also decreases the immunity and growth performance as a trade-off for three generations.

Key Words: transgenerational phenotypic and epigenetic inheritance, Poly(I:C), chicken, immune, reproduction

MT13 Genome-wide identification of imprinted genes and its methylation statuses in various tissues of Hanwoo. K.-T. Lee*, K.-S. Lim, B.-H. Choi, H.-H. Chai, J.-E. Park, E.-W. Park, G.-W. Jang, and D. Lim, *National Institute of Animal Science, RDA, Wanju, Jeonbuk, South Korea.*

Genomic imprinting is a epigenetic phenomenon in which only one of two alleles is expressed, resulting in a functional hemizygosity. Numerous genes showed species-specific and tissue-specific imprint patterns. Here, we aimed to identify imprinted genes transcriptome-widely and to find clues for relation between imprinting and methylation in Hanwoo. For three Hanwoo family trios, the transcriptome data of 17 kinds of tissues were generated, and methylation levels were estimated from methylome data in three offspring. A total of 62 imprinted genes expressed monoallelically in at least one tissue. Comparing genotypes among each family trio, the preference alleles of eighteen genes were identified (maternal expression, $n = 9$; paternal expression, $n = 9$), and the imprinting of random selected three genes were validated by direct sequencing. Imprinted genes were involved in gene regulation, metabolic process and immune response, and in particular six genes encode transcription factors (FOXD2, FOXM1, HTATSF1, SCRT1, NKX6–2 and UBN1) with tissue-specific expressions. Methylated reads were

obtained in 2kb upstream region of all imprinted genes, and IFITM2 showed highest methylation level. This is the first study to identify the imprinted genes in aspect of various adults tissues in cattle, and this results could contribute to elucidation of epigenetic effects for phenotype variations.

Key Words: genomic imprinting, cattle, adult tissues, Re-seq, RNA-seq

MT14 Effects of maternal nutrition on the transcriptome and epigenome of the offspring. H. Namous[1], F. Peñagaricano[2], M. Del Corvo[3], E. Capra[4], A. Stella[4], J. Williams[5], P. A. Marsan[3], and H. Khatib*[1], [1]*University of Wisconsin, Madison, WI, USA;* [2]*University of Florida, Gainesville, FL, USA;* [3]*Università Cattolica del S. Cuore, Piacenza, Italy;* [4]*Istituto di Biologia e Biotecnologia Agraria, Lodi, Italy;* [5]*University of Adelaide, Roseworthy, Australia.*

The objective of this study was to evaluate the impact of maternal nutrition of pregnant ewes on the epigenome and transcriptome of their fetuses. Ewes were naturally bred to a single sire, and from days 67 ± 3 of gestation until necropsy (day 130 ± 1) they were individually fed alfalfa haylage (HY; fibre) or corn (CN; starch). A total of 26 fetuses were removed from 15 dams and longissimus dorsi muscle, perirenal adipose depot, and subcutaneous adipose depot tissues were collected. Total RNA and genomic DNA were extracted from fetal tissues for transcriptomic and DNA methylation analyses. To assess the effects of maternal diets on the transcriptome of the fetal tissues, a total of 36 pooled samples (12 pooled samples per tissue with 4 biological replicates per diet) were analysed using RNA-sequencing. From 18,393 genes tested for differential expression in fetal longissimus dorsi muscle tissue, 823 genes showed differential expression between CN and HY maternal diets. Many of these genes are directly involved in embryonic and fetal development, skeletal muscle cell and tissue differentiation, and muscle myosin complex and sarcomere organisation. To assess the effects of maternal diet on the epigenome of the fetus, whole genome DNA methylation analysis was performed in 16 fetal longissimus dorsi muscle tissues using MethylMiner, a methyl binding-based method. A total of 61 differentially methylated regions (DMRs) between HY and CN diets were found in which 39 DMRs showed higher methylation levels in HY compared to CN and 22 DMRs showed higher methylation levels in CN compared to HY. Several differentially methylated genes were validated using bisulfite sequencing. In addition, the correlation between gene expression and DNA methylation was validated for differentially-expressed and differentially methylated gene. Overall, these findings provide evidence that maternal diet supplementation during pregnancy can modulate gene expression and epigenetic changes in the offspring.

Key Words: maternal nutrition, transcriptome, epigenome, sheep

MT15 The effect of histone acetyl-transferase inhibitor (trichostatin A) administration on porcine mesenchymal stem cells transcriptome. A. Gurgul*, J. Opiela, K. Pawlina, T. Szmatola, and M. Bugno-Poniewierska, *National Research Institute of Animal Production, Balice, Poland.*

The use of histone acetyl-transferase inhibitors such as trichostatin A (TSA) for epigenetic modulation of mesenchymal stem cells (MSCs) is an interesting approach in research involving somatic cell cloning of pigs and other mammalian species. Despite the effectiveness of TSA in cloning applications was already confirmed, the detailed mechanisms underlying this effect are not yet fully recognised, especially for pig MSCs. To add to this knowledge, in this study we performed a comprehensive transcriptome analysis using high-throughput RNA sequencing of pig bone-morrow derived MSCs, treated and untreated with TSA, and evaluated the effect of TSA administration on their transcription profile after 24 h of *in vitro* culture. Subsequently, the stability of introduced epigenetic modifications was evaluated after another 50–72 h of culture without TSA. The results showed a wide stimulating effect of TSA on MSCs transcription, affecting genes across the whole genome with some minor signs of site-specific acting in regions located on SSC2 and SSC6. TSA had stronger impact on already expressed genes with only minor influence on silenced genes. Genes with expression altered by TSA were related to a wide range of biological processes, however, we found some evidence for specific stimulation of genes associated with development, differentiation, neurogenesis or generation of muscles. The analysis of cell transcriptome after prolonged culture following the TSA removal, showed that the expression level of majority of genes affected by TSA is restored to the initial level. Nonetheless, the set of about six hundred genes was altered even after 50–72h of culture without TSA. TSA also enhanced expression of some of pluripotency marker genes (*FGF2, LIF, TERT*) but their expression was stabilised during further culture without TSA. The detailed analysis of factors connected with neuron-like differentiation allows us to assume that TSA mostly stimulates neurogenic differentiation pathway in the pig MSCs and thus seems to trigger mechanisms conducive of epigenetic reprograming. This research was supported by the Polish National Science Centre resources allocated on the basis of decision number 2014/15/B/NZ9/04288

Key Words: MSC, pig, transcriptome, trichostatin A

MT16 DNA methylation and microRNA modifications in scrapie. J. Toivonen[1], A. Sanz[1], O. López-Pérez[1,2], D. Sanz-Rubio[1], M. Salinas-Pena[1], J. Alejo[1], R. Bolea[2], J. Espinosa[3], J. Badiola[2], P. Zaragoza[1], J. Torres[3], and I. Martín-Burriel*[1,2], [1]*Laboratorio de Genética Bioquímica, IIS Aragón, IA2, Universidad de Zaragoza, Zaragoza, Spain;* [2]*Centro de Investigación en Encefalopatías y Enfermedades Transmisibles Emergentes, IIS Aragón, IA2, Universidad de Zaragoza, Zaragoza, Spain;* [3]*Centro de Investigación en Sanidad Animal, CISA-INIA, Valdeolmos, Madrid, Spain.*

Scrapie is a transmissible spongiform encephalopathy (TSE) of sheep and goats. Recent evidence suggests an important role for epigenetic mechanisms, such as DNA methylation or regulation of gene expression by microRNAs (miRNAs), in the pathogeny of neurodegenerative diseases. Studies on epigenetics may help in clarifying some of the molecular mechanisms of the scrapie associated pathology and could allow identification of molecules for diagnostics (biomarkers) and for therapeutic targets. We present here an analysis of global DNA methylation levels and miRNA profiling in the central nervous system of transgenic (Tg) murine models of scrapie in early (preclinical) and late (clinical) stages of the disease and a verification of these changes in sheep naturally infected with scrapie. Scrapie-induced alterations in small RNAs, including miRNAs, were determined by RNA sequencing in cervical spinal cord (SC) of Tg501 animals (Tg mice expressing the wild type ARQ goat *PRNP* allele) infected with scrapie in preclinical and clinical stages, and in their age-matched controls. After multiple correction, one and six significant miRNA alterations were found in preclinical and clinical stage, respectively. These miRNA alterations are currently being validated by quantitative PCR (qPCR) in the same Tg501 model, in Tg338 mice (Tg mice homozygous for the sheep VRQ allele) infected with ovine scrapie, and in sheep naturally infected with scrapie. On the other hand, global DNA methylation and hydroxymethylation was quantified using a colourimetric ELISA assay in SC from the three models. DNA methylation was significantly higher ($P < 0.05$) in SC from clinical Tg338 mice than in their age-matched controls. On the contrary, global DNA hydroxymethylation decreased in the early phases of the disease. Epigenetic changes are being validated in the remaining models, and the expression of genes encoding epigenetic enzymes (DNA methylases, histone deacetylases and ten-eleven translocation enzymes) is also being quantified by qPCR. This is the first time that DNA

methylation changes are described in any model of prion diseases and we are currently performing genome-wide studies to investigate the true depth of epigenetic changes in these diseases.

Key Words: sheep, epigenomics, microRNA, RNAseq, infectious disease

Animal Forensic Genetics

MT17 Comparison of the effectiveness of 19 STR and 22 STR panels for forensic DNA analysis of canine in Poland. A. Radko*, A. Podbielska, and M. Miszczak, *National Research Institute of Animal Production, Department of Animal Genomics and Molecular Biology, Balice, Poland.*

Dog DNA-profiling is of high importance for the investigation of accident and crime, especially for dogs that are strongly integrated into human society. Twenty-one STR loci and one sex determination locus, recommended for parentage verification by ISAG were tested in the Polish dog population providing allele frequencies necessary for the application of STRs to forensic genetic casework. Two panels STRs, one containing 19 loci and second containing three additional loci were tested on 452 randomly selected individuals of five dog breed groups (Herding, Greyhound, Terrier, Non-Sporting and Toy Group). To compare the efficiency of the panels for individual identification and paternity testing, we estimated for each panels the cumulative probabilities of parentage exclusion, when one parent is known (CPE_1) and two parents is known (CPE_2), the combined power of discrimination - CPD, the combined probability of identity - $CP_{ID(theoretical)}$ and random match probability - RMP. The cumulative probabilities of parentage exclusion CPE_1 and CPE_2 for 18 loci were 0.9998806 and 0.99999976, respectively and for 21 *loci* were 0.9999829 and 0.9999999901, respectively. The power of discrimination for each marker showed high values above 0.85, CPD values were near 1.0 for both of panels. The theoretical estimates of CP_{ID} for 18 markers were 4.02×10^{-20} and for 21 markers increased to 6.42×10^{-24}. The probability that a dog selected at random from the population (RMP) will have the same profile as the evidence sample, estimated for 19 STRs and 22 STRs ware 2.85×10^{-22} and 1.73×10^{-26}, respectively.

Key Words: canine, STR profiling, forensic parameters

Applied Genetics and Genomics in Other Species of Economic Importance

MT18 Camel milk protein polymorphisms and their potential use. Y. Öner and Y. N. Berhane*, *Uludag University, Özlüce Mahallesi, Nilüfer/Bursa, Turkey.*

Camels have a huge potential to use and benefits on marginal environmental conditions however there is shortage of information about both its production and genetics. In recent year it has been recognised that camel's milk has many therapeutic feature for human health. This situation depends on differences in milk component of camel milk and those of the other ruminants. Besides the other components as vitamins and minerals, casein fraction's composition is also different in camel milk. Caseins are principal ruminant milk proteins and are affected by genetic polymorphisms as much as environmental conditions. There are four major caseins in ruminant milk as α_{S1}-CSN, α_{S2}-CSN, β-CSN, κ-CSN. Although milk protein polymorphisms have been intensively studied on other ruminant's milk, information on camel milk protein polymorphism remains limited. In this study information on milk protein polymorphisms and their potential use on human benefits will be reported.

Key Words: milk protein genes, camel, polymorphism

MT19 Investigation of the ferret genome to identify evolutionary relevant markers and domestication related genomic features. V. J. Utzeri[1], L. Penso-Dolfin[2], G. Schiavo[1], A. Ribani[1], G. Etherington[2], L. Fontanesi*[1], and F. Di Palma[2], [1]*Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy;* [2]*Earlham Institute, Norwich, UK.*

The domestic ferret (*Mustela putorius furo*) is a species domesticated from the wild relative European polecat (*M. putorius*) belonging to the family Mustelidae (Order Carnivora). Ferrets are raised for multiple purposes in different fields such as the fur market and hunting. They are also bred as pets and as animal models for some human diseases (e.g. influenza, gastritis, cystic fibrosis). The domestication of the European polecat is still unclear and actually under study starting from the release of the domestic ferret genome assembly MusPutFur1.0. Recently the market of ferrets as a pet has increased and some main coat colours have been selected during the domestication process: Sable (wild type), Silver, Albino and Cinnamon. In this study, we mined re-sequenced domestic ferret genomes of animals with different coat colours and detected very recent evolutionary signatures associated to some putative causative coat colour mutations. In addition, we investigated the domestic ferret nuclear genome for the identification of integrated mitochondrial DNA (mtDNA) pseudogenes that might shed light on recent and more ancient evolutionary events including the differentiation from the wild European polecat. These pseudogenes are derived from the horizontal transfer of mtDNA fragments into the nuclear genome producing nuclear DNA sequences of mitochondrial origin, also called *numts* Using a pipeline that included LAST algorithm, a total of ~100 *numts* were detected in the MusPutFur1.0 genome version, including regions with homology ranging from ~65% to 100% with the corresponding mtDNA genome regions. These evolutionary relevant features (i.e. coat colour genes and *numts*) detected in the ferret genome might be important to understand the evolutionary processes that shaped and differentiated the domestic ferret and the European polecat genomes.

Key Words: ferret, *Mustela*, evolution, genome, domestication

MT20 Genetic differentiation of farm and wild populations of the red fox in Poland. M. Zaton-Dobrowolska*[1], A. Mucha[1], D. Morrice[2], H. Wierzbicki[1], M. Moska[1], and M. Dobrowolski[3], [1]*Department of Genetics, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland;* [2]*The Roslin Institute, University of Edinburgh, Edinburgh, Scotland, UK;* [3]*Institute of Animal Breeding, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland.*

The red fox in Poland lives in the wild and at fur farms. The aim of the study was to compare genetic diversity of wild and farm populations of the red fox. 30 canine-derived microsatellites were used to perform analyses of genetic differentiation of both studied populations. 22 microsatellite genotypes were detected in a group of

farm foxes (3 microsatellites were monomorphic), while in a group of wild foxes 24 microsatellite genotypes were detected (one was monomorphic). Polymorphic microsatellites differed significantly in several alleles per locus and allele frequencies. Three separate genetic clusters were distinguished in the farm foxes, while the wild foxes constituted genetically homogeneous group totally distinctive from the farm foxes.

Key Words: other species, red fox, biodiversity, popualtion identification

MT21 Genetic distances between farm and wild populations of the red fox in Poland. M. Zaton-Dobrowolska*[1], A. Mucha[1], D. Morrice[2], H. Wierzbicki[1], M. Moska[1], and M. Dobrowolski[3], [1]*Department of Genetics, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland;* [2]*The Roslin Institute, University of Edinburgh, Edinburgh, Scotland, UK;* [3]*Institute of Animal Breeding, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland.*

The farm and wild red fox populations in Poland are genetically differentiated. The microsatellite analysis showed significant differences between populations in the analysed loci. This indicates genetic distinctiveness of both populations most likely caused by selective breeding and different ancestry. Genetic distances calculated using three different methods showed significant genetic differences between both populations. Estimates of Fst ranged from 0.127 to 0.282. Values of Gst ranged from 0.162 (Nei's method) to 0.707 (Hedrick's method). Jost's D estimate reached 0.594. The results obtained suggest that farm and wild populations of the red fox are genetically different, and genetic distance between them is significant.

Key Words: other species, red fox, genetic distance

MT22 A *de novo* hybrid assembly of a dromedary camel. H. Holl*[1], D. Miller[2], S. Abdalla[3], B. Shykind[3], J. Malek[3], Y. Mohamoud[3], A. Ahmed[3], K. Pasha[4], A. Khalili[4], D. Antczak[2], and S. Brooks[1], [1]*University of Florida, Gainesville, FL, USA;* [2]*Cornell University, Ithaca, NY, USA;* [3]*Weill Cornell Medical College in Qatar, Doha, Qatar;* [4]*Tharb Veterinary Hospital, Doha, Qatar.*

The domestic dromedary camel is of economic and cultural importance in many countries in north Africa, the Middle East, and in parts of Asia. With a global population estimate of over fifteen million, many in developing nations, camels are often selected for meat and milk production, draught, riding, and racing traits. Camels possess an array of unique physiological adaptations that have enabled their survival in the arid desert environment. Assembled using only Illumina reads, the currently available reference genome contains 32,572 scaffolds with a N50 of 4.2Mb. Our goal was to produce a more robust set of genomic resources that will facilitate genomic selection and the study of desert adaptation in mammals using a comparative genomic approach. We selected a male dromedary camel from the US to serve as our genome animal. We established a fibroblast cell line from a skin biopsy for future cytogenetics work, as well as to prepare high molecular weight DNA for next-generation sequencing. A total of 74x paired end Illumina and 15x PacBio coverage was generated for a hybrid assembly strategy. The contigs were further assembled into scaffolds using 30x coverage of 10X Genomics sequencing. In addition, we performed a *de novo* transcriptome assembly from blood, cultured cells, and testis to assist with scaffolding and gene annotation. Existing alpaca genetic maps and dromedary radiation hybrid maps were used to anchor scaffolds to chromosomes. The resulting assembly is a valuable resource for future genomic studies in the dromedary camel. This study was made possible in part by NPRP Grant 6-1303-4-023 from the Qatar National Research Fund (a member of Qatar Foundation). The findings achieved herein are solely the responsibility of the authors.

Key Words: old world camelids, genome assembly

MT23 SNP genotyping of reindeer (*Rangifer tarandus)* using BovineHD BeadChips. V. Kharzinova*[1], A. Dotsev[1], V. Fedorov[2], G. Brem[1,3], K. Wimmers[4], H. Reyer[4], and N. Zinovieva[1], [1]*L.K. Ernst Institute of Animal Husbandry, Moscow, Russia;* [2]*Yakut Scientific Research Institute of the Agriculture Federal Agency Scientific Institutions, Yakutsk, Russia;* [3]*Institute of Animal Breeding and Genetics, VMU, Vienna, Austria;* [4]*Institute of Genome Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany.*

For most model species, SNP genotyping assays became a highly effective molecular genetic tool for biodiversity studies. In regards to non-model species (such as reindeer), these targets are currently limited by the lack of SNP arrays. Some recent research demonstrated the successful application of the medium density DNA chips for survey of genetic diversity and population structure of the reindeer populations, but high-density SNP arrays have not been applied for this purpose yet. In our study, the Illumina BovineHD BeadChip genotyping array was tested as a new tool for describing the biodiversity and estimating individual level of inbreeding within reindeer populations. Genomic DNA was isolated from tissue samples of 22 individuals belonging to three semi-domestic reindeer populations (POP1, n = 7; POP2, n = 12; POP3, n = 3) using a Nexttec column (Nexttec Biotechnologie GmbH, Germany). We used PLINK1.07, Admixture 1.3. Software and R packages ('VennDiagram', 'inbreedR' and 'diveRsity') were used for statistical analysis. After QC (GENO 0.1, MAF = 0.01) 9623 SNPs were taken for the further analyses. All populations had approximately equal polymorphism level, while POP1 was characterised by the highest level of genetic diversity (Ho = 0.227 ± 0.002, He = 0.202 ± 0.002 and Ar = 1.499 ± 0.003) and POP3 the lowest (Ho = 0.223 ± 0.003, He = 0.155 ± 0.002 and Ar = 1.392 ± 0.005). The F_{IS} values ranged from -0.055 in POP2 to -0.386 in POP3. The g2 estimator of identity disequilibrium (ID) significantly differed from zero (g2 = 0.00055, se = 0.00022, $P < 0.001$, based on 999 permutations) and the expected r2 between inbreeding level and heterozygosity was 0.64. Multi-dimensional scaling (MDS) components one (C1) and two (C2) explained ~10.5% and 9.0% of the variation, respectively. Admixture analysis revealed a clear differentiation of the studied reindeer populations, which indicates their unique genetic profiles. We demonstrated that SNP genotyping using Bovine 770K SNP BeadChips provides sufficient information on reindeer genetic profiles which might be used for creation of specific SNP panels for this species. This research was funded by Russian scientific foundation project number 14–36–00039.

Key Words: SNP, BovineHD BeadChips, reindeer

MT24 The population and landscape genetics of the European badger (*Meles meles)* in Ireland. A. Allen*[1], J. Guerrero[2], A. Byrne[1,3], J. Lavery[1], E. Presho[1], G. Kelly[1], E. Courcier[4], J. O'Keefe[5], U. Fogarty[6], D. O'Meara[7], G. Wilson[8], D. Ensing[1], C. McCormick[1], R. Biek[9], R. Skuce[1,3], [1]*Agri Food and Biosciences Institute, Belfast, Northern Ireland;* [2]*CEFE-CNRS, Centre D'Ecologie Fonctionelle et Evolutie, Montpelier, France;* [3]*School of Biological Sciences, Queen's University Belfast, Belfast, Northern Ireland;* [4]*Department of Agriculture, Environment and Rural Affairs, Belfast, Northern Ireland;* [5]*Department of Agriculture Food and the Marine, Dublin, Republic of Ireland;* [6]*Irish Equine Centre, Johnstown, Republic of Ireland;* [7]*Waterford Institute of Technology, Waterford, Republic of Ireland;* [8]*Animal*

and Plant Health Agency (APHA), Stonehouse, Gloucestershire, England; [9]University of Glasgow, Glasgow, Scotland.

The European badger (*Meles meles*) is an important member of the fauna of Britain and Ireland, not least because it acts as a wildlife reservoir for bovine tuberculosis. Genetic structure of the species is expected to have been influenced by anthropogenic activities and also landscape-level effects. The relative contribution of both factors is debated, but will conceivably have implications for both wildlife and disease management. Recent Europe-wide surveys of genetic diversity have suggested human-aided introduction of badgers into Ireland. These studies have not, however, indexed island-wide diversity of the species, nor comprehensively attempted to detail demographic and geographic factors which shaped the extant population. Herein, we detail the most comprehensive population and landscape genetic study of the badger in Ireland to date. Our data demonstrate that north-eastern and south-eastern counties of Ireland contain a badger sub-population genetically similar to its British contemporaries. Approximate Bayesian computation suggests this sub-population arose in Ireland ~250–3500 years ago through likely import of a small number of badgers from Britain, which then admixed with an already resident Irish badger population. Landscape genetic analyses determined that geographic distance and elevation were the primary drivers of genetic differentiation, in keeping with the philopatric nature of the species elsewhere in Europe. Other factors such as land cover type, earthworm habitat suitability and the River Shannon, had no detectable effect on gene flow. These data are likely to be useful in future efforts to better understand bovine tuberculosis epidemiology and spatial distribution in the Irish badger population.

Key Words: Badger, Ireland, colonisation, landscape genetics

MT25 Genomic selection for performance and reproduction traits in American mink. K. Karimi*[1], Y. Miar[1], and M. Sargolzaei[2,3], [1]*Department of Animal Science and Aquaculture, Dalhousie University, Nova Scotia, Canada;* [2]*Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada;* [3]*Semex Alliance, Ontario, Canada.*

Phenotypic relationships between performance and reproduction traits were explored using bivariate models on a dataset from a genomic study aimed at improving economically important traits in American mink. Performance and reproduction traits of 21,939 mink during the period of 2002–2016 were collected by Canadian Centre for Fur Animal Research at Dalhousie University. A study to implement genomic selection for these traits on 2,000 mink using genotyping-by-sequencing has been also initiated. Phenotypic variations and correlations between bodyweight at different ages, litter size, the number of weaned kits and mortality rate until weaning were estimated using SAS 9.4 program. The average number of kits born was equal to 6.12 ± 2.58 and varied between 0 and 17 kits per female. On average, 0.83 kit per female was dead at the first 24 h post whelping. Our results indicated that 84.19% of alive kits survived until weaning (6 weeks). The average number of weaned kits was 4.92 ± 2.54 per female and ranged from 0 to 13. The average birthweight was 11.20 ± 2.95 gr per kit and ranged from 5 to 23.8 gr. Furthermore, the average weight at 3 weeks and weaning weight were equal to 134.1 ± 17.4 gr and 389.8 ± 62.2 gr, respectively. Negative phenotypic correlations were found between birthweight with litter size (-0.47 ± 0.17), the number of kits born alive (-0.39 ± 0.14) and the number of weaned kits (-0.20 ± 0.07). Weaning weight was negatively correlated with litter size (-0.31 on average), the number of kits born alive (-0.32 on average) and the number of weaned kits (-0.36 on average). Additionally, the phenotypic correlation between pairing weights and the number of weaned kits was low (-0.10 ± 0.04). Relatively high phenotypic variation and moderate correlation between the studied traits in American mink warrants further investigation to estimate genetic parameters and to investigate the possibility of genetic improvement through implementation of multi-trait genomic selection.

Key Words: Mustelids, animal breeding, performance traits, phenotypic correlation, genomic selection

MT26 Plains bison triallelic SNPs for determining parentage, estimating cattle introgression, and inbreeding. T. Kalbfleisch*[1], J. Tait[2], V. Basnayake[2], B. Simpson[2], T. Smith[3], and M. Heaton[3], [1]*University of Louisville, Louisville, KY, USA;* [2]*GeneSeek, Lincoln, NE, USA;* [3]*USMARC, USDA, Clay Center, NE, USA.*

Bison producers are interested in availing themselves of genomic technologies to make well-informed decisions for breeding and herd management. Two pressing issues for them are parentage, and a measure of bovine introgression in their seed stock. Fortunately, whole genome next-generation sequencing data generated from bison lends itself well to mapping to the bovine reference genome for comparative analysis. In this work, we have mapped the whole genome sequence data from two plains bison. From this data, we have identified a panel of markers which are heterozygous in both bison where one allele is unique to bison by virtue of not appearing in the USMARC Bovine Diversity panel at 5% minor allele frequency or greater. The second allele does not appear in the Bovine Diversity Panel, and is consistent with the ancestral allele measured in 2 gaur, 2 banteng and 2 yak. A MALDI-TOF multiplex is being developed for this panel of markers that will measure all three alleles, the two found in the bison, as well as the cattle specific allele. This marker panel enables bison producers to inexpensively measure a unique genetic fingerprint for their animals that can be used in parentage, as well as the ability to quantify the amount of bovine introgression in the animal.

Key Words: bison, parentage, introgression

MT27 Characterisation of a family of alpacas exhibiting disproportionate dwarfism. K. A. Munyard* and T. Y. K. Tan, *School of Biomedical Sciences, Curtin Health Innovation Research Institute, Curtin University, Perth, Western Australia, Australia.*

An alpaca breeder noted that one of his herdsires (Sire X) produced cria which were noticeably smaller than normal ~50% of the time. Both male and female cria were affected, and there was also perceived mild dysmorphia. We report here the investigation of this trait, including phenotypic characterisation, inheritance pattern, and a preliminary genomic association study. The animals in the study were Sire X, cria out of normal females that were sired by Sire X, and cria out of these same dams by normal sires. Phenotype was characterised by measuring weight over time, and body proportions at age >1yo. Mode of inheritance was hypothesised from pedigree records. A genome wide association study was performed on seven of these animals. PLINK was used to analyse SNPs generated via double digest reduced representation Ion Proton sequencing, mapped to the vicpac2.0 genome. The affected alpacas conformed to a disproportionate dwarfism phenotype, namely a significant reduction in the length of the spine ($P = 0.002$), the length of elbow to withers ($P = 0.02$), spine to hind knee ($P = 0.03$), and hock to hind fetlock ($P = 0.01$). Weights were significantly lower at birth ($P = 0.01$) and approached significance at 1yo ($P = 0.056$). Pedigree analysis supported an autosomal dominant mode of inheritance. Between 142,831 and 396,069 SNPs were called for each animal, although only 41,261 SNPs were called at >20x coverage in all 7 animals. One SNP (scaff_19: 12429719) had a p-value of 0.000532, and 15 others also had high p-values. However, none of these reached genome-wide significance after correction for multiple testing. A candidate gene near the top SNP encodes for an enzyme that cleaves Amyloid Precursor Proteins (APP). Abnormal accumulation of APP has been linked to a disease which includes

deformity of the long bones as a symptom (Kimonis *et al*., 2007). Additional candidate genes will be examined. The genotyping by sequencing protocol proved to be successful, but still needs further optimization to maximise the common regions sequenced, and thus the number of SNPs able to be called across the whole cohort.

Key Words: New World camelids, genome-wide association, genotyping, genetic disorder, animal health

Avian Genetics and Genomics

MT30 Effects of dietary fiber content in interaction with a locus on GGA4 on growth, body composition and feed efficiency in chicken of an advanced intercross population. M. K. Nassar*[1], S. Lyu[2], J. Zentek[3], and G. A. Brockmann[2], [1]*Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt;* [2]*Albrecht Daniel Thaer-Institute for Agricultural and Horticultural Sciences, Humboldt-Universität zu Berlin, Berlin, Germany;* [3]*Department of Veterinary Medicine, Institute of Animal Nutrition, Freie Universität Berlin, Berlin, Germany.*

In this study, we examined the effect of different dietary fibre contents on growth, body composition and feed efficiency of chicken with different genetic background. We tested, if the traits were affected by a previously identified growth locus on chromosome 4 (GGA4) between 69.3 and 77.9 Mb (Nassar *et al*. 2015, *Animal Genetics* 46: 441–6; Lyu *et al*. 2017, *Animal Genetics* DOI: 10.1111/age.12532). A trail was conducted with male chickens of generation 12 of an advanced intercross population descending from the initial cross between New Hampshire (high growth) and White Leghorn (low growth). At four weeks, the chickens were randomly divided into two diet groups, which were fed either a low (LFD, n = 38) or a high fibre diet (HFD, n = 41) containing 0.8% and 5% Arbocel, a lignocellulose with a crude fibre content of 60%, respectively. Bodyweights were recorded weekly. Feed intake was analysed in weeks 6 and 13. Body composition was examined at 13 weeks. The effect of dietary fibre content on bodyweight gain and feed intake was age-dependent. Feeding HFD resulted in 1.1 times higher bodyweight gain (P = 0.0467) and feed intake (P = 0.0143) in week 6, 0.6 times lower visceral adipose tissue mass (P = 0.0079) and 1.1 times higher gizzard (P = 0.0125) compared to LFD. The lower fat deposition was associated with reduced intramuscular fat content in drumsticks-thighs (4.2 *v.* 5.1%, P = 0.0045). Bodyweight, muscle mass and feed conversion ratio were not altered by the dietary fibre content. The genotype of the GGA4 locus (SNPs between 69.3 and 77.9 Mb) affected bodyweight gain (P = 0.0416), visceral adipose tissue mass ($P \leq 0.0288$) and feed intake ($P \leq 0.0498$) in interaction with dietary fibre content. Our findings suggest that high dietary fibre content not only improves the quality, but also the productivity of chicken production, while feed efficiency did not change. Furthermore, the data suggest interaction of genes in the target GGA4 region with dietary fibre usage or linkage between genes affecting weight gain, fat content, feed intake and fibre usage.

Key Words: chicken, nutrigenomics, SNP, Arbocel, Growth and fat deposition

MT31 Sire influence on hatchability and heritability estimates of quails in a humid tropical environment. U. K. Oke*, O. C. Obi, and U. K. Ibe, *Department of Animal Breeding and Physiology, Michael Okpara University of Agriculture, Umudike, Nigeria, West Africa.*

Study on Japanese quail was undertaken to determine sire influence on hatchability and estimation of heritability values among three strains of the bird in a humid tropical environment in Nigeria. Fertility and hatchability traits; bodyweights (BW), shank length (SL), thigh length (TL), breast length (BL), body length (BL), keel length (KL), and wing length (WL) at 2nd,6th,10th weeks of age, were measured on 300 crossbred progeny from 270 cinnamon brown dam, mated with three different strains of sire namely; cinnamon brown (CB), Panda white (PW), and Silver brown (SB) in the mating ratio of 1:4. The data were subjected to analysis of variance appropriate for completely randomised block design (CRBD), and significant means separated with Duncan's multiple range tests. Heritability estimate was done using the sire component equation. The average percent fertility (71.64–75.76%), hatchability (50.41–57.17%), percent dead in germ (11.20 - 18.25%), dead in shell (11.201–18.25%) were significantly ($P < 0.05$) better in PW – sired progeny, while piped (13.06–20.11%), brooding (3.00–4.67%) and rearing (1.00–1.33%) mortality showed no significant different ($P > 0.05$) among the three sired – progeny. The analysis showed that at week 2,6,10, SB- sired progenies had superior heritability estimates in most of the linear traits and recorded lower indeterminate values as compared with other sired – progenies . Moderate to high heritability estimates (42% -83%) obtained for bodyweight at ages 6 and 10 weeks among the three progenies suggests that selection for bodyweight or growth rate in Japanese quail should be carried out at 6th and 10th weeks of age and that PW – sired progeny be selected for better hatchability for enhanced breeding program for both egg and meat production in the strains.

Key Words: sire, hatchability, heritability, quails and humid tropics

MT32 Mitochondrial DNA polymorphism in Nigeria indigenous turkey population. D. M. Ogah*, *Nasarawa State University keffi, Shabu-Lafia campus, Nasarawa State, Nigeria.*

MtDNA still represents a useful tool in the study of molecular genetic diversity, because it appears in multiple copies in the cells and the mitochondrial gene content is strongly conserved across generations. In order to understand the population diversity of Nigerian indigenous turkey breed and to preserve this genetic diversity, evaluation of mitochondrial DNA sequence was employed. The study was aimed at determining mitochondrial DNA (mtDNA) D-loop, HV1 region polymorphism of indigenous turkey population in Nasarawa State north central Nigeria. We analysed the complete mitochondrial DNA D-loop. To achieve this, blood samples were collected from 30 indigenous turkey, 10 each from three different populations separated by distance A 623-bp fragment of the mtDNA D-loop region was sequenced in the sampled turkey populations. The result obtained indicate that in total, 11 haplotypes were identified. Haplotype diversity and nucleotide diversity were 0.81 ± 0.07 and 0.15 ± 0.07 respectively. With 126 number of polymorphic sites. Analysis of molecular variance (AMOVA) based on partial D-loop sequences of the turkey population also indicates that 99.05% of the total sequence variation between haplotypes was present within the population and 95.00% between populations. These results show a high mitochondrial D-loop diversity and indicate multiple maternal origins for Nigeria indigenous turkey. The molecular information on genetic diversity revealed in this study may be useful in developing genetic improvement and conservation strategies to better utilise indigenous Nigerian turkey resources

Key Words: genetic diversity, turkey, indigenous, mitochondrial, population

MT33 Aflatoxin B_1 induced apoptosis in chicken hepatocytes and rescue effects of Selenium. M. Jameel*[1], X. Peng[2], A. A. Kamboh[3], and J. Fang[1], [1]*Sichuan Agricultural University, Chengdu, Sichuan, China;* [2]*ChinaWest Normal University, Nanchong, Sichuan, China;* [3]*Sindh Agriculture University, Tandojam, Sindh, Pakistan.*

Aflatoxin B_1 ($AFB_{1)}$ intoxication is a major concern in poultry industry resulting in decreased meat/egg production, hepatotoxicity, nephrotoxicity, disturbance in gastrointestinal tract (GIT) and reproduction, immune suppression, and increased disease susceptibility. AFB_1 provokes liver dysfunctioning by causing hepatocytes apoptosis via death receptor pathway. Selenium (Se) is an antioxidant trace element which effectively neutralize the toxic effects of $AFB_{1.}$ Yet the mechanism of AFB_1 induced apoptosis in chicken liver via death receptor pathway and rescue effects of Se in hepatocytes still remain elusive. Present study was performed to explore apoptotic mechanism initiated by death receptor pathway in liver of chickens fed with AFB_1 for 3 weeks and to explore rescue effects of Se. Two hundred and thirty four 1 day old Cobb broilers were divided in to 3 groups; control (0 mgAFB_1/kg of basal diet), AFB_1 (0.6 mg AFB_1/kg of basal diet) and Se (0.6 mg AFB_1/kg of basal diet + 0.4mg Se/kg of basal diet). Each group was further divided in to 3 weeks which were 7 days, 14 days and 21 days, with 26 chickens in each week. The histopathological, ultrastructural, biochemical, flow cytometrical and gene expression data were recorded at 7, 14 and 21 days. Histopathological and ultrastructural changes in liver such as hydropic degeneration, fatty vacuolar degeneration and proliferation of bile duct in hepatocytes in AFB_1 group, along with imbalance between reactive oxygen species (ROS) and antioxidant defence system upon AFB_1 ingestion were seen. Also AFB_1 intoxicated chickens showed excessive apoptosis, involving up-regulation of death receptors Fas, TNFR1 and down-regulation of inhibitory apoptotic proteins XIAP and Bcl-2. Interestingly Se as dietary supplement reversed toxic effects of AFB_1 at all 3 weeks, resulting in reduced anti-oxidative stress, improved biochemical and gene expression profiles. The results from this novel and comprehensive study will lead to better understanding of mechanisms and involvement of death receptor pathway in hepatocytes apoptosis induced by AFB_1 and rescue effects of Se and will offer a cost effective and efficient method for coping up with economic losses caused by AFB_1 to the poultry industry.

Key Words: poultry, aflatoxin B_1, selenium, apoptosis, death receptors

MT34 Scanning signals of artificial selection in the chicken genome. Y. Ma*, L. Gu, L. Yang, and S. Li, *Key Laboratory of Agricultural Animal Genetics, Breeding, and Reproduction, Ministry of Education, and Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture; Huazhong Agricultural University, Wuhan, Hubei, China.*

Artificial selection played an important role in the improvement of economical traits in chickens. Understanding the genetic mechanisms underlying specific phenotypic changes could contribute to further shaping the economically important traits. Based on Affymetrix chicken 600k SNP chip data, a total of 331 chicken individuals from 9 populations, including Jingfen (JF), Jinghong (JH), Araucana (AR), Italiener (IT), Zwerg-Cochin (ZW), Shamo (SH), Gallus Gallus Spadiceus (GA), Rheinlander (RH) and Vorwerkhuehner (VO), were sampled for this study. Through population structure analysis, five combining breed-pools (or breed), referred to as JH_JF, ZW, RH_IT, AR_VO, and GA_SH, were used to detect potential selection signals using the composite likelihood ratio (CLR) and the integrated haplotype score (iHS) tests. To further identify the signals of artificial selection, GA_SH with a little experience of artificial selection was defined as the common reference population and the F_{ST} statistic was calculated in the other breed-pools to identify the signals departing from the expected patterns under natural scenario. Finally, a total of 205, 136, 129 and 100 potential signals of artificial selection were identified in AR_VO, ZW, RH_IT and JH_JF, respectively. Enrichment analysis suggests that the regions of artificial selection are harboring genes underlying economic traits like fertility, growth, immunization and feed intake, including THSR, PTHLH, IGF2 and PMCH. Further analysis indicated that only a few putative artificial selection regions were shared among populations while the divergent selection signals extensively distributed in the whole genome, suggesting that genetic diversity should play an important role in the process of adaptive evolution, especially when the environmental change is rapid. Under a strict control, 16, 32, 17 and 39 putative hard sweep regions and 72, 50, 40 and 51 putative soft sweep regions were separately identified, which is more likely to support that many of artificial selection signals is based on the standing genetic variation rather than the newly arisen beneficial mutation in domestic chicken.

Key Words: chicken, artificial selection, divergent selection, soft sweep

MT35 Forty-two shades of pink: Sex assignment and parentage analysis in a captive colony of Greater flamingos (*Phoenicopterus roseus*). C. Biolatti*[1], C. Beltramo[1], A. Dogliero[2], V. Campia[1], S. Peletto[1], S. Colussi[1], P. Modesto[1], and P. Acutis[1], [1]*Regional Center for Exotic Animals, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy;* [2]*Department of Veterinary Science, University of Turin, Grugliasco, Turin, Italy.*

Sex assignment and parentage assessment of captive colonies' members has a crucial role in managing collections. DNA-based sexing techniques are required to avoid errors in species with little sexual dimorphism, such as Greater flamingos. Aim of the study was to assign sex and unravel parentage relationships among a group of 42 Greater flamingos. Secondary goal was to compare 3 molecular methods to sex Greater flamingos. DNA was extracted from blood samples. To sex animals, the PCRs described by Balzik *et al*. (2007) and by Itoh *et al*. (2001) were utilised. Moreover, the protocol described by Griffiths *et al*. (1998) was modified by labelling the P2 primer at 5′ end with a fluorophore (HEX), to detect the length of *CHD* gene by capillary electrophoresis. For parentage analysis, 23 microsatellites described by An *et al*. (2010) were evaluated. After the first screening, markers were selected and combined in multiplex PCRs. The forward primer of each marker was labelled at 5′ end with a fluorophore (FAM, HEX, ATTO550). Sizing of all fragments was carried out on a 3130 Genetic Analyzer (Applied Biosystems). Sexing was successful with all techniques: the colony was composed of 18 males and 24 females. The Itoh's PCR gave an internal control and a female-specific fragment of ~185-bp and 160-bp, respectively. The Balzik's PCR resulted in a male or in a female band at around 590-bp or 330-bp, respectively. The Griffiths' PCR produced a single 373-bp fragment on males and two (373-bp and 386-bp) on females. Parentage analysis is at its early stage. The following 18 microsatellites were chosen and combined in 4 multiplex PCRs: M1 (PrA3, PrA102, PrA110, PrD4, PrD5), M2 (PrC122, PrD3, PrD7, PrD102, PrD121), M3 (PrA105, PrA113, PrB102, PrD9), M4 (PrB3, PrB105, PrC101, PrC109). Since the absence of a studbook, resulting genotypes will be analysed with two parentage softwares (COLONY and CERVUS) and outcomes will be compared. All sexing methods gave overlapping results. The Griffiths' technique, that was formerly considered inapplicable to Greater flamingos, was able to clearly differentiate females from males, thanks to the high discriminative power of capillary electrophoresis.

Key Words: Greater flamingos, sex determination, parentage, microsatellite

MT36 The widespread introgression in Chinese indigenous chicken breeds from commercial breeds. C. Zhang*[1,2], D. Peng[2], R. Yung[2], J. Wang[2], and D. Lin[1,2], [1]*Beijing Advanced Innovation Center for Food Nutrition and Human Health, China Agricultural University, Beijing, China;* [2]*State Key Laboratory for Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing, China.*

Chinese indigenous chickens are world-renowned genetic resources for excellent traits. Unfortunately, introduction of a large number of commercial chicken breeds in past two decades caused adverse effects on Chinese indigenous chickens. Using the chicken 60K Single-Nucleotide Polymorphism (SNP) chip, we assessed the genetic diversity and population structure of 1374 chickens representing 12 Chinese indigenous chicken breeds and 2 commercial breeds. By employing a new method based on haplotype similarity, we found extensive gene introgression from commercial breeds to almost all Chinese indigenous chickens. Approximately 10% of the genome of Chinese indigenous chicken was introgressed ranging from 1.33% for Tibetan chicken to 15.87% for Shiqi Za chicken. Further analysis showed the introgression loci were only subjected to weak selections. For the first time, we systematically mapped and quantified and introgression from commercial breeds at whole genome level. Our results suggested a grim situation for protecting genetic resources for Chinese indigenous chickens.

Key Words: poultry and related species, genetic introgression, haplotype

MT37 Genetic association between carcass traits and DNA microsatellite marker genotype in chickens. K. Tatsuda*, *Hyogo Prefectural Institute of Agriculture, Forestry and Fishieries, Kasai, Hyogo, Japan.*

We developed a resource population to detect DNA microsatellite marker genotypes affecting carcass traits in meat chickens. Among the 23 pairs of Satsumadori males and Nagoya females (Japanese native fowl, respectively), 2 pairs were selected for the parents of the resource population based on their utility for association analysis. In total, 420 F2 birds were produced from 12 F1 birds (4 males and 8 females) at 16 hatcheries. Three hundred and eleven F2 birds were slaughtered at 140 days of age and 12 carcass traits were measured. The association of 104 marker genotypes with the carcass traits was analysed. The genotypes of 2 markers were significantly associated with 7 carcass traits ($P < 0.05$). Thigh meat, breast meat, drumstick, wing, liver and heart yield were associated with 5 genotypes of marker ADL0019 (mapped at 122 cM of Ch.1). Genotype frequency (%) of this marker in F2 animals was as follows: AA (30), AB (25), AC (15), BB (15) and CC (15). Thigh meat yield of the birds with AA or AB genotype was significantly higher than those with AC or BC genotype. Breast meat yield of the birds with AA, AC or BC genotype was significantly higher than those with AB genotype. Drumstick, wing and liver yields of the birds with BC genotype were higher than those with AA, AB or AC genotype. Heart yield of the birds with BC genotype was higher than those with AC genotype. Thigh meat yield and whole meat yield (thigh meat, breast meat and white meat) were associated with 3 genotypes of marker LEI0068 (mapped at 145 cM of Ch.1). Genotype frequency (%) of this marker in F2 animals was as follows: AA (45), AB (30) and BB (25). Thigh meat yield of the birds with AA genotype was significantly higher than those with AB or BB genotype. Whole meat yield of the birds with AA genotype was significantly higher than those with AB genotype. These results demonstrated the possibility of marker-assisted selection of carcass traits in chickens.

Key Words: chicken, carcass trait, DNA microsatellite marker, genotype

MT38 MtDNA D-loop genetic diversity of Middle East and North African indigenous chicken. A. Al-Jumaili*[1], S. Al-Bayatti[2], A. A. Essa[2], R. Alatiyat[3], A. Ahbara[1], J. Mwacharo[4], O. Hanotte[1], and R. Aljumaah[3], [1]*University of Nottingham, School of Life Sciences, Nottingham, Nottinghamshire, UK;* [2]*Ministry of Agriculture, Directorate of Animal Resources, Genetic Resources Division, Baghdad, Iraq;* [3]*King Saud University,Animal Science Department, College of Food and Agriculture, Riyadh, Kingdom of Saudi Arabia;* [4]*The International Centre for Agricultural Research in Dry Areas (ICARDA), Small Ruminant Genetics and Genomics Group, Addis Ababa, Ethiopia.*

Indigenous domestic chickens represent a major resource for agricultural communities around the world. In the Middle East and the North African regions, their populations are declining following increased demand for poultry meat and eggs favouring the more productive exotic commercial breeds. As a step towards the understanding of their diversity, we report here the analysis of the mtDNA D-loop from 293 chicken samples from Iraq ($n = 85$), Libya ($n = 23$) and Saudi Arabia ($n = 185$). A fragment of a 549 bp was PCR amplified and a 397 bp fragment, which include the hypervariable region of the D-loop, was subsequently analysed. Clustal X *v.*2.1 was used to align the sequences; DnaSP *v.*5 to calculate the genetic diversity, the number of polymorphic sites and haplotypes, pairwise Fst and Nst between populations. Network 5 was used for phylogenetic network analysis and Arlequin 3.5.2 for AMOVA. The results show the presence of at least three haplogroups (A, C and E; *sensu* Liu *et al.* 2006) suggesting more than one origin for the study populations.

Table 1 (Abstract MT36). Percentage and random test of candidate introgression region

Chinese indigenous breed	Introgression ratio from White Rock	Random test Z value	Introgression ratio from White Rock	Random test Z value
Beijing Fatty	11.51%	−2.004	4.78%	−2.374
Huiyang Bearded	14.04%	−3.812	2.62%	−3.38
Jinhu Black-Bone	12.72%	−2.467	3.49%	−0.042
Kuaida Silky	14.58%	−4.41	3.20%	−2.152
Langshan	10.37%	−0.655	5.02%	−2.576
Qingyuan	9.52%	−0.825	2.12%	0.302
Shiqi Za	15.87%	−6.431	5.56%	−1.46
Wenchang	11.59%	−2.585	2.07%	−0.76
Silkie	9.19%	−0.424	3.41%	−1.385
Tibetan	1.33%	−0.985	0.42%	−0.459

Haplogroup E, which occurred in 284 samples, is the most frequent in all the three countries and it may have been introduced from Indian subcontinent. Haplogroup A (n = 6) is also present in the three countries and may be of commercial origin. Haplogroup C (n = 3) was only detected in the south-west region of Saudi Arabia and may have arrived here following possibly maritime introduction. Most of the diversity occurs within rather than between populations within countries. Our results suggest that ancient trading networks, different origins and commercial introgressions have shaped the modern day diversity of indigenous chicken found in the Middle East and North Africa.

MT39 Diversity analysis of *AvBD* gene region in Japanese quail. T. Ishige*[1], H. Hara[2], T. Hirano[2], and K. Hanzawa[2], [1]*Tokyo University of Agriculture, Setagaya, Tokyo, Japan;* [2]*Tokyo University of Agriculture, Atsugi, Tokyo, Japan.*

The avian β-defensin (*AvBD*) gene region is an important component of the innate immune system, encoding a variety of antimicrobial peptides. The *AvBD* region forms a multigene cluster in a specific region of the chromosome. Comparison of the *AvBD* gene region among various birds suggests defects, duplication (i.e. copy number variation; CNV), and pseudogenesis at many loci. In the zebra finch, *AvBD1* and *AvBD3* overlap, and *AvBD14* is absent. The *AvBD* gene region from certain Galliform birds, namely chicken, turkey, and bobwhite quail, contains *AvBD3*, *AvBD6*, and *AvBD7*, and the latter shows CNV in chickens. We focused on the innate immune system of the Japanese quail (*Coturnix japonica*; *Cj*) with a subclinical infection. We identified the *AvBD* gene region in the Japanese quail (*CjAvBD*) and observed absence of *AvBD3* and *AvBD7*, and an orthologous duplication of *AvBD6* (*CjAvBD101*). We mapped the haplotype of the *CjAvBD* region to examine its diversity. DNA for genomic analysis was extracted from the peripheral blood of 99 randomly selected quails from six inbred lines. Nine alleles of *CjAvBD1* and eight alleles of *CjAvBD12* were detected, respectively. Ten haplotypes, including three that were strain-specific, were found in alleles from both the *CjAvBD1* and *CjAvBD12* loci. On these ten haplotypes, the nucleotide sequences of the *CjAvBD* region (56 to 70 kb) for seven homozygous diplotypes were determined by next-generation sequencing. These seven haplotypes contained between 12 and 16 *CjAvBD* genes, and were composed of 11 common loci: *CjAvBD1*, *-2*, *-4*, *-5*, *-8*, *-9*, *-10*, *-11*, *-12*, *-13*, and *-14*, but lacking *CjAvBD3* and *-7*. Furthermore, up to five CNVs of *CjAvBD101* were observed among the seven haplotypes. The *CjAvBD101* genes were also classified into five types (??, ??, ??, ??, and a pseudogene), based on alignment and phylogenetic analysis. In addition, we detected amino acid substitutions causing net charge mutations that could affect antimicrobial activity, at *CjAvBD4*, *-13*, *-14*, *-101α*, -101β, -101γ, and *-101θ*. These results suggest that the *CjAvBD* gene region is unique among Galliformes, and that its diversity results in functional variation in innate immunity.

Key Words: antimicrobial peptide, genetic diversity, Japanese quail, NGS

MT40 Signature of selection in domestic chicken comparison between the White Leghorn, Indonesian and Langshan chicken. T. Goto*[1,2], R. A. Lawal[1], D.-D. Wu[3], Y.-P. Zhang[3,4], P. M. Hocking[5], D. W. Burt[5], and O. Hanotte[1,6], [1]*The University of Nottingham, Nottingham, UK;* [2]*Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan;* [3]*Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China;* [4]*Yunnan University, Kunming, China;* [5]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK;* [6]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia.*

We report here the analysis of full genome sequences of four chicken populations (White Leghorn n = 3, Langshan n = 8, Sumatera n = 5 and Kedu Hitam n = 10) for the identification of candidate region under positive selection. With an average of 5.9-fold genome coverage per bird 16,495,644 bi-allelic single nucleotide polymorphisms (SNPs) on chromosomes 1–28 were detected in the dataset (GATK Best Practices). To detect signatures of selection, the Z transformations of pooled heterozygosity (ZHp) in 10 kb sliding 20-kb windows (total 92,168 windows) were used with a significant threshold ZHp > -4. Combining all populations together, 243 significant windows were detected. Excluding the White Leghorn, which produces pure white coloured egg in contrast to the other three breeds, the number of significant window was 245. By comparing them, we identified 100 significant non-White Leghorn specific windows. These windows define 33 candidate regions on chromosomes 1–5, 7, 10, 15, 20, 22–23, and 27. Of these 33 regions, three (chromosomes 1, 2, and 3) overlap with eggshell colour QTLs regions. These results illustrate the usefulness of comparative population genomics approaches to understand the genetic basis of phenotypic diversity in chicken.

Key Words: chickens, eggshell coloration, population genomics, signature of selection, genome sequence

MT41 Identification of candidate genes and variants associated with resistance to Marek's disease virus. J. Smith*[1], E. Lipkin[2], M. Soller[2], J. Fulton[3], and D. Burt[1], [1]*The Roslin Institute, Midlothian, Edinburgh, UK;* [2]*The Hebrew University of Jerusalem, Jerusalem, Israel;* [3]*Hy-line International, Dallas Center, IA, USA.*

Marek's disease (MD) is a major disease affecting poultry health and welfare, with estimated annual global losses of US$2 billion. MD virus (MDV) is a highly contagious, cell-associated, oncogenic α-herpesvirus associated with T-cell lymphomas. Having been controlled by vaccination for over 40 years, growing evidence indicates that intensive use of vaccines is driving the virus to increasing virulence, and new, more virulent strains of the disease continue to emerge. Other means of controlling the disease are therefore necessary. Identification of QTL regions (QTLR), genes and mutations responsible for genetic resistance would be a significant step towards direct selective breeding programmes for birds able to withstand viral infection. Genes encoded in the MHC locus have long been known to contribute to MD resistance with a rough hierarchy of resistance between haplotypes, with B21 most resistant and B19 generally most susceptible. Despite this, other genes also have a strong influence on MD resistance. However, to date, only a handful of genes have been implicated in resistance to MDV. Using a variety of genetic and genomic techniques (GWAS, genomic variation analysis, RNAseq, pathway/network analysis and genotyping and association analysis) within commercial lines, we have identified QTLR associated with resistance, defined potential candidate genes and pinpointed variants showing significant association with the resistant phenotype.

Key Words: poultry, QTL, functional genomics, animal health, disease resilience

MT42 Three differential expression clusters showed acclimation mechanism of White Pekin duck under heat stress. J.-M. Kim[1], K.-S. Lim[1], K.-T. Lee[1], Y.-R. Yang[2], H.-H. Chai[1], J. Hwangbo[1], B.-H. Choi[1], D. Lim[1], Y.-H. Choi[2], and J.-E. Park*[1], [1]*National Institute of Animal Science, Rural Development Administration, Wanju-gun, Jeollabuk-do, Republic of Korea;* [2]*Gyeongsang National University, Jinju-si, Gyeongsangnam-do, Republic of Korea.*

White Pekin duck is an important meat resourcein the livestock industries. However, the temperatureincrease due to global warming has become a serious environmental factor in duckproduction,because ofhyperthermia. Therefore, identifying the gene regulationsand understanding the molecularmechanismfor adapting to the warmer environment will provide insightful information on

the acclimation system of ducks. This study examinedtranscriptomic responses to heat stress treatments (3 and 6 h at 35°C) and control (C, 25°C) using RNA-sequencing analysis of genes from the breast muscle tissue. Based on three distinct differentially expressed gene (DEG) sets (3H/C, 6H/C and 6H/3H), the expression patterns of significant DEGs (absolute log2 >1.0 and false discovery rate < 0.05) were clustered into three responsive gene groups dividedintoup-regulated and down-regulated genes. Next, we analysed the clusters that showed relatively higher expression levels in 3H/C and lowerlevels in 6H/C with much lower or opposite levels in 6H/3H;we referred totheseclusters asthe adaptable responsive genes group. These genes were significantly enriched in theErbB signalling pathway, neuroactive ligand-receptor interaction and type II diabetes mellitus in the KEGG pathways ($P < 0.01$). According to the functional enrichment analysis and significant regulated genesobservations in the enriched pathways, we think that the adaptable responsive genes are responsible for the acclimation mechanism of ducks and suggest that the regulation of phosphoinositide 3-kinasegenes including*PIK3R6*, *PIK3R5*and*PIK3C2B*has animportant relationship with the mechanisms of adaptation to heat stress in ducks.

Key Words: Pekin duck, heat stress, acclimation, expression

MT43 Molecular genetics unveiled unknown family relationships and hybrids in an ex-situ colony of African penguins (*Spheniscus demersus*). P. Modesto*[1], C. Biolatti[1], L. Favaro[2], S. Colussi[1], S. Peletto[1], S. Piga[3], M. V. Riina[1], D. Pessani[2], E. Trincas[3], V. Isaja[3], and P. L. Acutis[1], [1]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy;* [2]*Laboratorio di Zoologia e Biologia Marina, Dipartimento Scienze della Vita e Biologia dei Sistemi, Turin, Italy;* [3]*Zoom Torino, Turin, Italy.*

The genealogical relationships among colony members, the inbreeding status and the presence of hybrids are crucial data and can assist zoo curators in captive colony management and decision-making on relocation for reproduction. Aim of this study was to employ molecular markers to study a large colony of African penguins hosted in an Italian biopark. DNA was extracted from blood samples of 56 penguins. Sex of each animal was determined through the amplification of the chromo-helicase-DNA-binding1 (*CHD1*) gene. A panel of 15 STRs was selected and statistics of gene variation at 15 loci and inbreeding coefficient were calculated. Genotype data were analysed through the COLONY software to determine parentage relationships and to compare the existing studbook information to a pedigree built from genetic analyses. Moreover, the existence of extra-pair mating and the presence of hybrids were investigated. Several discrepancies in kinship relationships emerged after molecular parentage analysis. Moreover, 11 unknown genetic relationships were revealed. Infidelity of one member of the pair was observed in six cases and, in two episodes, extra-pair copulation was assessed by genetic analysis. We found that a member of the colony was a hybrid and his progeny, derived by extra-pair copulation, was traced. Additionally, we found and assessed other three hidden hybrids using the identified candidate private alleles. Overall, our results demonstrated that the use of molecular methods to confirm parentage and to analyse relatedness among colony members could complement studbook-based genetic management of African penguin captive populations. Relying on studbook information only is not recommended considering that in some species a variety of behavioural dynamics (e.g. extra-pair mating) can make observations ineffective and that molecular markers outperform studbook in identifying the presence of hybrids.

Key Words: wild species, genotyping, population identification, parentage

MT44 Genomic variability in Mexican chicken population. M. G. Strillacci*[1], E. Gorla[1], M. C. Cozzi[1], S. I. Roman-Ponce[2], F. J. Ruiz[2], V. E. Vega-Murillo[2], F. Bertolini[3,4], L. Fontanesi[3], S. Cerolini[1], and A. Bagnato[1], [1]*Department of Veterinary Medicine, University of Milan, Milano, Italy;* [2]*Centro Nacional de Investigación en Fisiología y Mejoramiento Animal, Col. Centro Veracruz, Mexico;* [3]*Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Bologna, Italy;* [4]*Department of Animal Science, Iowa State University, Ames, IA, USA.*

Copy number variations are an important source of genetic polymorphism in populations. Genetic variation enables both adaptive evolutionary changes and artificial selection. In Mexico poultry population is a widespread Creole chicken (*Gallus gallus domesticus*), an undefined cross among different breeds brought to Mexico from Europe under natural selection and adaptation for almost 500 years. The aim of this study was to investigate the genomic variation in the Mexican chicken population using CNVs and SNP genotype data to reveal any underlying population structure. A total of 256 DNA samples coming from different rural production units located in 25 states of Mexico were genotyped with Axiom Genome-Wide Chicken Genotyping Array (Affymetrix) and used in the analyses. After stringent quality control the individual-based CNV calling was performed by the Hidden Markov Model (HMM) of PennCNV software on autosomes. CNV were summarised to CNV regions (CNVRs) at a population level (i.e. overlapping CNVs), using BEDTools. A total of 1,924 CNVs in the genome of 256 samples were detected (1,538 gains and 386 losses) resulting al population level in 1,216 CNV regions, of which 959 gains, 226 losses and 31 complex ones (i.e. containing both losses and gains), covering a total 5,12% (47 Mb) of the chicken galGal4 assembly autosome. A comparison among CNV lengths was performed. The highest number of gain CNVRs are those with a length of 10–100 Kb, while the loss CNVRs have generally a length of 1–10 and the complex CNVR have mainly a length of 10–100 Kb. Identity by Descent (IBD) estimation and the Principal Components Analysis (PCA) were performed using SVS Golden Helix 8.4 software. The ADMIXTURE *v.* 1.3.0 software was used to infer the most probable number of ancestral populations based on the SNP genotype data. ADMIXTURE analyses identified three ancestors and the proportion of each of them has been determined in each individuals. The results of the NJ analysis was consistent with the one obtained by the PCA. A total of 3,059 Run of Homozygosity (ROH), mainly short in length, indicated a low inbreeding level and selective pressure on in the Mexican chicken genome. Co-funded by project M01678 - Ministry of Foreign affairs of Italy and Mexico.

Key Words: poultry, CNV, ROH, genetic diversity

MT45 Response of the hepatic transcriptomes of domesticated and wild turkey to aflatoxin B_1. K. Reed*[1], K. Mendoza[1], J. Abrahante[1], and R. Coulombe[2], [1]*University of Minnesota, St. Paul, MN, USA;* [2]*Utah State University, Logan, UT, USA.*

Domesticated turkeys (*Meleagris gallopavo*) are one of the most susceptible animals known to aflatoxin B1 (AFB_1), which poses a worldwide animal health and food safety problem. This mycotoxin is a potent human and animal hepatocarcinogen and is hepatotoxic and immunosuppressive in animals causing significant economic losses to agriculture. Eastern wild turkeys (*M. gallopavo silvestris*) are far less susceptible than domesticated birds due primarily to functional hepatic glutathione S-transferases, the principal route of AFB_1 detoxication. Utilising RNA-sequencing (RNA-seq), the effects of AFB_1 on genome-wide expression in liver was compared in domesticated and wild birds. Acclimated poults were challenged with 300 ppb AFB_1 in the diet for 10 days then tissue samples collected. RNA libraries were prepared for challenged and control birds and sequenced to produce ~12 M 101 bp paired-end reads per library. Reads were mapped to the annotated turkey

genome (UMD 5.0) and differential expression analysis was used to contrast the effects of AFB_1 exposure in domesticated and wild birds. Transcriptome comparisons identified unique liver profiles in domesticated and wild turkeys with significant variation in the response to AFB_1. Over 5400 genes were significantly affected (FDR pval < 0.05 and $|Log_2FC| > 2.0$) by AFB_1 treatment with a greater number of transcripts altered in the domesticated birds. These results provide insight into changes in AFB_1 response that have occurred during domestication and selective breeding for production and will help elucidate ways to improve poultry resistance to AFB_1. Supported in part by Agriculture and Food Research Initiative Animal Genome competitive grant 2013–67015–21241 of the USDA National Institute of Food and Agriculture.

Key Words: turkey, aflatoxin, RNA-seq, transcriptome

MT46 Genetic diversity and LD size between commercial and native chickens in Korea. D. Seo*, D. Lee, N. Choi, S. Jin, P. Sudrajad, S. H. Lee, and J. H. Lee, *Division of Animal and Dairy Science, Chungnam National University, Daejeon, South Korea.*

Native chickens have low productivity than commercial broilers. However, these chickens become more popular due to the unique texture and taste characteristics. Genetic diversity and linkage disequilibrium (LD) size were investigated in this study for identifying genetic relationships in order to give basic information for the native chicken populations. 600K high-density SNP array was used using 187 native chicken samples from 14 different chicken lines. The results of genetic diversity indicated that most of the native chicken lines were independently clustered with commercial native chickens. The r^2 values of LD size in each population were calculated and the results indicated that most of the native chicken populations have higher LD values than that of the commercial native chicken populations. With further analysis, these results can provide valuable information for the breeding strategy using the native chicken breeds.

Key Words: genetic diversity, linkage disequilibrium, native chicken, commercial native chicken

MT47 Mapping QTLs affecting Marek's disease by selective genotyping in F6 of full-sib intercross population. E. Lipkin*[1], J. Smith[2], D. Burt[2], M. Soller[1], and J. Fulton[3], [1]*Dept. of Genetics, Silberman Life Sciences Institute, The Hebrew University of Jerusalem, Jerusalem, Israel;* [2]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Midlothian, UK;* [3]*Hy-Line International, Dallas Center, IA, USA.*

Marek's disease (MD), a lymphoma caused by an avian herpesvirus, is a major disease affecting the poultry industry. One way to reduce MD prevalence is genetic selection. Genetic progress can be accelerated by using genetic markers associated with the disease. The aim of the present study was to map QTLs, genes and mutations affecting chicken MD susceptibility. A full-sib intercross population was generated by two partially inbred commercial White Leghorn layer lines differing in genetic resistance to MD. At the F6 generation, a total of 1,615 chicks from five independent families were phenotyped for MD resistance. Phenotypic tails from the five families were used in a GWAS based on survival test. Moving average of -LogP and Log drop were used to define QTL regions (QTLRs). A total of 159 QTLRs were found among the five families, averaging 32 QTLs per family and ranging from 23 to 41. Overlap and proximity were used to condense the list to 138 QTLRs shared among families. RNASeq and bioinformatics analyses of the QTLRs identified differentially expressed and functional candidate genes, and protein Loss of Function (LoF) mutations. Putative quantitative traits genes (QTG) and candidate causative nucleotides (QTN) were identified. Markers from the candidate genes and the LoF mutations were used to genotype 9,391 males with MD progeny tests from eight lines across 16 generations. Association analysis confirmed most of the QTLs found in F6. An independent study based on selective DNA pooling, reported elsewhere at this meeting, confirmed most of the QTLs found in the F6 population.

Key Words: poultry, animal health, genome-wide association, complex trait, infectious disease

MT48 Copy number variation in *SOX6* contributes to chicken muscle development. X. Zhang*, *Department of Animal Genetics, Breeding and Reproduction, College of Animal Science, South China Agricultural University, Guangzhou, Guangdong, China.*

Copy number variations (CNVs), covering a large number of functional genes, were associated with the complex diseases and phenotypic diversity. In previous study, 5 individual variation of White Rock Resistance (WRR) chicken (CN = 1, CN = 3) and two individual variation of Xinghua (XH) (CN = 3) chicken were found in the CNP13 region (chr5:10500294–10675531) which overlaps with *SOX6*. It has been shown that Sox6 plays a key role in fast-twitch muscle fibre differentiation in the zebrafish. However, the role of *SOX6* on chicken skeletal muscle development is still unknown. In current study, Acucopy and CNVplex were used to characterise the CNVs phenotypes in XH and WRR chickens. Real-time quantity PCR (RT-qPCR) was used to verify the candidate copy number polymorphisms (CNPs) and to detect the relative expression of genes. The overexpression and knockdown of *SOX6* in QM-7 cells were used to study the function of *SOX6* in skeletal muscle development. As a result, 15 CNPs were significantly related to different traits on genome level, of which, 12 were associated with growth traits, 4 were with carcass traits, and 6 were with meat quality traits. Five WRR (CN = 1, CN = 3) variant individuals and 2 XH (CN = 3) variant individuals were found in the CNP13 region (chr5:10500294–10675531) which overlaps with *SOX6*. *SOX6* was expressed in 16 tissues of XH and WRR chicken, with higher expression levels in the chest, leg muscle, pituitary, heart, cerebellum and kidney. Notably, the expression of the *SOX6* mRNA was associated with *SOX6* copy number variation. Interestingly, bioinformatics analysis of SOX6 protein showed that the amino acid sequence (265–579 aa) region, coded by overlapping partial CNV region, is a disordered region. Moreover, the QM-7 cells were significantly decreased in G1 phase and arrested in S phase after overexpression of *SOX6*. The *SOX6* is highly expressed during the QM-7 cell differentiation, the same as muscle different marker genes *myognin* and *MYHC* are. Surprisingly, after the knockdown of the *SOX6*, the expression levels of *IGFIR1, MYF6, SOX9, SHOX* and *CCND1* were significantly down-regulated, indicating that *SOX6* can influence these genes to promote the proliferation of muscle cells.

Key Words: chicken, CNV, SOX6, skeletal muscle, disorder region

MT49 Phenotype and multi-tissue transcriptome response to diet changes in laying hens. M. Brenet[1,2], A. Rau[3], C. Désert[1,2], M. Boutin[1,2], K. Muret[1,2], S. Leroux[4], D. Esquerre[5], C. Klopp[6], D. Gourichon[7], F. Pitel[4], T. Zerjal[3], and S. Lagarrigue*[1,2], [1]*INRA, UMR1348, PEGASE, St Gilles, France;* [2]*Agrocampus-Ouest, UMR1348, Rennes, France;* [3]*INRA, AgroParisTech, Université Paris-Saclay, UMR GABI, Jouy-en-Josas, France;* [4]*INRA/INPT ENSAT/INPT ENVT, GenPhySE, Castanet Tolosan, France;* [5]*INRA, Plateforme GENOTOUL, Castanet-Tolosan, France;* [6]*INRA, SIGENAE, Castanet-Tolosan, France;* [7]*INRA, PEAT, Nouzilly, France.*

Adaptation to feed changes in laying hens is particularly important to promote innovation, in selection schemes and in dietary solutions, for the sustainability of the egg-production sector. In Eu-

rope and USA poultry feed is rich in cereals, while Asian countries privilege by-products to soybean meal, resulting in low energy diets. In this study, we investigated the effects of a sub-optimal low energy diet on different traits and multi-tissue transcriptomes of brown egg layers of 2 divergent lines selected for low (R-) and high (R+) residual feed intake. The 2 diets had a similar protein content, while the energy content was reduced by 15% as compared to the standard diet (2450 Kcal versus 2800 Kcal). The R+ and R- hens were fed *ad libitum*, with the standard diet until 17 wk of age; half of the birds were fed with the low energy feed until 33 wk of age when a subset of birds was slaughtered for tissue sampling (8 per line and diet). Food intake was increased in response to the suboptimal diet whereas egg number was unchanged showing that birds were able to adjust their energy intake by modifying feed intake. Nevertheless, hens fed the low energy diet had a higher feed efficiency (pval < 5%) and a higher residual feed intake (*P*-value < 1%). No diet × line interaction was observed for these traits. PolyA+ RNA from different tissues were sequenced resulting in 90 M reads per sample. After bioinformatics treatment and differential analysis, we observed in liver and adipose tissue only few differentially expressed genes (DEG) between diets (16 and 21 respectively). In contrast, we observed in blood 1179 DEG out of 17123 expressed genes with 463 and 716 over and under expressed in the suboptimal diet compared to the standard diet. No diet × line interaction was observed in the three tissues. GObp term enrichment revealed that under expressed DEG in blood were associated with glucose catabolism, cholesterol biosynthesis, mitotic cell cycle and protein catabolic process. Taken together, these results indicate an adaptation of birds to diet changes by increasing feed intake to maintain egg production, and a tissue-specific response with a limited role of metabolic tissues as liver and adipose tissue compared to the blood.

Key Words: poultry, functional genomics, RNA-seq, adaptation, diet

MT50 Addition to the chicken W-chromosome specific repetitive landscape. A. Saifitdinova, S. Galkina*, A. Dyomin, M. Kulak, E. Koshel, and E. Gaginskaya, *Saint-Petersburg State University, Saint-Petersburg, Russia.*

In many species, sex determination is genetic and often accompanied by the presence of dimorphic sex chromosomes in the karyotype. Recent progress in genomic sequencing gave the information about the gene content of sex chromosomes which allowed to reveal their origin from ordinary autosomes and to trace their evolutionary history. Unlike other sequenced sex chromosome chicken W chromosome does not contain genes specifically expressed in reproductive tissues. At the same time female-specific W chromosome in birds as well as mammalian male-specific Y chromosome is characterised by the degeneration of gene content and the accumulation of repetitive DNA. Despite the best efforts chicken W chromosome assembly includes only 7 Mb of expected 55 Mb, because tandem repeats complicate the analysis of genomic data and assembling. Repetitive DNA occupies more than two thirds of the chicken W chromosome. In the composition of chicken W chromosome three major repetitive families *Xho*I, *Eco*RI and *Ssp*I have been characterised. Recently we identified $(GGAAA)_n$ tandem repeat and assigned it by FISH into two sites in chromosome W. Here, we performed a structural analysis of the borders between assembled scaffolds and extended flanking areas enriched with $(GGAAA)_n$. For this, we used sequences of BAC clones CH261–114G22 (AC182258.2) and CH261–75N4 (AC175832.2) co-localised with the repeat by high resolution FISH on lampbrush chromosomes and the last version of W chromosome assembly. We studied also the distribution of $(GGAAA)_n$ short stretches in the chicken genome. The potential role of homopurine sequences as regulators of tissue-specific gene expression is discussed. Financial and technical support: RFBR #16–04–01823, Research Resource Centre 'Chromas' of Saint Petersburg State University.

Key Words: poultry and related species, sex determination, genome assembly

MT51 MicroRNAs associated with high rate of egg production in chicken ovaries. U. Gaur*, N. Wu, M. Yang, and D. Li, *Sichuan Agricultural University, Chengdu, Sichuan, China.*

Chicken (*Gallus gallus*) is an important agricultural and avian-model species, which is a major source of protein worldwide. However, a role for miRNAs in chicken ovarian development has not hitherto been reported clearly. The characteristics of ovarian tissue are highly related with reproductive and economic traits of chicken, so it is necessary to identify and characterise the miRNA in ovarian tissue. In this study, we performed the first miRNA analysis of low and high egg production chicken ovarian tissues at 300 days of age using high-throughput transcriptome sequencing. By comparing low egg production (LP) chickens with high egg production chickens (HP), 17 significantly differentially expressed miRNAs were found ($P < 0.05$), including 11 known and 6 novel miRNAs. We found that all 11 known miRNAs were mainly involved in pathways of reproduction regulation, such as steroid hormone biosynthesis and dopaminergic synapse. Additionally, expression profiling of randomly selected six differentially regulated miRNAs were validated by quantitative real-time polymerase chain reaction (qRT–PCR). Some miRNAs such as gga-miR-34b, gga-miR-34c and gga-miR-216b were reported to regulate processes such as proliferation, cell cycle, apoptosis and metastasis and expressed differentially in HP chicken ovaries, suggested they have an important role in ovaries development and reproductive management of chicken. Furthermore, we uncovered a significantly up-regulated miRNA: gga-miR-200a-3p, which is ubiquitous in reproduction regulation related pathways. This miRNA might play a special central role in the reproductive management of chicken.

Key Words: microRNA, chicken, ovary, Illumina sequencing, qRT-PCR

MT52 Genome wide association study of complex traits in response to Newcastle Disease Virus in chickens. K. Rowland*[1], H. Zhou[2], R. Gallardo[3], T. Kelly[2,3], A. Wolc[1,4], and S. J. Lamont[1], [1]*Iowa State University, Department of Animal Science, Ames, IA, USA;* [2]*University of California-Davis, Department of Animal Science, Davis, CA, USA;* [3]*University of California-Davis, School of Veterinary Medicine, Davis, CA, USA;* [4]*Hy-Line International, Dallas Center, IA, USA.*

Newcastle disease (ND) causes up to 80% mortality in chickens in developing countries where velogenic NDV strains are endemic. Genetic improvement of disease resistance, complementary to vaccination, has the potential to be an important tool to reduce the impact of NDV. We hypothesise that many genes regulate NDV response in chickens. Our specific objective was to identify genetic markers associated with NDV resistance (reduced viral load, high antibody titer) so these markers can be applied in a genetic selection program benefiting areas of endemic NDV challenge. The experiment was replicated across three hatches from 150 dams of a commercial egg-laying line, Hy-Line Brown. We inoculated with NDV, La Sota strain, on day 21 of age by an ocular-nasal route. Virus load was estimated from viral mRNA level in lachrymal fluid by qRT–PCR. Systemic antibody response to NDV in serum was measured by ELISA. Genomic DNA was genotyped via Affymetrix 600K chicken SNP array. Analyses of viral RNA and antibody levels confirmed response of challenge groups to the virus and lack of response in control groups. ASReml estimated heritabilities of 0.34, 0.34, 0.105, 0.117, 0.19, and 0.05 for hatch weight, day 31 body weight, 0 and 10 days post-infection (dpi) antibody levels, and 2dpi

and 6dpi viral load, respectively. Genome-wide associations with 250 kb windows were tested using the Gensel program. BayesC was used to estimate variance components, subsequently, BayesB (pi=0.999) tested associations. Several QTL regions were found to confirm previously reported QTL for these traits. Identification of novel SNPs and regions will provide insights into genetic control of response to NDV infection in chickens as well as the genetic architecture of these complex traits. Targeted genotyping and single SNP association tests may further elucidate genetic mechanisms that influence host response to NDV infection. Support: USAID Feed the Future Innovation Lab for Genomics to Improve Poultry, Hy-Line International, and Hatch project #5357.

Key Words: poultry and related species, genome-wide association, genotyping, disease resilience, genetic improvement

MT53 Goose transcriptome provides insights into novel mechanisms of adipogenesis. G. Wang*[2,1], Y. Liu[1], L. Jin[1], D. Shang[1], C. Gill[2], M. Li[1], and J. Wang[1], [1]*Sichuan Agricultural University, Chengdu, Sichuan, China;* [2]*Texas A&M University, College Station, TX, USA.*

Goose is a widespread poultry species with large markets in several countries including China and France. Because geese are migratory birds, their metabolism has adapted to acquire a highly tolerable balance between the rapid intake of massive amounts of glucose before flight and energy consumption during flight. An understanding of the molecular basis of metabolism in geese will strengthen development of the consumer market worldwide, and could provide insights into human obesity-related metabolic diseases. The objective of this study was to investigate the network of genes affecting lipogenesis in geese. Our approach was to use RNA-seq to compare the transcriptomes of 3 tissues involved in adipogenesis (liver, abdominal adipose and subcutaneous adipose) from 3 geese fed a control diet and 3 geese fed a high-energy diet. RNA-seq reads were aligned to the goose genome sequence with Tophat. Between the case and control groups, we identified ~1,900 differentially expressed coding genes for liver, ~800 and ~500 differentially expressed coding genes for abdominal adipose and subcutaneous adipose, respectively. We found there was significant enrichment for metabolic-related pathways among the up-regulated genes. Interestingly, several cancer pathways, including the PI3K-AKT signalling pathway were significantly enriched among the down-regulated genes. Using cuffcompare, CPC, and cmscan, we also identified ~2,000 long non-coding RNAs among the 3 tissues. Several of these lncRNAs were located near key genes in the enriched pathways, indicating they are potentially cis-acting regulatory elements. These results suggest there may be a self-protection mechanism involved in metabolic adaptation in the goose.

Key Words: goose, transcriptome, adipogenesis

MT54 The broiler chicken transcriptome. C. Schmidt*[1] and S. Lamont[2], [1]*University of Delaware, Newark, DE, USA;* [2]*Iowa State University, Ames, IA, USA.*

Next-generation RNA-seq transcriptome analysis provides a deep description of the genes expressed in tissues. We have sequenced over 10 billion Illumina reads from multiple tissues isolated from the Ross 708 broiler line of chickens. Tissues sampled include the pituitary, hypothalamus, retina, pineal, cerebellum, heart, breast muscle, liver, spleen, intestine and adipose tissue. Relative tissue analysis has been used to compare between tissues and identify known and novel sequences that are enriched in each examined tissue. In addition, we have analysed the impact of chronic heat stress on gene expression patterns in these broiler chickens. These studies have identified genes up or down-regulated by heat stress and the pattern of responsive genes varies across organs. Finally, a combination of transcriptomic and metabolomic approaches has provided an integrated description of the liver's response to chronic heat stress in the Ross 708 birds.

Key Words: chicken, transcriptome, metabolome

MT55 The evolving chicken genome reference. W. Warren*[1], L. Hillier[1], C. Tomlinson[1], P. Minx[1], M. Kremitski[1], T. Graves[1], S. Sullivan[2], I. Liachko[2], M. Delaney[3], J. Fulton[4], M. Abrahamsen[5], R. Hawken[5], M. Miller[6], and H. Cheng[7], [1]*Washington University School of Medicine, St Louis, MO, USA;* [2]*Phase Genomics Inc., Seattle, WA, USA;* [3]*University of California-Davis, Davis, CA, USA;* [4]*Hy-Line International, Dallas Center, IA, USA;* [5]*Cobb-Vantress Inc., Siloam Springs, AR, USA;* [6]*Beckman Research Institute, Duarte, CA, USA;* [7]*USDA-ARS, East Lansing, MI, USA.*

Given the continued interest in further improvements to the chicken genome reference (Gallus_gallus-5.0) we have built a version Gallus_gallu-6.0 from longer single molecule read technology (SMRT) reads. Most assembled contigs were ordered and oriented (i.e. scaffolded) using a proximity-based map into 39 scaffolds at 94% of the assembly size; 865 contigs remained as unplaced. The scaffolds and contig sequences were aligned to the Gallus_gallus-5.0 reference or the published chicken genetic linkage map in order to break chimeric scaffolds and contigs. We found 87 scaffolds and 28 contigs required manual breaks due to *de novo* assembly or scaffolding error. At this phase Gallus_gallus-6.0 assembled bases were 1.05 Gb, with an N50 contig and scaffold lengths of 18 and 35 Mb, respectively. Of the 1.05 Gb genome, ~94% of the assembled bases have been anchored to chromosomes. Next steps will be to assign large unplaced contigs to the remaining unknown autosomes after validating no genetic linkage exists to any known autosomes. In addition, we plan to add novel reference sequence to clusters of immune system genes (MHC) that are challenging to *de novo* assemble and genotype. Furthermore, copy number variation within MHC genes are likely to play an important role in the host immune responses, thus, we will present local assemblies of phased haplotypes from commercial birds chosen for the homozygous state of MHC-B and MHC-Y regions. The Gallus_gallus-6.0 reference is a substantial improvement in base contiguity and autosomal assignment. However, the use of more targeted approaches will be required to elevate the chicken genome to levels comparable to human.

Key Words: chicken, genome, MHC

MT56 Liver and whole blood transcriptome response to chronic heat exposure in laying hens. F. Jehl[1], A. Rau[1], C. Désert[2,3], M. Boutin[2,3], K. Muret[2,3], S. Leroux[4], D. Esquerré[5], C. Klopp[6], D. Gourichon[7], F. Pitel[4], A. Collin[8], S. Lagarrigue[2,3], and T. Zerjal*[1], [1]*GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [2]*INRA, UMR1348 Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'élevage, Saint-Gilles, France;* [3]*Agrocampus-Ouest, UMR1348, Rennes, France;* [4]*UMR INRA/INPT ENSAT/INPT ENVT - GenPhySE, Castanet Tolosan, France;* [5]*INRA, Plateforme GENOTOUL, Castanet-Tolosan, France;* [6]*INRA, SIGENAE, Castanet-Tolosan, France;* [7]*INRA-PEAT, Nouzilly, France;* [8]*URA, INRA, Nouzilly, France.*

Adaptation to heat exposure is required to maintain animal welfare and productivity under high ambient temperature (AT) conditions. In this study we investigate the effects of chronic heat exposure (5 weeks at a constant temperature of 32°C) on the liver and whole blood transcriptome of brown egg layers from 2 divergent lines selected for low (R-) and high (R+) residual feed intake. The R+ and R- hens were equally distributed among 2 temperature-controlled chambers and reared under thermo-neutrality (22°C). At 28 wk of age the AT of one chamber was increased to 32°C until 33 wk of age, when 32 animals (8 per line and treat-

ment) were slaughtered. Total RNA was obtained from the liver and blood and was sequenced using the Illumina HiSEqn 3000, yielding an average per sample of 90 million paired-end reads. The reads were mapped to the *Gallus gallus*-5 reference genome by STAR software and counted by RSEM software using the Ensembl V87 GTF annotation. Comparisons between the two AT groups were made using the *edgeR-robust* R/Bioconductor package. Patterns of AT-specific differential expression were largely shared by the two lines, and no evidence of temperature × line interactions were observed. In liver, a total of 229 differentially expressed genes (DEG) were identified (*adjusted p*-values < 0.05) with respectively 104 and 125 over and under expressed in the heat-exposed compared to the control group. In blood, 960 DEG were identified between the two AT groups with 479 and 481 over and under expressed. Most DEG were tissue specific, and only 18 genes were DE in both liver and blood. Ingenuity Pathway Analysis revealed that many of the DEG in liver were associated with amino acid and lipid metabolisms and energy production. Key genes involved in *fatty acid β-oxidation, ketogenesis, cholesterol biosynthesis were under-expressed in the heat-exposed animals.* In blood, many of the DEG were associated with cell related functions. Based on the DEG expression profile, down-regulation was observed for the PI3K/AKT, the VEGF and the PDGF signalling pathways involved in cell survival and growth, vasculogenesis and angiogenesis. Taken together, these results indicate a tissue-specific response to heat exposure.

Key Words: heat exposure, liver, blood, RNA-seq, laying hens

MT57 The not-so-missing genes in birds. T. Hron*, H. Farkasova, P. Pajer, J. Paces, P. Bartunek, and D. Elleder, *Institute of Molecular Genetics of the AV CR, v.v.i., Vídenská, Prague, Czech Republic.*

Thanks to the advances in high throughput sequencing methods and data analysis, genome of the huge number of species has been recently read and assembled. Despite of this, subset of essential and well-described mammalian genes, such as erythropoetin, leptin or TNF α, have not been identified in birds and their existence has been controversial for a long time. After thorough analysis of combined sequencing data with high coverage, we were able to identify some of these genes and thus prove their existence. Interestingly, all these 'missing' genes are characterised by exceptionally high GC content and long G/C stretches. Such characteristics cause difficulties in PCR amplification preventing efficient sequencing and can, therefore, lead to the absence of these sequences in databases and genome assemblies. This observation is probably general and seems to apply for a significant portion of avian genes thought to be missing. In all genes we analysed, the GC richness was observed exclusively in the avian orthologs and not in the orthologs of other species. This arises the question what is the cause of such an extreme evolutionary driven accumulation of GC nucleotides in this subset of avian genes. We hypothesise that these regions are important for formation of G-quadruplex structures involved in the pairing of homologous chromosomes during meiosis. However, this possibility has to be further investigated. Our work also demonstrates that sequences biased in their nucleotide content are often underrepresented in sequencing data. Thus, genome assemblies should be treated with caution.

MT58 Integrating genome and transcriptome profiling for dissection of the mechanism of muscle growth and lipid deposition in ducks. L. Wang*, X. Li, J. Ma, Y. Zhang, and H. Zhang, *Lab of Animal Genetic Resource and Molecular Breeding, China Agricultural University, Beijing, P.R. China.*

The objectives here were to detect candidate genes on muscle growth and lipid deposition in Pekin ducks through comprehensive analysis combining whole genome sequencing (WGS) and RNA-seq. Pekin duck is famous for fast growth and high intramuscular fat (IMF). Cherry Valley Pekin duck (CD) had higher growth rate, whereas native Pekin duck (BD) deposited more lipids. Combing of WGS and RNA-seq has been widely used to explain genetic basis of different phenotypes. 80 individuals (40 BD and 40 CD) were slaughtered at 3- and 6-weeks of age. Phenotypes of water and IMF content, density and diameter of breast fibre were measured (n = 20). Genomic DNA was resequenced on Illumina HisEqn 4000 platform with PE-150bp. Transcriptomic data of breast were sequenced in HisEqn 2000 platform. BD ducks displayed higher IMF content than CDs at 6-weeks of age, while CD ducks displayed significant differences in both density and diameter of muscle fibres than BDs at 3-weeks. After screening for genomic variations, 206 positively selected genes (PSGs) from 224 windows were detected. Analysis of differentially expressed genes (DEGs) between periods detected 195 and 243 DEGs in BD and CD, respectively. At 3-week, the 52 DEGs between breeds were mainly involved in muscle tissue development. At 6-week, the 206 DEGs were mainly involved in PPAR and adipocytokine signalling pathways. After integrated analysis, 7 genes, both PSGs and DEGs, including PODXL, KY, FABP5, FLT1, ENPP3, NGEF, and NEU2 were confirmed. Another seven PSGs (TTN, TNNI2, CDH2, MEF2A, INSIG1, PCSK1, and PLPPR1) and five DEGs (FoxO3, MAT1A, TNNT2, ADORA1, and ACSL1) were also detected. Finally, we screened 19 candidate genes, of which, 11 (PODXL, KY, FLT1, NEU2, TTN, TNNI2, CDH2, MEF2A, FoxO3, MAT1A, and TNNT2) were identified to regulate muscle growth involving in pathways like muscle organ development and muscle organ maintain. Eight genes (FABP5, ENPP3, NGEF, INSIG1, PCSK1, PLPPR1, ADORA1, and ACSL1) involving pathways like PPAR signalling were identified to regulate lipid deposition.

Key Words: ducks, integrative genomics, candidate gene

MT59 Genome-wide detection of SNPs and CNVs in chickens associated with beak deformity using chicken high-density 600K SNP arrays. H. Bai, Y. Y. Sun, D. L. Li, Y. F. Liu, F. G. Xue, Y. L. Li, S. S. Xu, A. X. Ni, and J. L. Chen*, *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Beak deformity (crossed beaks) was found in several indigenous chicken breeds including Silkies and Beijing-You (BJY) studied here. Birds with deformed beaks have reduced feed intake, poor production performance. The welfare was also severely inparied as the nature behaviour performed by the aiding of beak was not possible. Previous studies have shed light on the genetic regulations of this malformation. However, the detail genetic basis remains unclear. In this study, a total of 48 beak deformity (case) and 48 normal (control) birds were genotyped using the high-density 600K SNP genotyping array to detect genome-wide SNPs and CNV associated with beak deformity trait with the assistance of ROADTRIPS and PennCNV analysis software, respectively. As a result, 95 individuals and 429,539 SNPs were obtained after quality control. *P*-value was corrected by a Bonferroni adjustment based on linkage disequilibrium (LD) pruning. The case-control association study identified one associated SNP (5% genome-wide significance) and seven suggestively associated SNPs. Four high-confidence genes, *LOC421892*, *TDRD3*, *RET*, and *STMN1*, were identified as the most promising candidate genes for beak deformity. Two and 8 high confidence CNV regions covering 0.25 Mb and 1.9 Mb on autosomal chromosome were identified in beak deformity and normal birds, respectively. Within these regions, 3 and 22 known genes were associated with beak deformity, respectively. Further qRT–PCR studies validated 9 of the 10 CNVs. *MAGI3*, *SLC16A1*, and *LRIG2* genes were only identified in beak deformity birds. *LRIG2* gene could be a key factor due to its known functions in human orofacial syndrome. Moreover, bioinformatics analyses showed that the detected candidate genes were enriched in six GO terms (melanosome, pigment granule, integral to membrane, intrinsic to membrane, protein transport, establishment of protein localization processes) and one

pathway (antigen processing and presentation). Overall, our results provide deep insights into the genomic structure variation underlying beak deformity in poultry.

Key Words: chicken, genome-wide association study, SNP, CNV

MT60 *De novo* genome assembly of *Agapornis roseicollis* and SNP discovery for parentage verification. H. van der Zwan*[1], R. van der Sluis[1], and C. Visser[2], [1]*North-West University, Potchefstroom, North-West, South Africa;* [2]*University of Pretoria, Pretoria, Gauteng, South Africa.*

The African parakeet *Agapornis*, or lovebirds, are globally bred as pets. The main breeding selection criterion is plumage coloration. Birds with rare coloration and their heterozygous offspring (with wildtype coloration) are sold at a premium. Currently, there is no genetic test inferring parentage of the heterozygous offspring, nor has the genes or mutations linked to colour variation been identified. The aim of this study was to discover SNPs to develop a SNP-based parentage verification test for *A. roseicollis* by sequencing, assembling and annotating its *de novo* genome. One young male was selected and its genome sequenced at 100x coverage on the Illumina HiSeq platform. The size of the genome was 1.1Gbp and 15 045 genes were identified. This is comparable to other bird species such as the budgerigar (*Melopsittacus undulates)*. The genomes of the male's parents were sequenced at 30x coverage on the same platform. The parents' reads were mapped to the offspring's reference genome using the Burrow-Wheeler aligner. Making use of the command line Linux-based program Genome Analysis Toolkit (GATK), variants were discovered using the HaplotypeCaller module. Approximately 1.2 million raw SNPs were discovered for the mother and 800 000 for the father. Since it is a non-model organism, hard filtering parameters had to be applied to first extract SNPs and then indels from the raw SNP set. SNPs discovered in indels were discarded. These SNPs were further filtered to include only variants where both parents were heterozygous at that locus indicating heterozygosity from all grandparents. This resulted in a total of 614 700 SNPs after all the filters were applied. SNPs not complying with Mendelian inheritance patterns between the parents and offspring were rejected. Where SNPs was located less than 100bp apart only the SNP with the highest quality score was included. A panel of the top 400 SNPs was selected based on the quality scores obtained during the GATK analyses. This panel will form the foundation for the development of a commercial parentage verification test for lovebirds.

Key Words: lovebird, avian genomics, bioinformatics, *de novo* genome assembly, genomic selection

MT61 Low number of mitochondrial DNA sequences inserted into the turkey (*Meleagris gallopavo*) nuclear genome: Implications for evolutionary inferences. G. Schiavo[1], M. G. Strillacci[2], S. Bovo[1,3], A. Ribani[1], S. I. Roman-Ponce[4], S. Cerolini[2], F. Bertolini[1,5], A. Bagnato[2], and L. Fontanesi*[1], [1]*Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy;* [2]*Department of Veterinary Medicine, University of Milan, Milano, Italy;* [3]*Biocomputing Group, Department of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy;* [4]*Centro Nacional de Investigación en Fisiología y Mejoramiento Animal, Instituto Nacional de Investigaciones Forestales, Agricola y Pecuarias (INIFAP), Col. Centro Veracruz, Mexico;* [5]*Department of Animal Science, Iowa State University, Ames, IA, USA.*

Mitochondrial DNA (mtDNA) insertions have been detected in the nuclear genome of many eukaryotes. These sequences are pseudogenes that derives from the horizontal transfer of mtDNA fragments (both from coding and non-coding regions) into the nuclear genome, producing nuclear DNA sequences of mitochondrial origin, called *numts.* Some of these regions, that derives from recent insertion events in terms of evolutionary time, have high homology with the original mtDNA genome and may affect the interpretation of population genetic and phylogenetic studies based on mtDNA sequences. A few studies have shown that, in general, bird genomes contain a lower number of *numts* than mammalian genomes. At present in the turkey (*Meleagris gallopavo*) genome the frequency of these mtDNA originated pseudogenes is not known. In this study, we filled this gap providing a first picture of *numt* distribution in the genome of this avian species. The turkey reference genome (Turkey_2.01, GCA_000146605.1) was aligned with the reference linearized mtDNA sequence using LAST and BLASTN software. A total of at least 16 *numts* were identified using both software. A few of them were validated by amplifying and then sequencing the corresponding regions in a population of wild turkeys and in commercial and local lines or breeds. Identities between *numts* and the corresponding mtDNA sequences ranged from ~70% to 100%, spanning from ~60 to 500 bp and representing in total only 0.0003% of the whole nuclear genome. These fewer *numts* do not cover the whole mtDNA genome. The largest *numt* was located on chromosome 2 in an intronic region of the *UFL1* gene and was 100% identical to part of the NADH dehydrogenase subunit 5 mtDNA gene (*ND5*). These results confirm that the low number of *numts* in bird genomes probably derives by an evolutionary selection towards compact genomes and reduced repetitive DNA regions where these mitochondrial pseudogenes are preferentially integrated.

Key Words: avian, turkey, *numt*, mtDNA, evolution

MT62 Mapping QTLs affecting Marek's disease by selective DNA pooling in eight lines across 15 generations. E. Lipkin*[1], J. Smith[2], D. Burt[2], M. Soller[1], and J. Fulton[3], [1]*Dept. of Genetics, Silberman Life Sciences Institute, The Hebrew University of Jerusalem, Jerusalem, Israel;* [2]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Midlothian, UK;* [3]*Hy-Line International, Dallas Center, IA, USA.*

Marek's disease (MD) is a major disease affecting the poultry industry. The aim of the present study was to map QTLs, genes and mutations affecting chicken MD mortality. Selective DNA pools were made of 9,391 sires of 8 lines and 15 generations per line, that had progeny test results for MD mortality. The pools were genotyped using an Affymetrix 600 K chicken SNP array. Allele frequencies were obtained by raw intensities of alleles A and B, B% = B/(A+B). The significance of allele frequency differences between sires with low or high progeny mortality was obtained using empirical SE. Moving average of -LogP and Log drop were used to define QTL regions (QTLRs). A total of 42 QTLRs were found among the eight lines, averaging 32 QTLs per line and ranging from 0 to 11. Overlap and proximity were used to condense the QTLRs to 28 QTLRs shared across families. The shared QTLRs averaged 1.2 Mb in length and covered a total of 33.3 Mb (3.1% of the entire genome). RNASeq and bioinformatics analyses of the QTLRs identified differentially expressed and functional candidate genes. Markers from the candidate genes were used to individually genotype 9,391 individuals from the same lines. Association analysis of the individual genotyping confirmed most of the QTLs found by the pools. Putative quantitative trait genes (QTG) and candidate causative nucleotides (QTN) were identified. This study confirmed most of the QTLRs found by the F6 study reported elsewhere at this meeting.

Key Words: poultry, genome-wide association, quantitative genetics, animal health, complex trait

MT63 An evaluation of the ISAG recommended parentage and identification panel for the domestic pigeon (*Columba*

***livia domestica*).** M. de Groot*, *VHLGenetics, Wageningen, the Netherlands.*

In this study, the ISAG recommended panel for the identification of pigeon is characterised based on commonly used statistical parameters. The assessed marker panel for the domestic pigeon (*Columba livia*) genotyping is based on 16 short tandem repeat (STR) loci (PIGN15, PIGN10, PIGN57, PIGN26, CliμD16, CliμD19, PIGN12, CliμD17, CliμT17, PIGN04, CliμD01, CliμD11, CliμD35, CliμT02, CliμT13, CliμT43). The alleles of the 16 loci consist of a mixture of tri-, tetra- penta- and hexameric repeat patterns. A sex determination marker was included in the multiplex for quality control. The repeat sequence of the PIGN markers was previously unpublished and therefore sequenced to reveal the sequence pattern. In total, 1,421 pigeons were genotyped on all 16 STR loci to generate allele frequency data for each locus. All 16 markers combined a PE1 (combined non-exclusion probability, first parent) of 0.9986 and PE2 (combined non-exclusion probability, second parent) of >0.9999 was observed. Comparing both the alleged father and mother, a PE value of >0.9999 was observed. Two of the markers, CliμD19 and PIGN12, were found to have relatively high HWE and F(Null) values. Therefore it can be considered to replace these markers by other STRs. Another point of discussion can be to add a gender identification marker to the recommended ISAG panel. Not only can this serve as an extra identification marker, but also because it is very hard to determine the sex of an animal based on phenotypical characteristics, especially for chicks. The set of 16 STR markers can be used in routine parentage verification work as well as identification of individuals.

Key Words: STR, genotyping, *Columba livia domestica*, pigeon

MT64 Evaluation of semen characteristic of the high and low sperm motility groups in two different strains of chicken. M. Farahi, A. A. Masoudi*, and A. Ehsani, *Tarbiat Modares University, Tehran, Tehran, Iran.*

Successful fertility in males is very important in the poultry industry. One of the key factors affecting the fertility of males during the lifetime is semen quality. The aim of this study was to understand the trends of the semen parameters quality in two different groups of high and low sperm motility in two different strains (commercial and indigenous). An experiment was started by sperm motility evaluation of Arian dame line and Urmia native roosters at the age of 27 weeks. Then the samples were allocated to two High Motility level (HML) and Low Motility Level (LML) for next analyses. In the following sperm parameters, sperm viability and sperm integrity was performed individually for each bird at regular intervals every other week for the eight-month course. The results indicated that although the trend of sperm motility was diverse among the groups, the sperm motility in HML was greater than in LML in both strains throughout the time of the semen production. In addition the trend of sperm motility in high and or low groups of commercial and native strains was almost the same. The results of the sperm motility showed an acceptable quality of sperm motility (0.58 to 0.87) for all the groups during the period of semen production. The sperm viability, however, showed a little reduction but generally the tendency of sperm viability was extremely constant across the production course in HML and LML of both strains. Sperm membrane integrity was significantly lower ($P < 0.05$) in LML of the native strain. Results of this research indicated that the birds ranking based on the semen characteristics at the early breeding of the chicks will be fixed across of the course of the bird rearing and therefore it could be a useful indicator of male selection in the poultry industry.

Key Words: chicken, fertility, pedigree line, sperm motility, native

MT65 Discrimination of native chicken breeds using SNP marker combination. S. Jin[1], N.-R. Choi[1], D. Seo[1], P. Manjula[1], H.-Y. Kim[2], S. H. Lee[1], and J. H. Lee*[1], [1]*Chungnam National University, Daejeon, Republic of Korea;* [2]*Insilicogen Inc., Yongin, Republic of Korea.*

The consumption of chicken meat is steadily increasing in the world and the rate of increase is much higher in Korea because chicken meat has been recognised as healthy food. Recently, Korean government launched Golden Seed Project (GSP) for developing new chicken breeding stocks using native chickens. In this study, 600K high-density SNP array was used for the genetic verification of new chicken breeding stocks. Total, 192 native chickens were investigated for selection of SNP markers for breed discrimination. As the results, 128 SNPs were initially selected. Based on the validation study, highly significant 48 SNP markers were finally selected with 96% sensitivity and 98.4% specificity for identifying target population. These results can provide useful information for developing of new chicken breeding stocks with further verifications.

Key Words: SNP, new chicken breeding stock, native chicken, breed discrimination

MT66 A novel approach for mapping of animal genome assemblies to a chromosomal level applied to avian genomes. J. Damas[1], R. E. O'Connor[2], M. Farre[1], H. Martell[2], E. A. Slack[1], E. Allanson[1], L. Kiazim[2], R. Jennings[2], A. Mandawala[3], S. Joseph[2], K. E. Fowler[3], D. K. Griffin[2], and D. M. Larkin*[1], [1]*Department of Comparative Biomedical Sciences, Royal Veterinary College, University of London, London, UK;* [2]*School of Biosciences, University of Kent, Canterbury, Kent, UK;* [3]*Canterbury Christchurch University, Canterbury, Kent, UK.*

The ultimate aim of a genome assembly is to create a contiguous length of sequence from p- to q- terminus of each chromosome. Most assemblies are however, highly fragmented, limiting their use in studies of trait linkage, phylogenomics and genomic organisation. To overcome these limitations, we developed a novel scaffold-to-chromosome anchoring method combining Reference-Assisted Chromosome Assembly (RACA) and fluorescence *in* situ hybridisation (FISH) to position scaffolds on chromosomes. Scaffolds generated from *de novo* sequenced genomes were ordered and orientated using RACA against a reference and outgroup genome into 'predicted chromosome fragments' (PCFs) for 18 previously published avian genomes. PCFs were verified using PCR for 6 genomes and the second round of RACA was run for all genomes using settings derived from the PCR results. An universal set of FISH probes developed through the selection of conserved regions in 21 avian genomes were then used to map PCFs of the peregrine falcon (*Falco peregrinus*) and rock pigeon (*Columba livia*) genomes. We were able to physically map 87% of the peregrine and 84% of the pigeon genome, improving the N50 of both 7-fold, as well as identify a series of intra and interchromosomal rearrangements. Additional genomes mapped using this method include the ostrich (*Struthio camelus*), the saker falcon (*Falco cherrug*) and the budgerigar (*Melopsittacus undulatus*). Comparative analysis of evolutionary breakpoint regions identified in the upgraded genome assemblies demonstrated that positions of interchromosomal breaks in avian genomes are limited to the genome intervals with unusually low level of sequence conservation. This likely to shed light on why most avian species have very stable karyotypes. Our combined FISH and bioinformatics approach represents a step-change in the mapping of genome assemblies. This makes verification and upgrade of any avian genome assembly with long scaffolds to chromosomes an easy task allowing comparative genomic research at a higher resolution than previously possible.

Key Words: genome assembly, comparative genomes, fluorescent in situ hybridization, avian genomes, chicken

MT67 *In situ* and *in silico* improvement of the Japanese quail genome assembly. S. Galkina*[1], M. Kulak[1], A. Saifitdino-

va[1], A. Komissarov[1], A. Dyomin[1], V. Volodkina[1], J. Damas[2], M. Farre[2], D. Griffin[3], D. Larkin[2], and E. Gaginskaya[1], [1]*Saint-Petersburg State University, Saint-Petersburg, Russia;* [2]*Royal Veterinary College, University of London, London, UK;* [3]*University of Kent, Kent, UK.*

The Japanese quail *Coturnix coturnix japonica* is a biomedical model species and one of the highest-producing poultry species. It has a relatively small genome (≈1.41 Gb) packed into 39 chromosome pairs. The first draft of its genome assembly was released in 2013. Since 2016 the quail genome assembly comprising of 32 (of 38+Z and W) linkage groups is available (https://www.ncbi.nlm.nih.gov/genome/?term = japanese+quail). However, it contains a significant number of sequence gaps due to the presence of repetitive coding and non-coding DNA elements that complicate contiguous assembly. Importantly, the repetitive DNA elements play a crucial role in evolution of chromosome structure and regulation of gene expression. Here, (1) we identified new highly repeated sequences of Japanese quail genome within unassembled short raw reads and mapped them *in silico* and *in situ*. (2) We assembled the Japanese quail rRNA gene cluster on the basis of raw read library using Geneious 9.0.5 software package. Repeat searching and annotation were done by Repeatmasker 4.0.5. (3) To verify the quail draft genome assembly we performed systematic ZOO-FISH experiments on Japanese quail chromosomes with chicken BAC clone probes from CHORI-261 chicken BAC library. BAC clones were selected according to their chromosomal positions and assigned for specific sequence markers and high cross-species hybridization efficiency. All BACs used were positioned in the quail genome assembly. In some cases we have found discrepancies between positions of the markers in the quail genome assembly and physical maps, caused by the newly identified interspecific rearrangements. Support: 'Chromas' Research Resource Center and Theodosius Dobzhansky Center for Genome Bioinformatics (SPbSU), RFBR #15–04–05684 & #16–04–01823.

Key Words: *Coturnix coturnix japonica*, repeat, rRNA cluster, rearrangement

Cattle Molecular Markers and Parentage Testing

MT69 Identification of distribution single nucleotide polymorphism of Cytochrome b gene in Kebumen Ongole grade cattle and Brahman cattle. T. Hartatik*, D. Maharani, J. H. P. Sidadolog, A. Fathoni, and Sumadi, *Laboratory of Animal Genetic and Breeding, Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia.*

The purpose of this research was to identify the genetic variation in Kebumen Ongole grade cattle and Brahman cattle by using marker mt-DNA Cytochrome *b* gene. The material used for the amplification of a cyt b gene was derived from a female cattle which consists of 14 Kebumen Ongole grade cattle and 15 Brahman cattle. The analysis of this research used the method of Polymerase Chain Reaction (PCR) and sequenced the PCR product. The PCR product of mt-DNA cyt b 464 bp was amplified with forward primer (L14735): 5′-AAAAACCACCGTTGTTATTCAACT-3′ and reverse primer (H15149)5 '-GCCCCTCAGAATGATATTT GTCCTCA-3 '. The single nucleotide polymorphisms (SNPs) was determined using *BioEdit_ClustalW progam. Mitochondria DNA* Cyt b gene in Kebumen Ongole grade cattle showed 36 SNPs with 8 haplotypes. Smaller number of SNPs in Brahman cattle with 7 SNPs and 6 haplotypes indicate that homozigosity in Brahman cattle was higher than that of Kebumen Ongole Grade cattle. The conclusion of this research was Kebumen Ongole grade cattle was originally different with Brahman cattle. However 50% of Kebumen Ongole grade cattle had 85% similarity of mtDNA sequence with Brahman cattle. It was predicted from the dams of Brahman cattle that introduced into the territory of Kebumen.

Key Words: Cyt b gene, Kebumen Ongole Grade Cattle, Brahman cattle, SNPs, haplotype

MT70 Phylogenetic analysis of three yak breeds or populations based on several molecular markers. J. Gao, S. Y. Jin, L. Huang, and Y. C. Zheng*, *College of Life Science and Technology, Southwest University for Nationalities, Chengdu, Sichuan, China.*

Yak (*Bos grunniens*) is a unique cattle species adapt to the hypoxic environment of Qinghai-Tibetan Plateau. In Sichuan province of China, there exist two famous yak breeds (Maiwa yak and Jiulong yak) and a yak population (Changtai yak), their major distribution regions are all at a distance of ~300 km. Changtai yak was less studied and its genetic relationships with Maiwa yak and Jiulong yak is unclear. The current study analysed the genetic relationship of Changtai yak (n = 40), Maiwa yak (n = 40) and Jiulong yak (n = 40) using mitochondrial DNA (mtDNA) D-loop sequences, 15 microsatellite markers and two loci of nuclear genes, in order to provide scientific data for the utilisation of yak genetic resources. DNA was extracted from blood or muscle samples and PCR was carried out before gene sequencing or genotyping. The results showed that the three yak breeds/populations had a high degree of polymorphisms. The gene heterozygosity (H_o) and haplotype diversity (*Hd*) in Changtai yak were higher than in Maiwa yak and Jiulong yak. mtDNA D-loop sequencing revealed that the genetic distances were similar among the three yak breeds / populations, suggesting their very close maternal origin; microsatellite polymorphism assay showed that Changtai yak had a closer genetic association with Maiwa yak, which was also supported by the unique polymorphisms of the lactate dehydrogenase-1 (LDH-1) and the duplication fragment of κ-casein (κ-CN) in the three yak breeds / populations. Thus we propose that Changtai yak has a closer genetic relationship with Maiwa yak than with Jiulong yak, it may represent an independent yak breed based on the cluster analysis with the two yak breeds. The present data have provided useful information for yak breeding practice.

Key Words: yak, breed diversity, microsatellite, phylogeny

MT71 Improving accuracy of genomic prediction for economically important traits in North American Holstein dairy cattle. S. Nayeri*[1], M. K. Abo-Ismail[1,2], M. Sargolzaei[2,3], S. P. Miller[2,4], F. Schenkel[2], S. S. Moore[1,5], and P. Stothard[1], [1]*University of Alberta, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada;* [2]*University of Guelph, Centre for Genetic Improvement of Livestock, Department of Animal Bioscience, University of Guelph,Guelph, ON, Canada;* [3]*The Semex Alliance, The Semex Alliance, Guelph, ON, Canada;* [4]*Angus Genetic Incorporation, Angus Genetics Inc., American Angus Association, St. Joseph, MO, USA;* [5]*University of Queensland, Agriculture and Food Innovation, Centre for Animal Science, University of Queensland, St. Lucia, QLD, Australia.*

Genomic selection can be applied without knowledge of the underlying genes and pathways involved, however, an understanding of the underlying quantitative trait nucleotides (QTNs) may help to increase accuracy of genomic predictions. The objectives of this study were to identify candidate causal genes and variants for eighteen production, lifetime performance index (LPI), longevity, and fertility traits in Holstein dairy cattle by integrating information from genome-wide association analysis (GWAS), whole-genome

sequencing and a variety of bioinformatics tools. A new custom Affymetrix panel (~80K SNPs) was then constructed by including the identified variants, and the change in the accuracy of genomic predictions was evaluated using a GBLUP model. The effects of combining the new panel with the existing 50K panel (creating a 124K panel) and of using only those SNPs that overlap with transcribed sequences (transcriptome panel) were also investigated. The results showed that when the custom genotyping panel was combined with 50K panel (124K panel), only a small increase in the accuracy of genomic prediction (0.57% averaged across all traits) was achieved over the 50K panel. The most promising result was obtained from the transcriptome panel, which consisted of 74k SNPs within transcribed regions. The transcriptome panel worked slightly better for all the traits and on average led to an increase in accuracy of 0.72% over 50K genotypes. In conclusion, the results indicated that the inclusion of candidate QTNs in a GBLUP model slightly increased prediction accuracy, but that further efforts aimed at using candidate QTN genotypes and alternate models, such as weighted ridge regression, Bayes RC, or Bayes Cπ, are warranted.

Key Words: genomic prediction, North American Holstein dairy cattle, genome-wide association, DNA sequencing

MT72 Effect of single nucleotide polymorphisms in the *ABCG2, β-LG, CSN3, FASN, IGF1, PPARGC1A* and *SCD* genes on milk production traits in dairy cattle. M. A. Alim*[1,2], D. Sun[2], Z. Yi[2], Z. Yuan[2], Z. Qin[2], and L. Lin[3], [1]*Division of Animal Biotechnology, National Institute of Biotechnology, Ganakbari, Asulia, Savar, Dhaka, Bangladesh;* [2]*College of Animal Science and Technology, Key Laboratory of Animal Genetics and Breeding of Ministry of Agriculture, National Engineering Laboratory for Animal Breeding, China Agricultural University, Beijing, China;* [3]*Beijing Dairy Cattle Center, Beijing, China.*

The current study was designed to evaluate the associations between single nucleotide polymorphisms (SNPs) and milk production traits in Chinese Holstein cows. In this study, we estimated the effects of polymorphisms in the *ATP-binding cassette G2 (ABCG2), fatty acid synthase (FASN), insulin-like growth factors I (IGF1), κ–casein (CSN3), peroxysome proliferator-activated receptor-c coactivator-1a (PPARGC1A), Stearoyl-CoA desaturase (SCD)* and β-lactoglobulin (β-LG) genes on milk production traits by Matrix-assisted laser desorption/ ionization time of flight mass spectrometry (*MALDI-TOF*) and DNA sequencing methods. Here, we genotypted 752 lactating cows for $ABCG2_{g.1810C>T,}$ $FASN_{g.16907T>C,}$ $FASN_{g.17924G>A,}$ $IGF1_{g.10888T>C,}$ $CSN3_{g.10888T>C,}$ $CSN3_{g.10924C>A,}$ $CSN3_{g.10944A>G,}$ $\beta\text{-}LG_{g.10329C>T,}$ $PPARGC1A_{g.12703G>T,}$ $SCD_{g.10153A>G,}$ $SCD_{g.10213T>C}$ and $SCD_{g.10329C>T}$ polymorphisms, and estimated genotypic effects on five milk production (milk yield, fat yield, protein yield, fat percentage and protein percentage) traits. Out of twelve, five polymorphism ($FASN_{g.17924G>A,}$ $CSN3_{g.10888T>C,}$ $CSN3_{g.10924C>A,}$ $CSN3_{g.10944A>G,}$ and $SCD_{g.10329C>T}$) was predicted to result in an amino acid replacement in the respective protein. Our result showed that all identified polymorphisms had significant ($P < 0.0001$) effect on the milk production traits in Chinese Holstein cattle. Our result demonstrated that the identified SNPs possibly contributed to conducting association analysis and can be recognised as genetic marker in programs of gene-assisted selection for the genetic improvement of milk production traits in dairy cattle.

Key Words: dairy cattle, SNP, gene, genetic marker, association

MT73 Genome-wide association study for displacement of abomasum in dairy cattle. H. Huang*[1], J. Cao[2], G. Guo[3], X. Li[3], Y. Wang[1], Y. Yu[1], S. Zhang[1], Q. Zhang[1], and Y. Zhang[1], [1]*Key Laboratory of Animal Genetics and Breeding of Ministry of Agriculture, National Engineering Laboratory of Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, China;* [2]*Department of Clinical Veterinary Medicine, College of Veterinary Medicine, China Agricultural University, Beijing, China;* [3]*Beijing Sunlon Livestock Co Ltd, Beijing, China.*

Displacement of abomasum (DA) is a common and economically important disorder in dairy cattle. Previous studies have yielded evidence for a genetic predisposition to this disease. Here, we conducted a genome-wide association study (GWAS) for DA in Chinese Holstein cows based on 373 cases and 2316 controls. Single marker tests were performed by the genome-wide rapid association using mixed model and regression (GRAMMAR) approach. Two polygenic models (i.e. linear and logistic) were used to obtain residuals adjusted for polygenic covariation and fixed effects. Results from these two analyses were highly concordant. In total, 38 SNPs located on 22 bovine chromosomes (BTA) showed significant associations with DA. At the significance threshold there were 5 significant SNPs common to the two analyses (Table 1).The SNP with the most significant association was located on BTA 12, 9.8 kb from the candidate gene *NALCN*, which encodes a voltage-independent, nonselective, non-inactivating cation channel permeable to Na^+, K^+, and Ca^{2+}. The concentration of these cations could affect the circular muscle of the abomasal corpus and thus might play an important role in the pathogenesis of DA. To our knowledge, this is the first GWAS study for DA in the Chinese Holstein population. These significant SNPs and candidate genes for DA may provide deeper insights into the pathogenesis of DA and illuminate an important part of the genetic background of this disease. Our results add to understanding of the genetic architecture of this complex disease and suggest that genetic improvement of DA resistance is possible.

Key Words: Chinese Holstein, genome-wide association, complex trait, animal health

MT74 Genomic single-step national evaluation of Holstein cattle in the Czech Republic. A. Kranjcevicova*[1], J. Pribyl[1], J.

Table 1 (Abstract MT73). Single nucleotide polymorphisms significantly associated with displacement of the abomasum (-log10 *P*-value>4.0)

SNP name	BTA	Position (bp)	OR[1]	Major/ minor allele	Nearest gene	Distance (bp)
ARS-BFGL-NGS-18448	12	81540197	1.74	A/C	*NALCN*	9884
ARS-BFGL-NGS-7067	24	61958252	0.73	A/G	*BCL2*	within
Hapmap59976-rs29022169	8	105898019	1.49	A/G	*TNFSF8/TNC*	27388/67479
BTB-00054069	1	122606040	0.39	A/G	NA	NA
ARS-BFGL-NGS-41732	23	44897933	1.56	A/C	*NEDD9/ADTRP*	within/361065

[1]Odds ratios were determined based on the allele counts: OR >1: the minor allele increases the risk of disease; OR < 1: the major allele increases the risk of disease.

Splichal[2], J. Bauer[2], J. Motycka[3], and L. Vostry[1], [1]*Institute of Animal Science, Prague, Czech Republic;* [2]*Plemdat (Czech Moravia Breeders Corporation), Hradistko, Czech Republic;* [3]*Holstein Cattle Breeders Association, Hradistko, Czech Republic.*

Genomic national evaluation of dairy cattle using SNP markers on 50K Illumina Beadchip is routinely applied for production traits and somatic cells score where the test-day random regression model is used. Conformation and reproduction traits are evaluated by single trait animal model and longevity is evaluated by survival kit. Data included 1.3 mil of cows records with ~25 mil. of test-day records and 5,500 genotypes of animals. A single-step procedure is used and thus genotyped and non-genotyped animals are evaluated together across the whole population. National procedure is validated by Interbull organisation. A positive genetic trend is observed in last 35 years in milk production, but the negative one in reproduction traits. The genetic trends of evaluated traits are similar for period 1980 - 2010 but after 2010 when the genomic evaluation is used values increase. Distribution of genomic enhanced breeding value (GEBV) has similar range for subpopulations of cows, non-genotyped bulls, proven genotyped bulls, and young genotyped bulls. Average GEBV for proven genotyped bulls is slightly higher than for other groups in milk production. Czech population is strongly influenced by import of foreign bulls and therefore young animals have weak connection to domestic production records and low reliability of prediction of genetic evaluation. Evaluation is improved by implanting international MACE (Multiple Across Country Evaluation) values into domestic evaluation. Interbull MACE values were deregressed into test-day records and added to the database of domestic animals. Validated reliabilities of young bulls were calculated using for prediction partial data until the year 2012 and for verification a whole data until year 2016. Single-step evaluation on combined data including a whole domestic population and all available Interbull values of genotyped and non-genotyped bulls (MACE values of 100 000 bulls) has higher validated reliability of evaluation of young bulls then evaluation on only domestic population.

Key Words: single-nucleotide polymorphism (SNP), genomic prediction, milk production, animal breeding, population genomics

MT75 Identification of hypotrichosis in Kazakh Whiteheaded beef cattle breed in Kazakhstan. T. Yechshzhanov*[2], R. Uskenov[1], Y. Mukhanbetkaliyev[1], S. Zhamaliyeva[2], and A. Smakova[1], [1]*S.Seifullin Kazakh AgroTechnical University, Astana, Kazakhstan;* [2]*L.N.Gumilyov Eurasian National University, Astana, Kazakhstan.*

Hypotrichosis is autosomal recessive hereditary disorder, which has had significant economic effect on beef cattle breeding worldwide. The prevalence of carriers of disorder has not been reported before in Kazakhstan. The objective of the study was to identify carriers of this disease in population and artificial insemination centre. Previously, 54 animals of different beef breeds of domestic and imported breeds from Akmola and North Kazakhstan regions were screened for 8 SNPs of glycogen storage disease, hypotrichosis, maple syrup urine disease, male subfertility and dwarfism. Our previous results revealed that the Kazakh whiteheahed bull from artificial insemination centre is the carrier of a recessive allele for genetic disease: hypotrichosis with the SNP on HEPHL1 gene. In this study, to look for distribution of recessive allele among progeny 75 offsprings of carrier bull progeny were selected for further analysis. Parentage testing with 100 SNP was used for verification. Genomic DNA was extracted from hair follices by Qiagen DNA extraction kit with small modifications. Genotyping was performed for the following autosomal recessive diseases of beef cattle breeds: hypotrichosis with a SNP in the HEPHL1 gene. Genotyping of DNA samples for the SNPs carried out by using Illumina bovine bead chips at Labogena (France). According to the results of genotyping it revealed that the Kazakh whiteheahed breed bull is the carrier of a recessive allele for genetic disease: hypotrichosis with the SNP on HEPHL1 gene. This study demonstrates that carriers of hypotrichosis are present in the Kazakh whiteheaded breed population, although at a low frequency. As artificial insemination is used in cattle breeding, carriers of hypotrichosis, are likely present within progeny of breeding sires. Since the mutant alleles are invisible in phenotype of animals, it is very important to detect and eliminate carriers of these diseases from breeding stock.

Key Words: cattle, animal breeding, genotyping, genetic disorder, genetic improvement

MT76 Genomic profiles to assess accuracy of breed composition and hybrid vigor in crossbred beef cattle. M. K. Abo-Ismail*[1,2], J. Crowley[1,3], E. C. Akanno[1], G. Manafiazar[1], C. Li[1,4], G. Plastow[1], M. D. MacNeil[6], D. Berry[7], P. Stothard[1], and J. Basarab[1,5], [1]*Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada;* [2]*Animal and Poultry Production, Damanhour University, Damanhour, Egypt;* [3]*Canadian Beef Breeds Council, Calgary, AB, Canada;* [4]*Agriculture and Agri-Food Canada, Lacombe Research and Development Centre, Lacombe, AB, Canada;* [5]*Lacombe Research Centre, Alberta Agriculture and Forestry, Lacombe, AB, Canada;* [6]*Delta G, Department of Animal, Wildlife and Grassland Sciences, Bloemfontein, South Africa;* [7]*Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Co., Cork, Ireland.*

The objective of this study was to assess the effectiveness of different methods of single nucleotide polymorphism (SNP) marker selection and densities to predict breed composition for crossbred beef cattle and subsequently evaluate the use of those breed fractions to calculate retained heterozygosity (RH). A total of 8,792 individual genotypes were available from six breeds (Gelbvieh, Charolais, Angus, Simmental, Limousin, and Hereford), three commercial crossbred herds and two crossbred research herds (Kinsella composites and Lacombe Research and Development Centre [LRDC]) as well as a crossbred validation group (n = 102) from LRDC. Genomic breed composition was predicted for all individuals using the cross-validation procedure implemented in ADMIXTURE software using different approaches in terms of number of SNPs (including ISAG parentage assignment panel), methods of SNP selection and breed information. The results indicated a strong effect for number of SNPs and SNP selection methods on breed composition and RH estimates. Genomic estimated breed composition was regressed onto the pedigree recorded breed composition in the validation group to assess accuracy (R^2) of prediction. The ISAG SNPs had 13 percentile points lower accuracy than that obtained when using the maximum number of SNP (~7,616 SNP overlapped between SNP panels). The correlation between RH values estimated using the maximum number of SNP and other approaches ranged from 61 to 95%. Inclusion of informative SNPs using different approaches including pairwise Wright's FST and Delta statistic improved the accuracy of genomic-based breed composition and RH. Thus, predicting genomic-based breed composition was achieved using cost-effective marker panels without affecting the accuracy of estimation of RH required to optimize crossbreeding programs in beef cattle.

Key Words: beef cattle, admixture, breed diversity and composition, retained heterozygosity

MT77 Residual feed intake candidate gene associations. N. A. Zulkifli*[1,2], W. S. Pitchford[2], and C. D. K. Bottema[2], [1]*School of Environmental Science and Natural Resources, The National University of Malaysia, Bangi, Selangor, Malaysia;* [2]*School of Animal*

and Veterinary Sciences, The University of Adelaide, Roseworthy, Australia.

Residual feed intake (RFI) is a measure of net feed efficiency, an economically important trait in livestock. The RFI of an animal depends on the ability of the animal to consume less feed than expected based on their weight gain and weight maintained during the feed testing period. Recent work has implicated mitochondrial function as being involved in the feed efficiency of livestock. The objective of this study was to identify genes involved in mitochondrial function that may affect net feed efficiency in cattle. Several quantitative trait loci (QTL) affecting feed efficiency were previously mapped in Jersey × Limousin double backcross progeny in three sire families. Based on the QTL mapping results, ten candidate genes related to mitochondrial function and energy metabolism were identified: *ALDOB*, *AK1*, *CAT*, *GHRL*, *HADHB*, *NDUFA8*, *NDUFB5*, *SOD1*, *SOD2*, and *SUCLG1*. All ten genes were sequenced in three Jersey × Limousin sire families in order to locate DNA variants in the genes for association studies. A total of 58 DNA variants were discovered, which included six insertion/deletions (in/dels) and 52 single nucleotide polymorphisms (SNPs). Fourteen SNPs were selected for genotyping in the 366 progeny from the three sire families. Genotyping results were analysed to observe the effect of the SNPs with 27 RFI related traits and specific fat depot traits. Only four SNPs in the candidate genes were associated with residual feed intake, three of which were in the *HADHB* gene. The haplotype of *HADHB* from these three SNPs explained 8.5% of the variation in RFI. The other SNP was in the *SOD1* gene, which had a p-value < 0.001 for residual feed intake and explained another 3% of the variation in this trait. Thus, the results indicate that DNA variants in genes involved in mitochondrial function and energy metabolism may influence RFI.

Key Words: residual feed intake, feed efficiency, mitochondrial function, cattle, candidate genes

MT78 Impact of genomic selection for feed efficiency on heifer reproduction and calf performance traits. E. Akanno*[1], C. Ekine-Dzivenu[1], M. Abo-Ismail[1,2], M. MacNeil[3,4], C. Li[1,5], C. Fitzsimmons[1,5], J. Basarab[1,6], and G. Plastow[1], [1]*Livestock Gentec, AFNS, University of Alberta, Edmonton, AB, Canada;* [2]*Department of Animal and Poultry Production, Damanhour University, Damanhour, Egypt;* [3]*Delta G, Miles City, MT, USA;* [4]*Department of Animal, Wildlife and Grassland Sciences, University Free State, Bloemfontein, South Africa;* [5]*Agriculture and Agri-Food Canada, Lacombe Research and Development Centre, Lacombe, AB, Canada;* [6]*Alberta Agriculture and Forestry, 4Lacombe Research and Development Centre, Lacombe, AB, Canada.*

Genetic improvement in feed efficiency is economically important in cow-calf operations but measuring feed intake is an expensive and difficult process particularly on large numbers of cows. Thus, genomic selection (GS) offers opportunities to improve feed efficiency in cow-calf herds but the potential impact of selecting for improved feed efficiency on maternal traits is unknown. Therefore, the objective of this study was to evaluate a short-term correlated response in heifer reproduction and calf performance traits due to a GS scheme that incorporates genomic breeding values (GBV) for residual feed intake (RFI) in either a maternal or feedlot productivity index. A total of 981 replacement heifers in their first calving bred between 2012 and 2014 were collated from three experimental herds namely Angus (AN; 285), Charolais (CH; 182) and Kinsella Composite (KC; 514). All replacement breeding stock for AN and CH were selected for feed efficiency using GBV within multi-trait maternal and feedlot profitability indexes, respectively, while the KC population was randomly split into feed-efficient and control herds contributing 264 and 250 heifers, respectively. Replacement breeding stock for the KC control herd was subjected to traditional selection, while the KC-efficient replacements were selected similarly to the Angus herd. In order to examine the impact of GS on pre-breeding weight (PBW), calving date (CD), calf birthweight (CBW) and calf weaning weight (CWW), a multiple linear regression analysis was performed using R statistical software. Except for CBW (36 – 35 kg), the results showed a significant ($P < 0.01$) phenotypic trend for PBW (337 - 362 kg), CD (209 - 163 days), and CWW (202 – 223 kg), on average, in heifers from the efficient herds (AN, CH, KC) bred over the course of three years. Within a selection year, there was no difference ($P > 0.05$) observed for maternal traits between the control and efficient herd in the KC population. Despite the short-term selection experiment and the limited sample size used in this study, it appears that a genetic improvement program that incorporates RFI in a selection index will have no adverse effect on maternal traits in a cow-calf operation.

Key Words: beef cattle, feed efficiency, genomic selection, maternal traits

MT79 Bilateral iridal hypopigmentation in Holstein Friesian cattle. A. K. Hollmann[1], M. Bleyer[2], A. Tipold[3], J. N. Neßler[3], W. E. Wemheuer[1], E. Schütz[1], and B. Brenig*[1], [1]*Institute of Veterinary Medicine, University of Goettingen, Goettingen, Germany;* [2]*Pathology Unit, German Primate Center, Leibniz-Institute for Primate Research, Goettingen, Germany;* [3]*Dept. Small Animal Medicine and Surgery, University of Veterinary Medicine Hannover, Hannover, Germany.*

Eye pigmentation abnormalities in cattle are often related to syndromes like albinism, Chediak-Higashi or Tietz. However, also mutations that solely affect the pigmentation of body and eye have been described. These discolorations of the iris, either mono- or bilateral, complete or partial are usually referred to as heterochromia iridis. A total of 20 cattle (10 male, 10 female) with bilateral hypopigmentation of irises were observed. 18 cases were of Holstein Friesian (HF) breed, two other affected animals were crossings of Red cattle and Fleckvieh. Ophthalmological and neurological examination did not reveal any signs of an underlying syndrome or anomalies other than the iridal hypopigmentation. All cases showed an iris coloration with two differently coloured parts of varying colour intensity. The central regions were silvery-blue to grey-blue with darker and lighter parts and the peripheries were light brown to grey with occasional light grey zones. Coat colour of affected cattle was characteristic of the breed without any obvious colour deviations. Histological evaluation revealed a reduction in iris pigmentation mainly affecting the anterior border layer and the iridal stroma. Differences in pigmentation of other uveal structures, iridal thickness or stromal density were not detected. To analyse the genetics of the iris hypopigmentation, a genome-wide association study was performed using genotyping data of the 20 cases and 172 randomly selected HF cattle. Three loci with p-values above the Bonferroni genome-wide significance level of $-\log_{10}(p) = 6.65$ were detected on bovine chromosome (BTA) 8 (position 60,990,733, $-\log_{10}(p) = 9.2$), BTA4 (position 7,186,971, $-\log_{10}(p) = 6.81$) and BTA9 (position 77,442,550, $-\log_{10}(p) = 6.95$). Further analysis of genotypic and allelic dependences between the 20 cases and 316 randomly selected HF cattle using 3x2 or 2x2 contingency tables and Fisher´s exact or chi-squared statistics (df = 2) were performed. The presence of the A-alleles at each of the 3 loci are leading to a 6- (BTA8), 9.04- (BTA4) and 5.47- (BTA9) times higher chance to develop an iridal hypopigmentation.

Key Words: cattle, genome-wide association, animal health

MT80 The price of looking for something no one wants to talk about: The search, quantification, and cost of genetic diseases in Ireland. J. McClure*[1], P. Flynn[2], S. Waters[3], M. Mullen[4], T. Pabiou[1], R. Schnabel[5], J. Taylor[5], F. Kearney[1], R. Weld[2], and M. McClure[1], [1]*The Irish Cattle Breeding Federation, Highfield House, Shinagh, Bandon, Co. Cork Ireland;* [2]*Weatherbys DNA Laboratory, Johnstown, Co. Kildare;* [3]*Animal & Grassland Research and*

Innovation Centre, Teagasc, Grange, Co. Meath; [4]Department of Life and Physical Sciences, Faculty of Health and Science, Athlone Institute of Technology, Athlone, Co. Westmeath, Ireland; [5]Department of Animals Sciences, University of Missouri, Columbia, MO, USA.

It is estimated that every animal is a carrier for 20–100 genetic disease causing alleles, and that each new animal born may have 50 spontaneous mutations. These new mutations may be benign or may be lethal with no way to predict until they surface in a population. Mutations responsible for embryonic loss, or early mortality are especially troubling because of the high economic loss in production systems, and the extra stress on the ecosystem due to non-productive animals in a herd. Since 2013, over 650,000 Irish dairy and beef cattle have been genotyped on the International Dairy and Beef (IDB) SNP chip. Independently confirmed carrier or affected animal DNA was used to validate 65 disease and trait SNP probes on IDB chips. Allele frequencies from validated disease probes were analysed to calculate carrier frequency for 33 genetic diseases. The economic cost for some of these particular diseases has been calculated and found to be very high. With low cost genotyping a system will be put in place where superior mating strategies can be identified that minimizes genetic disease economic cost by including the genetic disease status of the sire and dam. By implementing this 'smart breeding plan' the industry can retain animals with high value while reducing the number of animals affected by known genetic diseases. The success of the above programs has incentivised ICBF to seek out new causative disease alleles in the Irish bovine population. To identify genetic diseases with the highest impact a survey was developed for farmers to report genetic defects and a system was designed to collect samples for genetic analysis. The caveat to a program like this is the stigma associated with having abnormal animals. While uptake has been slow, articles online, in trade magazines, and popular press have increased the frequency of reports. Once a few diseases were targeted, DNA from affected animals was sent off for whole genome sequencing to elucidate candidate causal mutations. This is an ongoing process which is poised to save the industry millions of Euros every year once diseases are identified and new SNPs are added to the next version of IDB.

Key Words: genetic, defect, disease, major gene

MT81 The OpenArray SNP parentage panel in cattle—Preliminary studies. A. Piestrzynska-Kajtoch*, D. Rubis, A. Fornal, and A. Radko, *National Research Institute of Animal Production, Balice, Poland.*

Cattle parentage verification is currently based on the 12 microsatellite (STR) genotyping. Increasing interest in the parentage testing based on the SNP markers and development of molecular biology techniques enabled accurate, large-scale SNP analysis. SNPs can be used as supporting tool for some parentage cases, when parentage STR verification is difficult or impossible. Among high-throughput SNP analysis techniques, microarray method fulfils most of the criteria necessary for large-scale genetic studies. However, not every bovine individual will undergo genomic evaluation in the nearest future. Therefore, other SNP techniques could be consider for cattle parentage verification. By using bioinformatic tools, we have designed 110 TaqMan Genotyping Assays for ISAG/ICAR core bovine SNP panel and 100 TaqMan Genotyping Assays for ISAG/ICAR additional bovine SNP panel. For some SNPs from both ISAG/ICAR panels, double TaqMan assays were designed to avoid wrong genotyping in case of neighbour SNP (variant assays). Additionally, 7 TaqMan Genotyping Assays were designed for SNPs from Y chromosome. The assays were placed on two sets of OpenArray plates (2 × 120 format) and tested on different cattle breeds and some reference sample from ISAG/ICAR International Cattle SNP Comparison Tests (2013–2014 and 2015–2016) with QuantStudio 12K Flex platform. We have also checked the samples of different quality and isolated with different methods. Among all assays studied, two connected with Y chromosome and a few variant assays have failed. However, before final rejection they should be tested and verified on a higher number of individuals. Most of the TaqMan assays had clear and easy-readable clusters. The preliminary analysis seems promising – the OpenArray method could be possibly used as a tool for parentage verification of individuals for whom genomic evaluation is not demanded and for medium-throughput research.

Key Words: bovine, parentage testing, SNP, OpenArray

MT82 Pedigree, microsatellite and genomic based inbreeding coefficient correlations in a highly fragmented population, the Lidia bovine breed. O. Cortés*, S. Dunner, P. Eusebi, and J. Cañón, *Department Animal Production. Veterinary Faculty. University Complutense of Madrid. Avda. Puerta de Hierro, s/n, Madrid, Spain.*

In local breeds with small population size, one of the most important genetic restriction is the increase of inbreeding coefficient (*F*). Traditionally, *F* has been estimated on the basis of pedigree information. Also, microsatellite markers have been commonly used to estimate inbreeding in absence of pedigree information. Recently, with the availability of high-density single nucleotide polymorphism (SNP) arrays, *F* can also be accurately estimated. The Lidia bovine breed has several peculiarities that make it one of the most successful of domestic breeds in Spain. The traditionally breeding management of the Lidia bovine breed has favoured their fragmentation in different lineages, also called 'encastes', characterised by a clear reproductive isolation among them. The aim of the present study was to analyse the inbreeding coefficient of the Lidia bovine breed based on either pedigree data, microsatellite markers and high density SNP array. A total of 307 animals belonging to 28 lineages were genotyped using bovine SNP50K v2 BeadChip (Illumina Inc., San Diego, CA, USA). We also have the geotypes of 1525 animals for 24 microsatellite DNA markers in which the 307 animals previously mentioned was included. All the pedigree information for all the genotyped animals was included in pedigree analysis. The different genetic diversity parameters estimated from microsatellite markers (proportion of heterozygous, standardized heterozygosity, internal relatedness and homozygosity by locus) showed a high correlation among them but low with pedigree information (15%). Initially three inbreeding estimations were calculated from SNP50K array based on the usual variance-standardized relationship minus 1, heterozygous estimate, the correlation between uniting gametes and inbreeding coefficient (*F*) using PLINK. The correlation of the inbreeding coefficients among the three different sources of information ranged from 7% to 44%. Finally, the correlation between inbreeding coefficients based on different length of runs of homozygosity with the inbreeding estimated using microsatellites showed medium values significantly different from 0.

Key Words: pedigree, microsatellite, SNP, inbreeding, Lidia breed

MT83 SNP data quality control in a National Beef and Dairy Cattle System and highly accurate SNP-based parentage verification and identification. M. McClure*[1], J. McCarthy[1], R. Weld[2], P. Flynn[2], M. Kean[1], K. O'Connell[1], and J. Kearney[1], [1]*Irish Cattle Breeding Federation, Bandon, Cork, Ireland;* [2]*Weatherbys Ireland, Johnstown, Kildare, Ireland.*

As single nucleotide polymorphism (SNP) genotyping costs decrease the level of genotyping in a country's national herd increases. A major use of this genetic data is parentage verification and identification as inaccurate pedigrees result in decreased genetic gain. Since 2012 the international standard for SNP-based verification in cattle has been the ISAG100 or ISAG200 SNP set for *Bos taurus* breeds. While these SNP sets have provided an increased level of parentage accuracy over microsatellite markers (MS), they can validate the wrong parent for an animal and can predict >1 sire

or dam at < 1% misconcordance rate levels, indicating that more SNP are needed if a more accurate pedigree is required. With rapidly increasing numbers of cattle being genotyped in Ireland representing 80 *Bos taurus* breeds from a wide range of farm types: beef/dairy, multiple breeds, pedigree/commercial, purebred/crossbred, AI/stock bulls, and large to small herd size the Irish Cattle Breeding Federation (ICBF) analysed different SNP densities to determine that at a minimum >500 SNP are needed to consistently predict only one set of parents at a < 1% misconcordance rate. ICBF currently uses 800 SNP for parentage validation and prediction selected according to SNP clustering quality, ISAG200 inclusion, call rate (CR), and minor allele frequency (MAF) in the Irish cattle population. Microsatellite imputation and genetic relationship matrixes are also used to assist parentage when the true parent is not SNP genotyped. When dealing with large datasets sample and SNP quality control (QC) is paramount. Most publications only deal with SNP QC via CR, MAF, parent-progeny conflicts, and Hardy–Weinberg deviation, but not much on the sample QC. Through trial and error ICBF has developed our own sample QC pipeline to deal with the unique challenges of genotypes from a national herd. We share this pipeline for the benefit of others so they can be proactive with their datasets in dealing with SNP genotype errors from mis-tagging of animals, laboratory errors, farm errors, and multiple other issues that can arise.

Key Words: SNP parentage, quality control, parentage prediction

MT84 Next-generation targeted sequencing panel for verification of bovine parentage. A. Burrell*[1], P. Siddavatam[1], A. Allred[1], C. Willis[1], R. Ferretti[2], and A. Raeber[1], [1]*Thermo Fisher Scientific;* [2]*Neogen GeneSeek Operations.*

Bovine parentage verification is a critical aspect of successful herd management. Due to its highly accurate and reproducible results, SNP genotyping is becoming an increasingly favoured tool for parentage verification. With the utilisation of high-throughput next-generation sequencing platforms like the Ion S5 sequencing system, laboratories can test hundreds of samples and thousands of SNPs simultaneously. We developed a targeted sequencing panel based on 200 bovine SNP markers selected by the International Society of Animal Genetics (ISAG) for the purpose of verifying bovine parentage. Utilising the AgriSeq™ sequencing workflow, a high-throughput targeted amplification and re-sequencing workflow, panel performance was tested on 115 diverse bovine DNA samples. Samples included a panel of 96 samples obtained from the USDA (MARC Beef Cattle Diversity Panel v2.9) as well as samples contained within the 2015 ISAG/ICAR 3rd SNP Typing Bovine Comparison Test panel. Samples originated from 20 different bovine breeds. Libraries were prepared using the high-throughput AgriSeq workflow. The resulting amplicons were ligated to unique barcodes and sequenced on a single run on the Ion S5 sequencing system using an Ion 540 chip. Utilising this system, up to 768 samples can be barcoded and run on a single sequencing run allowing for up to ~1500 samples to be tested a day (2 runs/day). Data was analysed using the Torrent Variant Caller (TVC) plugin as part of the Torrent Suite software package to determine the genotype call for each marker and sample. The mean call rate (the percent of markers generating a genotyping call) was >98%. As a comparison, the USDA gDNA was also hybridized to six replicate arrays to generate a consensus array genotype call. Genotype call concordance was >99% between the array and AgriSeq workflow calls. Accurate parentage determinations were made for the samples within the 2015 ISAG/ICAR 3rd SNP Typing Bovine Comparison Test panel as compared to provided reference genotypes. The data demonstrates that the bovine parentage panel tested with the AgriSeq workflow provides accurate and reproducible results for SNP-based parentage verification.

Key Words: NGS, GBS, bovine parentage, AgriSeq, SNP genotyping

MT85 High cross-platform genotyping concordance of Axiom high-density microarrays and Eureka low-density targeted NGS assays. M. A. Patil* and A. Pirani, *Thermo Fisher Scientific, Santa Clara, CA, USA.*

Microarrays ranging from mid- to high-plex have been developed on the Axiom Genotyping Solution to interrogate single-nucleotide polymorphisms (SNPs) and insertion/deletions (indels) for over 55 agrigenomic organisms. This technology has the flexibility to genotype populations exhibiting diploid to various levels of allopolyploid genetics. It also incorporates methods for accurately genotyping samples originating from normal and inbred populations. The high variant density possible on microarrays has the ability to facilitate multi-breed genomic selection, fine mapping of quantitative trait loci, and detection of copy number variation. The Eureka Genotyping Solution is an affordable, low- to mid-plex, high-throughput genotyping assay that uses common next-generation sequencing (NGS) platforms for signal readout. It enables the detection of tens to thousands of genetic markers which are increasingly in demand for routine animal agrigenomics testing. This routine testing can include parentage and sex validation and genomic evaluation, after imputation. High reliability and concordance across the Axiom and Eureka technologies is essential to allow researchers to migrate between the platforms seamlessly. Thermo Fisher Scientific has accomplished high genotype concordance and high genotype call rate (low missing data rate) by adapting the same genotype calling and SNP QC framework across both platforms. The developed genotype calling algorithm has been shown to work on both microarray intensity and counts of allele × locus barcodes of next-generation sequencing (NGS) reads. Various overlapping animal datasets have been evaluated across these microarray and targeted NGS technologies. A high level of genotype concordance is demonstrated, allowing for easy comparison across and migration between platforms.

Key Words: genotyping, next-generation sequencing, microarray, genomic selection

MT86 Effectiveness of SNPs genotyping assay as a tool for genetic traceability of cattle production chain. A. Pozzi*[1], C. Previtali[1], R. Capoferri[1], S. Arabi[1], A. Galli[2], and G. Bongioni[1], [1]*Istituto Sperimentale Italiano L. Spallanzani, Rivolta d'Adda, Cremona, Italy;* [2]*Centro di ricerca per le produzioni foraggere e lattiero-casearie CREA, Lodi, Italy.*

In the last years the concern of consumer about food safety is increased, so in the European Union great value is placed on accurate and safe animal identification; breeding plan, disease monitoring, and food safety depend heavily on conventional animal identification. For this reason, the protection of the integrity of these programs from fraud or accidental errors is necessary. Genetic traceability is presented here as a way to further enhance conventional traceability; it represents a powerful tool to verify the identity of animal products during every step of the cattle production chain. From the molecular point of view, single nucleotide polymorphism (SNP) markers have gradually replaced microsatellites (STRs), mostly due to their abundance, cost efficiency, and potential for automation. In this work, the amount of information provided by a 32 SNP panel, selected in a previous study was evaluated on different biological matrices. The main sources of genomic DNA are peripheral blood leukocytes, semen, hair follicles and tissues but sampling requires specialised staff and the animals may be subject to stress, influencing negatively welfare, health and productivity. A valid and cheaper alternative of genomic source can be the milk somatic cells (from 2×10^4 to 2×10^5 cells per millilitre of milk). In details, DNA from 50 semen, 30 meat, 30 hair and 30 milk samples were collected. DNA from 140 samples was processed by TaqMan PCR and scanning array on the Open Array platform, the genotypes were generated by SNP Genotyping and TaqMan Genotyper software. Allele frequencies for each SNP were determined by direct count-

ing. A software developed 'ad hoc' (PAF), was used to estimate the levels of genetic variability: expected heterozygosity (He) values ranging from 0.47 to 0.50 with medium value of 0,46, observed heterozygosity (Ho) ranging from 0,38 to 0,60 and medium value of 0,48. Probability of identity (PI) was calculated for the 32 SNPs and it was equal to 8.40×10^{-14} The 32 SNPs assay described in this study represents a valid and useful tool for DNA-based traceability employed in different applied research projects and in the major commercial cattle products.

Key Words: cattle, single nucleotide plymorphisms, product traceability

Companion Animal Genetics and Genomics

MT87 Complete mitogenome sequence of Lagomorphs from Upper Palaeolithic in the Balkan refuge. V. Brajkovic*[1], S. Radovic[2], D. Brajkovic[7], M. Girardi[3], S. Krebs[5], I. Medugorac[4], I. Curik[1], P. T. Miracle[6], C. Vernesi[3], and V. Cubric-Curik[1], [1]*Department of Animal Science, Faculty of Agriculture University of Zagreb, Zagreb, Croatia;* [2]*Institute for Quaternary Palaeontology and Geology, Croatian Academy of Sciences and Arts, Zagreb, Croatia;* [3]*Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, S. Michele all'Adige (TN), Italy;* [4]*Chair of Animal Genetics and Husbandry, LMU Munich, Munich, Germany;* [5]*Laboratory for Functional Genome Analysis (LAFUGA), Gene Centre Munich, LMU Munich, Munich, Germany;* [6]*McDonald Institute for Archaeological Research, University of Cambridge, Cambridge, UK;* [7]*Private, Zagreb, Croatia.*

Ancient DNA (aDNA) analysis is an important tool capable in resolving numerous questions related to the evolution and adaptation of animals and plants. Lagomorph phylogenetic has several open topics. Here, our interests referred to European rabbit (*Oryctolagus cuniculus*) where the Iberian Peninsula is considered as a main reservoir of genetic diversity as well as to migration dynamics and relation between brown hare (*Lepus europaeus*) and mountain hare (*Lepus timidus*) with respect to single (Balkans) versus multiple (Mediterranean Basin) refuges hypotheses. We have started our analysis with ancient bones of 54 lagomorphs, from deposits dated to different archaeological periods between the Upper Palaeolithic to Roman Period, that were taken from eight different archaeological sites in Croatia. After aDNA extraction, samples were prepared by multiplexed target capture enrichment method and genotyping by next-generation-sequencing (MiSeq Illumina Sequencing Platform). Complete mitogenome (around 16291 bp) was obtain for five lagomorphs that, according to taxonomic identification, exclusively based on morphometric and morphologic characteristics of bones, were previously classified as brown (2) and mountain (1) hares as well as rabbits (2). When phylogenetic analysis were performed with all complete mitogenome sequences available in the GenBank belonging to *Lepus sp*. and *Oryctolagus cuniculus* following results were obtained: (1) two different unique brown hare haplotypes were grouped with other brown hare haplotypes from Germany, Greece, Poland and Sweden; (2) unique mountain hare haplotype was in branches with mountain hare haplotypes from Finland and as well as with *Lepus granatensis* from Spain and (3) two unique haplotypes were identified as typical *Oryctolagus cuniculus* haplotypes. Despite their chronostratigraphic correlation within archaeological layers, additional radiocarbon dating of successful samples is required, which is of particular interest for better understanding of rabbit origin.

Key Words: ancient DNA, mtDNA, haplotype, genome sequencing, diversity

MT88 Genotype imputation in dogs. J. Friedrich*, R. Antolin, S. Hoj-Edwards, J. Hickey, and P. Wiener, *The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, UK.*

The domestic dog is a valuable animal model for the genetic analysis of complex traits due to their genetic and physiological features. An increasing number of genome-wide association studies for a range of traits are being carried out and the use of genotype imputation to infer higher density (*HighD*) genotypes will be an important tool for fine-mapping complex traits. Factors affecting imputation accuracy, such as marker density, and relatedness between animals, have been studied in other species, but not in dogs. These factors are of interest due to the acknowledged genetic structure and inbreeding within dog breeds. Furthermore, in contrast to livestock animals, pedigree information and genotyped ancestors are not always available for dogs. In this study, we used real data to simulate different genotyping strategies to provide recommendations for the design of imputation-based experiments where animals genotyped at low density (*LowD*) are imputed to *HighD* genotypes. The aspects covered different ratios between *HighD* and *LowD* genotyped dogs, different densities of the *LowD* array, inclusion of pedigree information, and relatedness between the *HighD* and the *LowD* population. Analyses were carried out on 1,179 Labrador Retrievers genotyped with the Illumina Canine High Density Beadchip. In this analysis, the 5,826 SNPs on the largest chromosome (CFA1) were used to represent the *HighD* array. The *LowD* array was simulated by masking different proportions of markers on the *HighD* array. For imputation from the *LowD* array to the *HighD* arrays, different methods were used (AlphaImpute, FImpute). The correlation between true and imputed genotypes (imputation accuracy) ranged from 0.77 to 0.96, depending on the scenario used. An imputation accuracy of 0.90 was found for a likely scenario (10% of animals in the reference set, 87% of *HighD* markers masked in the *LowD* array), which indicates that genotype imputation in dogs can be a valuable tool to reduce experimental costs while increasing sample size. Furthermore, genotype imputation can be performed successfully even without pedigree information and with low relatedness between animals in the reference and imputation sets.

Key Words: dogs and related species, imputation

MT89 Characterizing the proteic deposits in feline amyloidosis: A proteomic approach using FFPEs. F. Genova[1], M. Longeri*[1], L. A. Lyons[2], F. G. Scalvini[1], G. Tedeschi[1], G. Sironi[1], and S. Nonnis[1], [1]*Dept. of Veterinary Medicine, Università degli Studi di Milano, Milan, Italy;* [2]*Dept. of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA.*

Amyloidoses are a group of primary and secondary diseases characterised by abnormal deposition in different organs of insoluble fibrillar proteins, which affect cats, humans, cattle and poultry. In humans more than 28 types of the disease have been identified occurring via genetic mutations (hereditary), proteins increased production or decreased elimination and misfolding disorders (in Alzheimer). In cats, amyloidosis is diagnosed with certainty only by *postmortem* identification of the deposits. Aetiology is unknown and the fine characterisation of the protein deposits is not available yet. Preliminary results obtained from the proteomic analysis of few

affected and normal frozen tissues of Abyssinian cats, suggest the expression of some proteins is regulated in amyloid deposits. As it is difficult to obtain fresh tissues of affected cats, Formalin Fixed Paraffin Embedded (FFPE) samples are a valuable resource to characterise the components of the deposits with a proteomic approach. The current study is devoted to optimize the sample preparation protocol set up on frozen tissues to apply the mass spectrometry-based proteomics to the analysis of the proteins extracted from FFPEs. FFPEs from kidneys of the previously analysed frozen samples of Abyssinian cats, were used for the protocol optimization. Protein components from deposits and corresponding normal tissues were derivatized, digested and analysed by LC-nanoESI-LTQ Orbitrap Velos equipped with a RPC18 column for peptides separation before MS/MS analysis. For protein identification and quantification, raw data files were processed and analysed using MaxQuant 1.3.0.5. and Perseus software. The results obtained with FFPE and frozen samples were comparable. The protocol was then applied to 38 FFPEs (kidney and other tissues) from 12 Abyssinian, eight Siamese, 17 Europeans and one Charteux diagnosed as affected by necropsy together with corresponding normal tissues. Preliminary data confirmed a significantly abnormal prevalence of several proteins in the renal deposits in Abyssinian cats. These results will be furthermore integrated with the genomic data obtained via GWAS and WGS from the 99Lives project, to decrypt the mechanism of the disease.

Key Words: Felis Cat, familial amiloidosis, proteomics, FFPEs

MT90 Genetic dissection of complex traits in a popular dog breed. P. Wiener*[1], J. Ilska[2], E. Sánchez-Molano[1], M. J. Haskell[2], Z. Polgar[1], S. E. Lofgren[3], D. N. Clements[1], J. A. Woolliams[1], and S. C. Blott[4], [1]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, UK;* [2]*Scotland's Rural College, Edinburgh, UK;* [3]*Youth Science Institute, Los Gatos, CA, USA;* [4]*School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, UK.*

The wide range of phenotypic characteristics across dog breeds has been exploited to identify genes associated with breed-differentiated traits. However, there is also extensive variation within breeds, which encompasses substantial information for genetic analysis. This study investigated the genetic basis of various complex traits in the Labrador Retriever breed using population genomic and quantitative genetic approaches. Data on a large number of UK Labrador Retrievers was collected, including genomic (CanineHD SNP array), pedigree and trait information. Population genomic methods (e.g. Bayesian clustering methods, F_{ST} analysis, characterisation of Runs of Homozygosity, N_e estimation) were used to explore inbreeding and population structure in the breed. Two genetic clusters were identified, which were associated with the role of the dog (i.e. working dog *v.* show dog). Regions showing extreme genomic differentiation between clusters were investigated for functional associations. Several genes associated with cranial morphology were identified within these regions, suggesting selection may have occurred on related traits. Owner-assessed behavioural information was also collected for a large subset of dogs and analysed using quantitative genetic approaches (heritability estimation and GWAS). Principal components analysis was used to define 12 separate temperament traits, 10 of which showed evidence for genetic effects, 6 with moderate pedigree-based estimates of heritability (>0.20). Genomic estimates of heritability were generally lower than their pedigree-based counterparts, consistent with the 'missing heritability' identified in human studies. Regarding GWAS, there were no associations significant at the genome-wide level for the 12 traits, but several suggestive associations were identified, including those for fear of loud noises and fetching ability. This study demonstrates the utility of both population genomic and quantitative genetic approaches for the identification of candidate regions associated with complex phenotypes. It also exemplifies the power of dog as a model species for studying the genetics of complex traits.

Key Words: dogs and related species, inbreeding, population structure, heritability, genome-wide association

MT91 Identification of the mutation causing progressive retinal atrophy in Old Danish Pointing Dogs. P. Karlskov-Mortensen* and M. Fredholm, *Department of Veterinary and Animal Science, Faculty of Health & Medical Sciences, University of Copenhagen, Copenhagen, Denmark.*

Progressive Retinal Atrophy (PRA) is a disease characterised by degeneration of the photoreceptor cells of the retina resulting in nyctalopia and subsequent loss of vision. The condition is documented in numerous dog breeds and is known to be genetically heterogeneous between breeds. For the majority of breeds the mutation(s) responsible for PRA have yet to be identified. The Old Danish Pointing Dog is one of five national Danish dog breeds recognised by the international organisation for dogs FCI (Fédération Cynologique Internationale). It was once a very common dog in Denmark but in the middle of the 20th century the breed suffered a severe bottle neck and was reduced to less than 20 dogs. In 1961 the breed was accredited by the Danish Kennel Club and 161 dogs were registered in the club. Today, ~100 new puppies are registered by the Danish Kennel Club each year. The first cases of PRA in Old Danish Pointing Dog were registered in the late 1990s. The disease is typically diagnosed at around seven years of age and disease development and symptoms resemble the PRA variant known as progressive rod-cone degeneration in other dog breeds. Pedigree analyses suggest that the disease segregates as an autosomal recessive trait. Genotyping of 170K SNPs in 12 cases and 12 controls was performed to carry out association analysis and homozygosity mapping. Subsequently, whole genome sequencing of five cases and five controls was performed to identify the PRA causing mutation in Old Danish Pointing Dogs. In this work we investigate why genetic mapping based on SNP genotypes proved unsuccessful in this case and describe how whole genome sequencing was used to identify a frame shift mutation in C2orf71 in Old Danish Pointing Dogs. The identified mutation is the same as the one Downs *et al*. identified causing PRA in Gordon an Irish Setter breeds (Animal Genetics 44:169–77).

Key Words: dog, GWAS, genome sequencing, PRA

MT92 The impact of selection on genetics within the Siberian Husky breed. K. Ellis* and H. J. Huson, *Cornell University, Ithaca, NY, USA.*

The Siberian Husky (SH) was originally bred for its strength and endurance and served as a means of transportation, protection, and companionship for semi-nomadic arctic tribes. Modern SH remain proficient in sledding but are also bred as show and companion animals. Racing SH are selected for endurance and hardiness, while show dogs undergo more rigid selection for their appearance and stature. Companion dogs are often selected for behaviour and may not meet sledding or show requirements. While there is overlap in selection criteria among these three groups of sledding, show, and companion animal, each group prioritizes their selection criteria differently. In this study, we sought to explore the performance and conformation variation within SH in comparison to genomic variation. We collected 19 physical body measurements, DNA, health and usage information, and pedigrees from 178 dogs. Principal component analysis (PCA) on body size identified four body patterns distinguishing subpopulations including segregating sledding dogs based on chest width and wither height. Preliminary results are described for 61 dogs genotyped on the Illumina Canine HD beadchip. Quality control filtering of the SNPs (call rate >0.9 and MAF < 0.05) provided 105,872 SNPs for analysis. Genetic PCA

and marker-based F_{ST} of 21 sled, 23 show-sled, 8 show, and 7 companion SH identified 3 genetic subpopulations within the breed. PCA identified individuals competing as both sled and show dogs overlapping in these respective groups. Companion dogs showed the greatest amount of variation. Marker-based F_{ST} identified two SNPs on chromosome 10 with an $F_{ST} > 0.6$ when comparing companion SH to other populations. Genes in this region had neurological implications linked to serotonin release which correlated with decreased body fat and weight, and to anaemia and oxygen flow. F_{ST} also identified a SNP on chromosome 32 near four genes regulating iron metabolism and oxygen transport. These traits highlights the potential differences in genetic selection of subpopulations. The presence of these genes in show dogs is suggestive of the use of popular sires in both the sled and show communities. An additional 103 dogs are currently being genotyped and added to this analysis to improve the sample size.

Key Words: Siberian Husky, Fst, ROH, PCA

MT93 Combined clinical, genetic, and pathophysiological investigations of inherited pulmonary fibrosis in West Highland white terriers. E. E. Patterson*[1], K. M. Minor[1], D. A. Feeney[1], and P. B. Bitterman[2], [1]*University of Minnesota College of Veterinary Medicine, Saint Paul, MN, USA;* [2]*University of Minnesota School of Medicine, Minneapolis, MN, USA.*

Pulmonary fibrosis (PF) is a form of interstitial lung disease that causes unrelenting lung fibrosis leading to death, and in dogs primarily occurs in West Highland white terriers (WHWT). Objectives of these studies were to further define the clinical characteristics of WHWT PF, identify associated genetic mutations, and determine if WHWT PF has a similar pathophysiologic basis to idiopathic familial pulmonary fibrosis (FPF or IPF) in people. Control ($n = 17$) dogs were >8yrs old with normal arterial blood gas (ABG) oxygen (O2) levels. Presumptive cases were determined by ABG alone ($n = 2$) or ABG and radiographs ($n = 5$), and gold standard diagnosis cases ($n = 12$) were determined by high definition CT scan. Pulmonary mesenchymal progenitor cells (MPCs) were isolated from 3 cases immediately postmortem. Controls dogs mean age was 11.2 years and mean age of onset of cases was 11.5 years. Mean ABG PaO2 levels was statistically significantly lower in cases (72.4mmHg) *v.* controls (103.7mmHg) - $P = 0.0001$, with 67% of cases having a PaO2 < 80mmHg. Most affected dogs survived 1–3 years after diagnosis. Initial, whole genome sequencing (6 cases/6 controls) did not detect any coding variants in the Muc5B gene which is the most frequently mutated gene associated with disease in people. A follow-up GWAS was performed with 1.2 Million SNPs, and two strong signals stood out on CFA16 ($p_{raw} = 1.510–5$) and CFA21 ($p_{raw} = 3.0 \times 106$); potential positional candidates genes are being evaluated from the WGS. Cultured canine PF MPCs cells did not display the characteristic IPF/FPF fibroblast phenotype. WHWT PF appears to have a significant genetic component, with the potential for 1 or 2 major susceptibility genes based on initial GWAS analysis. WHWT terriers are typically diagnosed at a geriatric age and can live for several years. The underlying pathophysiology appears to be different than human IPF/FPF based on relative time of survival and MPC characteristics. WHWT PF, however, might potentially have pathophysiologic overlap with the less common human Idiopathic Nonspecific Interstitial Pneumonia (NSIP) which would need further study.

Key Words: dogs and related species, genome-wide association, DNA sequencing, respiratory system, biomedical model

MT94 Genetic variants in *ATP1B2* and *KCNJ10* in Belgian Shepherd dogs with ataxia. N. Mauri[1], M. Kleiter[2], M. Leschnik[2], S. Högler[3], E. Dietschi[1], C. Monney[4], A. Oevermann[4], D. Henke[5], M. Wiedmer[1], J. Dietrich[1], F. Steffen[6], S. Schuller[7], C. Gurtner[8], N. Stokar-Regenscheit[8], D. O'Toole[9], T. Bilzer[10], C. Herden[11], V. Jagannathan[1], and T. Leeb*[1] [1]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland;* [2]*University Clinic for Small Animals, Department for Companion Animals and Horses, University of Veterinary Medicine Vienna, Vienna, Austria;* [3]*Institute of Pathology and Forensic Veterinary Medicine, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria;* [4]*Division of Neurological Sciences, Department of Clinical Research and Veterinary Public Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland;* [5]*Division of Clinical Neurology, Department of Clinical Veterinary Medicine, Vetsuisse Faculty University of Bern, Bern, Switzerland;* [6]*Section of Neurology, Department of Small Animals, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland;* [7]*Division of Small Animal Internal Medicine, Department of Clinical Veterinary Medicine, Vetsuisse Faculty University of Bern, Bern, Switzerland;* [8]*Institute of Animal Pathology, Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland;* [9]*Wyoming State Veterinary Laboratory, University of Wyoming, Laramie, WY, USA;* [10]*Institute of Neuropathology, University Hospital Düsseldorf, Düsseldorf, Germany;* [11]*Institute of Veterinary Pathology, Justus-Liebig-University, Gießen, Germany.*

Ataxia is characterised by uncoordinated movements and may be caused by cerebellar, vestibular or proprioceptive dysfunction. In the Malinois variety of Belgian Shepherd dogs, puppies that showed a severe ataxia with an age of onset at 4–8 weeks had previously been reported. Neuropathologically, ataxic puppies suffered from spongy degeneration targeting the cerebellum, brainstem, and spinal cord in some cases. We performed a genetic investigation in six closely related families with ataxic puppies and seven additional isolated cases. Linkage analysis revealed an unexpected genetic heterogeneity within the families, which was correlated with variation of the neuropathological lesions. The affected dogs from four families and one isolated case shared a ~1.4 Mb common homozygous haplotype segment on chromosome 38. Whole genome sequence analysis revealed a missense variant in the *KCNJ10* gene encoding a potassium channel (c.986T>C; p.Leu329Pro) that was homozygous in the cases from four of the six families and one isolated case. Pathogenic variants in *KCNJ10* were reported previously in humans, mice, and dogs with neurological phenotypes. Therefore, we consider *KCNJ10*:c.986T>C the most likely candidate causative variant for one subtype of ataxia, which we propose to term spongy degeneration with cerebellar ataxia 1 (SDCA1). In all four affected puppies from another family and one of the isolated cases we detected a structural variant in the *ATP1B2* gene encoding a subunit of the Na^+/K^+-ATPase. *Atp1b2* deficient mice show early onset motor incoordination and severe progressive neurodegeneration. Thus, the detected *ATP1B2* variant is a compelling candidate causative variant for a second ataxia form in Malinois dogs. So far, no human patients with *ATP1B2* defects have been described. Therefore, we propose that this gene should be considered a functional candidate gene for unexplained human cases of ataxia. The investigation of the last of the six families is still ongoing and we will present an updated analysis during the conference.

Key Words: dog, molecular genetics, disease, nervous system, high-throughput sequencing (HTS)

MT95 Detection and characterisation of a genetic association with Norwich terrier upper airway syndrome. T. Marchant*[1], E. Dietschi[2], R. Harrington[1], M. Drögemüller[2], U. Rytz[3], T. Leeb[2], and J. Schoenebeck[1], [1]*The Royal (Dick) School for Veterinary Studies and Roslin Institute, The University of Edinburgh, Edinburgh, Midlothian, UK;* [2]*The Institute of Genetics, Vetsuisse Faculty, The University of Bern, Bern, Switzerland;* [3]*Department of Clinical Veterinary Medicine, Division of Small Animal Surgery, Vetsuisse Faculty, The University of Bern, Bern, Switzerland.*

In domestic dogs, the 'flat-faced' brachycephalic head shape is a risk factor for developing the respiratory defect, Brachycephalic

Obstructive Airway Syndrome (BOAS). As the popularity of breeds such as the French bulldog continues to increase in the UK, so too are the expected incidences of BOAS. For this reason, we became interested in the Norwich terrier, a non-brachycephalic breed which presents with Upper Airway Syndrome (UAS), a condition highly reminiscent of BOAS. Here, we have studied this single breed to identify genetic association(s) with UAS. Pathological assessments and grading from laryngoscopic examinations held at the Vetsuisse Faculty of the University of Bern, were used as phenotypes in conjunction with microarray genotypes to perform GWAS. In total, 233 Norwich terriers were examined. We identified the same QTL on canine chromosome (CFA) 13 to be associated with the abnormal positioning of laryngeal cartilage and everted saccules in the dogs most severely affected by UAS. We phased genotypes at the CFA13 QTL to conduct haplotype mapping, which led us to define a 413 kb critical interval which encompasses a single positional candidate gene. The derived haplotype within this interval is overrepresented: it is found to be homozygous in 61 of 81 (74%) severely affected cases. In contrast, this homozygous haplotype was identified among 7 of 86 (8.1%) mild/unaffected controls. We have resequenced four dogs representing phenotypic extremes to 16-fold depth to identify putatively causal variants. We will provide an update to this ongoing project, which is expected to guide Norwich terrier breeding and inspire additional exploration of the CFA13 locus to improve animal welfare.

Key Words: dog, animal health, genome-wide association, quantitative trait locus (QTL), candidate gene

MT96 Canine diversity and disease: Genome analysis in Australian dogs. S.-A. Mortlock, J. Marin-Cely, R. Booth, P. Soh, M.-S. Khatkar, and P. Williamson*, *The University of Sydney, Sydney, NSW, Australia.*

Dogs have tremendous natural diversity in form and stature, and are recognised as excellent models for genetic studies. There are also many documented diseases in dogs that represent comparative models of human disease and which are found within breed sub-populations. We have utilised genome technologies customised for dogs to study disease risk and variation in biological processes in Australian dogs, specifically in the Kelpie, Bullmastiff, Border collie, and German Shepherd dog (GSD) breeds. In this study we analysed natural variation in metabolomic profiles of GSD. Genotyping data was generated using the Illumina Canine HD array, and 52 metabolites were measured in plasma. Amino acid profile measurements measured by liquid-chromatography mass spectrometry were obtained from 82 dogs for 20 amino acids. A mixed linear model association analysis was conducted in GCTA for each of the 20 amino acids to detect any loci associated with these metabolite measurements. A single association was detected for threonine levels, the peak signal observed from a 5.3Mb region on CFA25 (p-value < 0.05, FDR < 0.05). Subsequently, a sliding window analysis identified haplotypes within the region that were associated with threonine levels. The most significantly associated haplotype contained six SNPs and two genes, *MTMR9* and *LOC477365*, and was shown to be predominantly homozygous in dogs with high threonine values. The *LOC477365* gene, which is now annotated as a threonine dehydrogenase, was the top candidate gene owing to its involvement in threonine metabolism and location within the top haplotype. Analysis of expression or structural polymorphisms within the gene are underway.

Key Words: canine, genome-wide association study, metabolomics, amino acids

MT97 Early events of cat domestication uncovered through ancient mitochondrial DNA analysis. E.-M. Geigl*[1], C. Ottoni[1,2], and T. Grange[1], [1]*Institut Jacques Monod, CNRS, University Paris Diderot, Paris, France;* [2]*KU Leuven, University of Leuven, Department of Imaging and Pathology, Center for Archaeological Sciences; University Hospitals Leuven, Laboratory of Forensic Genetics and Molecular Archaeology, Leuven, Belgium.*

The analysis of mitochondrial DNA in domestic, feral and wildcats showed 10 years ago that domestic cats are the descendants of the wildcat from Northern Africa (NA) and South-west Asia (SWA), *Felis silvestris lybica*. Little is known, however, about their domestication centre(s), process and spread owing to both hybridization between domestic and wild cats blurring the tractability of the genetic signature of wild cats as well as the scarcity of archaeological remains. Solely two archeological finds, a cat skeleton found in a 9,000-year-old child burial in Cyprus and some skeletons excavated from a 5,700-year-old Egyptian Elite cemetery, suggest a cat-human relationship evocative of a taming process. The richest source documenting this relationship is the iconography of ancient Egypt showing an evolution of the cat-human relationship towards an incorporation of the cat into the domestic context. To shed light on the domestication process of cats, data on the predomestication situation are required. In particular, knowledge of the phylogeography in the past and its evolution over time is a prerequisite to disclose the 'where, when and how' of this process. Analysing mitochondrial DNA from ancient specimens covering ~9,000 years, from the Mesolithic to the 19th century CE, and a large geographic area including Europe, SWA and NA, we were able to decipher crucial steps of the spread of wildcats in the ancient world. Our data indicate that *F. s. lybica* was tamed twice at different times, first in SWA and later in ancient Egypt, and that these tamed cats conquered the world as companions of merchants and soldiers on their voyages throughout the ancient world. These translocated ship's cats subsequently reshaped the diversity of wildcats in the corresponding areas.

Key Words: cat, ancient DNA, DNA analysis

MT98 AgriSeq targeted sequencing panel for determination of canine parentage and genetic health. M. Karberg*[1], A. Burrell[1], P. Siddavatam[1], A. Allred[1], M. de Groot[2], and W. van Haeringen[2], [1]*Thermo Fisher Scientific, Austin, TX, USA;* [2]*VHL Genetics, Wageningen, Netherlands.*

The objective of this presentation is to show the performance of canine parentage and genetic health AgriSeq primer panels, and demonstrate how the panels can be combined, or otherwise modified, without detrimental effects to detection. Ensuring an accurate pedigree is particularly important for purebreds, having both economic and animal health implications. Historically, microsatellites (short tandem repeats or STRs) have been used for genetic identification, traceability and paternity. In recent years, other DNA based tests such as single nucleotide polymorphism (SNPs) detection have become increasingly used for this purpose. High-throughput targeted amplification and re-sequencing, using the Applied Biosystems AgriSeq target enrichment technology and the Ion S5 sequencing system, allows for the simultaneous and accurate genotyping of a large number of SNPs to interrogate the heritage and genetic health of an animal. In addition, the AgriSeq approach is very flexible, allowing panels to be combined or modified easily, thereby helping both breeders and diagnostic laboratories stay relevant with evolving content needs. Here, we describe the development of two AgriSeq panels targeting canine SNP markers: a parentage panel based on 200 ISAG canine targets, and a genetic health panel based on over 140 well characterised genetic markers. To demonstrate the modularity of the AgriSeq approach, the performance of the genetic health and parentage panels were analysed independently and also combined and analysed simultaneously using 192 samples pooled on an Ion S5 540 chip. Variant calling was performed using the Torrent Variant Caller (TVC) plugin as part of the Ion Torrent Suite software package. The data show that the two panels work similarly, regardless of whether they are used separately, or combined together on one sequencing chip. The mean sample call rate was

above 95% and the sample concordance between the separate and combined panels was over 99.9%.

Key Words: dogs and related species, DNA sequencing, parentage

MT99 An analysis of canine caudal fossa morphology and its genetics. R. Harrington*, T. Marchant, D. Argyle, D. Clements, T. Liuti, T. Schwarz, K. Marioni-Henry, and J. Schoenebeck, *Royal (Dick) School of Veterinary Studies and Roslin Institute, The University of Edinburgh, Easter Bush, Midlothian, UK.*

The caudal fossa is the concavity of the occipital bone that surrounds the cerebellum. In dogs, abnormalities in caudal fossa (CF) morphology are suspected of contributing to the development of neurological conditions such as syringohydromyelia. This painful condition is a major animal welfare concern, particularly to small breed dogs such as the Brussels Griffon and Cavalier King Charles Spaniel. Although the exact causes of syringohydromyelia are unknown, its correlation with Chiari malformations of the occipital bone have led to speculation about the developmental interplay between the skull and its underlying soft tissues. To date, analyses of the canine CF have not determined the types and breadth of CF shapes observed both within and across dog breeds. We present our methodology to study CF morphology. Using 3D reconstructions of skulls derived from computed tomography (CT) imaging, we have landmarked 500+ skulls from a wide range of dog breeds of varying sizes and head shapes, including the CF morphology. Each patient used in our study was also genotyped on the Illumina Canine HD 170K SNP array. Our quantification of CF morphologies enabled us to conduct genome-wide association studies (GWAS) to investigate the basis of CF shape in a manner that is controlled for individual size. The morphological analysis and GWAS methodology presented can directly improve canine welfare through the identification of CF morphologies which are at higher risk for syringohydromyelia and their genomic associations.

Key Words: dogs and related species, genome-wide association, diagnostic imaging, quantitative trait locus (QTL), morphometrics

MT100 Revealing the genetic basis of diabetes mellitus in Burmese cats. G. Samaha[1], J. Beatty[1], L. Lyons[2], C. Wade[3], and B. Haase*[1], [1]*School of Veterinary Science, Faculty of Science, University of Sydney, Sydney, NSW, Australia;* [2]*College of Veterinary Medicine, University of Missouri, Columbia, MO, USA;* [3]*School of Life and Environmental Sciences, Faculty of Science, University of Sydney, Sydney, NSW, Australia.*

This study aims to identify the underlying genetic factors causing feline diabetes mellitus (FDM) in the Australian Burmese cat population. Feline diabetes mellitus (FDM) is a common feline endocrinopathy, characterised by insulin resistance, defective insulin secretion and β-cell loss. Among established breeds, the incidence of FDM is highest among Burmese cats [O'Neill et al. (2016) J. Vet. Intern. Med. 30:964–972]. Variation in the prevalence of this disease among breeding populations is well established. Australian, European, British and New Zealand-bred Burmese are more likely than US-bred Burmese to suffer from FDM [McCann et al. (2007) J. Feline Med. Surg. 9:289–299; Lederer et al. (2009) Vet. J. 179:254–258]. We performed a genome-wide association analysis using 76 samples, including 10 cases and 66 controls. All animals were genotyped on the Illumina Feline 63K DNA array (Illumina, San Diego). Affected cats were diagnosed by a veterinarian based on persistent fasting hyperglycemia, glycosuria with clinical signs; weight loss, polydipsia and polyphagia. Unaffected cats displayed no clinical signs of FDM at the time of sample collection. Association analysis and permutation testing was performed using PLINK [Purcell et al. (2007) Am. J. Hum. Gen. 81:559–575]. HAPLOVIEW [Barrett et al. (2005) Bioinformatics 21:263–265] was used to assess linkage disequilibrium of significant SNPs and to construct haplotype blocks. The GWAS revealed strong associations of FDM to loci on FCA4 ($P_{raw} = 3.72\times10^{-13}$) and FCA7 ($P_{raw} = 7.08\times10^{-12}$). A high degree of linkage disequilibrium was observed among significant SNPs within the associated regions on FCA4 and FCA7 ($r^2 = 0.77–1.0$ and $r^2 = 0.8$, respectively). The distribution of alleles on FCA4 and FCA7 among cases and controls suggests both regions play a role in the pathogenesis of FDM. Our findings provide new insights into the genetic basis of FDM in domestic cats and may have provided a basis for the identification of functional variants associated with the condition within the Australian Burmese breeding population.

Key Words: diabetes mellitus, feline, Burmese cat, GWAS

MT101 Insights into 115 domestic cat and 17 wild felid genomes. L. Lyons*, *University of Missouri, Columbia, MO, USA.*

The 99 Lives Cat Genome Sequencing Consortium was initiated in 2014 and has now reached its goal of over 100 high-quality genomes from domestic cats. Over 90% of genomes are 30x coverage, with a minimum of 20x coverage, and produced using only 100 – 150 bp paired-end reads from PCR-free libraries using Illumina HiSeq sequencing technology. The genome dataset comprises 115 domestic cats, including at least 22 breeds, a representative from the ten racial populations of cats, four parent-offspring trios, and a few affected sib-pairs. Over 20 investigators have contributed sequences to the consortium, as well as three industry partners. Funding has been provided by various contributors for each investigator, the Winn Feline Foundation, the National Geographic Foundation, and industry. Nearly 50% of wild felid species are represented including cats from six of eight lineages within Felidae, including domestic cat, domestic cat, leopard cat, puma, lynx, caracal, and panthera. The 99 Lives dataset has led to the discover of DNA variants likely causal for congenita Myasthenic syndrome, progressive retinal atrophy, bobtailed tail, and a novel form of Niemann-Pick Type C in domestic cats, and a retinal degeneration in Black-footed cats and as well as several other traits that are yet unpublished. The discovery of the Niemann-Pick Type C mutation is an example of whole genome sequencing of a clinical case and discovery of the likely causal variant during the course of the cat's disease. This success demonstrates thay Precision Medicine can be used in feline health care. The dataset has allowed the identification of a vast array of variants in the cat genome that can support the development of a high-density DNA array. Goals are to include at least one representative from each cat breed and species, and to continue variant discovery for diseases and traits in the felids. The success and the limitations of the 99 Lives project will be presented, including the Niemann-Pick Type C Precision Medicine effort.

Key Words: domestic cat, felids, whole-genome sequencing, disease, phenotypes

MT102 Characterization of Plakophilin-2 expression in canine skin and identification of differential gene expression in non-lesional skin from dogs affected by atopic dermatitis. G. Andersson*[1], K. Tengvall[2,3], B. Ardesjö-Lundgren[1,2], S. Kozyrev[2], M. Kierczak[2], M. Olsson[2,3], F. F. Farias[2], Å. Hedhammar[4], K. Bergvall[4], and K. Lindblad-Toh[2,5], [1]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [2]*Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, BMC, Uppsala University, Uppsala, Sweden;* [3]*Center for Molecular Medicine, Neuroimmunology Unit, Department of Clinical Neuroscience, and Reumatology Unit, Department of Medicine, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden;* [4]*Department of Clinical Sciences, Swedish University of Agricultural Sciences,*

Uppsala, Sweden; [5]Broad Institute of MIT and Harvard, Cambridge, MA, USA.

Canine atopic dermatitis (CAD) is an immune-mediated disease caused by interactions between genetic and environmental factors. We previously identified genetic risk factors for CAD located on CFA27 in German shepherd dogs (GSDs). Targeted re-sequencing and fine-mapping identified transcriptional regulatory variants within the *Plakophilin-2 (PKP2)* locus as likely major risk factors (Tengvall *et al*. *PLoS Genet.* 2013). Subsequent functional studies of these regulatory variants confirmed their differential transcriptional regulatory activities in epithelial- and immune-derived cells implicating a role for these risk variants in the development of CAD (Tengvall *et al*. *BMC Genet.* 2016). We performed immunofluorescence, electron microscopy immuno-labelling, and morphology analyses of PKP2, which is a crucial desmosome component, in non-lesional axillary skin biopsies collected from CAD-affected and healthy control GSDs to define the cellular expression pattern of PKP2 in epidermal cells and its intracellular localization. PKP2 was evenly expressed in keratinocytes and strong expression was also observed in Langerhans cells (LCs) and T-cells. PKP2 protein was located in nuclei and on keratin filaments attached to desmosomes (Ardesjö-Lundgren *et al*. *Vet. Dermatol.* 2017). Since LCs and T-cells are known to be involved in atopic disease, altered expression of PKP2 in these cell types may correlate to CAD pathogenesis. To gain further insights into gene expression patterns and pathways influenced in CAD, we performed mRNA sequencing of the transcriptome of non-lesional atopic axillary skin tissue and axillary skin from healthy dogs. Differential gene expression was identified and gene ontology analyses identified involvement of inflammatory as well as structural pathways. Our results will be used for improved annotation of the canine skin transcriptome. The implications of the observed differential gene expression for the development of CAD will be discussed. Funding was provided by the Swedish Research Council, the Swedish Research Council FORMAS and the European Research Council (ERC).

Key Words: dogs, functional genomics, RNA sequencing, disease genetics, allergy

MT103 Localizing the regions of causative mutations in feline amyloidosis: a next-generation genomic approach. F. Genova*[1], B. Gandolfi[2], A. Thomas[3], E. Creighton[2], L. Lyons[2], and M. Longeri[1], [1]*Department of Veterinary Medicine, Università degli Studi di Milano, Milan, Italy;* [2]*Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA;* [3]*Antagene, La tour de Salvagny, France.*

The term "amyloidosis" is referred to a heterogeneous group of metabolic diseases that arise as a consequence of the improper folding of some autologous proteins. Protein aggregation and deposition is reported in different organs. In the mutated system the amyloid protein precursors acquire a β - sheet secondary structure, forming fibrillary deposits that are insoluble and resistant to proteolysis. The amyloid fibrils can be deposited locally in one single organ, or they can be distributed systemically. In felids, amyloidosis was observed in fancy pedigreed breeds and random bred cats and cases were also reported in the black-footed cat, the Siberian tiger and the cheetah. In young Abyssinian/Somali and Siamese/Oriental breeds, a familial inheritance in developing the Inflammatory amyloidosis (AA) was described. The pathogenic pathway of this disease is still unknown in felids and the final diagnosis is only *postmortem*. In this study, the whole genome sequences (WGS) of two affected Abyssinians, two affected Siamese and one affected Black-footed cat, were used to identify mutations potentially related to amyloidosis, using additional 127 whole genome cat sequences in the 99 Lives Project context as controls for variant exclusion. Identified variants were prioritized based on a previous GWAS analysis carried out on affected/healthy cohorts of Abyssinians, which showed associations with cat chromosomes B4 and C2. Among all the genes within the two regions, one above all, on chromosome B4, is considered a good candidate as it is involved in controlling the levels of b-amyloid deposits, through the interaction with a zinc finger protein. Indeed, two mutations within the candidate gene were found, one unique to the affected cats and an additional variant unique to the affected black-footed cat. Other interesting variants were found in two zinc finger proteins as well as in several genes on cat chromosome C2. To confirm the causality link to the disorder, the identified variants will be genotyped on a larger population of pedigreed bred cats, as well as on random bred cats and four black-footed cats.

Key Words: cats and related species, genome sequencing, genome-wide association, candidate gene, animal health

MT104 Phylogenetic analysis of Angora, Van and stray cats of Anatolia. N. Bilgen*, B. C. Kul, M. Akkurt, O. Cildir, O. Ozmen, and O. Ertugrul, *Ankara University Faculty of Veterinary Medicine Department of Genetics, Ankara, Turkey.*

Unlike agricultural animals (cattle, sheep, pig etc.) or carrying animals (horse and donkey) the cat domesticated due to its feeding habit on rodents, which invade grain storage of farmers showing cats as 'commensal'. Earliest archeological evidence was found in Cyprus pointing that cat's domestication period was determined as between 9500–4000 years ago. Anatolia is considered as the cradle of domestication for many animal species. As an important species of Anatolia, there is not enough molecular study to reveal history of the Angora and Van cats. To shed light on domestic cat breeds of Anatolia, Angora (n = 28), Van (n = 49) and stray cats (n = 51) were sampled. The Cytochrome *b* gene (CYTb) and control region (CR or D loop) on mtDNA were aimed to investigate by Polymerase Chain Reaction (PCR) and sequencing methods, and data analysed by bioinformatics tools. Phylogenetic analysis revealed that the *F. silvestris lybica* was major maternal origin whereas Van, Angora and stray cats also shared branch with *F. silvestris ornata.* Network analysis and frequency calculations showed ~70% of the cats were represented by two major haplotypes, A and D for CR; Haplotype10 and Haplotype15 for CYTb. Unique sequences were found in %9.3 of the population (Van n = 1; Angora n = 3; stray cats n = 8). Haplotype diversity of CYTb and CR region were determined 0.71 and 0.77, respectively. Shared haplotypes were high, thus FST statistics revealed low genetic differentiation between groups. Obtained data and new mtDNA haplotypes provided in completion of the information about the genetic structure of the domestic Anatolian cat breeds. This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) grant number 114O768.

Key Words: Angora cat, control region, cytochrome b gene, mtDNA, Stray cat

MT105 Canine brachycephaly is associated with a retrotransposon-induced missplicing of *SMOC2*. T. Marchant[1], E. Johnson[1], R. Harrington[1], L. McTier[1], C. Johnson[1], A. Gow[1], T. Liuti[1], D. Kuehn[2], K. Svenson[3], M. Bermingham[4], M. Drögemüller[5], M. Nussbaumer[6], M. Davey[1], D. Argyle[1], R. Powell[7], S. Guilherme[8], J. Lang[9], G. Ter Haar[1], T. Leeb[5], T. Schwarz[1], R. Mellanby[1], D. Clements[1], and J. Schoenebeck[1]* [1]*Royal (Dick) School of Veterinary Studies and Roslin Institute, The University of Edinburgh, Midlothian, UK;* [2]*Friendship Hospital for Animals, Washington, DC, USA;* [3]*The Jackson Laboratory Bar Harbor, Bar Harbor, Maine, USA;* [4]*Institute of Genetics and Molecular Medicine, The University of Edinburgh, Edinburgh, UK;* [5]*Institute of Genetics, University of Bern, Bern, Switzerland;* [6]*Naturhistorisches Museum, Bern, Switzerland;* [7]*Powell Torrance Diagnostic Services, Hertfordshire, UK;* [8]*Davies Veterinary Specialists, Hertfordshire, UK;* [9]*Department of Clinical Veterinary Medicine, University of Bern, Bern, Switzerland;* [10]*Department of Clinical*

Sciences and Services, The Royal Veterinary College, Hertfordshire, UK.

The accentuated morphological features of the domestic dog have leant themselves to making this species a powerful model for identifying developmental programmes of bone formation and growth. Here, we have used computer tomography scans of 374 pedigree and mixed-breed dogs to generate high-resolution three-dimensional reconstructions of the canine skull. Geometric morphometric analyses of the neurocranium and rostrum enabled us to separate craniofacial size from shape in dogs that were genotyped by high-density SNP arrays. Using haplotype mapping, we identified a 187 kb critical interval on canine chromosome 1 present among the brachycephalic dogs used in our study. Leveraging whole genome sequencing, we identified candidate mutations that included a large structural variant and several other SNPs. All variants are found within introns of the SPARC-related modular calcium binding 2 (*SMOC2*) gene. The structural variant is a fragment of a long interspersed nuclear element (LINE-1), a family of retrotransposons. RNAseq analysis of *SMOC2* mRNA has revealed three alternative *SMOC2* transcripts present among individuals that carry the LINE-1 element. All alternative transcripts incorporate the LINE-1 element and a portion of preceding intron. This is predicted to introduce premature stop codons following exon eight of *SMOC2*'s canonical 13-exon transcript. RT-qPCR has revealed an 80% reduction in *SMOC2* transcript levels for individuals that are homozygous carriers of the LINE-1 which may be the result of nonsense mediated decay of the alternative transcripts. No differential expression was detected among transcripts of genes neighbouring *SMOC2*. Our models of phenotypic effect predict that the LINE-1 insertion explains up to 34% of the overall face length variation captured by our study. Endogenously expressed (mouse) *Smoc2* is observed in the pharyngeal arches during development and the viscerocrania of *Smoc2* null mice are dysmorphic. Our data provide a compelling picture that implicates *SMOC2* in mammalian craniofacial development. Our results are predicted to have health implications to both human and veterinary medicine.

Key Words: dog, craniofacial anomaly, genetics, brachycephaly, retrotransposon

Comparative MHC Genetics: Populations and Polymorphism

MT106 MHC Class III gene Tenascin-XB (*TNXB*) is associated with mature weight in U.S. Rambouillet, Targhee, Polypay, and Suffolk sheep. M. U. Cinar*[1,2], M. R. Mousel[3,4], M. K. Herndon[1], M. A. Highland[3,4], J. B. Taylor[5], D. P. Knowles[1,3], and S. N. White[1,3], [1]*Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA;* [2]*Erciyes University, Faculty of Agriculture, Department of Animal Science, Kayseri, Turkey;* [3]*Animal Disease Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Pullman, WA, USA;* [4]*Paul G. Allen School of Global Animal Health, Washington State University, Pullman, WA, USA;* [5]*Range Sheep Production Efficiency Research, Dubois, ID, USA;* [6]*Center for Reproductive Biology, Washington State University, Pullman, WA, USA.*

The major histocompatibility complex (MHC) class III region is located between the class I and class II regions, and contains genes with both immune and non-immune functions. Among these, Tenascin-XB (encoded by the TNXB gene) is a large extracellular matrix protein that plays a key role in matrix formation during wound healing. An association of the TNXB charged E2004 allele (glutamic acid) with increased bodyweight compared with the nonpolar G2004 allele was found in sheep from Uda, Yankasa, Balami, and West African Dwarf breeds in Nigeria. The present study was to investigate an association of *TNXB* charged amino acid substitution E2004G with growth and lifetime lamb production traits in prevalent USA breeds. A total of 891 Polypay, Rambouillet, Targhee and Suffolk sheep were genotyped, resulting in 292 E2004 homozygotes, 404 heterozygotes, and 236 G2004 homozygotes. Nineteen phenotypic traits including multiple measures of mature weight were analysed separately using mixed models with breed, age/birth year, and genotype as fixed effects and sire nested within breed as random. *TNXB* E2004 homozygotes had greater bodyweights at all adult time points (twice per year, ages 3 and 4; all $P \leq 0.05$). These data confirm a prior report showing an association of the ancestral E2004 allele with increased growth over the recent mutant G2004 allele in prevalent USA breeds. Sheep breeders have various breeding objectives, including increased growth or maintenance of specific mature body size. Thus, the *TNXB* E2004G may be useful in genetic selection programs for multiple objectives involving sheep growth.

Key Words: PCR-RFLP, extracellular matrix protein, ovine MHC, mature body weight, US sheep breeds

MT107 Characterization of the diversity of LEI0258 microsatellite locus in Ethiopian indigenous chicken populations. A. Kebede*[1,2], O. Hanotte[3], T. Dessie[3], N. Spark[4], G. Belay[1], K. Tesfaye[1], J. Jung'a[5], M. Kyalo[6], W. Ekaya[6], and R. Pelle[6], [1]*Addis Ababa University (AAU), Addis Ababa, Ethiopia;* [2]*Amhara Regional Agricultural Research Institute (ARARI), Bahir Dar, Ethiopia;* [3]*International Livestock Research institute (ILRI), Addis Ababa, Ethiopia;* [4]*Center for Tropical Livestock Genetics and Health (CTLGH), Scotland, UK;* [5]*University of Nairobi (UoN), Nairobi, Kenya;* [6]*Biosciences eastern and central Africa (BecA), Nairobi, Kenya.*

The microsatellite locus LEI0258 is located within the B region of chicken major histocompatibility complex (MHC-B) on the microchromosome 16. Classical MHC class I and class II are known to be involved in the development of protective cellular (T-cell-based) and humoral (antibody-based) immunities against infections in vertebrates. Furthermore, direct correlates have been reported between LEI0258 microsatellite alleles and local chicken performance (antibody responses to vaccination against Newcastle disease virus (NDV); bodyweight; resistance to worms). We report here the characterisation of the diversity at this locus in 281 indigenous chickens from Ethiopia. These birds originated from 25 populations from different agro ecological zones across the country. We identify 30 different LEI0258 alleles of sizes ranging from 285 to 569 bp. Allele frequencies were different across regions whereas the PCA analysis of alleles diversity distributed the birds in four major groups. These alleles are being sequenced for further analysis and the preliminary sequencing result reveals presence of single nucleotide polymorphisms (SNPs) in LEI0258.

Key Words: chicken, diversity, Ethiopia, LEI0258 microsatellite

MT108 Evolution by gene duplication of the horse major histocompatibility complex class II structure. A. Viluma*, S. Mikko, G. Andersson, and T. F. Bergström, *Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences (SLU).*

The major histocompatibility complex (MHC) is a gene dense genomic region that harbors multigene families encoding for class I and class II antigen presenting molecules. The MHC structure has been widely studied in primate species and it is known for variable number of paralogous loci and evolution by gene duplication. Re-

cently, a high quality assembly and annotation of the horse MHC class II region was generated. In comparison to six other mammals (human, mouse, cattle, pig, dog and cat), the corresponding region in horse showed increased number of the *Eqca-DRB*, *-DQA*, *-DQB* and *-DOB* loci, as well as different relative location and directionality of five *Eqca-DRB* loci. The aim of this study was to better understand the molecular mechanisms that have shaped the horse MHC class II region by defining size and order of the duplication events and by investigating break point sequences of the duplicated blocks. The duplication blocks were defined by comparing the horse MHC class II sequence to itself using PipMaker. Furthermore, the intronic sequences of the individual gene families (*Eqca-DRB*, *-DQA*, *-DQB* and *-DOB*) were aligned and the genetic distances were calculated using Jukes-Cantor model. The results of the horse MHC class II sequence self-comparison showed a block-wise duplications of tail-to-tail oriented *Eqca-DQA* and *-DQB* gene pairs. However, the *Eqca-DRB* and *-DOB* paralogous loci were more likely to be individual duplication events of a single gene. The genetic distances of all six *Eqca-DRB* loci suggested that the inversion of *Eqca-DRB* locus predates the rest of the *Eqca-DRB* duplication events. The comparison of genetic distances of all the multigene family members indicated that expansion of the horse MHC class II region occurred in a relatively short evolutionary time period following the inversion of *Eqca-DRB* locus.

Key Words: MHC, horse, evolution, duplication

MT109 Major update to the Swine Leukocyte Antigen (SLA) Nomenclature System of the International Society for Animal Genetics (ISAG) and the International Union of Immunological Societies (IUIS). S. Ho*[1], J. Lunney[2], A. Ando[3], C. Rogel-Gaillard[4], J.-H. Lee[5], L. Schook[6], and S. Hammer[7], [1]*Gift of Life Michigan, Ann Arbor, MI, USA;* [2]*USDA, Beltsville, MD, USA;* [3]*Tokai University School of Medicine, Isehara, Kanagawa, Japan;* [4]*INRA, Jouy-en-Josas, France;* [5]*Chungnam National University, Daejeon, Korea;* [6]*University of Illinois, Urbana, IL, USA;* [7]*University of Veterinary Medicine Vienna, Vienna, Austria.*

The SLA system is among the most well characterised major histocompatibility complex (MHC) systems in nonhuman animal species. The ISAG/IUIS SLA Nomenclature Committee was established 15 years ago with primary objectives to: 1) validate newly identified SLA sequences according to the guidelines established for maintaining high quality standards of the accepted sequences; 2) assign appropriate nomenclatures for new alleles as they are validated; and 3) serve as a curator of the IPD-MHC SLA Sequence Database (www.ebi.ac.uk/ipd/mhc/group/SLA), which is the repository for all recognised SLA genes, their allelic sequences and haplotypes. The newly released and improved IPD-MHC Database version 2.0 has incorporated the latest sequence updates, provide new tools that enhance database queries and improve the submission process. The SLA Nomenclature Committee met at last year's ISAG and made some major revisions to the allele naming system. The Committee decided to retire the provisional alphanumerical naming system for unconfirmed alleles and re-designate each allele an official number, adopting the HLA Nomenclature System with colons as field separators (e.g. SLA-1*01rh28 → SLA-1*01:03). Phylogeny will remain the primary approach for assigning SLA-1, -2, -3, DRA, DRB1, DQA and DQB1 alleles into allele groups with similar sequence motifs, while alleles of SLA-5, -6, -7, -8, -12, DMA, DMB, DOA, DOB1, DRB2, DRB3, DRB4, DRB5 are designated sequentially as they are discovered. Naming convention for alleles of other loci (SLA-4, -9, -11, DQB2, DOB2, DYB, MIC1, MIC2, TAP1, TAP2) is to be determined as sequences accumulate. There are currently 223 class I, 214 class II, 2 SLA-related and 2 non-SLA alleles officially designated. There are also 61 class I (SLA-1–3-2) and 49 class II (DRB1-DQB1) haplotypes designated at allele level resolution. This systematic nomenclature for SLA genes is critical to the understanding of the architecture and polymorphism of the SLA system and their role in swine diseases, vaccine development and allo- or xeno-responses in transplantation research.

Key Words: MHC, SLA, immunogenetics, polymorphism, nomenclature

MT110 A rapid, direct-sequencing based MHC genotyping system for populations with insufficient information on allelic variation. J. Buitkamp* and J. Semmer, *Bavarian State Research Center for Agriculture Institute of Animal Breeding, Grub, Bavaria, Germany.*

The individual repertoire of MHC molecules determines which antigenic peptides are presented to the immune system. MHC coding genes belong to the most polymorphic genes in vertebrates and show a high degree of heterozygosity. Usually some main alleles with moderate frequencies occur aside with many rare alleles. Multiple polymorphic positions and different number of genes per haplotype hamper the correct definition of alleles. Haplotyping of heterozygous is particularly complicated when information on allelic variation is missing. We developed an efficient, sequence based MHC-genotyping system for poorly studied populations. It combines the initial definition of main alleles with a reference library that allows the rapid identification of alleles from heterozygous animals. The animals were Merino sheep from Bavaria. All lambs were genotyped for the microsatellite upstream from *DRB1* exon 2. Selected rams were genotyped for exon 2 of *DRB1* and *DQB* using multiple primer pairs that ensure amplification of a complete set of genes. From 500 lambs 53 were homozygote at the *DRB1* microsatellite. From these, 11 lambs representing all main alleles were sequenced at *DRB1* and *DQB* genes. In addition *DRB1* and *DQB* genes were sequenced from heterozygote animals carrying a rare allele in combination with a main allele allowing the determination of a large number of alleles occurring in the population using comparatively few animals. We identified 16 (including 3 new) *DRB1* alleles, and 19 (including 2 new) *DQB1* alleles. Using the sequences from homozygote lambs as a starting point, 11 MHC class 2 haplotypes could be defined. A reference database was build using these alleles in combination with all published alleles. To derive the correct alleles from heterozygote sequences a pipeline based on the blast algorithm was used that allows the identification of published and population specific MHC alleles. This system provides the basis for fast genotyping of MHC haplotypes and the identification of new rare alleles for small to medium scale projects in populations with limited knowledge of MHC diversity.

Key Words: sheep, immunogenomics, genotyping, MHC, animal health

MT111 Molecular characterisation of Ovar-MHC class II region reveals novel alleles in the Djallonke and Sahelian sheep breeds of Ghana. M. Yaro*[1], K. Munyard[1], E. Morgan[1], M. Stear[2], and D. Groth[1], [1]*Curtin University, Perth, WA, Australia;* [2]*La Trobe University, Melbourne, VIC, Australia.*

The Djallonke is the most ancient sheep breed in Africa. It is more resistant to many livestock diseases including haemonchosis and trypanosomosis than the Sahelian sheep from the same region. Both sheep breeds are of immense socio-economic relevance in more than 14 countries within the sub Saharan region. Resistance to nematode infection in sheep has been associated with variations within genes in the MHC class II region. These genes encode glycoproteins that present antigen to circulating CD4+ T-cells, and the most polymorphic ones are reported to be the DRB1, DQA1 AND DQA2. In this study we conducted sequencing-based genotyping

and analysis of the MHC class II DRB1, DQA1 and DQA2 loci in a population of 200 sheep from the Djallonke and Sahelian breeds from Ghana. Genomic DNA was genotyped with standard MHC primers and sequenced. New alleles and unresolved sequences were cloned into pGem-T easy plasmid vector, which then were used to transform *E. coli* competent cells and were sequenced in both directions using M13 primers. Preliminary results for both breeds show animals harbouring multiple amino acid substitutions (greater than 4) within the coding region of the second exon of DRB1 in reference to closest matched allele accessions at the Immuno-polymorphism database (IPD), suggesting novelty. Interestingly, there is a high frequency of variants of the FN543119.1 (ovar-DRB1*0901) allele only in the Djallonke population, and of EU176819.1 allele in the Sahelian population. Of particular interest is the presence of the FR751085.1 (ovar-DRB1*1303) allele in both sheep populations which was previously identified in the red Maasai sheep breed from Kenya. This suggests an ancestral relationship between these three African sheep breeds. To the best of our knowledge, this the first analysis of MHC architecture and allelic diversity in both breeds. Our ongoing studies aim to provide information that will enhance the sustainable management of these important sheep breeds.

Key Words: Djallonke, Sahelian, sheep, MHC, sub-Saharan Africa

MT113 Large-scale analysis of the specificities of livestock MHC class I and II molecules. D. B. Steen-Jensen[1], T. Østerbye[1], M. Rasmussen[1], M. Nielsen[2], A. Stryhn[1], and S. Buus*[1], [1]*Laboratory of Experimental Immunology, University of Copenhagen, Copenhagen, Denmark;* [2]*Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark.*

It is well established that T-cells recognise peptide epitopes presented in the context of MHC molecules, however, the identification and validation of T-cell epitopes is still a challenge; something that in real life situations is complicated by the size of pathogen 'peptidomes' and the extreme polymorphic/polygenic nature of the MHC of outbred host populations. Peptide binding to MHC is the most selective event involved in processing and presentation of antigens to T-cells and is therefore essential for our understanding of T-cell immunogenicity. We have proposed that the peptide binding specificity of all human MHC molecules should be mapped. To this end, we have developed recombinant human MHC class I and II molecules and generated the corresponding peptide-binding assays and large-scale affinity binding data. We have also confirmed that peptide-MHC stability is a better correlate of immunogenicity than affinity is, and begun to generate large-scale stability data. We have used the resulting data to develop a series of accurate bioinformatics predictors (e.g. NetMHCpan, NetMHCIIpan and NetMHCStab) and made these predictors publicly available. An efficient T-cell epitope discovery approach has transpired: a) use overlapping peptides representing proteins (or smaller proteomes) of interest to search for T-cell stimulatory peptides in outbred populations, then b) use the above bioinformatics resources to identify T-cell epitopes and their restrictions elements, and finally c) generate the corresponding MHC class I or II tetramers to validate the proposed T-cell epitopes. To extend these efforts to other species, we have generated unbiased and generally applicable methods, which only depends on the sequence of the MHC in question being available, and successfully begun to address the specificity of pig, cattle, and avian MHC class I and II molecules.

MT115 IPD-MHC 2.0: An improved interspecies database for the study of the major histocompatibility complex. G. Maccari*[1,2], J. Robinson[2,3], K. Ballingal[4], L. Guethlein[5], U. Grimholt[6], J. Kaufman[7], C. Ho[8], N. de Groot[9], R. Bontrop[9], P. Flicek[10], J. Hammond[1], and S. Marsh[2,3], [1]*The Pirbright Institute, Pirbright, Woking, Surrey, UK;* [2]*Anthony Nolan Research Institute, Royal Free Hospital, London, UK;* [3]*UCL Cancer Institute, Royal Free Campus, London, UK;* [4]*Moredun Research Institute, Pentlands Science Park, Scotland, UK;* [5]*Stanford University, Stanford, CA, USA;* [6]*Norwegian Veterinary Institute, Oslo, Norway;* [7]*University of Cambridge, Cambridge, UK;* [8]*Gift of Life Michigan, Michigan, USA;* [9]*Biomedical Primate Research Centre, Rijswijk, Netherlands;* [10]*European Molecular Biology Laboratory, Wellcome Genome Campus, Hinxton, UK.*

The IPD-MHC Database (www.ebi.ac.uk/ipd/mhc/) is a key resource for the collection, study and comparison of the major histocompatibility complex sequences from nonhuman species, providing the infrastructure and tools to enable accurate analysis. Since the first release in 2003, IPD-MHC has grown and currently hosts several specific sections, with more than 8,000 alleles from 76 species. Data is expertly curated and made publicly available through an open access website, recording an average of 1,500 unique visitors and more than 5,000 page views per month. As the database has grown in size and complexity, it has created several challenges in maintaining and organizing information, particularly the need to standardize nomenclature, while incorporating new allele submissions. To address these challenges a new version of the IPD-MHC Database was released in 2016, with the key aims of developing a universal cross-species data submission and display tool, aligned with the streamlining and standardisation of curator workflows. A new data submission system has been implemented to facilitate the inclusion of extensive metadata regarding MHC sequence origin and features. In the first eight weeks following release more than 500 new sequences were successfully submitted and curated. The advent of high-throughput sequencing technologies has allowed the generation of large amount of high quality data from highly polymorphic genes, providing the potential for extending the database coverage to include genomic sequences, rather than individual exons. For this reason the IPD-MHC Database has recently introduced the ability to analyse and annotate genomic data. These developments also incorporate a new internal sequence analysis tool that allows the automatic annotation of genomic data, giving users the ability to compare and analyse sequences at different levels of complexity. Furthermore, new tools are presented, enhancing database queries of high quality MHC data from an increasing number of species disseminated to the wider scientific community.

Key Words: bioinformatics, MHC, databases/repositories, polymorphism, sequence variation

MT116 Chicken MHC-*B* diversity detected by a high-density SNP panel. J. E. Fulton*[1], B. Bed Hom[2], and M. M. Miller[3], [1]*Hy-Line International, Dallas Center, IA, USA;* [2]*GABI, INRA, AgroParisTech, Jouy-en-Josas, France;* [3]*3Department of Molecular and Cellular Biology, Duarte, CA, USA.*

The chicken MHC was initially identified as the B blood group locus with variation detected serologically. Most early MHC-*B* studies were done with inbred lines or limited breeds due to the difficulties of using alloantisera to define MHC-*B* in heterogeneous outbred lines. The tandem repeat LEI0258 marker located within MHC-*B* has been particularly useful in identifying MHC-*B* haplotypes in multiple sources, including outbred populations. However, the LEI0258 marker has limitations in that the same allele size can occur in serologically different haplotypes and mutations occasionally arise within lines that change LEI0258 allele size. To provide a better typing method a single nucleotide polymorphism (SNP) typing panel encompassing 210K of the chicken MHC-*B* region was developed. The MHC-*B* SNP typing methodology is based in allele-specific PCR, with fluorescence detection of endpoint reads. The panel comprises 101 SNP with average spacing of 2,300 bp. The SNP are found in exons, introns or intragenic regions. For inclusion in the panel, SNP had to detect both alleles, be reliable in revealing genotypes, and consistently reveal the haplotypes in parent and offspring among animals with serologically known MHC-*B* haplotypes. This SNP panel allows a large number of sam-

ples to be rapidly and inexpensively genotyped for variation within the MHC-*B* region. Genotypes were generated for over 7,500 samples sampled from diverse sources, including serologically-defined MHC recombinants, MHC-defined inbred lines, in populations held at universities within North America, heritage broiler lines, and elite layer lines used for commercial egg production. Genotyping with the SNP panel revealed 78 unique haplotypes and 44 additional recombinant haplotypes with this diverse sample set. The panel revealed hotspots of recombination as well as regions of gene duplication and deletion. Associations are often observed between MHC-*B* variation and disease resistance in the chicken. To date such studies have been done mostly with the small number of haplotypes defined originally by serology. The new haplotypes revealed by MHC-*B* SNP typing provides new opportunities for enhancing the understanding of MHC-*B* and disease resistance in chickens.

Key Words: chicken MHC, SNP, haplotypes, diversity

MT117 Studies of MHC class II content in three common Arabian horse haplotypes. D. Miller*[1], A. Case[1], L. Younger[1], J. Tseng[1], H. Holl[2], Y. Ali Mohamoud[3], A. Ahmed[3], J. Malek[3], S. Brooks[2], and D. Antczak[1], [1]*Cornell University, Ithaca, NY, USA;* [2]*University of Florida, Gainesville, FL, USA;* [3]*Weill Cornell Medicine, Doha, Qatar.*

Previous studies from our laboratory have identified microsatellite-defined haplotypes within the major histocompatibility complex (MHC) of the horse, also known as the Equine Leukocyte Antigen (ELA) complex. We have developed a panel of 11 polymorphic microsatellite markers in the MHC region that span from 28.9Mb to 33.5Mb on horse chromosome 20, for a total coverage of 5.6 Mbp. This panel includes two markers in the MHC class I region, two in MHC class III, and seven in MHC class II. In identifying new microsatellite-defined haplotypes our strategy has been to test family groups (sire, dam, and offspring, or paternal half-siblings and sires). Different horse breeds have very distinct complements of MHC haplotypes defined in this way. In a large sample of over 550 Arabian horses, we identified over 60 new haplotypes not observed previously in other breeds. We also found a small number of MHC homozygous Arabian horses that carried common Arabian haplotypes. In this study we examined ELA Class II gene and variant content in three microsatellite defined haplotypes commonly found in Arabian horses, namely COR007, COR008, and COR026. This study utilised Illumina sequencing of genomic DNA, SNP content from a commercial 670K genotyping array, and sequencing of full length cDNA transcripts from multiple Class II loci. Furthermore, we examined both homozygotes, and heterozygotes who shared a combination of these common haplotypes in both domestic (US) populations, and populations based in Qatar. We will report the sequences identified and compare them with MHC class II alleles that we described previously. The new full-length sequences will be deposited into the equine section of the IPD database. These data will increase our knowledge of ELA Class II gene polymorphism and structural variation with the Class II region. This study was made possible in part by NPRP Grant 6-1303-4-023 from the Qatar National Research Fund (a member of Qatar Foundation). The findings achieved herein are solely the responsibility of the authors.

Key Words: horses, immunogenomics, microsatellite, MHC

Domestic Animal Sequencing and Annotation

MT118 A population-scale discovery of large deletions in Holstein, Jersey and Nordic Red Cattle genome. M. Mesbah-Uddin*[1,2], B. Guldbrandtsen[1], J. Vilkki[3], D. J. De Koning[4], D. Boichard[2], M. S. Lund[1], and G. Sahana[1], [1]*Department of Molecular Biology and Genetics, Center for Quantitative Genetics and Genomics, Aarhus University, Tjele, Denmark;* [2]*UMR 1313 GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [3]*Green technology, Natural Resources Institute Finland, Myllytie 1, Jokioinen, Finland;* [4]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Structural variants (SVs) are DNA polymorphisms involving more than 50 base pairs, e.g. insertions, deletions, duplications and inversions, as well as more complex changes, have a wide-spectrum of phenotypic impacts ranging from beneficial to lethal in both humans and animals. Among SVs, large deletions are potential candidates for loss-of-function, which could be lethal as homozygotes when including essential genes. In this study, we scanned the whole-genome sequences (WGS) of 175 dairy cattle from three breeds (e.g. 67 Holstein, 27 Jersey, and 81 Red Dairy Cattle) to discover large deletions. We analysed population genetic properties of these SVs, and explored their functional impact. WGS reads were aligned to the bovine reference genome assembly *UMD3.1* using *BWA,* and SVs were detected and genotyped using *GenomeSTRiP-2.0*. Here we report 8,482 large deletions (overall FDR 8.82%) − 72% of which are novel compared to SVs in the *dbVar* database. The SV genotypes and variant allele frequencies (VAF) were used to study population diversity and stratification (V_{ST}), and also to distinguish the three cattle population structure using principal component analysis (PCA). The analysis of selective constraints (*dN/dS* of cow-mouse one to one orthologues) reveals that genes overlapping deletions are on average evolutionarily less conserved than known (mouse) lethal genes (*P*-value = 2.3×10^{-6}; Wilcoxon test). We also report 227 natural gene knockouts in dairy cattle that are apparently nonessential based on the occurrence of live homozygote individuals. These genes are functionally enriched in immunoglobulin domains, olfactory receptors, and MHC classes (FDR = 9.46×10^{-36}, 2.41×10^{-27}, 1.07×10^{-23}, respectively). Finally, we demonstrate that deletions are enriched for health and fertility related QTL (2 and 1.5 fold enrichment, Fisher's exact test *P*-value = 8.91×10^{-10} and 7.4×10^{-11}, respectively). This shows promise for the inclusion of SVs in genomic prediction and association studies. Our results will facilitate discovery, genotyping, and imputation of deletions in large cohorts of animals, and subsequent studies for gene mapping and genomic prediction of breeding values.

Key Words: whole genome sequence, dairy cattle, structural variants, population genetics, genome annotation

MT119 CNVcaller: An ultra-fast population copy number variation detector for animals and plants. X. Wang*, Z. Zheng, Y. Cai, T. Chen, and Y. Jiang, *College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China.*

Copy number variations (CNVs) have been validated as the causative mutations of many diseases and agronomic traits. However, it was until recently prevented by technological limitations, as precisely calling CNVs for GWAS studies using the fast accumulating animals and plants resequencing data. Both of the draft quality reference genome or pan-genome with lots of partial scaffolds, and the inconvenient processing pipeline strongly reduce the CNV detection efficiency. We report a software, CNVcaller, for the simplified CNVs identification from large population resequencing data. The speed of CNVcaller is up to two orders of magnitude faster than the current widely used software. CNVcaller implies an absolute copy number correction upon the BWA mapping generated in the SNP detection, to mitigate the assembly mistake and detects more

previously untouchable CNVs in segmental duplication regions. Validated by the resequencing data of human, livestock and crops, CNVcaller achieved 12–18% improvement for sensitivity, with a low false-discovery rate (2–5%). In conclusion, CNVcaller is a fast and robust CNV detector with high sensitivity and accuracy. The final output VCF files could be easily integrated in SNP based population genetic studies to facilitate the causative mutations detections which are the primary demand for precise gene editing and breeding. The open code of CNVcaller and genome correction files of human, livestock and main crops are freely available on the website http://animal.nwsuaf.edu.cn/software/.

Key Words: copy number variation (CNV), bioinformatics tools, high-throughput sequencing (HTS), population genomics, genome-wide association

MT120 Candidate variants confirmation by GWAS applied to large cow data sets. A. G. Marete*[1,2], M. Lund[2], and D. Boichard[1], [1]*INRA, Paris, France;* [2]*Aarhus University, Denmark.*

This research aims to validate putative candidate mutations in three French dairy breeds. The data used included 79,532 genotyped cows with full pedigrees and 42 phenotypes including various trait classes based on production, morphology, and functional traits. The animals were genotyped either with the 50K chip or with Eurogenomics custom chip (Euro10kG), which contains 17,232 SNPs. Of these, 7,232 SNPs are custom selected and contain SNPs believed to be highly informative to genomic selection whereas the remaining are generic markers from the Illumina BovineSNP50 beadchip. Imputation to 50K was then done using the FImpute software. The validation was 2-fold and done on the imputed genotypes. First association studies were carried out to analyse all SNPs separately. A Genome-Wide Association Study (GWAS) model was fitted per breed, per chromosome and per trait and analysis were done using GCTA software. Second, using GS3 software, all markers were analysed simultaneously using a bayesian approach (BaysCπ) with a chosen prior of 0.001 and including genotypes, phenotypes, and pedigree information. Results of the fitted GWAS model showed that 172 SNPs from the custom chip were significantly better in fifteen traits compared to SNPs from the 50K ($P < 0.001$). Comparison of SNPs within same traits and between breeds showed that a total of 6,386 SNPs were highly associated ($P < 0.001$). The stringent prior of the bayesian model was aimed at reducing noise in the model and allowing only the best SNPs to be selected on each iteration. The results were consistent with GWAS model such that 138 SNPs from the custom chip had higher probabilities of inclusion compared to 50K markers (Bayes Factor >130). The two results were consolidated and a validated SNP list generated.

Key Words: putative variants, custom chip, dairy cow, big data

MT121 Extensive sequencing of a tropically adapted breed—The Brahman sequencing project. L. Koufariotis[1], B. Hayes[1], M. Kelly[2], B. Burns[3], R. Lyons[4], and S. Moore*[1], [1]*Centre of Animal Science, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, QLD, Australia;* [2]*Australian Agricultural Company (AACo), Brisbane, QLD, Australia;* [3]*Queensland Department of Agriculture and Fisheries, Rockhampton, QLD, Australia;* [4]*4School of Veterinary Science, University of Queensland, Gatton, QLD, Australia.*

Brahman cattle are well adapted to tropical environments and are extensively used for beef production in Northern Australia. Identifying mutations in Brahman genomes associated with adaptation, fertility, meat quality and growth rates would facilitate genome selection and therefore accelerate genetic gain for these traits, in both Brahman cattle and composite cattle with Brahman ancestry. Thirty six million high quality variants (SNP and indels) were discovered from 46 whole genome sequenced Brahman bulls that represent key ancestors of the breed in Australia. As some infusion of *Bos taurus* into Brahman cattle has occurred during breed formation, we investigated if we could identify regions of the Brahman genome that are highly similar with either *Bos taurus* or Gir (a *Bos indicus* breed used in the creation of Brahmans). We identified multiple genome regions in Brahmans that were *Bos Taurus* in origin, and investigated the association of some of these regions with disease and production traits.

Key Words: cattle, tropical adaptation, gene introgression, whole genome sequence

MT123 Reference sequence of the horse Y chromosome. T. Raudsepp*[1], J. Janecka[2], B. Davis[3], S. Ghosh[1], N. Paria[4], P. J. Das[5], M. Nielsen[6], L. A. A. Orlando[7], M. Schubert[7], T. A. E. Stout[8], and B. P. Chowdhary[1], [1]*Texas A&M University, College Station, TX,USA;* [2]*Duquesne University, Pittsburgh, PA, USA;* [3]*National Institutes of Health, Bethesda, MD, USA;* [4]*Texas Scottish Rite Hospital for Children, Dallas, TX, USA;* [5]*National Research Centre on Yak, Dirang, India;* [6]*University of Kentucky, Lexington, KY, USA;* [7]*Natural History Museum of Denmark, Copenhagen, Denmark;* [8]*Utrecht University, Utrecht, the Netherlands.*

Our understanding of the Y chromosome relative to the rest of the genome is limited because of its complex structure, repeat content and male-specific inheritance. The dynamics governing Y evolution are unique compared to autosomes. Transcripts from the male specific region of the Y (MSY) are primarily expressed in testes, thus important candidates for male fertility and reproductive barriers in speciation. Here we present the first assembly (~90% complete) and annotation of the euchromatic portion of the horse MSY (eMSY). The eMSY is primarily composed of a 7 Mb single-copy region and a 2.5 Mb ampliconic region. A small portion of eMSY (~250 kb) is duplicated in the pseudoautosomal region. The most proximal part of eMSY contains highly amplified novel testis-specific transcripts (ETSTY7) that populate also the Y- and Xq-heterochromatin. Notably, ETSTY7 sequences were found in equine parasite *Parascaris* spp. suggesting horizontal transfer. The eMSY assembly was annotated though bioinformatics pipelines and by alignment with testis RNAseq data. The chromosome carries 52 unique genes (37 single-copy; 15 ampliconic). Ampliconic genes are mainly Y-borne, novel and testis expressed, while single-copy genes are either X-Y gametologs or transposed from autosomes. Among the species with sequenced MSY, horse has the highest number (29) of X-Y gene pairs. Evolutionary history of eMSY was studied by aligning eMSY genes with representative mammalian homologues, with short read data from other equids and with genomic data from 10 other horse breeds, the Przewalski's horse, a middle and a late Pleistocene horse, and the domestic donkey. Testes transcriptome comparisons between horse and donkey enabled identification of genes that may play a role of speciation, whereas comparison of testis RNAseq data from horse, donkey and mule identified genes that are likely critical for spermatogenesis. The reference sequence of eMSY fills a major gap in the horse genome assembly and provides novel insight into evolution and function of this important sex chromosome.

Key Words: Y chromosome, horse, evolution, fertility

MT124 SNP discovery in indigenous Afrikaner, Drakensberger and Nguni cattle breeds of South Africa. A. A. Zwane*[1,2], A. Choudhury[3], M. L. Makgahlela[1], A. Maiwashe[1,5], E. Van Marle-Koster[2], and J. F. Taylor[4], [1]*Department of Animal Breeding and Genetics, ARC-API, Irene, South Africa;* [2]*Department of Animal and Wildlife Sciences, University of Pretoria, Hatfield, Pretoria, South Africa;* [3]*Sydney Brenner Institute of Molecular Bioscience, University of the Witwatersrand, Parktown, Johannesburg, South Africa;* [4]*Division of Animal Sciences, University of Missouri, Columbia, MO, USA;* [5]*Department of Animal,*

Wildlife and Grassland Sciences, University of the Free State, Bloemfontein, South Africa.

Single nucleotide polymorphism arrays have created new possibilities for performing genome-wide studies to detect genomic regions harboring sequence variants that affect complex traits. However, the majority of validated SNPs for which allele frequencies have been estimated are limited to European breeds, and their application in indigenous South African breeds are limited. The objective of this study was to search for new SNPs in indigenous SA breeds (Afrikaner, Drakensberger and Nguni) using next-generation sequencing. DNA of 30 individuals from each of the three breeds were equimolar pooled and sequenced to identify putative SNPs. Approximately 1.8 billion sequence reads were aligned to the UMD3.1 reference genome generating an average depth of 21-fold sequence coverage for each breed. A total of 15.7 million SNPs were identified across the breeds with the highest number of SNPs identified in Nguni. Verification of SNPs against Run 5 data from the 1000 Bull Genomes project suggested that 15% of the SNPs were novel variants. Annotation of the detected variants indicated numerous variants classified within functional genes that may be associated with complex traits in these cattle breeds. Functional enrichment analysis of novel SNPs identified 1,481 genes enriched for novel variants. These discoveries provide a valuable genomic resource for predicting breeding values, identifying variants underlying adaptive traits and for genome-wide association studies in SA cattle breeds.

Key Words: indigenous breeds, sequencing, mapping, novel variants, annotation

MT125 Exploiting long read sequencing technologies to establish high quality highly contiguous pig reference genome assemblies. A. Warr[1], R. Hall[2], K. Kim[2], E. Tseng[2], S. Koren[3], A. Phillippy[3], D. Birkhart[4], B. Rosen[4], S. Schroeder[4], D. Hume[1], R. Talbot[5], L. Rund[6], L. Schook[6], W. Chow[7], K. Howe[7], D. J. Nonneman[8], G. A. Rohrer[8], N. Putnam[9], R. E. Green[9], R. O'Connor[10], D. Griffin[10], B. M. Skinner [11], C. A. Sargent[11], N. A. Affara[11], C. Tyler-Smith[7], M. Watson[1], T. P. L. Smith[8], and A. Archibald*[1] [1]*The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, UK;* [2]*Pacific Biosciences, Menlo Park, CA, USA;* [3]*National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA;* [4]*Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD, USA;* [5]*Edinburgh Genomics, University of Edinburgh, Edinburgh, UK;* [6]*University of Illinois, Urbana, IL, USA;* [7]*The Wellcome Trust Sanger Institute, Hinxton UK;* [8]*USDA, ARS, USMARC, Clay Center, NE, USA;* [9]*Dovetail Genomics LLC, Santa Cruz, CA, USA;* [10]*University of Kent, Canterbury, Kent, UK;* [11]*University of Cambridge, Cambridge, UK.*

The current pig reference genome sequence (Sscrofa10.2) was established using Sanger sequencing and following the clone-by-clone hierarchical shotgun sequencing approach used in the public human genome project. However, as sequence coverage was low (4–6x) the resulting assembly was only of draft quality. We have built new *de novo* genome assemblies from whole genome shotgun (WGS) sequence reads generated using Pacific Biosciences (PacBio) long read sequencing technology for two pigs – the original reference animal (Duroc sow 2–14) and a Duroc/Landrace/Yorkshire crossbred barrow. About 60–70x coverage WGS data per animal were assembled with the Falcon assembler and error corrected with Quiver/Arrow and Pilon using high coverage WGS PacBio and Illumina reads, respectively. The estimated accuracy (99.999%) of the Duroc assembly meets the requirement of a Gold standard finished sequence. The Duroc assembly was scaffolded with paired-end reads from isogenic BAC and fosmid clones and assigned to chromosomes based on Fluorescent *In Situ* Hybridisation using probes generated from BAC clones. The crossbred assembly was scaffolded using Dovetail's Hi-Rise. The current statistics for these assemblies are: Duroc 2–14 (Sscrofa11) for SSC1–18, SSCX (2.39 Gbp, 122 contigs; contig N50 = 58.5 Mbp; scaffold N50 = 107.6 Mbp); Duroc/Landrace/Yorkshire crossbred for SSC1–18, SSCX, SSCY (2.62 Gbp, 14,924 contigs; contig N50 = 6.5 Mbp; scaffold N50 = 132 Mbp). The Sscrofa11 assembly has been updated recently to Sscrofa11.1 by the addition of the SSCY sequence data from Skinner *et al*. 2016 (Genome Res 26:130–9). The BAC and fosmid clone resource from Duroc 2–14 will facilitate further targeted sequence closure. These improved genome assemblies will be a key resource for research in pigs and will enable applications in agriculture and biomedicine. The assemblies are being deposited in the public database under the pre-publication data release terms of the Toronto Statement (Nature 461:168–70).

Key Words: pig, reference genome sequence, long read sequencing technology

MT126 Beyond sequencing: Assigning function to novel ncRNAs. F. McCarthy*[1], A. Cooksey[1], C. Gresham[2], and B. Nanduri[2], [1]*University of Arizona, Tucson, AZ, USA;* [2]*Mississippi State University, Starkville, MS, USA.*

New genomic technologies are accumulating information about functional elements within genomic sequence at a faster rate than ever before, and the FAANG Project is expected to increase this rate of acquisition. Understanding how these elements contribute to traits and phenotypes requires a concomitant effort to understand the function of these regulatory genes and regions. However, functional annotation relies on curation of published data and computational prediction pipelines; genomic technologies routinely identify novel functional elements (with no existing body of literature) and there are no existing computational pipelines for functional annotation of ncRNAs. We describe here our approach to develop functional annotation workflows for miRNAs and lncRNAs, two classes of ncRNAs that are being annotated in species that are studied as part of the FAANG Project. We pair miRNA target prediction with proteomics data and GO enrichment analysis to (1) better identify true miRNA targets and (2) predict the function of novel miRNAs. For predicting function of novel lncRNAs we use a similar approach, identifying lncRNA-mRNA pairs expressed in the same tissues, identifying interaction networks for these pairs and investigating GO term enrichment to predict function. By linking experimental expression data with computational approaches, we expect to generate preliminary, high-throughput functional information about commonly identified classes on ncRNAs. This in turn will allow us to provide information that can then be incorporated into functional analyses of typical gene expression datasets (e.g. transcriptome data).

Key Words: noncoding RNAs, functional annotation

MT127 Profiling the landscape of transcription, chromatin accessibility and chromosome conformation of cattle, pig, chicken and goat genomes [FAANG pilot project ''FR-AgENCODE"]. S. Foissac[1], S. Djebali*[1], H. Acloque[1], FR-AgENCODE Consortium[2], M. H. Pinard Van der Laan[2], S. Lagarrigue[3], and E. Giuffra[2], [1]*GenPhySE, INPT, ENVT, INRA, Université de Toulouse, Toulouse, France;* [2]*GABI, AgroParisTech, INRA, Université Paris Saclay, Paris, France;* [3]*PEGASE, INRA, Agrocampus Ouest, Rennes, France.*

Functional annotation of livestock genomes is a critical and obvious next step to derive maximum benefit for agriculture, animal science, animal welfare and human health. The aim of the Fr-AgENCODE project is to generate multi-species functional genome annotations by applying high-throughput molecular assays on three target tissues/cells relevant to the study of immune and metabolic traits. An extensive collection of stored samples from other tissues

is available for further use (FAANG Biosamples 'FR-AGENCODE'). From each of two males and two females per species (pig, cattle, goat, chicken), strand-oriented RNA-seq and chromatin accessibility ATAC-seq assays were performed on liver tissue and on two T-cell types (CD3+CD4+ & CD3+CD8+) sorted from blood (mammals) or spleen (chicken). Chromosome Conformation Capture (*in situ* Hi-C) was also carried out on liver. Sequencing reads from the 3 assays were processed using standard processing pipelines. While most (50–70%) RNA-seq reads mapped to annotated exons, thousands of novel transcripts and genes were found, including extensions of annotated protein-coding genes and new lncRNAs (see abstract #69857). Consistency of ATAC-seq results was confirmed by the significant proportion of called peaks in promoter regions (36–66%) and by the specific accumulation pattern of peaks around gene starts (TSS) *v.* gene ends (TTS). Principal Component Analyses for RNA-seq (based on quantified gene expression) and ATAC-seq (based on quantified chromatin accessibility) highlighted clusters characterised by cell type and sex in all species. From Hi-C data, we generated 40kb-resolution interaction maps, profiled a genome-wide Directionality Index and identified from 4,100 (chicken) to 12,100 (pig) topologically-associating domains (TADs). Correlations were reported between RNA-seq and ATAC-seq results (see abstract #71581). In summary, we present here an overview of the first multi-species and -tissue annotations of chromatin accessibility and genome architecture related to gene expression for farm animals.

Key Words: multispecies, Functional Annotation of Animal Genomes (FAANG), ATAC-seq, RNA-seq, Hi-C

MT128 Integrative and differential analysis of transcriptomes and chromatin accessibility regions reveals regulatory mechanisms involved in pig immune and metabolic functions [FAANG pilot project 'FR-AgENCODE']. S. Djebali[1], K. Munyard[2], N. Villa-Vialaneix[3], C. Cabau[1], A. Rau[4], E. Crisci[4], T. Derrien[5], C. Klopp[3], M. Zytnicki[3], S. Lagarrigue[6,7], H. Acloque[1], S. Foissac*[1], and E. Giuffra[4], [1]*GenPhySE, INPT, ENVT, INRA, Université de Toulouse, Castanet-Tolosan, France;* [2]*Curtin University, School of Biomedical Sciences, CHIRI Biosciences, Perth, Australia;* [3]*MIAT, Université de Toulouse, INRA, Castanet-Tolosan, France;* [4]*GABI, AgroParisTech, INRA, Université Paris Saclay, Jouy-en-Josas, France;* [5]*UMR6290 IGDR, CNRS, Université Rennes 1, Rennes, France;* [6]*UMR PEGASE, INRA, Rennes, France;* [7]*UMR PEGASE, Agrocampus Ouest, Rennes, France.*

In the context of the FAANG pilot project 'FR-AgENCODE' to improve the functional annotation of livestock genomes, we characterised the transcriptome and the chromatin accessibility of pig hepatocytes and two types of lymphocytes. More specifically, CD3+CD4+ ('CD4') and CD3+CD8+ ('CD8') T-cells were sorted from the blood of two male and two female Large White adult pigs. These samples, along with liver samples from the same animals, were processed by strand-oriented RNA-seq and ATAC-seq experiments. Principal Component Analyses on log-transformed TMM-normalized read counts in genes (from RNA-seq) and regions of chromatin accessibility (from ATAC-seq) consistently highlighted the variability between liver and the T-cells, and to a lesser extent within T-cells (CD4 *v.* CD8), as well as between the male and female samples. Comparative analyses identified differentially expressed genes between cell types as well as potential regulatory sites from differentially accessible chromatin regions. As expected, ontology annotations of differentially expressed genes were enriched for either immunity- or metabolism-related terms. Interestingly, correlations between gene expression and promoter accessibility across samples were enriched for both extreme positive and negative values, which suggests that ATAC-seq can efficiently capture distinct regulatory mechanisms of gene expression. Candidate enhancers and repressors were identified by comparing ATAC-seq regions with predicted binding sites of 500+ transcription factors. By integrating these results with those from Hi-C chromosome conformation capture on the liver samples, we further characterised the differences between 'active' and 'repressed' topological domains in terms of functional features, including gene density and general chromatin accessibility. Altogether, these results lead to a better understanding of the molecular mechanisms involved in pig immune and metabolic functions, and illustrate a useful contribution to the functional annotation effort of the FAANG initiative.

Key Words: Functional Annotation of Animal Genomes (FAANG), pigs and related species, ATAC-seq, RNA-seq, Hi-C

MT129 Pinpointing causal mutations among imputed sequence variant genotypes in three cattle breeds. H. Pausch*[1,2], I. MacLeod[1], P. Bowman[1,3], R. Emmerling[4], R. Fries[5], B. Gredler-Grandl[6], H. Daetwyler[1,3], and M. Goddard[1,7], [1]*Agriculture Victoria, AgriBio, Centre for AgriBioscience, Bundoora, VIC, Australia;* [2]*Animal Genomics, Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland;* [3]*School of Applied Systems Biology, La Trobe University, Bundoora, VIC, Australia;* [4]*Institute of Animal Breeding, Bavarian State Research Centre for Agriculture, Poing-Grub, Germany;* [5]*Chair of Animal Breeding, Technische Universitaet Muenchen, Freising, Germany;* [6]*Qualitas AG, Zug, Switzerland;* [7]*Faculty of Veterinary and Agricultural Science, University of Melbourne, Melbourne, VIC, Australia.*

The whole-genome sequencing of key ancestors of many cattle breeds yielded genotypes at millions of polymorphic sites. These data can be used as a reference population to impute sequence variant genotypes for tens of thousands of animals that have dense array-derived genotypes. Accurately imputed sequence variant genotypes may improve genomic predictions and facilitate pinpointing causal mutations in genome-wide association studies because the polymorphisms that underlie phenotypic variation are included in the data. We assessed the accuracy of imputing sequence variant genotypes in 249 Fleckvieh and 450 Holstein bulls using 39,728,987 sequence variants of 1577 animals from the fifth run of the 1000 bull genomes project. Imputation was performed using either *Minimac* or *FImpute* considering either within- or multi-breed reference populations. Regardless of the composition of the reference population and imputation software tested, the overall accuracy of imputation was high in both breeds (0.898 – 0.952). However, several segments with poor imputation quality were detected particularly at regions where the bovine genome contains large structural variants. The highest accuracy of imputation was obtained when *Minimac* was used to infer sequence variant genotypes and when allele dosages rather than best guess genotypes were considered at the imputed sequence variants. Using a multi-breed reference population increased the accuracy of imputation particularly at low-frequency variants. Next, we inferred genotypes for more than 23 million sequence variants in 6778 Fleckvieh, 5204 Holstein and 1646 Brown Swiss bulls using 1577 sequenced animals from various breeds as a reference population. Association tests between the predicted allele dosages at imputed sequence variants and daughter-derived phenotypes for protein and fat percentages in milk revealed several QTL and candidate causal mutations that were mostly detected in more than one breed. Two causal mutations in the *DGAT1* and *GHR* genes were the most significantly associated variants at two QTL on chromosomes 14 and 20 demonstrating that highly precise QTL mapping is possible with imputed sequence variant genotypes.

Key Words: cattle and related species, genome sequencing, imputation, genome-wide association, complex trait

MT130 Characteristics of the two primitive horse breeds (Polish Primitive Horse and Hucul) maternal lines using mtDNA D-loop sequence variation. L. Wodas*[1], J. Cieslak[1], A. Borowska[1], E. G. Cothran[2], A. M. Khanshour[3], and M. Mackowski[1,4], [1]*Department of Horse Breeding, Poznan University of Life Sciences, Poznan, Poland;* [2]*Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Science, Texas A&M University, College Station, TX, USA;* [3]*Texas Scottish Rite Hospital for Children, Dallas, TX, USA;* [4]*Horse Genetic Markers Laboratory, Poznan University of Life Sciences, Poznan, Poland.*

The aim of this study was to describe the genetic diversity among the Polish Primitive Horse (Konik, PPH) and Hucul maternal lines using the mtDNA D-loop analysis. Both breeds are covered by conservation programs in Poland since the beginning of 21st century. In their breeding strategies, the significant part of attention is paid to the existing maternal lines preservation. However, till date there was no study showing the real maternal diversity in PPH and Hucul kept in Poland, using the mtDNA analyses. Altogether 173 PPH and 84 Hucul DNA samples were examined. All existing PPH dam lines (16) and 13 of 14 Hucul lines were studied. Sequencing harbored the D-loop fragment (510 bp) spanning the hypervariable region 1 (HVR1). Sequence diversity indices were calculated in DnaSP tool. Phylogenetic analyses were conducted using PHYLIP package. Obtained results were compared to the official pedigree data. Altogether 33 and 35 variable sites (for PPH and Hucul breeds, respectively) were found in the analysed mtDNA fragment. In the case of PPH they segregated as 19 haplotypes, whereas in the Hucul breed the presence of 13 different mtDNA haplotypes was observed. The overall haplotype diversity calculated for both breeds was identically high (HapD = 0.92), while the nucleotide diversity in Hucul was slightly lower if compared to the PPH ($\pi = 0.023$ *v.* $\pi = 0.026$). Only for 5 PPH maternal lines the single, specific haplotype could be assigned. Among remaining 11 lines the segregation of 2–5 haplotypes was noticed. In the Hucul breed the unique haplotype was matched to the 9 of 13 studied dam lines. In the remaining 4 dam lines, the presence of 2 haplotypes was recorded. Comparison of the results with PPH and Hucul pedigree data indicated the presence of numerous mistakes in both breeds pedigrees (especially in PPH). This convinces that conservation programs need an extensive revision and the application of mtDNA markers on larger scale should be considered.

Key Words: horses and related species, conservation genomics, DNA sequencing, breed diversity, conservation

MT131 Investigation of fine-scale recombination rate variability in the horse. S. Beeson*, J. Mickelson, and M. McCue, *University of Minnesota College of Veterinary Medicine, Saint Paul, MN, USA.*

Recombination events have been shown in eukaryotes to cluster along chromosomes in small regions called hotspots. In humans and mice, genetic map length and local recombination landscapes vary by sex as well as across populations, suggesting that selective pressures play a role in recombination rate distribution across the genome. Structural rearrangements such as inversions are also known to affect recombination, and variants in the *PRDM9* gene have been implicated in hotspot differences within and across species. To create a gender and breed-averaged recombination map, 513 horses representing 28 breeds were genotyped for ~1.8 million SNPs on the MNEqn 2M array and divided into 100 groups of 20 random individuals to estimate recombination rates using LDhat. Chromosomes were split into intervals of 2000 SNPs with a 200-SNP overlap between adjacent windows, and each analysis was run for 10 million iterations with a 100,000 burn-in period. This procedure was also performed in 12 individual breeds. Rate estimates were used to predict recombination hotspots both across and within breeds using the LDhot program. Pairwise recombination dissimilarity (1-Spearman rank coefficient for recombination rates) and fixation index (F_{ST}) between breed pairs were compared by Mantel test with 10,000 permutations, revealing a strong correlation ($P < 0.0003$) between recombination rate and population differentiation quantified by F_{ST}. Haplotypes in the region surrounding the myostatin (*MSTN*) gene have been shown to influence muscle fibre type proportions, and the region has been identified as a genomic signature of selection in the Quarter Horse. As expected due to haplotype homozygosity, detectable recombination appears to be suppressed around *MSTN* in the Quarter Horse but not in other breeds. In addition, a 13-Mb region on chromosome 7 in the Standardbred corresponds with low recombination and a large inversion predicted using inveRsion and invClust. Analysis of Motif Enrichment (AME) identified relative enrichment ($P = 1.02e\text{-}65$) for the *in silico* predicted 13-mer recognition motif for equine PRDM9 in hotspot regions extracted from the EquCab2 reference sequence, indicating that this gene may also play a role in equine recombination hotspot determination.

Key Words: horse, equine, recombination, PRDM9

MT132 Molecular genetic diversity of donkey (*Equus asinus*) by microsatellite marker and mitochondrial DNA analysis in Korea. S. Yun*[1], C. Park[2], J. Hwang[2], S. Kim[2], and G. Cho[2], [1]*Animal and Plant Quarantine Agency, Pusan, Korea;* [2]*of Veterinary Medicine, Kyungpook National University, Daegu, Korea.*

We analysed 179 horse samples (79 donkeys, 50 Thoroughbred, 50 Jeju Halla horse) using 15 microsatellite markers (AHT4, AHT5, ASB2, ASB17, ASB23, CA425, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG10, LEX3 and VHL20), and also analysed the mitochondrial DNA (mtDNA) base pairs of five donkeys. We observed genetic diversity from biostatic analysis of them. The total number of alleles was 90, from 1 (ASB17), 2 (HMS1) to 14 (AHT5). The observed heterozygosity (OHet) was from 0.0000 (ASB17, HMS1) to 0.8828 (AHT5) which was mean value of 0.4861, the expected heterozygosity (EHet) was from 0.0000 (CA425) to 0.9104 (AHT5) with mean value of 0.5915, and the Polymorphism Information Content (PIC) on each group of microsatellite marker was from 0.0000 (ASB17) to 0.8968 (AHT5) observed as a mean value of 0.5374. Among 15 markers, PIC of AHT4, AHT5, ASB23, CA425, HMS2, HMS3, HTG4, HTG10 and LEX3 markers was observed above 5.000. We could observe the results of 3 horse groups in addition to those of donkey's. In specific, the donkey had 0.5915 EHet, 0.4861 OHet, Thoroughbred had 0.6721 EHet, 0.6587 OHet, and the Jeju halla horse had 0.7898 EHet, 0.7093 OHet on average, respectively. Furthermore, the mean alleles value is observed as 6.00, 4.83, 8.00 in donkey, Thoroughbred, Jeju halla horse breed, respectively. We observed 16,670 base pairs of donkey mitochondrial DNA, within 38 genes (22 genes encoding tRNA, 2 genes encoding rRNA, 13 genes associated with supplying information on protein synthesis, and 1 control region). An analysis of the haplotype in the CytB region, our focus within the 38 genes, revealed that there were 5 polymorphisms, 3 haplotypes, a haplotype diversity value of 0.800, and nucleotide diversity value of 0.00211 (which is low). In conclusion, these results suggested that the Korean domestic donkey might be originated in the Somali wild ass species of China.

Key Words: donkey, haplotype, mtDNA, microsatellite marker, phylogenetic character

MT133 Unique gene expression patterns in equine articular chondrocytes. E. N. Adam and J. N. MacLeod*, *University of Kentucky, Lexington, KY, USA.*

Horses and other mammals have several different types of cartilaginous tissues including joint (articular) cartilage, non-articular structural cartilage, and cartilage that progresses to bone through the process of endochondral ossification. Although all types of cartilage have several cellular and matrix features in common, there are important individual characteristics as well. To advance our understanding of unique functional parameters of equine articular chondrocytes, we compared the mRNA transcriptome between adult articular and nasal septum cartilage, neonatal articular and epiphyseal cartilage, and fetal interzone and anlage tissue. A panel of genes was identified exhibiting an expression pattern that clearly distinguishes adult articular cartilage from the other cartilaginous tissues.

Key Words: cartilage, chondrocyte, RNA-seq, horse

MT134 Rate of sex reversal cases in horses of Argentina. M. Martinez*, M. Costa, B. Elguero, and C. Ratti, *Laboratorio de Genética Aplicada, Sociedad Rural Argentina, Buenos Aires, Argentina.*

This work describes the sex reversal cases found on 168,000 horses tested for different breeds in Argentina. Sex reversal syndromes describe sexual and development disorders in mammals, including horses. Affected animals show a disagreement between sex chromosome constitution, gonadal and phenotypic/behavioural sex. According to XX/XY constitution and the Sex-Determining Region Y gene (SRY) presence, the sex reversal cases fall in 4 categories: female XY SRY-negative or SRY-positive genotypes and male XX SRY-negative or SRY-positive genotypes. Our laboratory genotypes horses for parentage verification using ISAG STR panel and the sexing marker amelogenin (AME, X and Y linked). When the gender reported by the owner disagrees with the inferred by AME, then SRY is tested. Doing so, 38 females XY SRY-negative, 6 females XY SRY-positive and 5 male XX SRY-negative genotypes were detected. Even when there are not males XX SRY-positive horses reported in the literature, one case was found out at the laboratory. Percentage rates of 0.019 and 0.038 were estimated in Thoroughbred and Polo breeds, respectively, for females XY SRY-negative genotypes. The higher rate in Polo may be linked to artificial assisted reproductive technologies used in this breed. It also may be explained by the preferential use of some sire lines with higher tendency to produce XY female offspring. Some of these animals have offspring, an unexpected outcome related to the extreme heterogeneity of this condition. Female XY SRY-positive genotypes are very unusual and some of them have been linked to autosomal/X chromosome gene mutations. In those reports, the animals were related. Not relationship was found for our cases. Three from the 5 male XX SRY-negative cases were horses showing chimeric genotype profiles, suggesting fusion of fraternal twins during the embryo development. Similar findings were previously showed in horses with no hereditary brindle coat colour patterns. In all the cases, except one, described as hermaphrodite by the breeder, not phenotype abnormalities were reported by the owners. Therefore, all the findings were done during routine parentage testing. Gender discrepancy was then informed to the owners for further research on affected animals.

Key Words: sex reversal, X chromosome, Y chromosome

MT135 Characterization of equine STR panel '15 TKY system' by imputation from dense SNP genotypes in a Thoroughbred population. M. Kikuchi*, H. Kakoi, T. Tozaki, K. Hirota, and S. Nagata, *Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan.*

Single nucleotide polymorphisms (SNPs) are an informative resource for genetic studies and recently, SNP genotyping is being considered an alternative to short tandem repeat (STR) genotyping, as a tool for parentage testing in many animals. Imputation of STR alleles from SNP genotypes is well developed for some species; however, there is little information, based on SNPs, on equine STR parentage panels. Our group developed a panel with 15 STRs, namely 15 TKY system. The ISAG has sanctioned it as a secondary parentage panel in horses, and some laboratories routinely employ several TKY markers in parentage testing. Therefore, as a model for imputation of STR markers in horses, we characterised the 15 TKY system by identifying SNP haplotypes corresponding to STR alleles in a Thoroughbred population. For the STR analysis, 94 randomly selected Japanese Thoroughbred horses (48 males and 46 females) were genotyped, and 90 alleles were observed on the 15 TKY STRs. This covered 88% of the previously reported alleles. Subsequently, all horses were subjected to SNP genotyping using 670K Axiom Equine Genotyping Array (Affymetrix). We extracted 200–737 (average 278) SNPs within 500 kb on either side of each STR (1 Mb) and selected SNPs that were completely genotyped for all horses and exceeded minor allele frequency of 5%. Then, the SNP haplotypes with STR alleles were phased by SNPAlyze Ver. 9, using 1,000 iterations. The total number of SNPs required to impute STR alleles was minimized by referring to a previously reported process. The number of SNPs corresponding to each STR was 3–6, and the SNPs were located within 34–469 kb (average 170 kb). Finally, 90 alleles of the 15 TKY STRs were theoretically explained by 105 haplotypes derived from 69 SNPs in the analysed population. With this model for imputation from a high-density SNP platform within a Thoroughbred population, it was concluded that the 15 TKY system could be mostly imputed by using the 69 SNPs in the Thoroughbred population. Further analysis for larger population size and simulation for parentage verification using both 15 TKY system and SNP haplotypes are in progress.

Key Words: SNP, STR, haplotype, Thoroughbred, parentage verification

Genetics of Immune Response and Disease Resistance

MT136 Two novel and differentially expressed SNPs in the promoter of *CD4* gene are associated with mastitis resistance in dairy cattle. T. Usman*, S. Ayaz, S. Gul, and I. Khattak, *Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan.*

Mastitis is the inflammation of udder tissues of dairy animals characterised by physical, chemical and pathological changes in the milk and mammary gland. Somatic cell count (SCC) and somatic cell score (SCS) are useful indicators of udder health and has highly positive genetic correlation (r_g >0.7) with clinical mastitis. The effects of single nucleotide polymorphisms (SNPs) in the 2Kb promoter region of *CD4* gene were investigated on mastitis indicator traits in a population of 312 Holstein Friesian dairy cattle. Two novel SNPs (104010804G/A and 104010752C/T) were found in the promoter region of *CD4* gene by sequencing. Statistical model was applied by general linear model procedure of SAS considering the effects of SNPs, parity, herd, season and year of calving. Genotype and allele frequencies of the two SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$) in the population. The mutation type AA (104010804G/A) and TT (104010752C/T) genotype of both the SNPs were highly significantly associated with SCC and SCS ($P < 0.01$). The additive effect of the mutation type genotypes of both of the SNPs was found significant on SCC ($P < 0.01$). Moreover, the

analysis of the combination genotypes showed that mutation type genotypes were significantly associated with higher SCC level ($P < 0.05$). As for mRNA expression analysis, both the mutation type AA and TT genotype of the two SNPs showed higher mRNA expression level and were significantly different from other genotypes ($P < 0.05$). The results infer that *CD4* gene can be a useful candidate gene and the identified SNPs could be powerful genetic markers against mastitis development in dairy cattle.

Key Words: *CD4* gene, single nucleotide polymorphism, mastitis resistance

MT137 Comparative analyses of PBMC transcriptome profiles between German Landrace and Pietrain pigs following PRRSV vaccination. M. A. Islam[1], M. J. Pröll[1], S. Rony[1], C. Große-Brinkhaus[1], M. J. Uddin[2], D. Tesfaye[1], M. Hölker[1], E. Tholen[1], K. Schellander[1], and C. Neuhoff*[1], [1]*Institute of Animal Science, Animal Breeding and Husbandry group, University of Bonn, Bonn, Germany;* [2]*School of Veterinary Science, The University of Queensland, Gatton campus, QLD, Australia.*

Porcine reproductive and respiratory syndrome (PRRS) is a devastating viral disease affecting swine industry worldwide. The innate immune response to PRRS virus (PRRSV) infection varies among pig breeds. Therefore, the current study aimed to investigate the breed difference in innate immune response to PRRSV vaccination between German Landrace (DL) and Pietrain (Pi) pigs. For this we used a total of 12 Affymetrix GeneChip porcine gene 1.0 ST array for transcriptome profiling of peripheral blood mononuclear cells (PBMCs) collected before (0h) and 24 h after PRRSV vaccination from three female piglets of four weeks age. With FDR < 0.01 and log2 fold change 1.5 as cutoff criteria, 4269 transcripts were found to be differentially expressed among four contrast pairs tested (DL-24h *v.* DL-0h, Pi-24h *v.* Pi-0h, DL-0h *v.* Pi-0h and DL-24h *v.* Pi-24h). The number of vaccine induced differentially expressed genes (DEG) was much higher (DL-0h *v.* DL-24h, DEG = 2459) in DL pigs than that of Pi pigs (Pi-24h *v.* Pi-0h, DEG = 291). Before vaccination, 3255 genes showed differential expression between DL and Pi (DL-0h *v.* Pi-0h) which indicated the genetic variation between two breeds. After 24 h of PRRSV vaccination, 1046 genes were overexpressed in DL pig compared to Pi (DL-24h *v.* Pi-24h) which indicated the breed differences in vaccine responsiveness. The top most biological pathways enriched by differentially expressed genes are linked to immune response function e.g.: cytokine signalling in immune system, GPCR signalling, JAK-STAT signalling or interferon signalling. The network enrichment analysis identified STAT1, MMS19, RPA2, BAD, UCHL5 and APC as potential regulatory genes for the functional network of PRRSV vaccine response specific for DL and FOXO3, IRF2, ADRBK1, MTOR and EIF3I genes for Pi pigs. SLC9A2, BAG3, RRS1, DCTN3, BUD31, ARPC1B and ATP5J2 were found to be the most potential hubs of the shared network of transcriptome response in PBMCs of both DL and Pi pigs. In conclusion, DL pigs differ greatly from that of Pi in terms of PBMCs transcriptome profiles after PRRSV vaccination.

Key Words: PBMCs, PRRSV, breed, pig, German Landrace

MT138 Experimentally induced *S. aureus* or *E. coli* mammary gland infection impacts the liver transcriptome in heifers selected for divergent mastitis susceptibility. A. Heimes*[1], A. Hehl[1], W. Demasius[1], J. Guenther[1], R. Weikard[1], F. Hadlich[1], H.-M. Seyfert[1], L. Rohmeier[2], M. Meyerholz[2,4], W. Petzl[2], H. Zerbe[2], M. Hoedemaker[3], H.-J. Schuberth[4], S. Engelmann[5], C. Kuehn[1], [1]*Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany;* [2]*Clinic for Ruminants with Ambulance and Herd Health Services, Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-University, Munich, Germany;* [3]*Clinic for Cattle, University of Veterinary Medicine Foundation, Hanover, Germany;* [4]*Immunology Unit, University of Veterinary Medicine Foundation, Hanover, Germany;* [5]*Institute of Microbiology, Technical University Braunschweig, Braunschweig, Germany.*

Mastitis is the most costly single-animal disease in dairy cows. It also raises concerns in human and veterinary medicine due to potentially increasing resistance of pathogens against antimicrobial drugs. *S. aureus* is an important aetiological pathogen inducing chronic subclinical mastitis while *E. coli* often induces acute clinical mastitis. Previous linkage and association studies had identified a genomic region on BTA18 influencing somatic cell score, a surrogate for udder health. Our ChronMast project seeks to identify biomarkers for mastitis susceptibility. Therefore, pregnant half sib heifers were selected before first calving for inheriting a favourable (Q) or unfavourable (q) paternal haplotype in the respective chromosomal region. Thirty six animals (18 Q, 18 q) were closely phenotyped for clinical health and immunological performance before and after calving. Five weeks postpartum, their udders were infected by a mastitis causing pathogen [$S.\ aureus_{1027}$ (n = 24) or $E.\ coli_{1303}$ (n = 12)]. Liver samples were collected at the peak of inflammation as determined from previous experiments (*E. coli* 24 h, *S. aureus* 96 h). High quality RNA was used for stranded library preparation and paired-end sequencing. In total, 3.8 billion reads were generated with an average of 107 million reads per sample. Processed reads were aligned to the UMD3.1. bovine genome assembly guided by the Ensembl 86 annotation, with 97.9% of the reads mapping at least once. Transcript assembly and subsequent differential expression analysis yielded 4,636 genes differentially expressed between liver samples from *S. aureus* and *E. coli* infected animals. Across Q and q groups, mammary gland infection with *E. coli* down-regulated the expression of genes involved in gluconeogenesis compared to *S. aureus* infection. In *E. coli* infected animals, HPX and LBP, encoding acute phase proteins, were among the most highly expressed genes according to FPKM values. In *S. aureus* infected animals, apolipoprotein coding genes (APOA2, APOC3) were among the most highly expressed genes and showed significantly higher expression than in *E.coli* infected heifers.

Key Words: cattle and related species, immunogenomics, RNA-seq, adaptive immunity, animal health

MT139 Genetic variation and regulation of TLR4 in relation to bovine mastitis. F. Korkmaz* and D. Kerr, *University of Vermont, Burlington, VT, USA.*

Toll-like-receptor 4 (TLR4) is the main TLR that activates the innate response to Gram negative bacteria and may contribute to between-animal variation in the severity of *E. coli* mastitis. In the current study we identified, out of a cohort of 60 adult Holsteins, 8 animals that exhibit a genetic difference in exon 3 of the *TLR4* gene that results in a lack of PCR amplification (PCR-). To study the functional significance of this variation, primary dermal fibroblasts (pDFs) were isolated from 8 standard and 8 PCR- animals and challenged with 100 ng/ml of LPS. The PCR- pDFs exhibit a small, but significant ($P < 0.05$) 1.5-fold reduction in baseline TLR4 mRNA expression. Post LPS treatment, the pDFs isolated from PCR- cows secreted less (1.6 ± 0.7 *v.* 2.7 ± 0.7 ng/ml, $P < 0.05$) IL-6 protein and expressed 3-fold lower levels of TNF-a ($P < 0.05$). To determine other genes affected by the functional variation in *TLR4*, RNA-seq was performed on untreated mRNA extracted from 5 standard and 5 PCR- pDF cultures. RNA-seq libraries were sequenced across 2 lanes of the Illumina HiSEqn 2000, generating ~42 million reads per sample. Expression analysis revealed 10 differentially expressed (FDR < 0.10, CPM >1.0) genes between standard and PCR- pDFs. Of these, one single gene, *CD36,* was up-regulated in PCR- pDFs and 9 genes were up-regulated in standard pDFs, including *FOS*, *SAA3* and *m-SAA3.2*. Finally, 6 standard and 6 PCR- cows were selected for an intra-mammary challenge with 200 cfu of *E. coli* (strain P4). While all 12 animals had a robust response to the infection, PCR- animals had lower ($P < 0.10$) serum TNF-a levels and a faster recovery to pre-infection milk production in both infected (P

< 0.10) and uninfected ($P < 0.05$) quarters. Notably, PCR- animals had an overall lower burden and more rapid clearance ($P < 0.10$) of *E. coli* from the infected quarter. Results suggest that variation in exon 3 of the *TLR4* gene contributes to severity of *E. coli* mastitis and PCR based tests could be developed to detect exon 3 variants to better select animals with resistance to severe mastitis.

Key Words: immunology, epigenetics, RNA-Seq

MT140 Pneumonia, *Pithomyces* and Purkinje cells: Genomics and animal health in sheep. K. M. McRae*, S. J. Rowe, P. L. Johnson, H. J. Baird, R. Brauning, and S. M. Clarke, *AgResearch, Mosgiel, New Zealand.*

Genetic and infectious diseases are of major importance to livestock production worldwide. There is well-documented evidence for between-animal variation in the ability of sheep to resist multiple infectious diseases of economic importance, including both pneumonia and facial eczema. These heritable differences mean that improvement of animal health through genetic selection for enhanced resistance can be used as a complementary approach to current methods for disease control. Genomics can also be used to further increase our understanding of disease, be it differences in the ability to withstand infection or causative mutations for inherited disease. Multiple genomic tools, including SNP chips and whole-genome sequencing, are available to researchers to pinpoint the small genetic differences that produce a variety of animal health traits in livestock. Large phenotypic datasets from the New Zealand sheep population have been used in conjunction with high and low-density genotyping to interrogate the sheep genome for regions associated with variability in resistance or tolerance to pneumonia and facial eczema, respectively. In an alternative approach, whole-genome sequencing has been used to search for a causative mutation for Cerebellar Cortical Abiotrophy (CCA), a genetic neurological disease in Wiltshire sheep.

Key Words: sheep, animal health, genome-wide association, genotyping

MT142 MicroRNAs associated with antibody response to bovine leukemia virus in Holstein cattle. E. Casas* and T. M. Taxis, *USDA, ARS, National Animal Disease Center, Ames, IA, USA.*

The objective of this study was to identify microRNAs associated with antibody response to bovine leukemia virus (BLV) in Holstein cattle. Samples from fourteen Holstein females were collected at the National Animal Disease Center, in Ames, Iowa. At sampling, three were heifers and eleven were cows with at least one calving. Serum from each animal was collected to establish IgG reactivity to BLV using an ELISA. Seven animals were seropositive (positive group) and seven were seronegative (negative group) for BLV exposure. MicroRNAs were extracted from leukocytes and sequenced on the Illumina 3000 Hi-Seq next-generation sequencer. A total of 152,109,424 sequences were identified as microRNAs. MicroRNAs analysed had a total of at least 10,000 copies for all 14 animals. Bta-miR-2427 ($P = 0.008$), bta-miR-151–5p ($P = 0.01$), bta-miR-652 ($P = 0.01$), bta-miR-342 ($P = 0.02$), bta-miR-361 ($P = 0.03$), bta-miR-17–5p ($P = 0.04$), and bta-miR-425–5p ($P = 0.048$), were associated with differences in expression between positive and negative groups. In all instances, the positive group had an average higher count of the microRNAs, when compared to the negative group. Bovine Leukemia Virus has been associated with breast cancer in humans. In human breast cancer tissue, hsa-miR-151–5p, hsa-miR-652, hsa-miR-342, hsa-miR-17–5, are overexpressed; whereas hsa-miR-425–5p is down-regulated. These human microRNAs are proposed as useful biomarkers to identify breast cancer. The orthologous microRNAs in cattle could potentially be used to identify cattle exposed to BLV. Further studies are required to establish if microRNAs differentially expressed in cattle exposed to BLV are similar to those differentially expressed microRNAs in human breast cancer due to BLV exposure.

Key Words: bovine leukemia virus, cattle, ELISA, microRNA, sequencing

MT143 Polyphenols and the regulatory interplay between immunogenomics and the bovine microbiome. M. Worku* and S. Adjei-Fremah, *NC A&T State University, Greensboro, NC, USA.*

The therapeutic potential of flavonoids derived from the cowpea and their impact on the effect and composition of the metagenome and host innate and adaptive immunity was studied *in vitro*. The composition of the hosts immune armory and the microbial metagenome have been associated with diseases. Plant derived polyphenols modulate the immune response and are being studied for mitigation of methanogenesis. Pathway analysis of microarray data was conducted on the effect of polyphenols from Cowpeas on the transcription of genes involved in innate and adaptive immunity. *In vitro* studies were was also conducted to evaluate their impact on rumen microbes by conducting a metagenomics analysis of the microbiome following Illumina HISeq platform sequencing. Methane emission was measured on a Picarro gas analyzer. Pattern recognition receptors such as Toll like receptors (TLR) and NoD-like receptors (NLRs) which sense microbial ligand and host derived signals of cell damage were activated. The Toll-like receptor pathway, inflammation response pathway, MAPK cascade pathway were identified among 66 pathways affected by exposure to polyphenols. Expression of immune markers such as CD40, CD68, TLR changed. Pathways involved in pathogen recognition involving signalling molecules such as the NFK b and MAP kinase were activated in response to treatment. Treatment impacted host genes involved in innate and adaptive immunity, rumen microbial diversity and reduced methane production in the rumen. Combining the techniques of metagenomics and immunogenomics may contribute to increased understanding of the action of feed based interventions such as polyphenols for better definition of the bidirectional interplay between the hosts genome and the microbiome for homeostasis in dairy cows.

Key Words: dairy, polyphenol, immunogenmics, microbiome, metagenome

MT144 Declining genetic trend of lameness health in North American Holsteins. G. Abdel-Azim* and R. Fourdraine, *CRI International Center for Biotechnology.*

Genetic trend for lameness health observed in traditional and genomic bull breeding values were validated to rule out inconsistencies in health data collection or bias in trend estimation. Nearly 1.8 million records of individual cow performance obtained from 480 farms with conditions recorded from 2010 to 2016 were studied. Phenotypes were recorded for 970 thousand daughters of ~20,000 sires born from 1990 to 2014. An animal model with herd-year-season, and parity fit as fixed factors was utilised in estimating breeding values. The model included three random effects, animals with full relationships, permanent environmental effects and sire by herd interaction. A strong negative genetic trend for lameness health was observed for birth years 2000 to 2014. The trend was validated using different approaches. First, phenotypic records were adjusted for non-genetic factors using a linear model that fit health records against fixed factors in the model. The residuals of the model showed an identical genetic trend to those obtained from sire evaluations. Second, daughter yield deviations were utilised in testing bias in the genetic trend estimate with a fixed model of individual deviations fit against sires and the year of sire use. The model showed no significant effect for the year of sire use, indicating that the negative estimate of genetic trend was unbiased. Third, condition year was fit within sire for a group of influential sires which showed a positive instead of negative trend for condition year. This

indicated that lameness health improved from a farm management perspective despite the deterioration observed on the genetic side. Finally, a full model encompassing all data was fit with both condition year, to adjust for managerial and environmental trends, and bull birth year, to adjust for genetic trends. The model was clear in finding a positive environmental trend but negative genetic trend.

Key Words: genetic trend, genomic evaluations, lameness, health, trend validation

MT145 Identification of a genomic region with a major effect on IgM antibodies binding autoantigens in chickens. M. Bao*[1], H. Bovenhuis[1], M. G. B. Nieuwland[2], H. K. Parmentier[2], and J. J. van der Poel[1], [1]*Wageningen University & Research Animal Breeding and Genomics, Wageningen, Gelderland, the Netherlands;* [2]*Adaptation Physiology Group, Wageningen, Gelderland, the Netherlands.*

In healthy humans and mice, auto-immune B-cells constitute the majority of the B-cell repertoire, suggesting an important role of these cells and their autoantibodies. Natural autoantibodies (NAAb) are involved in maintaining physiological homeostasis by removing aging cells, tumour cells and cellular debris. In healthy layers, levels of NAAb binding different auto-antigens were shown to be heritable. To identify genomic regions associated with NAAb in White Leghorn layers, we performed a GWAS and fine mapped the most promising region using imputed sequence variants. GWAS revealed a region on GGA4 ranging from 69.5 to 70.5 Mb with highly significant effects on IgM binding ovalbumin (OVA), cardiolipin (CAR) and keyhole limpet hemocyanin (KLH), and this region was targeted for fine-mapping. In total, 19 candidate genes were identified in the region of interest. The most likely candidate gene for the observed effects is TLR1A. One of the SNPs is located in an exon of TLR1A and has a deleterious effect (SIFT score 0.01) on the protein structure. The TLR1A gene belongs to *Toll*-like receptor genes, which play a crucial role in host immune responses. Furthermore, in humans, TLRs were found to activate and regulate B-cells for NAAb production.

Key Words: chicken, natural autoantibodies, TLR1A

MT146 Transcriptomic analysis reveals the potential of Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus to modulate immune system activation related to host-pathogen and damage associated signaling in infected porcine monocytes. L. C. Miller*[1], D. S. Fleming[1], X. Li[2], F. Blecha[3], and Y. Sang[4,3], [1]*USDA, Agricultural Research Service, National Animal Disease Center, Virus and Prion Research Unit, Ames, IA, USA;* [2]*National Research Center for Veterinary Medicine, China, Luo Yang City, China;* [3]*Kansas State University, Manhattan, KS, USA;* [4]*Tennessee State University, Nashville, TN, USA.*

One of the largest risks to the continued stability of the swine industry is by pathogens like porcine reproductive and respiratory syndrome virus (PRRSV) that can decimate production as it spreads among individuals. These infections can be low or highly pathogenic, and because it infects monocytic cells, PRRSV can undermine the innate immune response. This is especially true of the interferon stimulated inflammatory response. To shed light on the effect of PRRSV pathogenicity on activation status of the immune response, we polarized infected and control monocyte-derived cells (mDCs), applied transcriptomic analysis, and predicted protein–protein interactions as networks to elucidate the interconnected biological processes related to low and highly pathogenic PRRSV infection. Differentially expressed genes (DEGs) from each polarized/unpolarized and PRRSV infected/ uninfected comparison was used to predict networks and subsequent molecular functions affected by the viruses and mediators. The analysis uncovered previously uncharacterized evidence of the ability of PRRSV to affect M1 activation by altering expression of genes related to viral defence, the extra-cellular matrix (ECM), toll-like receptor (TLR) signalling, and damage induced inflammatory signalling. Genes showing variability in expression, as well as the most common predicted biological network from the comparisons, were related to cellular structure and inflammatory immune responses. These results supply additional insight into the interplay of PRRSV pathogenicity and immune system evasion by affecting multiple routes of host inflammatory signalling, as well as mDC polarization.

Key Words: PRRSV, monocyte-derived cells, RNA-Seq, predicted networks

MT147 Validation of polymorphisms in the GBP1, Mx1, and CD163 genes on host responses to PRRSV infection in pigs. B. Lim*[1], W.-I. Kim[2], C. K. Park[3], S.-W. Kim[1], and K.-S. Kim[1], [1]*Chungbuk National University, Cheongju, Korea;* [2]*Chunbuk National University, Iksan, Korea;* [3]*Kyungpook National University, Taegu, Korea.*

Porcine reproductive and respiratory syndrome (PRRS) is the most economically important disease to the swine industry, and effective prevention strategy for this disease is still required. Guanylate-binding proteins are important proteins belonging to the GTPase superfamily that have been previously described to show antiviral effects. CD163 is considered the most important receptor for PRRSV attachment and internalization. Therefore, the aim of the present study was to evaluate the effects of these genes on host resistance against PRRSV infection in conjunction with the host immune response following PRRSV challenge. 80 pigs experimentally challenged with a PRRSV have been assessed for viral load and growth performance between 21 and 50 days of age and are now planned to be genotyped. Validation of the previously identified gene polymorphisms will be presented.

Key Words: candidate genes, immune response, PRRSV

MT148 Single nucleotide polymorphisms in the bovine MHC region of Japanese Black cattle are associated with bovine leukemia virus proviral load. S. Takeshima*[1], S. Sasaki[2], P. Meripet[1], Y. Sugimoto[2], and Y. Aida[1], [1]*Viral Infectious Diseases Unit, RIKEN, Wako, Saitama, Japan;* [2]*Shirakawa Institute of Animal Genetics, Japan Livestock Technology Association, Nishigo, Fukushima, Japan.*

Bovine leukemia virus (BLV) is the causative agent of enzootic bovine leukosis, a malignant B-cell lymphoma that has spread worldwide and causes serious problems for the cattle industry. The BLV proviral load, which represents the BLV genome integrated into host genome, is a useful index for estimating disease progression and transmission risk. Here, we conducted a genome-wide association study to identify single nucleotide polymorphisms (SNPs) associated with BLV proviral load in Japanese Black cattle. The study examined 93 cattle with a high proviral load and 266 with a low proviral load. Three SNPs showed a significant association with proviral load. One SNP was detected in the *CNTN3* gene on chromosome 22, and two (which were not in linkage disequilibrium) were detected in the bovine major histocompatibility complex region on chromosome 23. These results suggest that polymorphisms in the major histocompatibility complex region affect proviral load. This is the first report to detect SNPs associated with BLV proviral load in Japanese Black cattle using whole genome association

study, and understanding host factors may provide important clues for controlling the spread of BLV in Japanese Black cattle.

Key Words: Bovine leukemia virus, whole genome association study, major histocompatibility complex

MT149 *Staphylococcus aureus* genotype modulates the *in vitro* immune response of bovine mammary epithelial cells. O. M. Keane*, D. Niedziela, and M. Murphy, *Teagasc, Grange, Dunsany, Co. Meath, Ireland.*

Intramammary infection or mastitis results in inflammation of the mammary gland and is primarily caused by bacterial infection. Genetic selection for mastitis resistance is performed indirectly using traits genetically correlated with mastitis, such as somatic cell count (SCC). However, the genetic correlation between SCC and *S. aureus* mastitis is only moderate. Host-pathogen co-evolution is a paradigm of infection biology with hosts adapting to recognise pathogens and mount an effective immune response while pathogens evolve to avoid such recognition. Mastitis pathogens that evade the host immune system and fail to induce a significant inflammatory (SCC) response will have a selective advantage. *S. aureus* can evade the host immune response in several manners, including forming a biofilm, internalising within mammalian cells and modulating the host's ability to produce pro-inflammatory cytokines, designed to attract immune cells into the udder. The strains of *S. aureus* that cause mastitis predominantly belong to several well-described bovine-adapted lineages. The objective of this study was to determine if a variety of potential virulence traits were associated with lineage. Bovine-adapted *S. aureus* isolates (n = 120), belonging to lineages CC71, CC97, CC151 and ST136, were tested for their ability to form a biofilm, adhere to and internalise within cultured bovine mammary epithelial cells (MAC-T) and to induce an immune response from MAC-T-cells. There were significant differences between the lineages in ability to form a biofilm with ST136 forming the strongest biofilm while CC151 formed the weakest biofilm. There were significant differences between the lineages in ability to internalise within MAC-T-cells with ST136 and CC151 displaying poor internalisation. Lineages also differed in their ability to elicit an immune response from the MAC-T-cell line, with CC97 eliciting a stronger immune response than CC151 and ST136. These data indicate the potential for an interaction between *S. aureus* genotype and the host response to intramammary infection.

Key Words: mastitis, *Staphylococcus aureus*, immune response

MT150 Suppression of proviral load by the bovine leukemia virus vaccine targeting susceptible cattle. Y. Aida*[1,2], L. Bai[1,2], P. He[1], N. Okimoto[3], J. Yamagishi[3], J. Kohara[4], and S.-N. Takeshima[1,2], [1]*Viral Infectious Diseases Unit, RIKEN, Wako, Saitama, Japan;* [2]*Bovine Leukemia Virus Vaccine Laboratory, RIKEN Innovation Center, RIKEN, Wako, Saitama, Japan;* [3]*Computational Biology Research, Core, Quantitative Biology Center, RIKEN, Suita, Osaka, Japan;* [4]*Animal Research Center, Hokkaido Research Organization, Shintoku, Hokkaido, Japan.*

Bovine leukemia virus (BLV), the etiological agent of the enzootic bovine leucosis (EBL), was continually spread worldwide. Our previously study demonstrated that the cattle with bovine major histocompatibility complex (*BoLA)-DRB3*1601/*1601* genotype tended to develop the terminal disease and high proviral load. One of the major problems to develop the BLV vaccine is unstable effect of the vaccine because of the individual difference for disease susceptibility. We here tried to develop the BLV vaccine for disease susceptible cattle and evaluated its vaccine effect in cattle. First, we determined the Th1 epitope in BLV against disease susceptible cattle using a total 118 kind of peptides corresponding to GAG p15, p24 and p12, and ENV gp51 and gp30. Next, we optimized our determined Th1 epitopes by antigen peptide modelling system using *in silico* screening to improve low affinity binding between peptide and susceptible BoLA DR molecule. To further resolve low stability of peptide and induce effectiveness of Th1 immunity, we encapsulated our optimized peptides, p12–4R1 and gp51R1 by Carbonate apatite (CO_3Ap) because CO_3Ap strongly induced Dendritic cell incorporation, antibody production and cellular immunity. Moreover, we demonstrated the induction of antigen-specific cell-mediated immune response in mice with both of subcutaneous and intradermal vaccination of p12–4R1/gp51R1 peptides-conjugated CO_3Ap. Finally, to demonstrate effect of this vaccine in cattle, we produced six heads of disease susceptible cattle that had *BoLA-DRB3*1601/*1601* genotype, by fertilized ovum transplantation technique. All of six animals were infected by BLV and intradermally immunized three times with either p12–4R1/gp51R1 peptides-conjugated CO_3Ap or PBS as a control at two weeks post infection. The proviral load, lymphocyte count, and antibody titer were measured for 119–161 days. Among six BLV-infected susceptibility cattle, vaccinated cattle (N = 3) significantly decreased the level of proviral load, lymphocyte count, and $CD5^+$B-cell count, comparing with PBS control cattle (N = 3). This vaccine successfully immunizes cattle and suppresses proviral load.

Key Words: MHC, bovine leukemia virus, vaccine, proviral load, cattle

MT151 Polymorphism of innate immunity genes in the conserved and selected populations of the Czech Red Pied cattle. K. Novák*[1], M. Bjelka[2], A. Kalashnikov[3], T. Valcíková[4], and V. Mátlová[1], [1]*Institute of Animal Science, Prague-Uhríneves, Czech Republic;* [2]*Breeding Cooperative Impuls, Bohdalec, Czech Republic;* [3]*L.K. Ernst Research Institute of Animal Husbandry, Dubrovitsy, Russia;* [4]*Czech University of Life Sciences, Prague-Suchdol, Czech Republic.*

The Czech Red Pied is a Simmental breed of dual-purpose type originating in the XIXth century. Extensive crossbreeding with Fleckvieh and Montbéliarde after 2000 led to the reduction in the number of original genotypes. Consequently, a nucleus herd was established in 2011 for *in vivo* conservation using the last 30 original animals. The diversity of the innate immunity genes was compared between the conserved population and the current production population of this breed. A designed amplicon panel covered 15 innate immunity genes comprising *TLR1–10* family coding for Toll-like receptors. This set was completed with the genes coding for the TLR interactors. The mixed amplicon samples of 60 animals from the conserved nucleus herd on one side and 200 bulls from the production population on the other were sequenced with PacBio (SMRT) technology in order to detect the general diversity present. An independent pipeline for variant detection included primary data quality check (FastQC), assembly (Ugene), duplicate PCR removal (PICARD), variant calling (SAMtools) and filtration (VCFtools). In view of the long reads of the PacBio technology, it was possible to directly determine haplotype organisation in most cases. The diagnostic SNPs (tagSNPs) were subsequently ascribed to individual animals with developed genotyping techniques. The primer extension was the first choice applied in 80 tag SNPs. As exemplified for the conserved population, the numbers of polymorphisms and haplotypes (in brackets) in the antibacterial *TLR*s were 6 (4) in *TLR1*, 16 (4) in *TLR2*, 8 (10) in *TLR4*, 26 (6) in *TLR5*, and 4 (6) in *TLR6*. The antiviral *TLR*s comprising *TLR3*, *-7*, *-8*, *-9* and *-10* harbored another 40 SNPs and indels. The diversity is comparable to the diversity in *TLR*s reported for the panel of world breeds. On the other hand, allelic richness found in the production population was reduced. Therefore, the conserved population might become a source of additional functional variants for the breeding program. The screening is being extended to the association study of the health effects of the monitored alleles.

Key Words: cattle, innate immunity, diversity, NGS

MT152 *PRNP* gene polymorphism in Polish native sheep breeds. A. Piestrzynska-Kajtoch*, G. Smolucha, A. Kawecka, M. Koscielny, and A. Miksza-Cybulska, *National Research Institute of Animal Production, Balice, Poland.*

The neurodegenerative scrapie disease is caused by prions, pathogenic forms of host-encoded prion proteins. The polymorphism of ovine *PRNP* gene influence the scrapie susceptibility. Alleles coding alanine (codon 136) and arginine (codons 154 and 171) are associated with resistance to classical scrapie, allele coding valine (codon 136) is associated with susceptibility to classical scrapie and allele coding phenylalanine (codon 141) is associated with increased susceptibility to atypical scrapie. The *PRNP* polymorphism was analysed in six Polish native breeds: old-type Polish Merino (MST), Blackhead Polish sheep (CZG), Wrzosówka (WRZ), Swiniarka (SW), Polish Mountain sheep (POG) and Cakiel Podhalanski (CP). All breeds, except POG, are included in the Programme of Genetic Resources Conservation. The genotypes were analysed in 1200 sheep by using combined techniques: Allelic Discrimination Assay (TaqMan probes) and RFLP (BspHI). Eleven different genotypes were found in the whole group studied. The most frequent genotype was ARR/ARR (34,7%) and the least frequent genotypes were ARH/ARQ and VRQ/VRQ (~0,2%). The allele frequencies were: ARR - 55,92%, AHQ – 6,00%, ARH – 0,08%, ARQ – 33,96% and VRQ – 4,04%. About 72% of sheep were in the G1 and G2 susceptibility classes (the least susceptible to classical scrapie). Allele F was observed in 10 sheep among 853 studied (1,17%). There were differences in the genotype frequencies between breeds. Not all genotypes were observed in every breed. In the MST and WRZ breeds, which have been under regular scrapie genotypes monitoring, alleles ARH and VRQ were not present. Allele ARR was the most frequent in CZG (86,2%), SW (66%) and WRZ (63,2%). Allele VRQ was the most frequent in SW (19%), which is indigenous primitive breed. Allele F was present in MST (2%), CP (0,7%) and POG (4,5%). A few atypical scrapie cases were found among POG, WRZ and SW breeds. Therefore, *PRNP* genotypes should be monitored regularly in sheep breeds with atypical scrapie occurrence. The study, financed by BIOSTRATEG2/297267/14/NCBR/2016, has been continued.

Key Words: sheep, scrapie, *PRNP* polymorphism

MT153 Expression profiles of immune genes in milk somatic cells and MIR predicted mineral contents in milk as indicators of mastitis. C. Marchitelli*[1], F. Signorelli[1], F. Napolitano[1], L. Buttazzoni[1], C. Grelet[2], A. Vanlierde[2], F. Dehareng[2], H. Soyeurt[3], M. Crowe[4], and GplusE Consortium[5], [1]*Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA)-Centro di Ricerca per la Produzione delle Carni e il Miglioramento genetico, Monterotondo, Italy;* [2]*Centre Wallon De Recherches Agronomiques, Gembloux, Belgium;* [3]*University of Liège, Gembloux, Belgium;* [4]*UCD School of Veterinary Medicine, Dublin, Ireland;* [5]*GplusE Consortium, Dublin, Ireland.*

Despite rigorous management practices, mastitis remains a problem in high producing dairy cows. To identify early indicators of mastitis, the following parameters were evaluated: 1. expression profiles in milk somatic cells of L-Selectin (SELL), Interleukin 8 (IL8), and Gelsolin (GSN) genes; 2. MIR predicted mineral contents in milk. We used milk somatic cell samples collected at 3 lactation stages (DIM 7, 14, 21) from seventeen Holstein cows. Total RNA was extracted by using Maxwell 16 RNA purification kit and DNAse treated. RNA quality and quantity were assessed before performing RT and qPCR. Expression analyses, normalized on two reference genes selected by geNorm (ATPase and RPS9), were performed by using the qBase^{PLUS} software. Furthermore, individual milk MIR spectra were collected at the same DIM, and prediction equations (Soyeurt *et al*., 2009) were used to predict Ca, K, Mg, Na, and P milk contents. Least squares means for gene expressions and predicted mineral contents in healthy *v.* mastitis cows (Positive California Mastitis Test and SCC >100,000 cells/ml in each of three samplings) were computed by Proc GLM in SAS. Correlations among all response variable and regressions of gene expressions on mineral contents were estimated by CORR and REG procedures in SAS. The 3 considered genes showed no differential expression at the 3 lactation stages, while their expressions were significantly different in mastitis *v.* healthy cows ($P < 0.05$) in all of the 3 considered lactation stages. Mineral contents were significantly more concentrated at 7 day than in the other two days ($P < 0.05$) and in mastitis *v.* healthy cows ($P < 0.05$). A significant correlation was found between GSN expression and Na, Mg and K contents (R^2 = 0.47, 0.34, 0.30, $P < 0.05$). Also regressions of GSN expression on mineral contents were significant ($P < 0.05$). Gene expression results corroborate our hypothesis of SELL, IL8 and GSN expression as indicators of mastitis, because of their roles in innate immunity. Results about mineral concentrations confirmed their concentration changes in milk during a mastitis. Therefore they could be reliable indicators of subclinical mastitis.

Key Words: cattle, animal health, milk production

MT154 Comparison of the cattle leukocyte receptor complex with related livestock species. J. C. Schwartz*[1], D. Heimeier[1], D. M. Bickhart[2], T. P. L. Smith[3], J. F. Medrano[4], and J. A. Hammond[1], [1]*The Pirbright Institute, Pirbright, Surrey, UK;* [2]*USDA-ARS, Madison, WI, USA;* [3]*USDA-ARS, Clay Center, NE, USA;* [4]*University of California, Davis, Davis, CA, USA.*

The natural killer (NK) cell receptor gene complexes are highly variable between species and their repetitive nature makes genomic assembly and characterisation problematic. As a result, most reference genome assemblies are heavily fragmented and/or misassembled over these regions. However, new long-read sequencing and assembly strategies promise to overcome these complications. Thus, using new genome assemblies for cattle, goats (divergence from cattle ~30 mya), and pigs (divergence from cattle and goats ~60 mya), we have greatly improved the assembly and characterisation of NK cell receptor complexes. In particular, the leukocyte receptor complex (LRC) is highly variable in gene content between these three species. In cattle, for which more published information is available, we identified three distinct haplotypes of killer immunoglobulin-like receptors (*KIR*) which vary greatly in their gene content. In addition, cattle have greatly expanded their leukocyte immunoglobulin-like receptor (*LILR*) content in comparison to goats. Furthermore, in the current pig reference assembly (Sscrofa10.2), we identified a large 280 kb sequence gap which in the new assembly contains a large number of novel *LILR* genes. We also identified a third group of novel, yet closely-related immunoglobulin genes downstream from the *KIR* region in all three species. These novel genes vary in gene number between species, are highly similar to one another, and encode both activating and inhibitory receptors. The large amount of variation between species that we observed is consistent with a quickly evolving LRC under strong selection pressures. These observations are a necessary step towards understanding the NK cell receptor repertoire and will help inform future studies investigating immune response variation in these important species.

Key Words: natural killer cells, cattle, goats, pigs, KIR

MT155 Genetic variants in the innate immune system associated with *Salmonella* shedding and colonization in Ontario pigs. M. H. Ainslie[1], V. Farzan[1,2], M. Jafarikia[3,4], and B. N. Lillie*[1], [1]*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada;* [2]*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada;* [3]*Canadian Centre for Swine*

Improvement Inc., Guelph, Ontario, Canada; [4]*Department of Animal Biosciences, Ontario Agricultural College, University of Guelph, Guelph, Ontario, Canada.*

Salmonella is an important foodborne pathogen and pigs are often asymptomatic carriers. Short nucleotide variants in the porcine innate immune system have been shown to alter the host response to pathogens including *Salmonella*. Genetic improvement programs may be able to exploit these variants to decrease the risk of an animal becoming an asymptomatic carrier. The objective of this study was to identify associations between genetic variants of the porcine innate immune system and *Salmonella* shedding and colonization. A total of 809 pigs were selected from 14 cohorts on 8 commercial farms in South-western Ontario. Fecal samples were collected before 4 days of age, at weaning and at the end of the nursery, grower and finisher periods; tonsil and lymph nodes were collected at slaughter. Pigs were weighed at each collection and farms were surveyed about management practices and herd health. All samples were cultured for *Salmonella* using selective media. Genomic DNA was extracted from blood, tail, or ear tissue and pigs were genotyped using MALDI-TOF mass spectrometry for 42 variants previously demonstrated to play a possible role in *Salmonella* pathogenesis and/or infectious disease. A mixed-effects multi-level logistic regression modelling method was used to analyse the association between variants and *Salmonella* shedding and colonization. The T allele in a synonymous exonic variant (Chr. 14:88687126 C>T) in the collagenous lectin gene *MBL1* increased the risk of shedding *Salmonella* ($P = 0.02$) while the A allele in a non-synonymous exonic variant in *NOD1* (Chr. 18:47016343 G>A), an intracellular pattern recognition receptor, increased the risk of tissue colonization at slaughter ($P = 0.04$). Importantly, the identified variant alleles did not impact growth performance as measured by average daily gain ($P > 0.1$). These findings are consistent with previous findings on the *MBL1* variant and *in vitro* research on the *NOD1* variant. The current research increases the value of these variants for genetic improvement programs towards breeding pigs with a more robust immune response to *Salmonella* and other pathogens.

Key Words: pigs and related species, innate immunity, food safety, *Salmonella*, single nucleotide variants (SNVs)

MT156 Serum IL10 response to *Eimeria* challenge in commercial and backcross chickens. K. Boulton*[1], M. Nolan[2], A. Psifidi[1], Z. Wu[1], K. Harman[2], R. Hawken[3], M. Abrahamsen[3], F. Tomley[2], D. Blake[2], and D. Hume[1], [1]*Roslin Institute, University of Edinburgh, Easter Bush, Midlothian, UK;* [2]*Royal Veterinary College, University of London, Hatfield, UK;* [3]*Cobb-Vantress Inc., Siloam Springs, AR, USA.*

Eimeria spp. are responsible for coccidiosis, a disease that costs the global poultry industry almost $3Bn per year. As such, efforts to identify early-life biomarkers for natural resistance in chickens are underway. In a recent large-scale study ($N = 1200$) of commercial broilers that sought evidence of selectable variance in response to *Eimeria tenella* infection significant correlations were found between serum interleukin-10 (IL10), an anti-inflammatory cytokine and pathology (lesion scores). Eigenanalysis to dissect the relationships among these variables and a production trait (percentage bodyweight gain) produced three distinct vectors. Visualisation of these vectors confirmed the difference between resistant and susceptible chickens, both defined by high levels of IL10 production, while a third, tolerant subpopulation produced little or none. In a similar study, equal numbers ($n = 250$) of a backcross population produced from two White Leghorn lines known to be susceptible to either *E. tenella* or *E. maxima*, were infected with 250 oocysts from a single species. This dose allowed quantification of parasite replication at the expense of producing variance in weight gain. While there was no sex difference in parasite replication or pathology in the group infected with *E. tenella*, males infected with *E. maxima* experienced significantly higher pathology and parasite replication than females. In both species, Eigenanalysis highlighted good dissociation between pathology and parasite replication and a subpopulation of resilient birds. IL10 production is high in all Eigenvectors in both groups, apart from a vector representing tolerance in the group infected with *E. maxima*, recognisable by a lack of IL10 production as was seen as in the *E. tenella* infected commercial birds. Preliminary analyses of the chicken genome have uncovered potential selective sweeps in the regions of the IL10 gene and a receptor (IL10R2) that is essential for the production of IL10 in innate immune response to infection. Thus, these results support the potential of IL10 as an early-life biomarker for resistance to infection.

MT157 Mammary epithelial cells, rather than professional immune cells dictate the pathogen species-specific immune reaction of the udder. J. Hehl*[1], M. Koy[2], A. Berthold[1], H.-J. Schuberth[2], M. Weinert[3], S. Engelmann[3], C. Kühn[1], H.-M. Seyfert[1], and J. Günther[1], [1]*Leibniz Institute for Farm Animal Biology (FBN), Institute for Genome Biology, Dummerstorf, Germany;* [2]*Immunology Unit, University of Veterinary Medicine Foundation, Hannover, Germany;* [3]*Institute of Microbiology, Technical University Braunschweig, Braunschweig, Germany.*

The aetiology determines extent and quality of the immune response after an udder infection (mastitis). Gram-negative bacteria (e.g. *Escherichia coli*) will quickly elicit strong inflammation of the udder, fully activate its immune defence and often eradicate the pathogen. In contrast, Gram-positive bacteria (e.g. *Staphylococcus aureus*) will slowly elicit a much weaker inflammation and immune response frequently resulting in chronic infections. It was unclear which of the different cell types residing in the udder determines the pathogen-species specific norm of immune reaction of that organ. Therefore, we diagnosed the pathogen-specific immune response of different relevant cell types from udder and blood. We challenged primary cultures of bovine mammary epithelial cells (pbMEC), fibroblasts from udder, bovine monocyte-derived macrophages (boMdM) and the established MEC line MAC-T with heat-killed *E. coli*, *S. aureus* and *S. uberis* and analysed their immune responses. *E. coli,* but not the other pathogens fully activated the immune response in pbMEC, fibroblasts and MAC-T-cells, including increased cytokine and chemokine expression and NF-kB activation. *S. aureus* and *S. uberis* induced weak or no immune reactions respectively. Yet, the macrophage models (boMdM and murine RAW 264.7 cells) responded strongly to all three pathogens including activation of IkB/NF-kB signalling. The models for MEC and fibroblasts responded with distinctly graded immune reactions to each of the three pathogens. *E. coli* induced a strong, transient cytokine storm in the models but neither of the Gram-positive bacteria did. This distinction was caused by the failure of MEC to activate TLR-mediated signalling upon challenges with *S. aureus* or *S. uberis*. Hence, the pathogen-species dependent immune reaction norm of MEC complies best with - and by inference - dominates *in vivo* that of the udder. We are now scrutinizing on humoral and genetic factors modulating the immune responsiveness of the MEC. Therefore, we isolated pbMEC and factor secreting boMdMs from cows have been selected for an inherited divergent susceptibility against mastitis causing pathogens.

Key Words: bovine mastitis, pbMEC, *E. coli*, *S. aureus*, immune response

MT158 Combining targeted next-generation sequencing and microarray analysis for cis expression quantitative loci analysis of the porcine innate immunome. R. S. Fraser[1], H. N. Snyman[1,2], A. Meyer[1,3], J. D. Hammermüller[1], and B. N. Lillie*[1], [1]*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada;* [2]*Animal Health Centre,*

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Infectious diseases are a major source of economic loss, antibiotic use, and animal welfare impact in the swine industry. The innate immune system is an important first line of defence against a variety of different infectious diseases, and mutations in innate immune genes can impair the ability of pigs to respond to pathogens. While mutations in the coding region of genes can have deleterious effects on protein function, mutations in regulatory regions can impact the level of expression of adjacent or distant genes. Previous work has shown that sequence variants in genes of the innate immune system, including the collagenous lectin genes, are more common in animals suffering from infectious diseases. The objective of this study was to identify sequence variants associated with variability in innate immune gene expression of pigs. In a previous study, we used a 4 × 44k Agilent microarray and identified 112 genes of the innate immune system with variable hepatic expression. In this study, we used targeted, high-throughput re-sequencing to comprehensively identify mutations in these 112 innate immune genes and their surrounding regulatory DNA. Genomic DNA was obtained from the liver of 87 healthy, market weight pigs sourced from a large Ontario abattoir, with the same animals used in both the microarray and sequencing experiments. The coding regions, introns, and up to 50 kb upstream and 3 kb downstream of each gene were targeted for re-sequencing. Target capture was performed using custom probes from Roche Nimblegen and DNA was sequenced on an Illumina HiSeq. Overall, 6.4 Mb of DNA was sequenced in each pig at an average depth of 14 reads per base. After applying quality control filters, 41 894 sequence variants were identified (41 556 SNPs, 338 indels). Cis expression quantitative trait loci (cis-eQTL) were identified using Matrix eQTL. In total, 320 significant ($P < 0.05$) eQTL were identified, associated with 18 different innate immune genes. These loci are interesting targets for further study, and provide helpful information for the development of future breeding strategies.

Key Words: pigs, innate immunity, eQTL, targeted gene sequencing, infectious disease

MT159 Haplotype resolution of leukocyte receptor complex in cattle through targeted enrichment and SMRT sequencing. D. Heimeier*[1], J. Schwartz[1], D. Bickhart[2], T. Smith[3], and J. Hammond[1], [1]*The Pirbright Institute, Woking, Surrey, UK;* [2]*Cell Wall Biology and Utilization Research, USDA-ARS, Madison, WI, USA;* [3]*Meat Animal Research Center, USDA-ARS, Clay Center, NE, USA.*

The highly repetitive nature of cattle leukocyte receptor complex (LRC) has made it difficult to assemble and fully characterise this region with short reads used by second generation sequencing. Previously, we reported the first two cattle killer immunoglobulin-like receptors (KIR) haplotypes; one complete and framed by leukocyte immunoglobulin-like receptor (LILR) and Immunoglobulin α Fc receptor (FCAR) genes (263kb), the other shorter and incomplete, that were resolved from combined Sanger and 454-pyrosequencing sequencing of BAC clones. Through subsequent targeted genome enrichment with Roche Nimblegen probes and Illumina sequencing of different cattle breeds and related species the haplotype variability and gene polymorphism of further haplotypes has been predicted to be gene variable with additional KIR genes. This data has now been combined and validated with another complete and larger KIR haplotype (350kb) that has been assembled using long-read single molecule real time (SMRT) sequencing with Pacific Bioscience technology. More recently, we have developed this targeted sequencing approach for use with SMRT sequencing to resolve the KIR region in more detail from further two individuals. Initial results show between 87 and 91% average base coverage when mapped to the shorter complete KIR haplotype at a maximum divergence of 10%. Average coverage was more than 20x with certain regions reaching more than 100x, which could be indicating additional or missing genes in the investigated individuals. This preliminary data is clearly indicating towards significant structural variation as well as polymorphisms. We are currently developing a bioinformatics pipeline to *de novo* assemble and phase each haplotype, so we can process a large number of individuals and identify gene variable haplotypes and polymorphic variants with confidence.

Key Words: cattle, immunogenetics, targeted enrichment sequencing, leukocyte receptor complex, haplotype

MT160 Identification of putative key transcription factors in canine macrophages after infection with *Leishmania infantum* and stimulation with a Toll like receptor-2 agonist. L. Solano-Gallego[2], S. Montserrat[2], F. Mayer[1], A. Castello[1], L. Alborch[2], S. Heath[3], A. Esteve-Codina[3], J. Gomez-Garrido[3], R. A. Cigliano[4], and A. Clop*[1], [1]*Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Cerdanyola del Valles, Catalonia, Spain;* [2]*Universitat Autonoma de Barcelona, Cerdanyola del Valles, Catalonia, Spain;* [3]*Centre Nacional d'Anàlisi Genómica CNAG-CRG, Barcelona, Catalonia, Spain;* [4]*Sequentia Biotech, Barcelona, Catalonia, Spain.*

Leishmaniosis is a common zoonotic disease in dogs and humans caused by the parasite Leishmania infantum. PAM3CSK4, a TLR2 agonist, promotes inflammation and reduces the parasite load in macrophages. In order to understand the molecular changes occurring in relation to L. infantum infection and after PAM3CSK4 stimulation, we compared the RNA-seq profiles of a canine macrophage cell line (DH82) (i) before infection, (ii) after infection with L. infantum promastigotes, (iii) after stimulation with the TLR2 agonist and (iv) after infection and TLR2 stimulation. We also performed ChIP-seq to map two histone modifications: H3K4me3 and H3K27ac, which mark promoters and genomic activity, respectively. We used an improved dog genome annotation built in-house with RNA-seq data from this project and from NCBI. In total, 530 million 75bp × 2 paired-end reads were generated in an Illumina HiSeq 2000 system. RNA-seq profiles were determined with STAR and RSEM and differential expression was assessed with DESeq2. These analyses show that TLR2 stimulation but not infection has an impact (FDR ≤ 0.05) on gene expression. Taking into account any of the comparisons involving TLR2 stimulation, 281 genes were differentially expressed (DE). 257 of these genes were DE when (i) and (iv) were compared. There was an enrichment of immunoinflammatory genes. Of note, 19 of the 257 genes are transcription factors (TF). ChIP-seq reads were mapped to the dog genome with BWA and peaks were called with MACS2. The peaks occurring between 4Kb upstream and 2Kb downstream of the transcription start site (putative regulatory regions) of the 257 DE genes were further investigated to identify potential binding sites for the 10 DE TFs with annotated motifs using Transfac/Jaspar. Cognate binding sites were present in 198 DE genes. RelA, Rel and Foxj1, three key immune-related TFs, displayed the largest number of binding sites. Further analysis will help determining whether these TFs orchestrate the gene dysregulation caused after stimulating the cells with a TLR2 agonist.

Key Words: leishmaniasis, TLR2 stimulation, RNA-seq, ChIP-seq, differential expression

MT161 Genetic basis for resistance to avian influenza in commercial egg layer chicken lines. W. Drobik-Czwarno*[1], A. Wolc[2,3], J. Fulton[3], T. Jankowski[4], J. Arango[3], P. Settar[3], N. O'Sullivan[3], and J. Dekkers[2], [1]*Department of Animal Genetics and Breeding, Faculty of Animal Science, Warsaw University of Life Sciences, Warsaw, Poland;* [2]*Department of Animal Science, Iowa*

State University, Ames, IA, USA; [3]Hy-Line International, West Des Moines, IA, USA; [4]Nutribiogen, Poznan, Poland.

A 2015 outbreak of H5N2 Highly Pathogenic Avian Influenza (HPAI), resulting in mandatory euthanization of millions of chickens, was the most fatal in US history. The aim of this study was to identify genomic regions associated with survival following natural infection with HPAI. Blood samples were obtained from 274 individuals from three commercial White Leghorn varieties. Survivors and age and genetics matched non-infected controls from each variety were included in the comparison. All individuals were genotyped on the 600k Affymetrix SNP array. A genome-wide association study was performed within the varieties with standard frequency test in PLINK, while logistic regression with the first three multi-dimensional scaling (MDS) components of SNP genotypes as covariates was used for all varieties together. Several SNPs located within three regions in two varieties were significant at a 5% Bonferroni genome-wide threshold ($P < 3.87E-06$): on chromosomes 5 and 18 for variety 1 and on chromosome 11 for variety 2. Genome wide scan with F_{ST} was also performed as an alternative method of analysis, using windows of 40, 100 and 500kb. The regions with highest F_{ST} values between cases and controls were located on chromosomes 1 and Z and overlapped several genes with immunological function and previously identified quantitative trait loci for health. Only few regions were consistent between the GWAS analysis (approaching significance) and at the same significance level in the F_{ST} genome wide scan. This study confirms that resistance to HPAI is a complex, polygenic trait and that mechanisms of resistance can be population specific. This study was supported by the Iowa Egg Industry Center.

Key Words: chicken, highly pathogenic avian influenza, resistance, GWAS

MT162 The ligands and polymorphic residues of chicken MHC YF class I-like molecules. R. M. Goto[1], G. Gugiu[1], B. M. Stadtmueller[2], P. J. Bjorkman[2], and M. M. Miller*[1], [1]*Beckman Research Institute, City of Hope, Duarte, CA, USA; [2]California Institute of Technology, Pasadena, CA, USA.*

Polymorphic amino acid residues in the binding groove of classical polymorphic MHC I molecules confer specificity for the types of peptides that bind to MHC class I and shape adaptive immune responses of conventional T lymphocytes. Non-classical MHC class I molecules direct responses of other lymphocyte populations by other means. Of particular interest among non-classical molecules are those encoded by polymorphic *YF* genes within MHC-*Y*, the second region of MHC genes in the chicken. It may be that MHC-*Y* genetics influences the incidence of infectious disease in chickens. Previous structural studies of YF revealed a binding groove too narrow to accommodate peptide ligands, but ligands have not been identified and how the polymorphic amino acid residues of different YF isoforms might affect ligand binding is not known. To define YF ligands, chicken gene sequences for YF1*7.1 heavy chain and β2 microglobulin (β2m) were expressed in *E. coli* using a recombinant expression vector to produce inclusion bodies. Inclusion bodies were purified, then YF1*7.1/β2m renatured first without added candidate ligands and then, in later experiments, renatured with added lipid extract from *E. coli* or with selected candidate ligands. To screen for bound ligand, renatured YF1*7.1/β2m was FPLC purified then analysed by ultra high performance liquid chromatography. Candidate ligands were identified in a Thermo Orbitrap Fusion mass spectrometer. YF1*7.1 ligands were found to be *lyso*-phospholipids including 17:1 *Lyso*-PE, 18:1 *Lyso*-PE and 17:1 *Lyso*-PG. The optimum fatty acid chain-length for ligand binding was 17 but *lyso*-phospholipids with 16 to 19 carbons were also bound. A second YF isoform also bound *lyso*-phospholipids even though differing from YF1*7.1 by 25 residues across the domains forming the ligand binding groove. Mapping the polymorphic residues for this second YF isoform and additional isoforms onto the original YF1*7.1 structure revealed that the side chains of polymorphic residues mostly point away from the ligand binding groove, suggesting that the polymorphic residues may be more important in recognition of different YF isoforms than in selection of ligand bound within the groove.

Key Words: chicken, major histocompatibility complex class I-like molecules, lyso-phospholipids, polymorphism

MT163 Polymorphism in TLR2 in different dairy cattle breeds suggests immune functional modulation. M. Bartens*, K. Tombacz, A. Gibson, and D. Werling, *Department of Pathobiology and Population Sciences, Royal Veterinary College, University of London, Hatfield, UK.*

There is strong evidence that high yielding cows are highly susceptible to infectious diseases. As the innate immune system is the first barrier for pathogens entering an organism, its activation by pattern recognition receptors (PRRs) is crucial for an adequate immune response. Within these PRRs, Toll-like receptors are the most important and polymorphisms within TLRs are suggested to be associated with disease resistance traits in farm animals. TLR2 plays a major role in recognising multiple pathogens, therefore, we compared TLR2 sequences of two popular dairy breeds by cloning and sequencing their coding TLR2 sequences from PBMC-derived macrophages. Assembled TLR2 contigs were translated to amino acid sequences and aligned. A total of 18 SNPs within the coding sequence of TLR2 were detected across the breeds. Of note, two were found in the crucial ligand-binding domain of the extracellular domain of the TLR2 receptor, H326Q and T63G. A functional role of the detected SNPs, was investigated using a cellular reporter assay for TLR2 receptor activation. Human embryonic kidney (HEK) 293 T-cells were stably transfected with the NF-kB-inducible reporter gene secreted embryonic alkaline phosphatase (SEAP) and our selected TLR2 receptor constructs. The transfectants were stimulated with TLR2-specific ligands such as Pam_3CSK_4 and FSL-1 as well as with heat-killed *E.coli*, *S.dublin* and *M.bovis BCG* as these are common pathogens in the farm environment. Significant increases in TLR2 activation were measured in TLR2 constructs containing H326Q, (SNP rs68343167). This variation was also previously found in an indigenous, resistant, Bos taurus Anatolian breed, thus we hypothesise the presence of this SNP to confer Brown Swiss (and Anatolian Black) to be less susceptible to infections than the Holstein Friesian breed. We suggest that immune function is compromised in those breeds under strong selective pressure for high production traits. Our results suggest that the variations seen may be of functional relevance leading to a stronger immune response within the Brown Swiss breed.

Key Words: bovine TLR2, cattle breeds, innate immunity, disease resistance, reporter assay

MT164 Porcine bloodomics: Identification of porcine neutrophil-specific genes through gene expression correlations to neutrophil abundance and comparative expression data. G. Vella[1,2], M. Schroyen[2], H. Beiki[2], C. L. Loving[3], and C. K. Tuggle*[2], [1]*College of Veterinary Medicine, Iowa State University, Ames, IA, USA; [2]Department of Animal Science, Iowa State University, Ames, IA, USA; [3]Food Safety and Enteric Pathogens Research Unit, USDA-ARS-NADC, Ames, IA, USA.*

Identification and expression profiling of porcine immune cell-specific genes will provide researchers with a more vivid portrait of healthy and diseased states in individual pigs, permit more accurate biomarker screening, and will improve porcine genome annotation. Cell-specific gene profiling has been conducted in human and mouse cells under both resting and inflammatory models. Evidence of conservation of lymphoid and myeloid signature genes in mice and humans has been published, but there is a lack of pig

cell-specific data. As an initial study to begin cataloging porcine cell-specific patterns, we used existing human granulocyte-specific expression data in combination with correlation of gene expression to neutrophil proportion in porcine whole blood RNA-seq data to predict neutrophil-specific pig genes. The RNA-seq datasets included collections from healthy as well as lipopolysaccharide-treated pigs. Correlation network comparisons were also used to visualise neutrophil-correlated modules. Pig neutrophil-correlated genes were significantly enriched ($P < 0.01$, Fisher's exact test) in a published list of human granulocyte-specific genes. A subset of these genes ($n = 26$) were tested using Fluidigm q-RT–PCR with RNA from neutrophils versus peripheral blood mononuclear cells (PBMC, monocytes and lymphocytes only), as well as the original blood samples to verify RNA-seq data. Quantitative PCR experiments verified 80–100% of these genes to be 2 fold higher in expression in neutrophils compared to PBMC, depending on the porcine whole blood RNA-seq dataset used for correlation, while 60–80% of tested genes were 10-fold higher in neutrophils over PBMC. To extend this work, we are currently analysing neutrophils by using RNA-seq and comparing human and porcine neutrophil-specific networks to expand our understanding of porcine immunogenomics and increase functional annotation of the porcine genome.

Key Words: pig, FAANG, comparative genomics, immunogenomics, biomarker

MT165 Analysis of the genomic regions associated to response to coccidiosis caused by *Eimeria maxima* in broiler chickens. B. Bed Hom*[1], P.-v. d. L. Marie-Hélène[1], R. Hawken[2], and H. Edin[1], [1]*INRA, Jouy-en-Josas, France;* [2]*Cobb-Vantress Inc., Siloam Springs, AR, USA.*

Coccidiosis is an intestinal parasitic disease caused by species from *Eimeria* genus, widespread in poultry farming, leading to important losses by its direct impact on production and the associated control measures (such as vaccines or cocciostats). The integrative management against this disease should lead to include new parameters of animal's response to coccidiosis in selection schemes. Our goal was to study the variability of broilers' response to coccidiosis caused by *Eimeria maxima*, to identify associated genomic regions and putative candidate genes and to understand underlying biological mechanisms. For this aim, we performed an experimental infection with 2 024 animals (Cobb500 broilers) and measured phenotypes of animals' response (such as bodyweight, temperature, lesion scores and many blood parameters). Animals have been genotyped using the Affymetrix 580K SNP panel, and the genome-wide association study has revealed genomic regions highly significantly associated to some traits of interest (bodyweight gain, plasma colour and plasma β2-globuline concentration). However some genetic markers have contrasted effects on phenotypes between control or infected animals, indicating putative trade-offs between production and health. The analysis of biological functions of genes from regions associated to phenotypes has also shown the major role played by biological pathways of metabolism and some innate immune parameters. Moreover, the inference of interaction networks between genes highlighted very significant networks centred on gut repair or cardiovascular functions. The genetic markers identified during this study can be used in selection programs.

Key Words: chicken, genome-wide association, disease resilience, coccidiosis

MT166 Enhanced genetic disease control with selection for low susceptibility and infectivity. S. Tsairidou*, O. Anacleto, J. A. Woolliams, and A. Doeschl-Wilson, *Roslin Institute, Edinburgh, UK.*

Genetic heterogeneity in host infectivity and susceptibility to infectious diseases has a great impact on spread and severity of livestock epidemics. Current genetic selection schemes exploit heritable variation in susceptibility to reduce disease prevalence. However, increasing evidence suggests that there is also variation in infectivity and super-spreaders have been documented in disease outbreaks. This study examined if combined selection for both low infectivity and susceptibility can be more efficient in reducing epidemic severity and risk compared to selection on susceptibility alone. Infectivity depends on genetics and disease epidemiology. Thus, a stochastic SIR (Susceptible, Infected, Recovered) epidemiological model was used to model disease dynamics accounting for polygenic genetic heterogeneity in infectivity and susceptibility. A population of 5,000 animals was generated and divided in groups of 100. Epidemics were simulated within each group and different scenarios for genetic variance and reproductive ratios R_0 were tested. Selection on infectivity and susceptibility was simulated over 20 generations following standard quantitative genetics theory and alternative selection schemes were tested by varying selection accuracy and intensity. There was a greater reduction of epidemic severity and risk by combined selection for susceptibility and infectivity compared to selection only for susceptibility. After 4 generations of combined selection with accuracy of 0.7 for both traits the proportion of infected animals was 44% lower compared to selection only on susceptibility. After 12 generations of combined selection the percentage of groups producing an epidemic was reduced by 50% for diseases with R_0 of 1.5, and by 20% for severe epidemics with R_0 of 6, while selection on susceptibility alone required more than 20 generations. Finally, even for an accuracy for infectivity of 0.2, there was substantial improvement from combined selection. In conclusion, combined selection for susceptibility and infectivity was more efficient than selection only on susceptibility. Further studies are needed to estimate genetic effects for both traits.

Key Words: animal breeding, infectious disease

MT167 Genome-wide CRISPR knockout screening for host factors involved in bovine herpes virus type 1 infection. W. Tan*[1], I. Dry[1], S. Lillico[2], C. B. A. Whitelaw[2], and B. Dalziel[1], [1]*Division of Infection and Immunity, the Roslin Institute, University of Edinburgh, Edinburgh, Scotland, UK;* [2]*Division of Developmental Biology, the Roslin Institute, University of Edinburgh, Edinburgh, Scotland, UK.*

Bovine herpes virus type 1(BHV-1) causes infectious bovine rhinotracheitis, fatalities in calves and pregnancy abortions in cows, leading to huge economy loss in Ireland and the UK. Unfortunately, little is known about how host factors interact with this virus, and our lack of knowledge is impeding vaccine and drug developments. CRISPR/Cas9 are novel molecular scissors that cut DNA in a site-specific manner and have been used to edit many genes in livestock. It relies on base pairing between the small CRISPR guide RNA (gRNA) and target DNA, an activity that leads the gRNA-bound Cas9 protein to exert DNA cutting, often resulting in gene inactivation or conversion changes. Since the specificity of CRISPR/Cas9 is largely determined by the base pairing, it is straightforward to introduce many guides together to achieve knockout of multiple genes in parallel. Various groups have fully utilised this strategy and generated CRISPR libraries that target every gene in given organisms. Cells treated with such libraries lead to dissections of host factors involved in infections such as those from flaviviruses and HIV. To enable whole genome screening in cattle, we are creating a CRISPR library with ~120,000 guides targeting every gene in the bovine genome. We will then use it to screen for host factors involved in BHV-1 infection. To achieve this, MDBK cells that stably express Cas9 from the *rosa26* locus will be transduced by a lentivirus library that expresses all guides at low titre, ensuring single knockout events in the majority of the cells. The resulting cell pool encompassing knockout events in all genes will then be challenged by recombinant BHV-1 virus with mCherry. Cell populations with early and late mCherry signals and cells devoid of mCherry post infection will be obtained by FACS sorting. Integrat-

ed gRNA in these populations will be amplified and sequenced by Illumina Hiseq which will reveal copy numbers of guides; enriched guides indicate inhibitory roles of corresponding genes whereas depleted guides imply facilitator roles of targeted genes. These candidates will then be validated by transfecting individual guides into MDBK cells which will also be challenged by the virus.

Key Words: BHV-1, CRISPR-Cas9, whole-genome screening, gene discovery

MT168 Genetic individual variability of vaccine responses in pigs. F. Blanc*[1], G. Lemonnier[1], J. Leplat[1,2], E. Bouguyon[3], Y. Billon[4], J. Estelle[1], and C. Rogel-Gaillard[1], [1]*GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [2]*CEA, DRF/IRCM/SREIT/LREG, Jouy-en-Josas, France;* [3]*VIM-INRA-Université Paris-Saclay, Jouy-en-Josas, France;* [4]*GenESI, INRA, Surgères, France.*

Impact of host genetic variation in shaping innate and adaptive immune responses is an emerging lever to be included in new vaccination strategies. Our aim was to analyse the genetic control of individual vaccine responses in pigs and we addressed this question by studying the variation of antibody responses induced by vaccination against *Mycoplasma hyopneumoniae* (*M. hyo*) or Influenza A Virus (IAV). Large White pigs housed in a conventional facility were vaccinated at weaning (around 28 days of age) with a booster vaccination 3 weeks later. Forty-eight families were produced in five batches. 190 and 192 piglets were vaccinated against *M.hyo* or IAV, respectively, and 64 non vaccinated piglets were included as controls for the two vaccine experiments. The humoral vaccine response was measured by following the dynamics of seric *M. hyo*- or IAV-specific IgGs, prior vaccination on the vaccine day, every week during five weeks post-vaccination, and before slaughtering. In addition, haemagglutination inihibition (HAI) assays were performed for IAV-vaccinated pigs. The individual variability of responses to vaccination was revealed by the differences in the levels of *M. hyo*- or IAV-specific IgGs in the sera of vaccinated animals. One week after the booster vaccination, *M. hyo* Ab levels (S/P values) ranged from 0.012 to 2.151 (mean = 1.322, s.d. = 0.337) and IAV-specific IgGs levels ranged from 0.59 to 128.5 µg/mL (mean = 33.39 µg/mL, s.d. = 2.475). For IAV-vaccinated animals, HAI titres ranged from 10 to 2560 (geometric mean = 332.2). Interestingly, females exhibited a higher humoral immune response to vaccination against *M. hyo* compared to males, with significant differences (unpaired *t*-test) two weeks post booster vaccination ($p = 0.024$) and also before slaughtering ($p = 0.0002$). This experimental design and the wide range of individual variabilities obtained for both vaccines will allow us to estimate the heritability of the phenotypes and to perform genome wide association studies (GWAS) to identify the genomic regions and candidate genetic markers associated with individual variability of vaccine responses.

Key Words: pigs and related species, immunogenomics, immune system, animal health

MT169 The association of copy number variations with tick count in South African Nguni cattle. L. Pickering*[1], K. Dzama[1], F. Muchadeyi[2], and M. Wang[2], [1]*Animal Science Department, University of Stellenbosch, Stellenbosch, Western Cape, South Africa;* [2]*Agriculture Research Council Biotechnology Platform, Pretoria, Gauteng, South Africa.*

Ticks and tick-borne diseases pose a major threat to livestock industries worldwide. The South African Nguni is a locally adapted cattle breed that is known for its resilience to ticks and tick borne diseases. Copy number variations regions (CNVR) comprise insertions, duplication and deletions within the genome that are larger than 1kb and are reported to play a possible role in adaptation. A preliminary investigation to determine the association between CNVR and tick resistance in South African Nguni cattle was performed. Tick count data was collected from 347 randomly selected Nguni cattle from three different locations within South Africa over a period of two years. Data was split per location and summary statistics were used to determine quartile and interquartile ranges of tick counts. If an animal had a tick count average lower than or equal to the first quartile it was classified as resistant (1), animals with a tick count average greater than or equal to the third quartile were classified as susceptible (0) and those with values in between were grouped as unclassified (2). DNA extracted from hair and blood samples was genotyped using the Illumina BovineSNP50 assay. Quality control and sample pruning was performed using Plink (Version 1.07) leaving 41193 high quality SNPs. LogR ratios and B allele frequency data of filtered SNPs was extracted and PennCNV software was utilised to identify 1831 unique CNVR. A data file containing respective copy number states (0, 1, 2, 3) of CNVR loci for each animal was generated. A general linear model testing the hypothesis that tick resistance is associated with CNVR was run using R Studio. Sixty-six CNVR located on chromosomes 4, 5, 6, 13 and 14 demonstrated a significant ($P < 0.05$) association with tick count. Associated CNVR covered 16 genes that played a role in multiple molecular functions and biological processes including catalytic activity, binding functions and immune, metabolic, cellular, reproduction and developmental processes respectively. This study is the first of its kind to demonstrate a significant association between tick count and copy number variations in South African Nguni cattle.

Key Words: cattle, tick resistance, copy number variation region, bovine 50K BeadChip

MT170 Integrated network analysis for mRNAs and miRNAs expressed in PRRSV vaccinated peripheral blood mononuclear cells of pigs. M. A. Islam[1], C. Neuhoff*[1], S. Rony[1], C. Große-Brinkhaus[1], M. J. Uddin[2], M. Hölker[1], D. Tesfaye[1], E. Tholen[1], M. J. Pröll[1], and K. Schellander[1], [1]*Institute of Animal Science, Animal Breeding and Husbandry group, University of Bonn, Bonn, Germany;* [2]*School of Veterinary Science, The University of Queensland, Gatton campus, QLD, Australia.*

MicroRNAs, posttranscriptional regulators of gene expression, have been emerged as potential tools for evaluating host immune response to infection or vaccination. The current study aimed to investigate the expression profiles of mRNA and miRNA in peripheral mononuclear cells (PBMCs) to uncover the miRNA-mRNA regulated host immune response to porcine reproductive and respiratory syndrome virus (PRRSV) vaccines. For this we analysed the global miRNA profiles of PBMCs collected at before (0 h), and 6, 24 and 72 h post PRRSV vaccination in German Landrace (DL) and Pietrain (Pi) pigs. Expression analysis identified 12, 259 and 14 differentially expressed (DE) miRNAs in PBMCs of DL and 0, 222 and 13 DE miRNAs in PBMCs of Pi at 6, 24 and 72 h post vaccination, respectively. The mRNA expression analysis of PBMCs of pigs was performed from the same sample pools at 0, 6, 24 and 72 h post vaccination. The list of predicted target genes of DE miRNAs were overlaid onto the list of vaccine induced differentially expressed gene (2,920) and a total of 1,397 matched mRNAs were found as true differentially expressed target genes (TDETGs) in PBMCs after PRRSV vaccination. The TDETGs are involved with regulation of biological processes including response to signal transduction, innate immune response regulation of MAPK kinase activity and apoptosis. The miRNA-mRNA co-regulatory network was drawn between down-regulated miRNA and their up-regulated true mRNA targets. The miRNA and gene co-regulatory network revealed that miR-6762, miR-23a-5p, miR-181b-5p, miR-4454 and miR-125–5p are the putative regulators of PRRSV vaccine induced genes including SIRT1, FOS, ARNTL, PKM, CD9, WNT1, CDKN1A, ABCG2, VEGFA and TNFAIP3 in PBMCs. In conclusion, the results of this study evidenced the immune response during PRRSV

vaccination is associated with co-regulatory miRNA-mRNA networks in PBMCs.

Key Words: PRRSV, miRNA, PBMCs, vaccination, pig

MT171 QTLs associated with resistance to MAP infection in Holstein-Friesian cattle. S. Mallikarjunappa*[1,3], M. Sargolzaei[4], K. Meade[2], N. Karrow[3], and S. Pant[1], [1]*Graham Centre for Agricultural Innovation, Charles Sturt University, Wagga Wagga, NSW, Australia;* [2]*Teagasc Animal and Bioscience Research Department, Grange, Co. Meath, Ireland;* [3]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada;* [4]*The Semex Alliance, Guelph, ON, Canada.*

The objective of this study was to perform genome-wide association (GWA) analysis using high-density (HD) imputed genotype data in order to identify putative Quantitative Trait Loci (QTLs) associated with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection that causes Johne's disease in cattle. The study was based on the hypothesis that the use of imputed HD SNP genotypes leads to identification of additional associations especially in the regions with low coverage on 50k panel used in the previous study. Therefore, previously analysed 50k genotypes of 232 Holstein-Friesian cows, comprised of a MAP-positive (n = 90) and MAP-negative cohort (n = 142), were imputed to a 777k SNP chip panel using the software package FImpute. Subsequently, principal component regression analysis was used for GWA of imputed genotypes, which revealed 15 putative QTLs (P = 1.99E-6) associated with the MAP infection phenotype on 8 different chromosomes (BTA 5, 7, 10, 14, 15, 16, 20 and 21) of which the QTLs identified on BTA 15, 16, 20 and 21 constituted the new additional QTLs identified in this study. Post-GWAS bioinformatic analysis identified several novel candidate genes underlying these QTLs including *NLRP3*, *IFi47*, *TRIM41, TNFRSF18, TNFRSF4*. Several of these genes have pro-inflammatory properties, which could be indicative of the role of phagocytic cells in eliciting an anti-MAP response during early stages of Johne's disease pathogenesis. To our knowledge, this is the first study to carry out Johne's disease GWA analysis using high density SNP genotypes. Our analysis revealed potential QTLs that were associated with resistance to MAP infection, which will now be functionally investigated. Once validated, associated QTLs could be exploited via marker-assisted selection to breed for Johne's disease resistance in cattle.

Key Words: cattle, genome-wide association, imputation, genetic marker, genetic improvement

MT172 Investigating genetic control of resistance to avian pathogenic *Escherichia coli* colonization in chickens. M. Monson*[1], M. Kaiser[1], A. Wolc[1,2], and S. Lamont[1], [1]*Iowa State University, Ames, IA, USA;* [2]*Hy-Line International, Dallas Center, IA, USA.*

Colibacillosis in poultry is caused by extraintestinal infections with avian pathogenic *Escherichia coli* (APEC) and impacts commercial production worldwide. Initial exposure to APEC occurs primarily through the respiratory tract, after which the bacteria can spread rapidly throughout the host. Poultry infected with APEC exhibit decreased growth and egg production and increased mortality and carcass condemnation. Improving host resistance to APEC colonization would maintain better poultry health and reduce the economic losses for the industry. This study aims to identify quantitative trait loci (QTLs) in chickens (*Gallus gallus*) linked to variation in the bacterial load after APEC challenge. These QTL regions could provide a first step towards developing markers for genomic selection and could point to novel target genes for resistance. In this study, 324 birds from the F_{24} generation of an advanced intercross line (AIL) and 46 from the parental lines (a closed broiler line and an inbred Fayoumi line) were challenged at 14 days of age with 10^7 CFU of APEC O1:K1:H7 via right-side intra-airsac injection. Blood and tissue (right lung, left lung, spleen, and liver) samples were collected after 1 day of exposure. Bacterial loads [$\log_{10}$(CFU/g)] in each sample were determined by plating 10-fold serial dilutions of tissue homogenates. The bacterial load differed significantly between tissues (right lung > spleen > left lung and liver > blood). For each AIL individual, genotypes were collected on the Affymetrix Axiom 600K Genome-wide Chicken Genotyping array, providing ~200,000 usable SNPs (call rate ≥ 95%; minor allele frequency ≥ 2%). Tissue colonization levels were determined to be lowly heritable traits (h^2 < 0.3) and used as quantitative phenotypes for Bayesian genome wide association analysis (GWAS). Association of specific regions of the genome with resistance to APEC colonization could inform future efforts to reduce colibacillosis in commercial chickens. Support: USDA-NIFA-AFRI US-UK Collaborative grant, Hatch project #5424.

Key Words: poultry, genome-wide association, genotyping, infectious disease, animal health

MT173 Integrative network genomics of the bovine host response to infection with M*ycobacterium bovis*. T. J. Hall*[1], K. E. Killick[1,2], M. P. Mullen[3], K. E. McLoughlin[1], N. C. Nalpas[4], I. W. Richardson[5], D. A. Magee[1], C. N. Correia[1], J. A. Browne[1], H. M. Vordermeier[6], B. Villarreal-Ramos[6], D. P. Berry[7], E. Gormley[8], S. V. Gordon[2,8], D. E. MacHugh[1,2], [1]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland;* [2]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland;* [3]*Department of Life and Physical Sciences, Athlone Institute of Technology, Athlone, Ireland;* [4]*Quantitative Proteomics and Proteome Centre Tübingen, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany;* [5]*IdentiGEN Ltd., Blackrock Business Park, Blackrock, Dublin, Ireland;* [6]*Animal and Plant Health Agency (APHA), Weybridge, Addlestone, Surrey, UK;* [7]*Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Cork, Ireland;* [8]*UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland.*

Bovine TB (BTB), caused by infection with *Mycobacterium bovis*, is a major endemic disease affecting global cattle production, particularly in many developing countries. In the current study we used correlation- and interaction-based network approaches to analyse the host response to infection with *M. bovis* at the level of the transcriptome to identify core disease response pathways. These networks were then integrated with genome-wide association study (GWAS) datasets to enhance detection of genomic variants for susceptibility/resistance to *M. bovis* infection. The host gene expression data consisted of bovine RNA-seq data generated using peripheral blood samples from cattle infected with *M. bovis* across a 14-week infection time course. These data were then used for weighted gene correlation network analysis (WGCNA) to construct a correlation-based network to identify gene modules associated with disease response. A base gene interaction network of the mammalian host response to mycobacterial infection was also generated using 213 genes identified from a GeneCards (www.genecards.org) search for relevant keywords and the network was constructed using InnateDB (www.innateDB.com). Differential gene expression data were superimposed on this base network and the JActiveModules Cytoscape (www.cytoscape.org) plugin was used to extract functional modules. Bovine GWAS data was obtained from a published BTB susceptibility/resistance study. SNPs from genes within the top functional modules (5 kb up- and downstream of each gene) were used with single-SNP regression and Bayesian approaches to analyse GWAS data from 841 Holstein-Friesian bulls with composite multi-relative BTB susceptibility/resistance phenotypes. These analyses identified new genomic variants associated with susceptibility and resistance to BTB, demonstrating that integration of tran-

scriptomics and GWAS data is a useful method for studying the host response to mycobacterial infection.

Key Words: integrative genomics, cattle, systems biology, bioinformatics

Genome Edited Animals

MT174 The role of leptin in nonalcoholic obesity, diabetes and hepatic fibrosis. T. Tan*[1,2], Z. Song[1], Y. Xing[1,2], X. Hu[1,2], and N. Li[1,2], [1]*College of Biological Sciences, China Agricultural University, Beijing, China;* [2]*State Key Laboratory for Agro-Biotechnology, Beijing, China.*

The studies of human obesity disease and related metabolic complications mainly based on rodents models. However, there are significant differences in physiology between human and rodents. In contrast, pig shows many similarities not only in the physiological structure and function, but also in the metabolic characteristics and genetic background. In this research, we generated a pig model of obesity via ZFN-mediated knockout system. The ZFN mutation rate reached 8.3%. Off-target effects have not been detected in *Leptin*$^{-/-}$ individuals. *Leptin*$^{-/-}$ pigs showed largely increased obesity related phenotypes corresponding to human. It has significantly improved appetite and body index especially weight. MSCT examination showed a significant increase of body fat rate in mutant pigs, which reflected in the subcutaneous fat and visceral fat. The *Leptin*$^{-/-}$ pigs showed significant increase in blood glucose concentration in the age of 12 months, and developed severe insulin resistance. By IVGTT assay, the blood glucose regulatory function is disordered in *Leptin*$^{-/-}$ pigs. Moreover, by HE and IHC analysis, we found that the *Leptin*$^{-/-}$ pigs show serious liver lesions, such as fatty liver and hepatitis particularly hepatic fibrosis, which is different from the results previously reported based on mouse models. This may imply that leptin plays different roles in the development of liver fibrosis in pigs. We were surprised at finding the expression of cytochrome oxidase was up-regulated in *Leptin*$^{-/-}$ pigs. A series of markers also confirmed the enhanced oxidative stress in the *Leptin*$^{-/-}$ pigs'liver contrary to the results in ob/ob mice. We have confirmed that leptin deficiency enhanced the βoxidation and triglyceride accumulation via JAK-STAT pathway and inhibited the expression of sirt1 via AMPK pathway. So parkin-mediated mitochondrial autophagy which activated by down-regulated sirt1 and enhanced βoxidation played common role in making liver injury secondary hit significantly enhanced. It resulted in deterioration from adipose degeneration to fibrosis in liver. There are some similarities between the pig and human in hepatic fibrosis caused by obesity, so our research provided a new model and a new approach to treat hepatic fibrosis disease.

Key Words: leptin knockout, pig, obesity model, fibrosis, oxidative stress

MT175 Editing the future of the domestic pig. S. Lillico*[1], C. Proudfoot[1], C. Burkard[1], F. Urnov[2], J. Oatley[3], B. Telugu[4], A. Mileham[5], and B. Whitelaw[1], [1]*The Roslin Institute, Roslin, Midlothian, UK;* [2]*Sangamo Biosciences, Richmond, CA, USA;* [3]*Washington State University, Pullman, WA, USA;* [4]*University of Maryland, Beltsville, MD, USA;* [5]*Genus Plc, DeForest, WI, USA.*

Selective breeding and more recently genomic selection have had huge impacts on the productivity of domestic swine. However, these approaches are limited to variation that can be readily identified in the breeding population. The genome editing revolution allows facile manipulation of mammalian genomes. Using these tools we have created multiple lines of modified domestic pig, with focus on the creation of novel traits of utility to the swine industry. Our current work encompassing traits such as germ cell ablation or resistance to viral diseases will be presented.

Key Words: pigs and related species, genome editing, CRISPR-Cas9, genetic improvement

MT176 Pest-off: Could gene drive help drive out Australia's invasive pest animals? M. Tizard*[1], T. Strive[2], P. Brown[3], S. Henry[2], A. Sunarto[1], K. McColl[1], C. Cooper[1], D. Moro[4], M. Byrne[4], and T. Doran[1], [1]*CSIRO Health & Biosecurity, Australian Animal Health Laboratory, Geelong, Victoria, Australia;* [2]*CSIRO Health & Biosecurity, Black Mountain Laboratory, Canberra, ACT, Australia;* [3]*CSIRO Agriculture & Food, Black Mountain Laboratory, Canberra, ACT, Australia;* [4]*Department of Parks and Wildlife, Kensington, Western Australia, Australia.*

The CRISPR/Cas9 tool has had a dramatic impact across biomedical research and is opening new opportunities for biotechnology in animal agriculture. Its adaptation as the driving force of synthetic, RNA guided, gene drives has initiated innovative thinking about approaches to dealing with invasive pest animals. Rabbits and cane toads are probably the best known of Australia's problem animals but the list is long; for example, feral cats and common carp (*Cyprinus carpio*) are less well known but arguably of similar importance. Current control measures are often insufficient to mitigate the impacts of these and other pest animals on Australia's native wildlife, landscape and agricultural systems. New approaches and new tools are needed and genetic technologies, particularly gene drive, present a significant opportunity, worthy of investigation. There has been a great deal of international debate involving, scientist, ethicists, biosafety experts, government regulators and non-governmental organisations about a range of concerns emerging from the potential power of the technology. As a result there are new international frameworks being adopted for regulation of gene drive research from funding through to execution of research projects, with a particular focus on the biocontainment of any animals that might be created. It is critical that balanced community engagement and appropriate research be undertaken to provide the substrate for informed public, scientific and regulatory debate. It is against this background that the pests of greatest consequence to Australia are being evaluated with particular reference to the practicality and desirability of the solutions that might be proposed. Social licence to operate is perhaps the most critical issue in the development and future of this technology. Dialogue, regarding the safety and efficacy of any proposed gene drive solution to a pest problem, with the public, with policy makers and with regulators will be as important as the development of the technology itself.

Key Words: CRISPR/Cas9

MT177 Developing and exploiting new technologies to advance understanding of the avian immune system. A. Balic*, H. Sang, and M. McGrew, *The Roslin Insitute, University of Edinburgh, Edinburgh, Scotland, UK.*

Global poultry production is increasing rapidly, especially in developing countries where poultry are a major source of animal protein. While we have a good understanding of how the mammalian immune system develops, this is not the case for birds. Indeed

while birds face similar pathogen challenges to mammals, they have a different repertoire of organs, cells, molecules and genes of the immune system. To address this deficit in our understanding we have generated several transgenic cell reporter lines of chickens which allow the avian immune system to be visualised and manipulated, including transgenic chickens in which either all haematopoietic cells (RUNX1-eGFP line) or macrophages (CSF1R-mApple) can be visualised in embryos and post-hatch birds. In addition, we have produced several additional lines of chickens which utilise the *Cre-loxP* system to allow single- or multicolour cell lineage tracing. Using these novel bird lines lines have identified different populations of antigen-presenting cells (APC), such as macrophages and classical dendritic cells and are currently using these lines of birds to further elucidate the development and function of the avian immune system. More recently we have developed gene editing technologies for modifying chicken primordial germ cells (PGCs), as a route to production of genome-edited birds, utilising either transcription activator-linked nucleases or the CRISPR-Cas9 system. In our hands CRISPR/Cas9 gene editing technology has proven to be a relatively simple, highly efficient and precise method for genetic modification of chickens which opens up many potential applications and benefits for avian researchers.We have developed an efficient method for the long-term culture and cryopreservation of PGCs, demonstrated that these PGCs can used to efficiently produce site-directed genome knockout chickens and crucially, has used these technologies to produce a gene knockout chicken in which homozygous mutant chickens are sterile due to the absence of PGCs. We will discuss further applications of these genetic engineering and genome editing approaches to further the reduction of production losses and enhance the selective breeding of more robust, healthy chickens

Key Words: chicken, transgenic, immune, CRISPR-Cas9, PGC

Horse Genetics and Genomics

MT178 A genome-wide association study for body weight in Japanese thoroughbred racehorses identified candidate regions near *LCORL*, *ZFAT*, and *MSTN* genes. T. Tozaki*, M. Kikuchi, H. Kakoi, K.-I. Hirota, and S.-I. Nagata, *Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan.*

Bodyweight is one of the most important traits in confirming growth and the management of physical condition in humans and animals. In thoroughbred racehorses, bodyweight is measured at various stages, such as postnatal development, training, and racing periods, and can be used for confirming growth and the management of physical condition. The bodyweight of mature thoroughbred racehorses generally ranges from 400 to 600 kg. This broad range suggests that both environmental and genetic factors are involved in determining this variable. Therefore, a genome-wide association study (GWAS) using Equine SNP70 BeadChip (65,156 SNPs) was designed to identify genomic regions associated with bodyweight in Japanese thoroughbred racehorses. In this study, 851 horses (3-years-old) registered as racehorses in Japan were used for GWAS. The average bodyweight of the horses was 473.9 kg (standard deviation: 28.0) at the age of 3. GWAS led to the identification of statistically significant SNPs for ECA3 (*BIEC2_808466*, $P = 2.32E{-}14$), ECA9 (*BIEC2_1105503*, $P = 1.03E{-}7$), and ECA18 (*BIEC2_417274*, $P = 1.44E{-}14$) associated with bodyweight. The candidate genomic regions of ECA3, ECA9, and ECA18 included the *LCORL*, *ZFAT*, and *MSTN* genes, respectively. The *LCORL* and *ZFAT* genes have been reported to be associated with withers height in several breeds, and the *MSTN* gene affects muscle mass in thoroughbreds. Therefore, it is considered that the genes predicted in this study may affect the bodyweight of thoroughbred racehorses through physiological mechanisms. Although the findings in this study would be useful for breeding plans and the growth management of thoroughbred racehorses, the information could also be used for the production of genetically modified animals and for gene doping (abuse/misuse of gene therapy), which should be prohibited to maintain the integrity of horseracing

Key Words: equine, horse, GWAS, body weight, gene doping

MT179 Investigation of the association between the *DMRT3* 'Gait keeper' mutation and early career performance in Coldblooded trotters. K. J. Fegraeus*[1], C. Olsson[2], L. Andersson[3,4], C. F. Ihler[5], E. Strand[5], G. Lindgren[1], and B. D. Velie[1], [1]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [2]*The Swedish Trotting Association, Bromma, Sweden;* [3]*Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden;* [4]*Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA;* [5]*Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway.*

The Swedish-Norwegian Coldblooded trotter (CBT) is a local breed in Sweden and Norway mainly used for harness racing. Previous studies have shown that a mutation in the *doublesex and mab-3 related transcription factor 3* (*DMRT3*) gene has a major impact on the harness racing performance of Standardbreds and Finnhorses. An association of the *DMRT3* mutation with early career performance in CBTs has also been suggested. The aim of the current study was to investigate this proposed association in a larger group of CBTs. A random sample of 769 CBTs (485 raced, 284 unraced) was genotyped for the *DMRT3* mutation. The association with racing performance was investigated for 13 performance traits at three different age intervals: 3 years, 3 to 6 years, and 7 to 10 years of age, using the R software. Each performance trait was analysed for association with *DMRT3* using linear models. The results suggested no association of the *DMRT3* mutation with precocity (i.e. performance at 3 years of age). Only two traits (race time and number of disqualifications) were significantly different between the genotypes at 3 years of age, with AA horses having faster times than CC horses, and CC horses having more disqualifications than CA horses. For the age interval 3 to 6 years of age there were no significant differences between the AA and CA genotype. However, the CA horses performed significantly better than the CC horses for the majority of traits, with AA horses also tending to outperform CC horses. For the older age interval, 7 to 10 years of age, the AA horses had the lowest number of starts, placings, earnings and the slowest race times, of the three genotypes. Additionally, the frequency of the AA genotype was significantly lower among raced horses compared with unraced horses - less than 50% of AA horses had participated in a race. Although demonstrated to be the most favourable genotype for racing performance in Standardbreds and Finnhorses across all ages, the current study suggest no association between the AA genotype and superior performance, early or late, in CBTs. Overall, the CA genotype appears to be the most advantageous for CBTs at all ages.

Key Words: harness racing, d*oublesex and mab-3 related transcription factor 3*, gene

MT180 Whole-genome sequencing of the Jeju, Jeju cross-bred, and Thoroughbred horse breeds. H. Seong[1], N.-Y. Kim[2], D. C. Kim[3], N.-H. Hwang[1], I.-C. Yang[1], A. Jang[1], J.-D. Kim[1], J.-W. Choi*[1], W.-H. Chung[4], and J.-B. Kim[1], [1]*Kangwon National University, Chuncheon, Republic of Korea;* [2]*Subtropical Animal Research Institute, National Institute of Animal Science, RDA,*

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The Jeju horse is a Korean indigenous horse breed registered with DAD-IS of the FAO. Nonetheless, there is severe lack of genomic studies available for the Jeju horse. Here we sequenced six stallion genomes from two Jeju horse, two Thoroughbred, and two Jeju crossbred (Jeju × Thoroughbred) – using the Illumina HiSEqn 2500 platform. Using the EquCab 2.0 assembly, we achieved averages of 48.7-, 56.2-, and 56.4-fold coverage of the Jeju, Jeju crossbred, and Thoroughbred horse breeds, respectively, and detected a total of 14.1 million SNPs, of which 73.8% were found to be novel. Of the total SNPs, 19.0% (Jeju), 12.6% (Jeju crossbred), and 7.8% (Thoroughbred) were located specifically in each of the three breeds. The homozygous-to-heterozygous SNP ratios of the breeds were 1:2.06 for Jeju, 1:2.76 for Jeju crossbred, and 1:2.28 for Thoroughbred, well exhibiting effects of the crossbreeding shown by the increased ratio of heterozygous SNPs on the Jeju crossbred. To assess SNP quality, transition-to-transversion (TS/TV) ratios were calculated to generate 1.85, 1.84, and 1.80 (excluding unplaced scaffolds), showing that most SNPs derived from this study could be regarded as correctly called SNPs. Annotations of the variants showed that most of the SNPs were located in intron (29.5%) and intergenic regions (68.4%), while we retrieved a total of 87,310 nonsynonymous SNPs that could influence phenotypic variations in traits of interest in the horse breeds. In addition, we detected several potential selective sweeps that may be implicated with selection in the horse breeds, using SNPs derived from this study.

Key Words: Jeju horse, Thoroughbred, genome sequencing, SNPs, selective sweeps

MT181 Changes in the gene expression profile of equine arthritic joint tissues induced by the administration of allogeneic mesenchymal stem cells. L. Barrachina*[1,2], A. R. Remacha[1], A. Romero[1,2], F. J. Vázquez[1,2], A. Vitoria[1,2], P. Zaragoza[1], and C. Rodellar[1], [1]*Laboratorio de Genética Bioquímica LAGENBIO (Universidad de Zaragoza), Instituto Agroalimentario de Aragón (Universidad de Zaragoza-CITA); Instituto de Investigación Sanitaria de Aragón (IIS), Zaragoza, Spain;* [2]*Servicio de Cirugía y Medicina Equina, Hospital Veterinario, Universidad de Zaragoza, Zaragoza, Spain.*

Osteoarthritis (OA) is a highly prevalent pathology in both human and horses with huge impact on health and economy. The lack of effective treatments has led to the study of mesenchymal stem cells (MSCs) application with promising results. The aim of this study was to assess the effect of repeat administration of allogeneic MSCs on the gene expression of cartilage and synovial membrane (SM) in an equine arthritis model. Arthritis was induced by intraarticular (IA) injection of Amphotericin-B in one radio-carpal joint of 11 horses divided into 2 groups: control (n = 4) and MSC-treated (n = 7). Two IA administrations of allogeneic MSCs or substance vehicle (control) were performed on weeks 2 and 5 after lesion in corresponding animals. Four months later, the contralateral joint of each animal was subjected to the same procedure. Six months since the beginning of the study, animals were killed and cartilage and SM samples were collected to study the short-term (second lesion, 2 months progression) and long-term (first lesion, 6 months progression) effects of MSC treatment on the gene expression of joint tissues by RT-qPCR. At 2 months, differences between MSC-group and control were not found in cartilage but MMP-13 was down-regulated in SM of MSC-group. At 6 months, the MSC-group showed up-regulation of COL1A1 and down-regulation of TNFα in cartilage and up-regulation of TIMP-2 in SM, compared to control group. When comparing the gene expression between 2 and 6 months within each group, differences were not found in control group. In MSC-group, down-regulation of MMP-13 and COX-2 in cartilage and of MMP-3, MMP-13 and TNFα in SM was found at 2 months. These results show that MSCs induced a change in the gene expression profile of joint tissues at both studied points, suggesting a more obvious effect at short-term. The up-regulation of genes coding for components of the cartilage matrix and the down-regulation of genes coding for inflammatory and catabolic mediators suggest a potential therapeutic benefit of allogneic MSCs in equine joint pathology.

Key Words: horses and related species, cell biology, cell culture, athletic performance, animal health

MT182 Using IgE levels as phenotypes to identify genomic regions associated with insect bite hypersensitivity. C. Lamberigts*[1], L. François[2], B. Velie[3], A. Stinckens[2], H. Hoskens[4], S. Blott[5], S. Tinel[2], L. Peeters[6], H. Savelkoul[7], E. Tijhaar[7], G. Lindgren[3], S. Janssens[2], B. Ducro[8], N. Buys[2], A. Schurink[8], [1]*Research Group Livestock Physiology, Department of Biosystems, KU Leuven, Leuven, Belgium;* [2]*Research Group Livestock Genetics, Department of Biosystems, KU Leuven, Leuven, Belgium;* [3]*Department of Animal Breeding & Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [4]*Laboratory for Genetic Epidemiology, Department of Human Genetics, KU Leuven, Leuven, Belgium;* [5]*School of Veterinary Medicine & Science, University of Nottingham, Leicestershire, UK;* [6]*Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium;* [7]*Cell Biology and Immunology Group, Wageningen University & Research, Wageningen, the Netherlands;* [8]*Animal Breeding and Genomics Centre, Wageningen University & Research, Wageningen, the Netherlands.*

Insect bite hypersensivity (IBH) is one of the most common skin diseases in horses, with symptoms like hair loss, excoriation, crusting, scaling and skin thickening. IBH is defined as a cutaneous allergic reaction against saliva allergens of *Culicoides spp* and is currently diagnosed by scoring clinical symptoms. Several case/control genome-wide association studies reported interesting regions that could underlie this allergic reaction but no causal variant has been identified as of yet. As the use of a scoring system likely increases the risk of misdiagnosis, using IgE levels against antigens of *Culicoides spp* as an objective and qualitative phenotype could improve the power to detect genetic defects substantially. Within this study 200 Shetland ponies (103 cases, 97 controls) and 146 Icelandic horses (73 cases, 73 controls) were genotyped using the 50k SNP chip while 223 Belgian Warmblood horses (116 cases, 107 controls) were genotyped using the 670k array. The examined phenotype consisted of the results of a diagnostic ELISA employing recombinant *C. nubeculosus* (Belgian Warmblood horse) or *C. obsoletus* (all populations) antibodies. Several SNPs passed the nominal significance threshold across breeds, though none surpassed Bonferroni correction. The results of this study confirm previously identified regions (SNPs located 3 Mb distant of the major histocompatibility region (MHC) region (ECA20) in Shetland ponies) in addition to providing novel regions of interest, notably in the Belgian Warmblood horse population. The lack of common regions across breeds could be ascribed to differences in SNP density, differences in the recombinant allergens used between populations, and differences in linkage disequilibrium (LD) structure within the breeds. As many novel regions of interest were found by using the higher density array on the Belgian Warmblood horse, our results suggest that high density SNP arrays are essential when using allergen-specific IgE levels as a phenotype. Although these results should be validated in a large independent dataset, the analysis shows the value of using an objective and continuous phenotype.

Key Words: horses and related species, genome-wide association, complex trait, allergy

MT183 The curly hair trait in the American Bashkir Curly Horse. A. Thomer*[1], K. Jung[1], M. Hewicker-Trautwein[2], O. Distl[1], and J. Metzger[1], [1]*Institute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover Foundation, Hannover, Niedersachsen, Germany;* [2]*Department of Pathology, University of Veterinary Medicine Hannover Foundation, Hannover, Niedersachsen, Germany.*

In this study, we investigated the molecular genetic determination of the curly hair trait in the American Bashkir Curly Horse (ABCH) representing a unique horse breed predominantly characterised by its curly hair. We collected EDTA blood and hair samples from 220 horses including 143 curly coated ABCHs, 38 straight-coated ABCHs and 39 straight coated Quarter Horses (QHs) as controls. By the means of scanning electron and light microscopy we identified morphological and morphometrical features characteristic for macroscopically curly hair fibres such as a rougher surface and a restricted medulla. Based on this morphological classification, a genome-wide association analysis (GWAS) was performed in 25 curly coated versus 23 straight coated individuals using genotypes from the Axiom Equine Genotyping Array 670K. GWAS resulted in a genome-wide significant peak region of 1 Mb ($-\log_{10} P_{max} = 7.958$), which was validated in a case-control analysis with 187 horses. Whole-genome sequencing (WGS) of three curly coated ABCHs and imputation of the genotyping data on WGS data followed by subsequent chromosome-wide association analysis confirmed the peak region for curly hair ($-\log_{10} P_{max} = 18.414$). A whole-genome RNA-seq for tail hair root samples in nine curly coated and six straight-coated horses revealed 125 significantly up-regulated genes in the targeted region. In total, 21 significantly differentially expressed genes in the candidate region were further validated in 13 curly coated and 25 straight-coated horses on RT2 Profiler PCR Array. Delta-delta Ct expression levels were analysed for their effects on hair type and degree of curliness using a generalized linear model (GLM). This analysis showed two candidate genes with strong differences in expression levels. In conclusion, we mapped a candidate region for the curly hair trait in ABCHs. RNA-seq and expression analysis broke down the number of candidates to two genes. Their role in the development of curly hair will be further investigated.

Key Words: horses and related species, genome-wide association, RNA-seq, candidate gene, breed/population identification

MT184 Expression patterns of equine skin tissue neoplasia combined with motility and invasiveness of corresponding primary skin fibroblasts. W. Witarski*, P. Podstawski, K. Ropka-Molik, K. Kowalska, T. Szmatola, A. Gurgul, and M. Bugno-Poniewierska, *National Research Institute of Animal Production, Krakow, Poland.*

Bovine Papillomaviruses are bovine pathogens that can affect horses, causing locally invasive, fibroblastic skin sarcoids. In sporadic cases, these sarcoids can turn into invasive form and induce metastasis. Neoplastic tissues were obtained from three horses. The control group consisted of three healthy individuals. RNA purification and quality control, preparation of cDNA libraries, evaluation of the quality of cDNA libraries were performed before sequencing (RNAseq on HiScanSQ), that was performed with 75 single-end cycles (Illumina). The raw data was analysed by using dedicated software. Results obtained from sequencing were validated with the selected genes expression analysis performed by Real-Time PCR method. Moreover, a presence of BPV1 or BPV2 genome in experimental samples were confirmed by Real-time PCR. Using additional filtering criteria (p-adjusted < 0.05; |fold change | >2), we detected 579 differentially expressed genes: 153 up-regulated and 426 down-regulated in neoplastic samples. Overrepresentation analysis test with Bonferroni correction showed several enriched processes including regulation of cell communication, regulation of signalling, regulation of signal transduction, with most notable changes in PI3-AKT and regulation of actin cytoskeleton pathways. It is worth mentioning that it is for the first time noted, that RIF-1 protein might be involved in viral-induced neoplastic changes in horses. Primary cell cultures were isolated from surgically removed skin fragments with the use of cell migration method. Each experiment was performed on a mix of three neoplastic cell lines (BPV-positive) and three control fibroblasts derived from BPV-free tissues (both tested with RT–PCR techniques for viral genome fragments L2E5). Motility tests, analysed with two different motility tests, showed statistically higher ($P < 0,05$) motility of control cell line over neoplastic. In contrary, neoplastic cell lines showed statistically significant higher invasiveness potential ($P < 0.05$) in matrigel-coated Transwell Plate migration assay. Funded by IZPIB:07-4.13.7; NCBiR: BIOSTRATEG2/297267/14/NCBR/2016.

Key Words: horse, sarcoid, RNAseq, motility, invasiveness

MT185 Circulating miRNAs in serum as noninvasive biomarkers for equine asthma. M. Kraft*[1,2], A. Pacholewska[1,2], V. Gerber[1], and V. Jagannathan[2], [1]*Swiss Institute of Equine Medicine, University of Bern and Agroscope, Bern, Switzerland;* [2]*Institute of Genetics, University of Bern, Bern, Switzerland.*

MicroRNAs (miRNAs) are small (~18–22 nt) single-stranded non-coding RNA molecules that regulate gene expression at the post-transcriptional level. Deregulated expression of miRNAs has been implicated in pathological mechanisms of numerous diseases across many diverse species. Growing evidence indicates that circulating miRNAs can be used as reliable disease biomarkers for either diagnostic or prognostic purposes. In the course of this study we investigated sRNA-sequencing data derived from sera of 35 healthy horses and 37 horses affected by severe equine asthma that is also called recurrent airway obstruction (RAO). RAO is an incurable, allergy-like obstructive pulmonary disease that shows many similarities to human asthma and affects ~10–20% of the adult horse population in temperate climates. By applying our bioinformatic pipeline to sRNA-seq data that uses the mirdeep2 tool and the DESEqn 2 library, we were able to systematically perform miRNA profiling and identify differentially expressed miRNAs between cases and controls. Four known miRNAs were identified as differentially expressed: eca-miR-128 (p-value = 0.004, FDR = 10%), eca-miR-107a (p-value = 0.088, FDR = 10%), eca-miR-103 (p-value = 0.088, FDR = 10%), eca-miR-744 (p-value = 0.081, FDR = 10%). Potential target genes of (down-regulated) eca-miR-128 include RAB20, BAZ2B, and HLX. Previous reports utilising RNA-seq data have revealed these genes to be up-regulated in RAO horses relative to controls in peripheral blood mononuclear cells. Up-regulation of the transcription factor HLX is known to induce a high expression of INFγ which was previously associated with severe asthma in humans. Additionally, mir-103 and mir-107 belonging to the mir-15/107 superfamily have been shown to control several processes including inflammation, stress response, cell-cycle regulation, and metabolism. We will present our results of the investigation on these four miRNAs as potential biomarkers for RAO pathophysiology.

Key Words: miRNAs, biomarker, sRNA-seq, horse, asthma

MT186 Assembly and analysis of subchondral bone and articular cartilage transcriptomes in neonatal and adult horses. A. Kemper*[1], M. McCue[2], and A. McCoy[1], [1]*University of Illinois, Champaign, IL, USA;* [2]*University of Minnesota, St Paul, MN, USA.*

Developmental orthopedic disease (DOD) encompasses several conditions related to abnormal growth and development of skeletal structures in young horses. Although there is a known heritable component to DOD, specific genes underlying risk in the horse are completely unknown. In part, the difficulty in identifying candidate risk genes may be due to lack of knowledge regarding tissue-specific gene expression for key tissues including articular cartilage and

with smaller size. While genetic characteristics of the northern horses have been intensively investigated, those of southern mountainy pony remain unclear. Since native horses of Myanmar and Laos are predicted to have typical features of the southern mountainy pony, we investigated genetic characteristics of the Myanmar and Laos native horse. We collected blood samples of 160 and 71 native horses from northern Myanmar and northern Laos, respectively, and genomic DNA was extracted from these blood samples. To determine haplotypes of mtDNA, D-loop region of the mtDNA were sequenced. To determine haplotype of Y chromosome, Y-chromosomal markers were genotyped by PCR-RFLP or sequencing. By sequencing the D-loop region, we found 38 and 14 haplotypes of the mtDNA in the Myanmar and Lao native horses, respectively. The mtDNA haplotypes of modern horses have been classified into 16 haplogroups. The haplotypes of Myanmar native horses were classified into 9 groups and those of Lao native horses were classified into 10 groups, indicating high level of genetic diversity of the maternal lineages of these horse population. Moreover, the haplogroup constitutions of these populations were different from those of European and East Asian populations, indication unique haprogroup constitutions of Myanmar and Lao native horses. The Haplotype diversity of horse Y chromosome has been reported to be very low. In the Lao native horse, we found only one haplotype, which is the ancestral type of domestic horse. However, 3 haplotypes including those specific to European breeds was observed in Myanmar native horse. In particular the haplotypes specific to European breeds was frequently observed in the horse raised in plane area but they were rarely observed those in mountain area. These findings suggest that the Myanmar and Lao native horses have a unique genetic characteristic and Lao native horses, in particular, might retain those of the ancestral Asian native horse.

Key Words: horses and related species, DNA sequencing, conservation genomic, animal domestication

MT190 Genetic characteristics of Kazakhstan native horses. H. Ezoe*[1], Y. Okuda[1], M. Nishibori[2], H. Mannen[3], Y. Takahashi[4], K. Nomura[4], T. Yamagata[5], Y. Yamamoto[2], K. Tsunoda[6], M. Bakhtin[7], P. Kazymbet[7], M. Alykhan[8], M. Suleimenov[8], O. Safronova[9], T. Kunieda[1], [1]*Okayama University, Okayama, Okayama, Japan;* [2]*Hiroshima University, Higashihiroshima, Hiroshima, Japan;* [3]*Kobe University, Kobe, Hyogo, Japan;* [4]*Tokyo University of Agriculture, Setagaya, Tokyo, Japan;* [5]*Nagoya University, Nagoya, Aichi, Japan;* [6]*Showa University, Shinagawa, Tokyo, Japan;* [7]*Astana Medical University, Astana, Akmola, Kazakhstan;* [8]*Institute of Zoology, Almaty, Almaty, Kazakhstan;* [9]*LLP Kazak tulpar, Kostanay, Kostanay, Kazakhstan.*

Horses were domesticated in Central Asia ~5500 years ago and spread throughout the Eurasian Continent, thereafter. However, the origin and migration pathway of horse domestication remain unclear. In order to clarify them, it is important to identify the populations that are expected to retain the genetic characteristics of ancestral population and to investigate the genetic characteristics of these populations. Kazakhstan is one of the Central Asian countries, but genetic characteristics of the Kazakhstan native horse remain unclear. In the present study, therefore, we investigated the genetic characteristics of Kazakhstan native horses, to obtain the information regarding to the origin and migration pathway of horse domestication. We collected 106 DNA samples of native horses in 6 regions of Kazakhstan, and examined haplotypes of mtDNA by direct sequencing of the D-loop region, and haplotypes of Y chromosome by genotyping the Y-chromosomal markers by PCR-RFLP or direct sequencing. We also genotyped functional genes *DMRT3*, *MSTN*, *LCORL*, which are related to body composition and physical performance of horse, by PCR-RFLP. As results of mtDNA sequencing, we found 25 haplotypes in Kazakhstan native horses indicating relatively high level of genetic diversity. The composition of these haplotypes was similar to those of the domestic horse populations in the neighboring area. By genotyping of Y-chromosomal markers, we found that haplotype widely distributed in European breeds was frequently observed in Kazakhstan native horses. In addition, the haplotype specific to English Thoroughbreds was also observed. The genotyping results of *MSTN* and *LCORL* which are involved in muscular development and withers height, respectively, indicate a significant difference in the allele frequencies of these genes among the local populations of Kazakhstan native horses. In conclusion, Kazakhstan native horse populations have relatively high level of genetic diversity in maternal lineages, suggesting a possibility of retaining genetic characteristics of the ancestral population. On the other hand, our findings also suggested that the Kazakhstan native horse was genetically influenced by European breeds in paternal lineage.

Key Words: horses and related species, conservation genomics, DNA sequencing, genotyping, animal domestication

MT191 The most over-represented molecular pathways regulated during training in Arabian horse muscle tissue. K. Ropka-Molik*[1], M. Stefaniuk-Szmukier[2], K. Zukowski[1], and K. Piórkowska[1], [1]*National Research Institute of Animal Production, Balice, Poland;* [2]*University of Agriculture in Cracow, Cracow, Poland.*

Arabians breed has versatile usage including endurance riding, flat racing, and different equestrian activities. The aim of the present study was to identify the global gene expression modification occurring in skeletal muscle following training regime preparing for flat racing. Such genetic variation can be potentially related with responsiveness to training and can determine exercise-related phenotypes. The transcriptome sequencing was performed using RNA-seq approach in total on 23 muscle samples (*gluteus medius)* collected from purebred Arabian horses at three time points: untrained horses (T_0; n = 5; 2.5-year-old horses); horses after intense gallop phase (before flat racing season) (T_1; n = 10; 3-year-old horses), and at the end of the racing season (T_2; n = 8; 3-year-old horses). The RNA-seq was performed in 50 single-end cycles on HiScanSQ (Illumina). The differentially expressed genes (DEGs) were determined using DesEqn 2 software and gene expression profile was compared between untrained horses and two training periods. Our results allowed to identified 1168 differentially expressed genes between untrained horses and horses in gallop phase (T_0*v.* T_1) and 763 DEGs between untrained horses and horses at the last stage of training schedule (T_0*v.* T_2). The 60 genes were up-regulated and 84 were down-regulated in both training periods (T_1 and T_2) compared to untrained horses (T_0). In order to pinpoint the most important genes regulated during training schedule the pathways analysis based on KEGG database was performed. The obtained results showed that the most over–represented up-regulated pathways were: PPAR signalling pathway, Dilated cardiomyopathy, and Cardiac muscle contraction. Significant down-regulated pathways were: Insulin and Focal adhesion pathways. Our research allowed to identify the set of DEGs whose expression was modified following exercise in horse muscle tissue. We identified several growth factors (*IGF1R; TGFBR2; EGF; HGF; FIGF*), signalling proteins and cytoskeletal proteins (*ATP2A2; SLC16A1; MYL2; MYH7; MYL3; PPARα; TNNC1; TNNI3; PLN*), which probably play a key role in significant metabolic processes critical for adaptive response during training. Supported by project 2014/15/D/NZ9/05256.

Key Words: Arabian horse, muscle tissue, training, transcriptome

MT192 Genomic and pedigree analysis of population structure of Old Kladruber horse. L. Vostry[1,2], H. Vostra-Vydrova[2], J. Bauer[3], A. Kranjcevicová*[1,2], and J. Pribyl[2], [1]*Czech University of Life Sciences Prague, Prague, Czech Republic;* [2]*Institut of Animal*

subchondral bone (SCB). We hypothesise that tissue-specific gene expression will help elucidate important skeletal development pathways likely disrupted in DOD. Therefore, the aims of this project are to 1) assemble complete transcriptomes of adult and neonatal articular cartilage and subchondral bone; 2) perform differential gene expression analysis between age groups within each tissue; and 3) perform network analysis of differentially expressed genes to identify key cellular pathways that may be disrupted in DOD. Articular cartilage and SCB was obtained from the fetlock, hock, and stifle of neonatal ($n = 4$; ≤ 4 weeks old) and adult ($n = 4$; 9–12 years old) horses. RNA was sequenced using an Illumina HiSeq, yielding ~22–38 million 100bp paired-end reads per sample. Approximately 74% of reads mapped to the reference EqCab2 assembly. After quality control and read quantification, differential expression analysis was performed using 4 software platforms (DEsEqn 2, edgeR, limma, Sleuth). 1831 genes in SCB and 2026 genes in cartilage were significantly differentially expressed between age groups across all analyses ($q < 0.05$). Isoform-specific differential transcript expression between neonates and adults was also detected. Subsequently, transcriptomes were assembled *de novo* using Trinity; ~94% of reads mapped back to this assembly. Annotation was performed using TransDecoder and Trinotate. *De novo* assembly with Oases and SOAPdenovo-Trans is ongoing and these will be compared with a variety of metrics to identify the best assembly. Differential expression analysis will then be repeated. This pilot work demonstrates proof-of-concept; additional sample collection and analysis is ongoing.

Key Words: horses and related species, HTS, skeletal system

MT187 Design and use of the MNEc670k SNP array for precision SNP imputation to millions of markers in 15 horse breeds. R. Schaefer*[1], S. Beeson[1], J. Mickelson[2], and M. McCue[1], [1]*Department of Veterinary Population Medicine, University of Minnesota, St Paul, MN, USA;* [2]*Department of Veterinary and Biomedical Science, University of Minnesota, St Paul, MN, USA.*

Single nucleotide polymorphism (SNP) genotyping arrays containing 54K-74 thousand (K) markers for the horse have enabled genome wide association studies examining disease and performance traits, as well as quantitation of variation within and between populations. We recently designed the MNEc670k array for denser genotyping capability, as well as genotype imputation, or the statistical inference of sample haplotypes from a smaller set of markers. As part of this design a cohort of 485 horses having 2 million (M) SNP genotype data ($n = 332$) or whole genome sequence ($n = 153$) was used to select 'tagging SNPs' that were informative for differentiating haplotypes both across and within 15 *breed tagging groups*. Across all breed tagging groups, 355,903 SNPs were needed to reconstruct haplotypes with minor allele frequency (MAF) >0.01 with an r2 >0.99. In each of the 15 breed tagging groups, between 144,175 and 387,279 SNPs were required for haplotype reconstruction. All SNPs that were informative across breed groups, as well as SNPs that were informative in five or more breed tagging groups, were included on the MNEc670k SNP array. The performance of the MNEc670k array for SNP imputation was assessed in several scenarios. Genotypes of the 485 horses with either 2M or WGS data were masked down to the MNEc670k density, as well as legacy array 54/75K SNP density, in a random 1/3 subset of individuals in each of the 15 breed tagging groups. After removing the imputation targets, the remaining horses were used as a reference population. Imputation concordance from 54/65K SNPs to 2M SNPs in breed tagging groups ranged from 82–96% depending on breed group, while concordance from 670k to 2M SNPs ranged between 97–99%. Imputation from 670K to 14M SNPs (WGS) was assessed in a cohort of 38 Standardbred and 20 Thoroughbred horses yielding a concordance of 96 and 97% respectively. Additionally, we report the gains in accuracy of imputation using breed-specific haplotype and recombination maps, which improve SNP accuracy in scenarios where breed specific parameters can be reliably estimated.

Key Words: horses, genome-wide association, single nucleotide polymorphism (SNP), imputation, genetic marker

MT188 Genome-wide analyses of the Jeju, Thoroughbred, and Jeju crossbred horse populations using the high-density SNP array. N. Y. Kim*[1], H. Seong[2], D. C. Kim[4], I. C. Cho[1], P. N. Seong[1], J. K. Son[1], S. M. Shin[1], S. H. Park[1], J. H. Woo[1], N. G. Park[1], W.-H. Chung[3], and J.-W. Choi[2], [1]*Subtropical Animal Research Institute, National Institute of Animal Science, RDA, Jeju, Jeju, Republic of Korea;* [2]*College of Animal Life Science, Kangwon National University, Chuncheon, Kangwon, Republic of Korea;* [3]*Division of Nutrition and Metabolism Research, Research Group of Gut Microbiome, Korea Food Research Institute, Sungnam, Gyeonggi, Republic of Korea;* [4]*Jeju Special Self-Governing Province Livestock Promotion Agency, Jeju, Jeju, Republic of Korea.*

The Jeju horse is a Korean indigenous horse breed that is currently registered with the FAO. Nonetheless, there is severe lack of genomic studies available for Jeju horses. In this study, we compared in genome-wide three horse populations including Jeju ($n = 341$), Thoroughbred ($n = 139$), and Jeju crossbred (Jeju × Thoroughbred) ($n = 297$) using the Equine SNP 65K genotyping panel. We performed extensive quality control (QC) on the initial datasets, resulting in a total of 754 horses and 44,721 markers remained for further analysis. To assess population differentiation between the three populations, values were calculated between those populations across the genomes, showing that the highest level of genetic differentiation was observed between the Jeju horse and Thoroughbred (= 0.123). The lowest level was found between the Jeju crossbred and Thoroughbred (= 0.034), while the Jeju and Jeju crossbred populations had a value of 0.062. The genetic relationship between the three populations was further assured by the Principle Component Analysis (PCA), showing that the clear genetic difference between the Jeju horse and Thoroughbred. The Jeju crossbred looks more genetically close to the Thoroughbred than the Jeju, corresponding the results derived from this study. We also estimated linkage disequilibrium (as r^2) for the all possible SNP pairs lower than 4Mb apart. The r^2 values dropped most drastically in the Jeju horse shown as low value of the r^2 (>0.2) within 40 to 45kb. Initial LD was the highest in the Thoroughbred, where r^2 values dropped below 0.2 within 250 to 300kb. The long-range LD was highest in the Thoroughbred compared to other populations. Furthermore, we found several interesting loci that might be influenced by selection, such as *NCAPG* gene that has a SNP with one of the highest values. This suggests that *LCORL*/*NCAPG* genes is a potential indicative of signature of selection in body size of the Jeju horse.

Key Words: Jeju horse, Thoroughbred, Jeju crossbred, HD SNP chip array

MT189 Genetic characterization of native horses in Myanmar and Laos using haplotypes of mitochondrial DNA and Y-chromosomal markers. Y. Okuda*[1], H. Moe[2], K. Moe[2], B. Kounnavongsa[3], S. Keonouchanh[3], B. Bouahom[3], T. Shimogiri[4], H. Mannen[5], M. Kanemaki[6], Y. Yamamoto[7], and T. Kunieda[1], [1]*Okayama Univercity, Okayama, Okayama, Japan;* [2]*University of Veterinary Science, Yezin, Nay Pyi Taw, Myanmar;* [3]*National Agriculture and Forestry Research Institute, Xaythany, Vientiane, Lao PDR;* [4]*Kagoshima University, Kagoshima, Kagoshima, Japan;* [5]*Kobe University, Kobe, Hyogo, Japan;* [6]*Institute of Animal Science, Nagoya, Aichi, Japan;* [7]*Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan.*

The Asian native horses are classified into two types, namely northern horses with a middle size and southern mountainy ponies

formation. Lower expression of this gene in four sarcoid tumour samples with respect to the samples of healthy skin, was identified using the Horse GE Two-colour gene expression microarray (4x44, Agilent) and then confirmed by real-time qPCR. The aim of this study was to investigate whether the previously identified changes in the expression level of *S100A14* gene may be associated with the difference in DNA methylation level of its potential regulatory sequences in sarcoids - a locally invasive skin tumour of equids. To quantify DNA methylation levels of the *S100A14* gene region in 10 sarcoids and 10 tumour-distant skin samples, BSPPCR method (bisulfite sequencing of PCR products) was used. The analysis of Sanger sequencing results with the use of Mquant method revealed that the group of sarcoids exhibited higher average level of methylation (78.5%) compared to the control tissues (42.3%). An additional quantitative analysis based on clones' sequencing of the BSPCR products revealed the same trend of methylation changes in the analysed tumour and healthy tissue samples. Furthermore, a similar DNA methylation percentage was also observed compared to the mean methylation percentage of CpG sites determined with Mquant method (the differences ranged from 1.2% to 37.9%). The obtained results may indicate some connection between changes in the expression level of *S100A14* gene with the identified differences in the level of DNA methylation between the healthy and tumour tissues. Knowledge on the changes taking place in the process of DNA methylation may provide a basis for the development of new alternative diagnostic or therapeutic approaches to equine sarcoids.

Key Words: horses and related species, epigenomics, DNA sequencing, animal health

MT196 Genetic diversity of Malopolski horse stallions. A. Fornal*, A. Radko, and A. Piestrzynska-Kajtoch, *National Research Institute of Animal Production, Krakow, Poland.*

Malopolski horse is a Polish warmblood breed strongly influenced by Thoroughbred horse. It is a one of the seven breeds in genetic resources conservation programme and because of that genetic variability of this native breed should be monitored. The genetic structure was tested on randomly selected 251 Malopolski horse breed stallions by using seventeen microsatellite markers recommended by ISAG. Mean number of alleles was 7,706 ± 0,460 and frequency of alleles less than or equal to 5% was 4,471 ± 0,273. Private alleles were not detected and number of effective alleles was 4,077 ± 0,321, PIC was estimated 0,687. Model-based clustering was analysed with Structure software. Nine runs were conducted and the analysis involved admixture model and correlated allele frequencies. There were slightly differences between individuals in the randomly selected population of Malopolski horse, without divergent patterns and distinctly formed clusters. Nevertheless, there is no inbred in tested population (F_{is} from -0.49 (HTG6) to 0.084 (CA425)). Malopolski horse Structure analysis showed no differences in this population. This study presents our preliminary results of analysis and requires proceeding and verifying a higher number of individuals, research would be conducted with using other Polish warmblood horse breeds.

Key Words: Malopolski horse, genetic diversity, horse

MT197 The known variants of the *TBX3* gene do not explain the variability of grullo coat color shades in Polish Primitive Horse (Konik). J. Cieslak*[1], L. Wodas[1], and M. Mackowski[1,2], [1]*Department of Horse Breeding, Poznan University of Life Sciences, Poznan, Poland;* [2]*Horse Genetic Markers Laboratory, Poznan University of Life Sciences, Poznan, Poland.*

The main goal of this study was to evaluate the influence of the known *TBX3* Dun/Non-dun causative variants on the variability of grullo (blue dun) coat colour shades observed in Polish Primitive Horse (Konik, PPH). Despite the PPH are selected for grullo coat colour since the number of years, their phenotype is not homogenous. Thus, in the official PPH breeding documents the three shades of grullo pigmentation are distinguished (light grullo, grullo and dark grullo). Altogether 247 samples were genotyped using gel electrophoresis (*D/Nd2* InDel) and direct sequencing (*Nd1* SNP). In the light grullo group most (94%) of horses were *D/D* homozygotes. In the cohorts of darker horses, the tendency towards the increased frequency of heterozygous *(D/Nd2)* animals was observed (13% and 46% for grullo and dark grullo groups, respectively). The overall frequency of the *Nd1* allele in PPH was very low (1%). Several novel SNPs were found in the region spanning the 1.6 kb insertion. Their association with grullo shades variability is now intensively studied. Our results seem to partly undermine the fully dominant effect of Dun mutation. However besides the role of 1.6 kb insertion zygosity, the presence of an additional genetic modifier of Dun dilution is very likely. Study was funded by the Polish Ministry of Science and Higher Education, grant number: IP2014 006873 (*Iuventus Plus* program)

Key Words: horses and related species, functional genomics, DNA sequencing, coat colour, other application

MT198 Transcriptome profiling and comparison of whole blood and gluteus medius muscle of Arabian horses during 24 weeks race training. M. Stefaniuk-Szmukier*[1], K. Ropka-Molik[2], K. Zukowski[2], K. Piórkowska[2], and M. Bugno-Poniewierska[2], [1]*University of Agriculture in Cracow, Cracow, Poland;* [2]*National Research Institute of Animal Production, Balice, Poland.*

Polish Arabians are commonly known from their beauty and performance ability, improving endurance and halter populations all over the world. It is well established that Arabians are mainly endurance horses. Endurance training requires maturity that is reach at least at 4 y.o. Before that, 2,5 y.o horses are introduced to race tracks and competing at least one racing season before they get involved in further endurance type training. The blood based transcriptomics profiles are powerful tolls to explore disease pathogenesis and physiological homeostasis, therefore might be useful to observation changes in musculoskeletal modifications occurred during training. Thus the aim of presented research is identification of the transcriptomic profiles, which might reflects muscle response to training in whole blood. The RNA-seq analysis has been performed for 5 2,5 y.o. horses introduced to race track training Centre. Total of 20 samples of whole blood (WB) and *m. gluteus medius* (M) have been collected in two periods: T_0 -unbroken and T_1 - after 24 weeks of training schedule which include 12 weeks' light work period and 12 weeks of heavy canters period required to fitness sufficient to compete in races. The RNA sequencing was performed in 75 single-end cycles on HiScanSQ platform (Illumina). Validation of obtained results was performed by real-time PCR method. The performed analysis showed DEG's ($P < 0.05$ and fold change >1.5) between analysed periods for investigated tissues (WB and M). Among up-regulated DEG's (503 for M; 568 for WB) 31 genes were common for both tissue. For deregulated DEG's (917 M; 498 B) – total of 138 genes were common. The common genes corresponding to pathways involved in i.e. glycerophospolipid metabolism, tight junctions, MAPK signaling, cGMP-PKG signaling, FoxO signaling. Furthermore, the genes common for WB and M (SLC16A3; SLC4A7; ATP2A1; NDUFAF5; MYO9A, PPARGC1B; FOXO3; ETFDH) due to their function, will be further explore as potentially biomarkers of training induced changes in young horses. The study was supported by the Polish Ministry of Science and Higher Education (project no. 2014/15/D/NZ9/05256).

Key Words: Arabians, RNA-seq, muscle, blood, racing

MT199 Characterisation of a congenital cardiac defect in an Arabian horse family. N. A. Hamilton*[1], C. Halliday[2], R. Crisman[3], C. M. Wade[1], and N. Beijerink[2], [1]*School of Life and Environmental Sciences, Faculty of Science, University of Sydney, Sydney,*

Science, Prague, Czech Republic; [3]3Czech-Moravian Breeders Corporation, Hradistko, Czech Republic.

The Old Kladruber horse, along with the Lipizzaner, Andalusian and Lusitano is of the original Italo-Spanish type. The Old Kladruber horse is kept in grey and black colour varieties. The population is closed and there is a concern about the loss of genetic variation. The genetic diversity and population structure were analysed according the pedigree information and according SNP Chip. Pedigree records collected from 1729 to 2013 contained 7971 animals. The pedigree depth was up to 33 generations, with an average of 15.1 complete generations. The average values of the inbreeding coefficient were 13% (maximum 29%) for the reference population, 11% for the grey variety (maximum 25%) and 15% for the black variety (maximum 29%). The proportion of animals with some level of inbreeding was 99%. The average rate of inbreeding in the reference population was 1%: 0.8% for the grey variety and 1.1% for the black variety, and the respective estimates of the effective population sizes were 52 for the reference population, 62 for the grey variety and 45 for the black variety. The total loss of genetic diversity in the reference population, in the grey variety and in the black variety was 11%, 13% and 17%, respectively. The active population of 145 horses were genotyped by Illumina Equine SNP70 BeadChip. High amount of homozygous and close to homozygous loci were detected – 14% of SNP loci have higher than 95% of homozygosity and 44% of loci higher than 70% of homozygosity. For estimation of genomic relationships was used VanRaden method I. Estimated correlations between elements of pedigree and genomic relationships matrix were 0.96 for off-diagonal and 0.57 for diagonal elements. Comparison between pedigree and genomic relationships matrix documented the quality of pedigree records and very deep genealogy. In spite of high general similarity of pedigree and genomic relationship, due to Mendelian sampling have some proportion of animals different relationship according pedigree and genomic data. These individuals need special care in breeding. The results of this study will be used for breeding strategies, which consider classical as well as recent achievements of population and conservation genetics.

Key Words: horse, conservation genetics, population structure

MT193 Population structure and admixture in closely related Czech draft horse populations. H. Vostra-Vydrova[2], L. Vostry[1,2], N. Moravcikova[3], B. Hofmanova[1], Z. Vesela[2], A. Svitakova*[2], J. Schmidova[2], and R. Kasarda[3], [1]*Czech University of Life Sciences Prague, Prague, Czech Republic;* [2]*Institut of Animal Science, Prague, Czech Republic;* [3]*3Slovak University of Agriculture in Nitra, Nitra, Slovak Republic.*

The study was conducted in order to examine the population structure, admixture and genetic relationship between historically related Czech draft horse populations: Silesian Noriker (genetic resource), Noriker and Czech-Moravian Belgian (genetic resource). A total of 157 Silesian Norikers, 267 Norikers and 353 Czech-Moravian Belgians were evaluated. A set of 13 microsatellite markers has been used for analysis of genetic variability. All loci used in this study reached good level of polymorphism (PIC = 0.68 ± 0.10) and therefore considered as informative. The average number of alleles per locus was the highest in the Czech-Moravian Belgian (7.31) and the lowest in the Silesian Noriker (6.77), whereas the observed and expected heterozygosities per breed ranged from 0.673 (Czech-Moravian Belgian) to 0.709 (Noriker) and 0.677 (Silesian Noriker) to 0.710 (Noriker), respectively. As expected due to the origin and historical breeding strategy, the intra-population genetic diversity parameters reached similar values in all three analysed breeds. Observed heterozygosity and level of inbreeding coefficient (-0.01–0.00) indicated the sufficient proportion of heterozygotes within each population. The degree of differentiation estimated based on F_{ST} (from 0.01 to 0.07) and genetic distances were low. The membership probability outputs showed that the frequencies of alleles varied across the two main regions represented by Czech-Moravian Belgian *v.* Silesian Noriker and Noriker. The migration rate indicates a continuous gene flow between the Silesian Noriker and Noriker breeds (24%)(crossing). Our results show high genetic variability, low inbreeding and low genetic differentiation, especially between Silesian Noriker and Noriker. This low genetic differentiation was in accordance with geographical location, history, and breeding practices of analysed breeds. The results of this study will be useful for the development of breeding strategies, which consider classical horse breeding as well as conservation genetics. Supported by the project QJ1510141.

Key Words: conservation genetics, population structure, microsatellite, admixture, horse

MT194 Analysis of DNA methylation profiles of genes encoding miR-101 and -200a in equine sarcoids. K. Pawlina*[1], E. Semik[1], A. Fornal[1], T. Zabek[1], C. Koch[2], K. Mählmann[2,3], M. Witkowski[4], and M. Bugno-Poniewierska[1], [1]*National Research Institute of Animal Production, Balice, Poland;* [2]*University of Bern and Agroscope, Bern, Switzerland;* [3]*Free University of Berlin, Berlin, Germany;* [4]*University of Agriculture, Krakow, Poland.*

In recent years increasing attention has been paid to microRNAs, which have ability to orchestrate gene expression and thus influence many vital biological processes. Alterations in DNA methylation patterns occurring in the process of neoplastic transformation may result in disruptions of miRNA expression profiles, which in turn might cause further progression of cancer phenotypes. Our previous studies on sarcoids – the most common equine skin tumours – revealed many deregulated microRNAs as well as altered patterns of DNA methylation. To shed light on possible mechanisms underlying changes in miRNA expression in equine sarcoids we made an attempt to investigate if aberrant methylation may be one of them. To address this issue we carried out an analysis aimed at identifying DNA methylation levels of two miRNA coding genes chosen on the basis of our previous research, namely miR-101 and -200a. The research material consisted of 10 samples of sarcoid tissue and tumour-distant skin tissue (control). The DNA methylation profile of 28 CpG sites located within potential regulatory regions of the analysed genes was determined using bisulfite sequencing polymerase chain reaction (BSPCR). The analysis of BSPCR sequencing results revealed high level of methylation of CpG sites in miR -101 as well as miR- 200a promoter regions. However, the application of Mquant method proved the same trend in DNA methylation of the analysed sarcoid and skin samples as well as a similar DNA methylation percentage (differences between groups were 4.27% and 5.92% for miR-101 and miR-200a, respectively). In conclusion, despite numerous reports describing the aberrant methylation of the promoters of the analysed genes in human cancers, the obtained data did not confirm the existence of such relationships in the examined tumour tissues.

Key Words: horse, epigenomics, microRNA, animal health

MT195 Analysis of the influence of DNA methylation on altered expression of S100A14 gene in equine sarcoids. E. Semik*[1], T. Zabek[1], A. Fornal[1], K. Pawlina[1], J. Klukowska-Rötzler[2], C. Koch[2], K. Mählmann[2,3], and M. Bugno-Poniewierska[1], [1]*National Research Institute of Animal Production, Balice, Poland;* [2]*University of Bern and Agroscope, Bern, Switzerland;* [3]*Freie Universität Berlin, Berlin, Germany.*

DNA methylation plays an important role in the regulation of many biological processes including stabilisation of chromatin structure or embryonic development. Recent studies have shown that epigenetic modifications also take part in other processes, like viral infections and development of cancer. *Locus S100A14,* is presumed to be involved in processes associated with malignant trans-

NSW, Australia; [2]*Sydney School of Veterinary Science, Faculty of Science, University of Sydney, Sydney, NSW, Australia;* [3]*Somersby Animal Hospital, Somersby, NSW, Australia.*

Cardiac defects account for around 3.5% of equine congenital disorders. Of these, ventricular septal defects (VSD) alone or as part of a more complex disease are the most commonly observed, and breed predisposition is suspected. We are investigating the probable genetic origin of septal defects in an Arabian horse family presenting with a high prevalence of these defects. During cardiac embryonic development the atria and ventricles comprise a common chamber. The heart is subsequently partitioned into the normal four chambers by the growth of cardiac septa. A fault in the development of the embryonic ventricular septum may result in a VSD and other conotrunctal septal deformities. Although VSDs are common, the precise molecular mechanisms underlying these defects remain to be determined in both humans and horses. In humans, the aetiology of congenital heart defects has a strong genetic component; however, there is rarely a clear inheritance pattern, and the occurrence of VSDs has been associated with variation in many genes. Often, *de novo* mutations appear to be responsible. It has also been suggested that haplotype insufficiency, epigenetic and copy number variation may also be contributing factors. We have been approached by a breeder that has recognised an increased incidence of VSDs on their farm. One stallion has produced nine affected offspring from seven different mares; while two related mares have produced affected foals by different stallions. Several of the foals have large membranous defects and a poor prognosis for life. The asymptomatic stallion himself has a small VSD which is haemodynamically nonsignificant. We have performed full cardiovascular examinations on all available family members and collected DNA samples for analysis. The samples are from ten affected and 20 twenty related but unaffected horses. The mode of inheritance is likely simple recessive, and common ancestors exist. The samples will be genotyped on the high density equine SNP array and a genome wide association study performed. If a causative mutation is found, we will determine its distribution in the wider population, allowing breeders to reduce the incidence of VSDs by making more informed choices.

Key Words: horse, genetic disorder

MT201 Evidence for introgression of a *CXCL16* allele from non-caballine horses to caballine horses. E. Bailey*[1], T. Kalbfleisch[2], J. C. Stevens[3], J. Eberth[1], J. S. DePriest[2], and U. B. R. Balasuriya[1], [1]*University of Kentucky, Lexington, KY, USA;* [2]*University of Louisville, Louisville, KY, USA;* [3]*Genomics GPS, Guilford, CT, USA.*

Two alleles were identified for the scavenger receptor protein gene, *CXCL16*. One serves as a receptor for equine arteritis virus (EAV) infection of equine cells while the other does not. The phenotypes were described as susceptible (S), for virus binding, and resistance (R), for non-binding. The two phenotypes differed by 4 non-synonymous mutations in the first exon of the gene with susceptibility dominant to resistance. Polymorphism was observed in diverse horse breeds including Thoroughbred, Standardbred, Saddlebred, Warmblood, Miniature, Icelandic, Ahkal-teke, Caspian and Quarter Horses. The gene was sequenced for other closely related species to determine the natural history of the gene. DNA sequences of *CXCL16* exon 1 from zebras and asses were found identical to the susceptibility allele in horses.More extensive comparisons were made for the 2kb region surrounding CXCL16 exon 1 based on comparison of 9 horses (*Equus caballus*) (2 R/R, 2 R/S and 5 S/S) and 4 non-caballines [2 asses (*E. africanus asinus, E. africanus somaliensis*), 1 kiang (*E. kiang*) and 1 Grevy zebra (*E. grevyi*) ; all S/S]. A strong similarity was observed for sequences within a 947 bp region for 7 horses (R/S & S/S) and 4 non-caballine equids. Within this region we observed 13 SNPs common to the susceptible horse haplotypes and all 4 non-caballine equids. This same region also contained 2 SNPs common to the non-caballine equids and 1 SNP common only to the susceptible horses. The 1000 bases sequences flanking this region are specific for caballine *v.* non-caballine. These results suggest a hypothesis of cross-hybridization of caballine and non-caballine species; based on the number of SNPs and the size of the conserved fragment, the hybridization event may have occurred between 400,000 and 600,000 years ago. This would be a remarkable hybridization event considering that the divergence of caballine and non-caballine equids is thought to have taken place over 4.5 million years ago.

Key Words: equidae, hybridization, evolution, phylogeny, scavenger protein

MT202 Genetic diversity and population structure in Chinese Mongolian horses. H. Han*[1], K. Bryan[1], W. Shiraigol[2], M. Dugarjaviin[2], and E. Hill[1], [1]*UCD School of Agriculture and Food Science, Dublin, Ireland;* [2]*College of Animal Science, Inner Mongolia Agricultural University, Hohhot, China.*

A landrace population, the Mongolian horse occupies a diverse range of habitat, is less managed than modern breeds of Western Europe and North America, and originated in the geographic region where domestication likely occurred. Long-term selection during the last 6,000 years by herdsmen for adaptation to local environments has led to four distinct Mongolian sub-types, with adaptations including the ability to survive in extreme temperatures (e.g. -40 ?) and physical adaptations to the hoof for traversing rugged terrain (i.e. 'Iron-hoof' horse). In this study, the impact of long-term selection on the genetic diversity and population structure of the Mongolian horse was evaluated using genome-wide single nucleotide polymorphisms obtained from 94 locally bred horses representing three typical Chinese Mongolian subtypes (steppe type Wuzhumuqin horse, n = 21; desert type, Wushen horse, n = 22; and mountain type, Baicha 'Iron Hoof' horse, n = 15), one cultivated breed (Sanhe horse, n = 20) and an indigenous breed (Abaga Black horse, n = 16). Heterozygosity values as well as inbreeding coefficients were calculated across all autosomes for each population. Expected heterozygosity (He) ranged from 0.318 in the Sanhe horses to 0.345 in the Abaga Black horses. The Wushen was the only population with a positive average inbreeding coefficient value (F = 0.003). After merging the five datasets, standard quality control (QC) and LD_pruning, 167,210 SNPs remained for further analyses. Principle component analysis and F_{ST} calculations were used to visualise individual relationships within and between populations. The results suggest that different ecological environments are not likely to have caused genetic distinctiveness between Wuzhumuqin and Wushen or between Wuzhumuqin and Sanhe, however, Wushen and Sanhe had clear genetically separate clusters. All of the Abaga Black horses were separated from the other breeds forming a unique cluster, while outliers were identified in Baicha 'Iron Hoof' population. These results will facilitate investigation into genomic regions associated with extreme environment adaptation.

Key Words: Mongolian horse, single nucleotide polymorphisms, genetic diversity

MT203 Cuanhama horses from Angola as a possible expression of centuries of slave trade. J. L. Vega-Pla*[1], C. Ribeiro[2], O. Cortes[3], F. T. P. S. Sereno[4], M. R. T. Costa[5], and L. Telo da Gama[6], [1]*Laboratorio de Investigacion Aplicada. Servicio de Cria Caballar de las Fuerzas Armadas, Cordoba, Spain;* [2]*Instituto Superior Politecnico da Tudavala, Lubango, Angola;* [3]*Departamento de Produccion Animal, Universidad Complutense de Madrid, Madrid, Spain;* [4]*Faculdade de Agronomia e Medicina Veterinaria, Brasilia, Brazil;* [5]*Empresa Brasileira de Pesquisa Agropecuaria,*

Amazonia Oriental, Belem, Brazil; [6]*Faculdade de Medicina Veterinaria, Universidade de Lisboa, Lisboa, Portugal.*

Between 1441 and 1888 the transatlantic slave trade initiated a forced migration of ~12 million people from many societies and cultures in west and west central Africa to European colonies in the Caribbean Islands, in Central and South America, and in North America. Initially, most slaves were captured on the west coast of Africa with the cooperation or assent of African kings and merchants that received various trade goods including beads, textiles, brandy, guns and even horses in return for slaves. Cuanhama horses are a population of horses currently existing in the Province of Cunene, in the South of Angola. This population could be the result of groups of horses brought from Brazil by the Portuguese during the period of slave trade. The aim of this study is the analysis of an extensive genetic survey of the Cuanhama horses and 9 additional Iberian, International, Brazilian and Uruguayan breeds using DNA polymorphisms in 25 microsatellites. Highly significant fixation indexes were obtained for all pairwise comparisons between the Cuanhama population and all other breeds. A population neighbour-joining breed phenogram was built, the Cuanhama population clustered near the Iberoamerican clade highlighting its hypothetic historic influence. Furthermore, assignment tests and the individual Q-matrices obtained with the program Structure grouped the Cuanhama breed with Brazilian and Uruguayan breeds until K = 6 groups. In conclusion, a relationship among the Cuanhama horses and some Brazilian horse breeds could support the historical records about horse movements associated with slave trade.

Key Words: biodiversity, horses, population identification, genetic markers

MT204 Imputation of microsatellite alleles from dense SNP genotypes of Mangalarga Marchador Horses. K. T. Souza*[1], C. T. F. S. Diniz[2], R. M. G. Lima[1], C. L. P. Meneses[1], and C. B. N. Campos[1], [1]*Linhagen Biotecnologia, Belo Horizonte, MG, Brazil;* [2]*Pontificia Universidade Catolica de Minas Gerais, Belo Horizonte, MG, Brazil.*

Mangalarga Marchador equines are the largest breed in Brazil. Traditionally microsatellite markers (MS) have been used for parental verification and are still the international standard for horses. Actually, there are ~500000 Mangalarga Marchador with ISAG MS genotyping. However, transition to SNP genotyping is essential because the future use of SNPs in parentage maybe a reality (like bovine). The affordable way to make a transition is to have a method of imputing MS alleles from SNP haplotypes on this breed. We study 107 animals of Mangalarga Marchador breed. All animals were genotyped using the Equine 70k Illumina array. We study flanking SNPs within 500kb on each ISAG microssatellite for horses. The BEAGLE program was used to phase the genotypes in this preliminary haplotype study. This approach may be applicable to additional horse breed to migrate from MS to SNP based parental verification.

Key Words: microsatellite imputation, SNP haplotype

MT205 Novel *KIT* variants underlying dominant white phenotypes in horses. R. Hoban[1], K. Castle[2], N. Hamilton[3], and B. Haase*[1], [1]*Sydney Schoold of Veterinary Science, Faculty of Science, University of Sydney, Sydney, NSW, Australia;* [2]*Practical Horse Genetics, Redfern, NSW, Australia;* [3]*School of Life and Environmental Science, Faculty of Science, University of Sydney, Sydney, NSW, Australia.*

Variants in the equine *KIT* gene have been shown to underlie dominant white patterning in equine species. To date, 20 functionally different alleles (W1-W19, W21) have been characterised in horses. While the amount of white can vary between individuals, all 20 alleles have the potential to cause a complete or almost complete lack of skin and coat pigmentation under heterozygous conditions. Despite this large number of candidate causative variants, there are still horses where the white coat phenotype cannot be explained by existing knowledge. We analysed 14 horses, representing two horse breeds and three families, where a white coat colour phenotype could not be explained with existing *KIT* alleles. DNA was extracted form hair roots or blood and all 21 *KIT* exons including flanking sequence was generated by direct sequencing of PCR products. Sequences were compared the equine reference sequence (EquCab2) and sequences obtained during previous studies. Comparative sequencing revealed four variants associated with the phenotype under investigation. Among these, we identified three new variants affecting the coding sequence of the *KIT* gene and a *KIT* allele that has previously been identified in a different horse breed. We designated *W22* to *W24* to these newly identified functional *KIT* alleles. The new mutations comprise one frameshift mutation (c.2536delA; p.S846Vfs*15), and two missense mutations (c.668T>C; p.L223P; c.1473T>G; p.C491W). We further identified the *W13* allele, previously described to cause a dominant white phenotype in Quarter Horses, in a family of Miniature Horses.

Key Words: KIT gene, horse, variants, dominant white

MT206 A genome-wide association analysis of exercise-induced pulmonary haemorrhage in Thoroughbred racehorses. S. C. Blott* and H. L. Cunningham, *University of Nottingham, School of Veterinary Medicine and Science, Sutton Bonington, Leicestershire, UK.*

Exercise-induced pulmonary haemorrhage (EIPH), defined as presence of blood in the respiratory tract following exercise, is a significant issue in the horse-racing industry world-wide for both economic and welfare reasons. Prevalence of EIPH depends on the method or criteria used to make a diagnosis. Prevalence measured by the observation of blood at the nostrils ranged from 0.25% to 2.5% (Raphel and Soma, 1982 Am. J. Vet. Res. 43:1123–1127), while prevalence of EIPH, when measured using a flexible endoscope to perform trancheobronchoscopic examination following exercise, ranged from ~43% to 75% (Sullivan and Hinchcliff, 2015 Vet. Clin. North Am. Equine Pract. 31:187–198). A genetic basis for susceptibility to EIPH has previously been identified through pedigree analysis, and the heritability of lifetime epistaxis has been estimated at 0.27 – 0.33 (Velie *et al*. 2014 Vet. J. 202:274–278). In this study a genome-wide association analysis was carried out in UK thoroughbreds to identify genome regions and candidate genes associated with EIPH. Tracheal wash samples were collected on a weekly basis from 107 thoroughbred horses in flat racing training. Scores from cytology analysis of samples for both haemosiderophages (HF) and red blood cells (RBC) were used to identify the occurrence of EIPH. Genotyping was performed using the Illumina Equine SNP50 BeadChip and linear regression association analysis was carried out using PLINK (Purcell *et al*. 2007 Am. J. Hum. Genet. 81:559–75) Covariates incorporated into the association analysis included gender, age of horse at first race start and the average number of days between race starts within the period of time tracheal wash sampling occurred. Significant SNPs were found on ECA 3, 13 and 23 for phenotypic measures of haemosiderophage score. Two SNPs on ECA 29 reached genome-wide significance for the phenotypic measure of red blood cell score. Several candidate genes affecting blood pressure control and vascular remodelling were identified.

Key Words: horses, genome-wide association

MT207 Signatures of airway hyper-responsiveness in the equine pasture asthma-restricted lung transcriptome. S. Mack[1], T. Mansour[1], J. Bowser[2], A. Eddy[2], C. Mochal[2], A. Claude[2], A.

Cooley[2], M. Johnson[2], and C. Swiderski*[2], [1]*College of Veterinary Medicine, University of California, Davis, CA, USA;* [2]*College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, USA.*

Equine pasture asthma (EPA) is a spontaneous asthma-like disease affecting horses in the southeastern United States. Affected horses exhibit seasonal respiratory distress in association with grazing pasture during conditions of high heat and humidity. Disease remits during winter. EPA possesses key facets of the severe asthmatic phenotype, including reversible airway obstruction, airway hyperresponsiveness (AHR) and chronic neutrophilic airway inflammation. We hypothesised that molecular signatures of AHR in human asthma could be identified in the lungs of horses with EPA. We performed short read RNA sequencing of serial thorascopic lung biopsies acquired from eight EPA horses and eight nondiseased controls. Biopsies were collected from individual horses during seasonal disease exacerbation and remission. RNAseq reads were trimmed, filtered (FastQC), and mapped to public equine transcriptomes and to a self-assembled, novel lung-specific transcriptome with Salmon (Patro) in order to accurately and thoroughly identify all differentially expressed genes (DEGs). Nomenclature for the DEGs was assigned using the Vertebrate Gene Nomenclature Committee orthology prediction tool, and pathway enrichment analysis was performed, defining molecular signatures of AHR. This self-controlled design, together with identification of an EPA-restricted transcriptome, create powerful tools for deciphering the pathophysiology of AHR in horses.

Key Words: equine, pasture asthma, EPA

MT208 Mapping transcriptional regulation at multiple layers using ChRO-seq. T. Chu[1,2], L. Choate[1,2], Z. Wang[1,2], E. Rice[1,2], and C. Danko*[1,2], [1]*Baker Institute for Animal Health, Ithaca, NY, USA;* [2]*Cornell University, Ithaca, NY, USA.*

The annotation of functional non-coding regions in our genomes has proven challenging, requiring large numbers of time-consuming molecular assays to exhaustively identify a pantheon of distinct varieties of functional elements. Here we show that genome-wide maps of nascent transcription can recognise multiple varieties of activating functional elements in mammalian genomes. In addition to providing robust measurements of gene and lincRNA expression levels, transcription can be used to accurately impute the levels of activating histone modifications, DNase-I hypersensitivity, and transcription factor binding when used in combination with sensitive machine learning tools. To facilitate the use of this technology across a wide variety of sample types we developed chromatin run-on and sequencing (ChRO-seq), a novel molecular tool that maps the location of RNA polymerase starting with virtually any cell or tissue sample. Notably, because ChRO-seq measures transcription through intact protein-DNA interactions, our strategy can be used to measure gene expression in tissue samples even after RNA degradation. To illustrate the applications of these tools for understanding the molecular basis of complex disease, we used ChRO-seq to analyse dozens of primary human brain tumours. Our integrative analysis revealed that whereas malignant brain tissue largely retained enhancers that were DNase-I hypersensitive in the tissue of origin, a rare population of ectopic enhancers resembled fetal tissues isolated from the nervous system, consistent with the cancer stem-cell hypothesis. We used these maps to identify transcription factors driving gene regulatory changes in the tumour. Our new technologies have implications for efficiently annotating mammalian genomes as well as for understanding the molecular basis of disease.

Key Words: functional genomics, genome annotation, epigenomics, machine learning, gene expression

MT209 EquCab3. T. Kalbfleisch*[1], M. DePriest[1], L. Orlando[2], and J. MacLeod[3], [1]*University of Louisville, Louisville, KY, USA;* [2]*University of Copenhagen, Copenhagen, Denmark;* [3]*University of Kentucky, Lexington, KY, USA.*

EquCab3 is now complete and has been released to NCBI and ENSEMBL for annotation in their respective pipelines. This is the culmination of 3 years work on the project, yielding significant improvements in both contiguity and composition. Additional sequence data for Twilight (the Thoroughbred mare on which the reference is based) has been incorporated to the previous assembly comprised of Sanger data. New datasets were produced using Illumina, PacBio, 10X Genomics, Chicago (Dovetail), and HiC platforms/libraries. We will report on the repeat structure of the genome, as well as the amount of structural variation found within Twilight's genome both of which challenged our efforts to produce a haploid representation of her genome.

Key Words: equine, horse, reference genome

MT210 Epigenetic characterization of centromeric chromatin in equids. S. G. Nergadze[1], R. Gamba[1], F. M. Piras[1], E. Cappelletti[1], M. Corbo[1], F. Gozzo[1], D. Miller[2], D. Antczak[2], E. Raimondi[1], K. Sullivan[3], and E. Giulotto*[1], [1]*University of Pavia, Department of Biology and Biotechnology, Pavia, Italy;* [2]*Cornell University, College of Veterinary Medicine, Ithaca, NY, USA;* [3]*National University of Ireland,Centre for Chromosome Biology, Galway, Ireland.*

Mammalian centromeres are typically associated with highly repetitive DNA (satellite DNA), which has so far hindered a detailed molecular analysis of this chromatin domain. A large body of evidence indicates that centromeres are epigenetically specified and that binding of the CENP-A protein is their main determinant. Previously (Wade *et al*. Science 2009; Piras *et al*. PLoS Genetics 2010) we showed that, during the evolution of the genus *Equus*, several centromeres moved to new sites lacking satellite DNA. In this system the epigenetic marks related to the centromere can be studied by comparing the centromeric domain of a species with the noncentromeric orthologous locus in other species. We also demonstrated (Purgato *et al*. Chromosoma 2015) that the location of the CENP-A binding domain can vary in different individuals giving rise to *epialleles*, proving that centromeres are autonomous relative to the DNA sequence and are characterised by positional instability. Here we present the results of ChIP-seq experiments with anti-CENP-A antibodies in horse, donkey and zebra cell lines, which led to the precise localization of several satellite-less centromeric domains in the genome of these species. Thanks to this powerful model system we were able to evaluate the possible role of DNA composition, sequence amplification and DNA breakage in centromere specification. In addition, we characterised the association of the centromeric function with several epigenetic features such as DNA methylation and histone modifications. The transcription of satellite-less centromeric domains was also tested. The possible implications of these findings will be discussed. Taking advantage of hybrid families of horse, donkey and mule individuals, the inheritance of centromeric domains through generations was studied. The transmission of centromeric domains in clonal cell populations was also evaluated. Taken together, our findings demonstrate that, in the genus *Equus*, centromeres are extraordinarily plastic and represent an important driving force in genome evolution.

Key Words: horse genome, genus *Equus*, centromere, epigenetic marks, genome evolution

MT211 Zooming in on chronic progressive lymphedema using a high-density array in the Belgian draught horse. L. François*[1], A. Schurink[2], B. Velie[3], A. Stinckens[1], S. Blott[4], B. Ducro[2], C. Lamberigts[5], S. Tinel[1], K. De Keyser[1], M. Oosterlinck[6], G. Lindgren[3], S. Janssens[1], and N. Buys[1], [1]*Research Group Livestock Genetics, Department of Biosystems, KU Leuven, Leuven,*

Belgium; [2]*Animal Breeding and Genomics Centre, Wageningen University & Research, Wageningen, the Netherlands;* [3]*Department of Animal Breeding & Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [4]*School of Veterinary Medicine & Science, University of Nottingham, Leicestershire, United Kingdom;* [5]*Research Group Livestock Physiology, Department of Biosystems, KU Leuven, Leuven, Belgium;* [6]*Department of Surgery and Anesthesiology of Domestic Animals, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*

Historically, the Belgian draught horse has been globally indispensable in its role as a working horse. However, due the increasing mechanization of agricultural production, the demand for a such a powerful horse has declined rapidly and the breed is now a recognised living heritage breed. One of the issues threatening the future of this breed is the occurrence of chronic progressive lymphedema (CPL) which leads to progressive lower limb swelling and deformation of the soft tissue. While the underlying cause of this condition is still under debate, two diverging hypotheses have been formed. The first hypothesis considers alteration in the skin elastic system as the initial cause, yet the second hypothesis regards CPL as an inflammatory dermatitis. The current study used 301 Belgian draught horses genotyped on the Affymetrix 670K array to assess the genetic background of this disease by performing genome-wide and homozygosity association analyses. The genome-wide association study reached genome-wide significance on ECA10 as well as nominal significance on several additional chromosomes. Several candidate genes have been proposed previously using a candidate-gene approach or whole-genome scan based on microsatellites. None of these can be found in the vicinity of ECA10; however, three are located in the proximity of regions reaching nominal significance: *FOXC2* (ECA3), *HET/MET* (ECA4), *ubiquitin protein ligase E3A* (ECA1), and *CD109* (ECA20). These candidate genes cannot exclude either hypothesis. All regions passing nominal significance in the genome-wide and homozygosity association analyses were subsequently analysed using PANTHER which ultimately suggested the involvement of several processes of the immune response. This seems implies an inflammatory dermatitis as the primary cause of CPL although the presence of alterations in the skin elastic system as cause cannot be excluded. These results provide the first insight into the genetic background of CPL using a dense marker set. Additional studies are necessary for confirmation and look deeper into the genetic mechanisms underlying CPL.

Key Words: horses and related species, genome-wide association, complex trait

MT212 Unraveling gene function using co-expression networks in the domestic horse. R. Schaefer*[1], E. Norton[1], J. Mickelson[2], and M. McCue[1], [1]*Department of Veterinary Population Medicine, University of Minnesota, St Paul, MN, USA;* [2]*Department of Veterinary and Biomedical Science, University of Minnesota, St Paul, MN, USA.*

Genome-wide studies in the horse have successfully been used to describe domestication and selection, clinical disease, athletic performance, and population structure and dynamics. Despite their success, the causal genes and functional mechanism underlying many of these studies remain unknown. For example, our recent GWA study of Equine Metabolic Syndrome (EMS) identified >2,000 SNPs representing 183 regions of the genome that were associated with 11 different monomorphic, biochemical and hormonal traits defining EMS. Identifying genes and alleles within the ROIs these contributing to EMS pathophysiology is an arduous task as: 1) these ROIs span ~98.5M base pairs and contain >3,000 genes; 2) little is known about EMS cellular and molecular pathophysiology, thus prioritization is biased towards genes characterised in human/model organisms; and 3) many genes have entirely unknown or unanticipated functions. To mitigate these issues, here, we systematically integrate whole genome SNP data, tissue specific RNAseq, and serum metabolomic data in order to better describe and inter-relate putative genomic loci associated with EMS. Independently, tissue specific gene co-expression networks were built from skeletal muscle and tail-head adipose depot in 28 horses from four breeds that displayed varying signs of EMS. Differential expression analysis between extreme phenotype scores quantifying EMS in these 28 horses identifies 529 genes among muscle and adipose tissues indicating these tissues are biologically active for our trait. In muscle/adipose networks, there were 6.5X and 8.3X enrichment for co-expression among genes within AgriGO terms demonstrating these networks capture biologically relevant information. Functional analysis for EMS was performed by directly extracting co-expression interactions among genes that were within breed specific haplotype windows containing associated GWAS SNPs. From our starting set of 2,375 associated GWAS SNPs we discover 259 genes with co-expression evidence related to EMS. Future work will focus on building disease specific, differential co-expression networks as well as corroborating metabolite abundances from Welsh Ponies affected by EMS.

Key Words: horses and related species, functional genomics, network analysis, bioinformatics tools, genome wide association

MT213 Progress toward functional annotation of the equine genome. J. Petersen*[1], E. Burns[2], M. Bordbari[2], E. Scott[2], B. Ming-Whitfield[2], V. Affolter[2], C. Ramirez Alanis[2], M. Barro[2], M. Mack[2], G. Gianino[2], F. Gianino[2], E. Giulotto[3], K. Hilburger[2], T. Kalbfleisch[4], J. MacLeod[5], M. Mienaltowski[2], S. Katzman[2], T. Leeb[6], T. Raudsep[7], P. Saelao[2], S. Vig[2], H. Zhou[2], R. Bellone[2], and C. Finno[2] [1]*University of Nebraska-Lincoln, Lincoln, NE, USA;* [2]*University of California-Davis, Davis, CA, USA;* [3]*University of Pavia, Pavia, Italy;* [4]*University of Louisville, Louisville, KY, USA;* [5]*University of Kentucky, Lexington, KY, USA;* [6]*University of Bern, Bern, Switzerland;* [7]*Texas A&M, College Station, TX, USA.*

High-quality reference genomes have accelerated the discovery of variants functioning to alter phenotype. However, significant phenotypic variation of traits associated with animal health and performance still cannot be explained. It is hypothesised that unexplained variation is due, in part, to alterations in genome regulation. As part of the international Functional Annotation of Animal Genomes (FAANG) initiative and with the overarching goal of understanding genome regulation in the horse, a biobank of tissue was generated from two adult Thoroughbred mares for tissue-specific assays to elucidate regulatory elements of the genome. As the objective of FAANG is to 'accelerate genome to phenome,' emphasis was placed on extensive phenotyping to allow for downstream data analyses with full knowledge of any pathology. Antemortem phenotyping included full physical examinations, lameness, ophthalmologic and neurologic evaluations, complete blood counts and serum biochemistries. At postmortem, all tissues were grossly and histologically evaluated to identify any subclinical pathology and characterise the cellular makeup of each tissue. Multiple aliquots of 86 tissues, 3 cell types, serum, whole blood, plasma, cerebrospinal fluid, synovial fluid, gastrointestinal contents, and mucosal samples were collected. Nuclear extraction was performed on 16 tissues for future DNase-I hypersensitivity assays. Initial funding, providing for RNA- and ChIP-sequencing of 8 tissues has been supplemented by the equine research community to allow for RNA-seq of 20 additional tissues in the first phase of this project. The involvement of the equine community has also led to additional data collection including: cell culture, centromere mapping, SNP genotyping, reduced representation bisulfite sequencing, karyotyping, and microbiome sequencing. Data generated to date includes whole-genome sequence (gDNA) and RNA-seq (small and mRNA). Datasets are

made publically available as they are generated. ChIP-seq assays to investigate 4 histone marks are currently being optimized. These data represent a valuable advancement for connecting genome to phenome in the horse and will also provide for cross-species analyses of genome regulation.

Key Words: horses and related species, functional genomics, RNA-seq, ChIP-seq, genome regulation

MT214 The potential of Y-chromosomal markers for individual lineage tracking in horses. S. Felkel[1], D. Rigler[1], C. Vogl[1], M. Neuditschko[2], S. Rieder[2], V. Jagannathan[3], T. Leeb[3], T. Rattei[4], C. Schlötterer[5], G. Brem[1], and B. Wallner*[1], [1]*Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Vienna, Austria;* [2]*Agroscope, Swiss National Stud Farm, Avenches, Switzerland;* [3]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland;* [4]*Department of Microbiology and Ecosystems Science, Division of Computational Systems Biology, University of Vienna, Vienna, Austria;* [5]*Institute of Population Genetics, University of Veterinary Medicine Vienna, Vienna, Austria.*

Like mitochondrial DNA, the Y chromosome is a powerful marker for studying the sex-specific history of populations. They enable to investigate the history of male and female foundation lines in a pedigree independent way. Stallion lines have an enormous role in horse breeding, but the low sequence diversity of the horse Y-chromosome has so far hindered the genetic tracing of male genealogies in a fine-grained way. We overcame this limitation by generating an extensive horse Y-chromosomal reference genome for horses. This reference enables calling of variants in a region covering 1.46 Mb of the male-specific region of the Y-chromosome using NGS data. Here we show two examples for elucidating the ancestry of founder studs within a breed. Based on a backbone Y-chromosomal phylogeny consisting of 24 haplotypes ascertained from 52 horses of 21 modern breeds, we first assign the two founder lineages of the American Saddlebred (Harrison Chief and Gaines' Denmark) to their respective English Thoroughbred ancestors by simply genotyping present Saddlebred stallions for already defined polymorphisms. In the second example we ascertained markers specific for four founder lineages in the Franches-Montagnes breed using NGS and pedigree data from 21 stallions. In addition to specific haplotypes ascertained for each lineage, we also detect three de-novo mutations that occurred during the time frame of written records. This de-novo mutations result in derived haplotypes and therefore distinguish even sub-branches within a lineage. Our setup can be performed in any breed. Combined with a target-enriched sequencing approach it has potential for high-throughput development of lineage characteristic Y-chromosomal markers.

Key Words: horses and related species, evolutionary genomics, genotyping, breed/population identification, Y-chromosome

MT215 Genetic contributions to measured speed in Thoroughbred racehorses during early training. G. Farries*, B. A. McGivney, K. F. Gough, L. M. Katz, and E. W. Hill, *Universitty College Dublin, Belfield, Dublin, Ireland.*

Higher speed during sprint bouts in two year old horses has been associated with improved racing career outcomes in racing Thoroughbreds (Santschi *et al*., 2017). We hypothesised that variation in early measures of speed is heritable. Using GPS and heart rate monitoring during high intensity sprint bouts (work days, WD) we derived speed indices (V_{peak}, Acc, aveSpr, Dist6a, Dist6b, Dist6) to be used as phenotypes for genome-wide association studies using n = 131 horses (69 male, 72 female) genotyped across 49,720 SNPs. All horses were less than three years of age (mean = 2.12 years; range = 1.67–2.96) and had completed less than four WDs before measurement. Sex, jockey and track condition were used as covariates. The speed phenotypes were refined by performing principal component analysis—principal component 1 (PC1) explained 67.8% of the variance across the speed indices. PC1 was largely determined by: V_{peak} (0.41), aveSpr (0.32), Dist6a (0.47), Dist6b (0.46) and Dist6 (0.49). Using PC1 as a phenotype, no SNP reached genome-wide significance ($P_{UC} < 3.2 \times 10^{-5}$). However a candidate 19kb region on chromosome 14 was identified, containing three genes: *PCDHGC5 (Protocadherin Gamma-C5)*, *PCDHGB5 (Protocadherin Gamma-B5)*, and *SLC25A2 (Solute Carrier Family 25 Member 2)*. Protocadhedrins are involved in calcium ion binding and the *PCDHB15* (*Protocadherin Beta 15)* gene, 25kb downstream from the candidate region, was found to be differentially expressed ($P < 0.05$) in equine skeletal muscle in response to acute exercise (RNA-seq, n = 27). Several other protocadhedrins were differentially expressed ($P < 0.05$) in equine skeletal muscle in response to both acute exercise and training. Incorporating field exercise measurements along with genomic and transcriptomic data from racing Thoroughbreds will provide insight into the genetic regulation of exercise responses in a highly-adapted athletic animal model.

Key Words: horses and related species, genome-wide association, functional genomics, athletic performance

MT216 Genetic diagnosis of sex chromosome aberrations in horses based on analysis of microsatellite and X- and Y-linked markers. J. A. Bouzada*, J. M. Lozano, M. R. Maya, A. Trigo, I. Bonet, F. Castillo, J. Fernández-León, T. Mayoral, E. Anadón, and L. B. Pitarch, *Laboratorio de Genética y Control, Algete, Madrid, Spain.*

Equine sex chromosome aberrations are often associated with clinical signs affecting health and reproduction. However, abnormal manifestation with sex chromosome aberration usually appears at maturity and potential disorders may be suspected infrequently. A reliable survey at an early stage is therefore required because detect and characterise sex chromosome aberrations in newborn has important economic effects. Through the routine DNA genotyping of animals, it is possible to identify profiles that are indicative of chromosome abnormalities. Including additional DNA markers in usual panels for pedigree and parentage verification can be useful identifying animals possessing chromosomal abnormalities. Abnormal profiles of genetic markers located on sex chromosomes can help identify animals with chromosomal defects. Markers panel used for horse DNA testing by Laboratorio Central de Veterinaria of Algete (Madrid) consisting of seventeen autosomal microsatellite markers (AHT4, AHT5, ASB2, ASB17, ASB23, CA425, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, LEX33 and VHL20), two microsatellite markers linked to sex chromosomes (LEX3 and LEX27) and the Amelogenin marker, a gene with distinct X and Y alleles, has proved be very useful for genealogical control and detection of chromosomal abnormalities. Additional sex-linked markers (LEX22, LEX24, LEX28 and TKY598) are also available at the laboratory for extended analyses of suspect cases. Detection at an early age and understanding of the prevalence of sex chromosome aberrations should assist in the diagnosis and management of horses kept for breeding. Further, the parental origin of the X chromosome of each disorder could be proved by the results of genetic analysis, thereby contributing to cytogenetic characterisation.

MT218 Genetic diversity, evolution and selection in the major histocompatibility complex *DRB* and *DQB* genes in the family Equidae. M. Klumplerova[1], P. Splichalova[1], J. Oppelt[2], P. Musilova[3], S. Kubickova[3], R. Vodicka[4], J. Vahala[5], L. Orlando[6], and P. Horin*[1], [1]*Ceitec VFU, University of Veterinary and Pharmaceutical Sciences, Dept. of Animal Genetics, Brno, Czech Republic;* [2]*Ceitec MU, Masaryk University, National Centre for Biomolecular Research, Faculty of Science, Brno, Czech Republic;* [3]*Veterinary Research Institute, Dept. of Reproduction and Genetics, Brno, Czech Republic;* [4]*Zoo Prague, Prague, Czech*

Republic; [5]*Zoo Dvur Kralove nad Labem, Dvur Kralove nad Labem, Czech Republic;* [6]*Centre for GeoGenetics Natural History Museum of Denmark University of Copenhagen, Copenhagen, Denmark.*

The objectives of this study were to study the genomic diversity, evolution and selection of the major histocompatibility complex (MHC) class II *DRB* and *DQB* genes in the family *Equidae*. Two individuals of *Equus caballus, Equus przewalskii, Equus asinus asinus, Equus africanus somaliensis, Equus kiang, Equus hemionus kulan, Equus quagga burchellii, Equus quagga boehmi, Equus quagga chapmanni, Equus quagga borensis, Equus grevyi* and *Equus zebra hartmannae* were used for this purpose. All currently available genomic resources were used for phylogenetic and selection analyses. Due to their functional importance, exon 2 sequences were analysed. Locus specific horse primers were designed and used for amplifying genomic exon 2 sequences in all species studied. Next-generation and Sanger sequencing combined with cloning were used for assessing the genomic sequence variation. Maximum likelihood phylogenetic trees were constructed and site-specific selection analyses were carried out using standard bioinformatic tools. Three *DRB* and two *DQB* genes were identified in the genomes of all equids. A third DQB locus was found in all members of the family except the asses *Equus hemionus kulan* and *E. h. onager.* The loci *DRB2*, *DRB3* and *DQB3* showed little differences, while *DRB1* and *DQB1* seemed to be less similar across all species analysed. The *DQB2* locus showed large differences among individual genomes. Some of the data obtained could be explained by within-species copy number variation contributing to the MHC diversity. Evidence for recombination was found for the *DQB1*, *DQB2*, *DRB1* and *DRB2* loci. It thus seems that with their at least three *DQB* genes, equids are an example of mammals characterised by a complex *DQB* MHC sub-region. Allele sharing was identified in all loci with the exception of *DRB1*. Site-specific selection analysis predicted loci under positive selection both in *DRB* and *DQB* loci. No selected amino acid sites were identified in *DRB2* and in *DQB3*. These data, along with phylogenetic trees clearly deviating from neutrality support the assumption that important pathogen-driven positive selection formatted the MHC class II *DRB/DQB* sub-regions in the *Equidae.*

Key Words: horses and related species, comparative genomics, DNA sequencing, MHC, biomedical model

MT219 Whole-genome sequencing reveals two Shetland pony specific variants affecting body size and shape. J. Metzger*, F. Naccache, A. Christmann, and O. Distl, *Institute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover Foundation, Hannover, Niedersachsen. Germany.*

The Shetland pony represents a particularly small horse breed with a characteristically large head and strong built often accompanied by thick mane and tail hair and a general robustness. This study aimed at investigating whole genome sequencing data from Shetland ponies for potential signatures of selection and deleterious variants in these highly selected regions, which might be responsible for the expression of a reduced body size and a Shetland pony specific shape. Runs of homozygosity (ROH) detection performed in three Shetland ponies, revealed 460 shared ROH regions harboring 1494 genes and 5826 variants with predicted high or moderate effects. Filtering for variants in these ROH regions with homozygous mutant genotypes exclusively found in Shetland ponies revealed two missense variants located in genes involved in growth regulation and bone development. The variant effects were predicted to be tolerated (0.07) for the missense/splice region variant as well as deleterious (0.00) for the missense variant. Further validation of these variants in ponies and horses of different breeds confirmed that both variants could be exclusively found in the Shetland pony and in the closely related Classic pony. In one of these variants the homozygous mutant genotype was only present in Shetland ponies smaller than 87.9 cm height at the withers and could therefore be proposed to be fixed in the Miniature Shetland pony population. None of the other investigated pony and horse breeds, Przewalski horses and donkeys harbored the mutant allele. In conclusion, our study revealed two Shetland pony specific variants of which one variant was private for Miniature Shetland ponies and potentially fixed by targeted selection of this specific pony type during domestication. Thus, these investigations add further two candidate genes to the genetically complex development of body size and shape in horses.

Key Words: horses and related species, homozygosity, DNA sequencing, candidate gene

MT220 Preliminary results of genetic monitoring of the occurrence of three genetic diseases (CA, SCID; LFS) in Arabian horses from Poland. M. Bugno-Poniewierska*[1], M. Stefaniuk-Szmukier[2], A. Piestrzynska-Kajtoch[1], A. Fornal[1], and K. Ropka-Molik[1], [1]*National Research Institute of Animal Production, Department of Animal Genomics and Molecular Biology, Krakow, Poland;* [2]*University of Agriculture, Department of Horse Breeding, Kraków, Poland.*

Polish horse breeding industry is well known from Arabian horse breeding tradition. The origin of the horse lines traces back more than 200 years which can be confirmed by historical sources. Polish Arabians are commonly known from their desirable beauty and performance ability. Balanced selection is based on preserving traits combining unquestionable appearance and utility. Therefore, the Polish population of Arabian horses are undoubtedly one of the most influential in the world. Genetic screening for heritable disorders in Arabians is an extremely important step for population management to avoid the production of affected offspring, reducing the incidence of carriers and preventing economic losses. There are three common genetic disorders tested in Arabian horses: Cerebellar Abiotrophy (CA); Severe Combined Immunodeficiency Disorder (SCID) and Lavender Foal Syndrome (LFS). The genetic background of causative mutations responsible for each conditions has been previously described (Brault *et al*. 2011, Shin *et al*. 1997, Brooks *et al*. 2010). The aim of the presented study is to determine the occurrence of mutant alleles of the three diseases in a sample population of Arabians from Poland using DNA-based test. Analysis included 448 healthy horses that were introduced into Polish Arabian Stood Book. The scan against mutant alleles reveled an absence of SCID and LFS carriers among investigated individuals. However, the investigation for CA alleles showed 11,4% frequency and all of them were in heterozygous state. Although the carriers of the mutant alleles do not show clinical signs and there is a lack of reports describing negative consequences for health and athletic performance in heterozygous individuals, further monitoring of active population is essential. The absence of SCID carriers in Polish Arabians has been reported previously (Terry *et al*. 199) which resulted in an increased efforts of breeders to maintain the population clear. The LFS is most common linked to Egyptian origin lines, even though that polish breeding program introduce stallions of Egyptian origin, the mutation causing LFS is not determined. Financed: BIOSTRATEG2/297267/14/NCBR/2016.

Key Words: horse, genetic diseases, CA, SCID, LFS

MT221 Protein-coding gene and transcript sequences quantify progress toward the new equine reference genome assembly. M. S. DePriest*[1,2], J. N. MacLeod[2], and T. S. Kalbfleisch[1], [1]*University of Louisville, Louisville, KY, USA;* [2]*University of Kentucky, Lexington, KY, USA.*

The current version of the equine reference genome (EquCab2) was assembled entirely from Sanger sequence reads and published

in 2009. Due to limited sequence read coverage and technological issues with Sanger data, some functional genes that are highly conserved in other mammals are not present in EquCab2. The new reference assembly, EquCab3, incorporates newer sequencing technologies that offset the weaknesses of Sanger data. Based on a comparison of mammalian protein-coding gene lists, we identified 1,430 protein-coding genes present in other mammals but not listed in EquCab2. We used tblastn to search for these genes in EquCab3 and found that the new reference assembly incorporates 758 of these genes. When we mapped RNA-Seq data to both EquCab2 and EquCab3, the mapping rate was ~2.5 percentage points higher for EquCab3 than for EquCab2. Also, 7,426 transcripts generated from the EquCab3 RNA-Seq mapping had truncated alignments in EquCab2, indicating that they had been extended in EquCab3. Finally, the distances between these transcripts and their nearest 5′ gaps tended to be longer in EquCab3 than in EquCab2, suggesting that missing 5′ regulatory regions of these genes had been captured in the new reference. These comparisons of functional loci demonstrate that EquCab3 is more complete than EquCab2.

Key Words: horses and related species, genome annotation, comparative genomics, genome sequencing, RNA-Seq

ISAG-FAO Genetic Diversity

MT222 Genome-wide assessment of genetic diversity and population structure in Chinese indigenous cattle. W. Zhang*, J. Li, Y. Chen, L. Xu, L. Zhang, X. Gao, and H. Gao, *Institute of Animal Science, Chinese Academy of Agriculture Science, Beijing, China.*

To explore genetic diversity and population structure in Chinese indigenous cattle, we conducted population genetic analysis at both individual and population level, and performed genome-wide selection signatures. We genotyped 572 samples from 20 Chinese indigenous cattle breeds and downloaded the published data for worldwide breeds. A total of 558 qualified samples with 17,821 SNP loci were left for subsequent analysis. Neighbour-joining (NJ) tree was constructed using PHYLIP, and population structure was analysed using STRUCTURE. TreeMix was employed to investigate interpopulation migration and gene flow. Genome-wide selection signatures (Fst) were calculated by Genepop. In PCA and NJ tree analysis, samples belonging to the same breeds were grouped together, leading to clear separation among other breeds. And Chinese indigenous cattle were clustered into two groups- Southern breeds and Northern breeds- with the Central breeds branched at an intermediate position. In STRUCTURE K = 2, the results showed a clear transition from the Northern breeds to the Southern breeds. And the Northern breeds contained less *E. taurus* (62.5%) proportion than previous studies reported (more than 90%). In STRUCTURE K = 3, a unique ancestry was detected in Southern breeds, which can reflect an initial admixture event between Chinese cattle and wide dispersed *Zebu*, followed by different selection pressures that leading to differentiation between Chinese and *Zebu*. TreeMix with four migrations events and *f3* statistic results provided the evidence of admixture history between Southern breeds and Northern breeds. According to the proportion of descent, we found the natural barrier– Qinling Mountain and Taihang Mountain– to gene admixture. In selection signatures analysis, 48 genes are annotated, some of which associated with production traits, participated in immune response, nervous system development, and other biological process. The results revealed the population structure and levels of admixture among Chinese indigenous cattle, shedding light on the origin and evolutionary history of these breeds. Candidate genes detected in this study provided the understanding of difference between Southern cattle and Northern cattle.

Key Words: cattle, population genetics, genotyping, admixture, selection

MT223 Phylogenetic analysis of Kazakhstani goats using mtDNA HV1 and SRY gene sequences. R. Tabata*[1], S. Sasazaki[1], M. Bakhtin[2], P. Kazymbet[2], M. Alyan[3], M. Suleimenov[3], M. Nishibori[4], and H. Mannen[1], [1]*Graduate School of Agricultural Science, Kobe University, Kobe-shi, Japan;* [2]*Astana Medical University, Radiobiological Research Institute, Astana, Kazakhstan;* [3]*Institute of Zoology, Almaty, Japan;* [4]*Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan.*

To understand the phylogenetic relationships and genetic variability in Kazakhstani goat population, we investigated mtDNA HV1 (481bp) and *SRY* gene 3′UTR (543bp) sequences. We sequenced 127 Kazakhstani goats from five different regions (Central, North, Southeast, South, West), and examined genetic structure. Further, we analysed these data in conjunction with previously published data from Eurasian and African populations. In previous study, mtDNA HV1 sequences of domestic goats revealed six haplogroups (A, B, C, D, F, G; Naderi *et al*. 2007). In present study, mtDNA sequences in Kazakhstani goats revealed 71 haplotypes and 3 haplogroups. The dominant haplogroup A was observed in all regions ($n = 123$), while haplogroup C was observed in Central ($n = 1$) and Southeast ($n = 1$), and haplotype D ($n = 2$) was only in the South-east region. The nucleotide and haplotype diversities ranged from 0.01859 (South) to 0.02781 (Southeast) and from 0.916 (South) to 0.989 (Southeast), respectively. Although the haplogroups C and D in Central and South-east regions were observed, AMOVA analysis indicated that most of mtDNA variations in Kazakhstani goats were shown within regions (>95%), suggesting the weak phylogeographic structure among five Kazakhstani regions. Our previous study indicated four representative *SRY* haplotypes (Y1A, Y1B, Y2A and Y2B) based on the 3′UTR sequences (Waki *et al*. 2015). In Kazakhstani goats ($n = 50$), 2 SRY haplotypes (Y1A and Y2A) were observed. The predominant haplotype was Y1A (86%, $n = 43$) and second one was Y2A (14%, $n = 7$). To investigate the haplotypic variations among Eurasian, African and Kazakhstani goats, we included 3827 mtDNA and 792 *SRY* sequences obtained by DDBJ database. From the mtDNA sequence data, Central and Western Asian goats showed extremely high frequency of haplogroup A and the presence of haplogroups C and D with low frequency. *SRY* sequence data suggested the genetic similarity between Western Asia, Kazakhstan and Mongolia in terms of predominant haplotypes of Y1A and Y2A. In conclusion, our results suggested the weak phylogeographic structure among five regions in Kazakhstani goats and the genetic similarity in Central and Western Asia.

Key Words: diversity, goats, mitchondrial DNA, phylogeography, SRY gene

MT224 Genetic diversity and admixture of the Mexican Lidia population inferred from medium-density genotypic data. P. G. Eusebi*, O. Cortés, S. Dunner, and J. Cañon, *Universidad Complutense de Madrid, Madrid, Spain.*

The bovine Lidia breed is selected upon behavioural features associated with aggressiveness, which makes it have a reduced ecological exchangeability. Moreover, Lidia breeders have been selecting for more than 250 years their animals according to different types of behaviour, establishing closed family trees that prompted to a fragmentation of the racial group into small lineages. Lidia

breed livestock diversity is still largely unexplored in spite of the genetic relationship of two populations historically related as are the Spanish and Mexican; both demographically well stablished, but with low effective population sizes that places them at a risk of extinction. This study investigated, by using genome-wide single nucleotide polymorphisms, the genetic diversity and population structure of the Mexican Lidia population, and, in order to assess for admixture patterns, the relationships of the most prominent Lidia breed populations (the Mexican and the Spanish), with Spanish native and American creole breeds which may provide insights of the genetic constitution of the Lidia breed. Illumina medium density genotypes were used, and after pruning, 37,148 NSPs spanning all the autosomal genome were selected on 532 purebred animals from eleven breeds; The Spanish (n = 349 from 28 lineages) and Mexican (n = 119 from 3 lineages) Lidia breed populations; Corriente (n = 5) and Texas Longhorn (n = 20) from America; and Berrenda en Negro (n = 5), Retinta (n = 4), Cárdena (n = 5), Terreña (n = 5), Negra Andaluza (n = 5), Morucha (n = 5), Berrenda en Cárdeno (n = 5) and Mostrenca (n = 5) from Spain. The admixture analysis revealed a strong genetic differentiation of the Lidia breed populations from the rest of the American creole and Spanish native breeds analysed. Moreover, the multi-dimensional scaling analysis applied just on the Lidia breed populations, indicated a separation among the Mexican population from the Spanish population, with some exceptions on a few Spanish Lineages which are closer to the Mexican lineages than to the rest of the Spanish population. In addition, we analysed Runs of Homocigosity (ROH) patterns among Lidia breed populations to better explain the origins of the Mexican Lidia population.

Key Words: genome-wide, Lidia breed, runs of homocigosity, admixture

MT225 Novel Y chromosomal haplotype of domestic sheep (*Ovis aries*) in China. W. Yan* and L. Xu, *Faculty of Animal Science and Technology, Gansu Agricultural University, Lanzhou, China.*

Investigations on the variation present at the male-specific Y chromosome region provide strong information to understand the origin and evolution of domestic sheep. One SNP OY1(g.88A>G) in the upstream region of *SRY* gene, and the microsatellite SRYM18 locus within ovine Y chromosome were analysed in one hundred and forty five samples drawn from eleven breeds (Kazakh, Gansu alpine fine-wool, Tibetan, Chinese Merino, Tan, Duolang, Tashikuergan, Texel, White Suffolk, Bond and Australia Merino) in China. SNP OY1 was analysed using PCR-SSCP method and sequencing. Two different PCR-SSCP patterns were observed, while SNP A-OY1 showed the most common frequency (76.6%). Sequencing of the compound structure of SRYM18 region revealed one novel size fragment (137bp) based on different repetitive units including (TTTTG)m, Indel-/G and (TG)n. Seven haplotypes (H4, H5, H6, H7, H8, H9 and H12) and two novel haplotypes (Ha and Hb) were established using combined genotype analysis. H6 showed the highest frequency (44%) across all breeds, and H8 showed the second frequency (24.1%). Ha was only found in one breed (Tan), while Hb was present in three breeds (Gansu alpine, White Suffolk and Duolang). In conclusion, we observed a novel allele in SRYM18 region and two novel haplotypes in ovine Y chromosome. Our findings indicate that the paternal gene flows between sheep breeds from both of China and foreign countries probably affect the development of breeds in China.

Key Words: sheep, Y chromosome, microsatellite

MT226 Analysis on geographical distribution and characteristics of Chinese indigenous chicken breeds. Z. Mengmeng*, X. Ran, and W. Keliang, *China Agricultural University, Haidian District, Beijing, China.*

China is one of the leading countries in terms of domestic chicken genetic resources, having more than one-third (107 breeds) of the total number of Asia. These native breeds have formed specific genetic characterisation through long-term selection and breeding. Herein, we analysed the breed characteristics according to the geographical distribution of breeds, further provided fundamental understandings for utilisation of those indigenous chicken breeds. Overall, the number of indigenous breeds had increased from 27 in 1988, to 81 in 2004, and reached 107 in 2011. Then, we ordered 107 indigenous breeds into 7 production areas in terms of the various geographical features and climates in China, including North-east(NE), Montenegro(MT), Huanghuaihai(HH), Loess Plateau(LP), Qinghai-Tibet(QT), South-east(SE) and South-west(SW) regions. The breeds in different regions exhibited their respective instinctive merits. About half (53 breeds) of the total Chinese local breeds originated from the SE, however, NE and QT only have 2 breeds. With comparing the characteristics of breeds distributed from the same region, we found the breeds located at NE and MT easily adapted to the alpine climate. QT breeds could adapt to hypoxia, high-altitude and cold temperature and they usually like climbing and flying. Most of the breeds from SW performed high nutritional, healthy and medical properties, such as Silky Fowl. Relative to the above regions, the advantage of SE breeds was their higher productivity, which provided the most chicken products (egg, meat and breeding stock) in China. Although the Chinese chicken market was influenced by commercial breeds, the local breeds with excellent quality has also been preferable for customers. For example, a popular high-quality commercial broiler breed, yellow-feathered broiler, is bred by crossing with Chinese indigenous chicken breeds.The characteristics of Chinese indigenous chicken breeds was significantly correlated with the geographic distribution of those breeds. Furthermore, establishing different breeding strategies according to distribution regions would provide a new insight into utilising the domains of those indigenous breeds in the long-term.

Key Words: characteristics, breed distribution, indigenous genetic resources, chicken, China

MT227 Genetic structure and relationships among 11 cattle populations using indel markers. H. Yamanaka*[1], S. Sasazaki[1], M. Lwin[2], H. Moe[3], T. Shimogiri[3], and H. Mannnen[1], [1]*Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Japan;* [2]*University of Veterinary Science, Yezin, Myanmar;* [3]*Faculty of Agriculture, Kagoshima University, Kagoshima, Japan.*

In genetic analysis using DNA markers, there are few studies using indel polymorphisms. The indel marker may be useful as novel DNA marker. Since large indel polymorphisms (>20bp) are considered to have low possibility of recurrence by mutation, it could be suitable for identification of IBD and accurate estimation of genetic admixture. In the present study, we developed 8 indel markers and investigated genetic structure, relationships and diversity in 11 cattle populations, representing 4 zebu and 7 taurine. We selected each one indel of 29 autosomes in the dbSNP database. Out of the 29 indels, we genotyped high polymorphic 8 indels with clear band detected by agarose gel in 11 cattle populations (4 *B.indicus* populations: native cattle in Cambodia, Bangladesh, Laos and Bhutan. 7 *B.taurus* populations: native cattle in Mongolia and Kazakhstan, and cattle breeds of Hanwoo, Japanese Black, Japanese Holstein, Angus and Hereford). Genetic index of average gene diversity over loci showed higher genetic diversity in Mongolian (0.337) and Kazakhstani (0.379) native cattle than the other populations. *B.indicus* populations (0.172 – 0.241) and Japanese Black (0.217) indicated low genetic diversity. UPGMA-tree and the PC1 (58.3%) of principal component analysis (PCA) clearly distinguished between *B.taurus* and *B.indicus* populations. The result of PCA reflected to

the geographical location of the cattle populations. STRUCTURE analysis demonstrated the distinct separation between *B.taurus* and *B.indicus* (K = 2) and between European breeds and Asian breeds of *B.taurus* (K = 3). At K = 4 (maximum likelihood estimation), Hereford separated from the other populations. In addition, at K ≥ 4, Mongolian and Kazakhstani native cattle showed genetic admixture derived from multi-ancestors. In conclusion, our study using indel markers could explain the genetic structure, relationships and diversity well, including: (i) low genetic diversity in *B.indicus*; (ii) low genetic diversity in Japanese Black cattle; (iii) high genetic diversity in Mongolian and Kazakhstani native cattle; (iv) the genetic distribution reflected to geographical location of cattle populations; (v) the genetic admixture derived from multi-ancestors in Mongolian and Kazakhstani native cattle.

Key Words: cattle, indel, diversity, structure

MT228 Genetic analysis of fauna from the Early Neolithic site of Chaves, Huesca, Spain. I. Ureña*[1,2], H. Bolívar[3], M. A. Galindo-Pellicena[2], J. L. Arsuaga[2], C. Ginja[1], and C. Valdiosera[4,2], [1]*CIBIO-InBIO–Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, Vairão, Portugal;* [2]*Centro Mixto UCM-ISCIII de Evolución y Comportamiento humanos, Madrid, Spain;* [3]*Laboratorio de Evolución humana, Departamento de Ciencias Históricas y Geografía, Universidad de Burgos, Burgos, Spain;* [4]*Department of Archaeology and History, La Trobe University, Melbourne, VIC, Australia.*

The genetic identification of species in archaeological studies of faunal remains is important namely when morphologic or biometric classification is difficult. This is the case of ovicaprine for which adequate taxa identification is key to infer some of the economic strategies of past human populations. Furthermore, studying past genetic diversity of domestic species helps to clarify the dispersion routes from domestication centers of origin and the demographic processes that underlie current biodiversity. The archaeological site of Chaves is a cave located in the Pre-Pyrenees, Huesca, Spain, which was used by humans mainly for hunting wild goats during the Upper Paleolithic and later on during the Early Neolithic. Chaves is considered a key site for understanding the introduction of the Neolithic culture in North-Eastern Iberia. In order to investigate the paleo-genetic history of the Pyrenean wild goat and the domestic ovicaprine of the Neolithic, ancient DNA methods were combined with Next-generation Sequencing technologies to analyse the Cytochrome *b* gene of 10 ancient specimens collected at this site, comprising both Paleolithic and Neolithic layers. Publically available mitochondrial sequences of wild goats, domestic goats and sheep were used for comparisons. Five remains were identified as Iberian wild goats of which one was from the Neolithic layer suggesting a mixed subsistence strategy (livestock and hunting) during this period. The other remains belong to domestic animals, four sheep and one goat. We identified the maternal haplogroup of this domestic goat showing that lineage C was present in the Iberian Peninsula at Chaves 7428–6795 cal BP years ago. This haplogroup has also been found in two contemporaneous samples from Early Neolithic levels at Baume d'Oullen, in the South East of France, together with three samples of lineage A. Currently, haplogroup A is the most frequent in Europe, whereas lineage C is restricted to populations from Switzerland and Slovenia at very low frequency. These results suggest that diversity of past domestic goats was distinct, but genetic studies of more specimens are needed to fully understand the patterns of goat dispersal through time.

Key Words: goats and related species, ancient DNA, animal domestication

MT229 Genetic analysis of the Estonian Native horse. C. Castaneda[1], R. Juras*[1], T. Raudsepp[1], I. Randlaht[2], and E. G. Cothran[1], [1]*Texas A&M University, College Station, TX, USA;* [2]*Estonian Native Horse Conservation Society, Estonia.*

The Estonian Native horse, also known as Klepper, is thought to be one of the few surviving direct descendants of the Northern forest horse Tarpan that populated Eastern Europe a few centuries ago and was announced extinct in 1909. Because of the geographic location and turbulent political history, Estonian stallions and mares have been deported to neighbouring territories - to Sweden as military horses and to Russia to improve their local horse breeds. In the 19th century, native horses were crossed with Hackney and Ardennais, to increase body size and improve performance, and in the early 1900s Arabians and Finn horses were introgressed with the breed. The Estonian Native horse is among endangered domestic animal breeds by FAO. This study was undertaken because despite the long cultural history and endangered status, genetic studies in the breed are negligible. The goal was to gain information on the genetic make-up of the Estonian horse for better insight into the breed's history and to improve its management and conservation. The study involved 33 Studbook registered Estonian Native horses, 320 horses from 9 different historically or geographically related breeds, and 70 Exmoor ponies as an outgroup. All animals were genotyped using a set of 15 microsatellite markers from 14 horse chromosomes. The data were analyzed for genetic diversity indicating that the level of heterozygosity, as well as the mean number of alleles per locus in the Estonian Native horse is above average for domestic horses. Genetic relationship of the Estonian horse with other breeds was determined by principal component analysis, STRUCTURE analysis, and construction of RLM trees. According to these, the Estonian Native horse groups with the Polish Primitive, Gotland, Hucul and Finn horses, with the closest genetic relationship to the Finn horse. The genetic data are consistent with the expectations based upon historical information and geography. In addition, due to anecdotal claims that Klepper is a gaited breed, we tested Estonian Native horses for the presence of the 'gait' mutation in *DMRT3* and showed that none of the 33 horses studied carried the mutation. The results are consistent with known characteristics of the breed and the everyday observations by breeders and owners.

Key Words: horse, diversity, microsatellite, conservation

MT230 Genetic relationships and admixture between European and American local pig breeds. A. M. Martinez*[1,2] and B. Consortium[1], [1]*University of Cordoba, Cordoba, Spain;* [2]*Animal Breeding Consulting, S.L, Cordoba, Spain.*

According to the historical records, Creole pig breeds descent from the Spanish and Portuguese pigs brought to the Americas in the 15th century. Afterwards, other European domestic and wild pig were introduced in America. The aim of this study is to investigate the genetic relationships and possible admixture among the current Creole and local pigs from Western Europe using the Chinese Meishan as outgroup. A total of 1715 animals representing 42 domestic pig breeds and 4 wild boar populations were analysed with 24 microsatellites. The dataset includes 12 Spanish, 3 Portuguese, 1 Hungarian, 1 Chinese, 18 North, Central and South American Creole and 8 European breeds. Wild boar populations were sampled in Portugal, Spain, Italy and Poland. The Neighbour-Net method as implemented in SPLITSTREE software was used to compute a network based on Reynolds distances. To assess the relative genetic contributions of breeds from different regions (Mediterranean + Wild boar, Celtic, British or Duroc) in the Creoles, a maximum likelihood estimation of admixture proportions was carried out with the LEADMIX software. The admixture estimates indicated that, for the Creole pigs considered as a single group, Mediterranean and wild boar pigs contributed ~37% to the genetic pool, Celtic populations ~26%, local British ~16% and Duroc 21%. When the analysis was performed for three different Creole groups according to their distribution in the Network-Net, the highest Mediterranean + Wild boar contribution to the genetic pool was observed in Creole group

G2 (51%), being its contribution of 40% in group G3 and 30% in G1. This group displayed the highest proportion of Celtic populations, ~37.5% and British (29.54%). The highest contribution of Duroc breed was observed in the group G3. In conclusion, different levels of signals of European pig breeds could be found into the great genetic diversity of the current Creole pig populations.

Key Words: microsatellites, creole pigs, biodiversity, wild boar

MT231 Genetic diversity in a Colombian population of Brahman cattle breeds Mantel and spatial autocorrelation analysis. D. A. Montano*, V. Castaneda, and G. Acuna, *Escuela de Artes, Letras y Ciencias, Bogotá, Colombia.*

Individuals of Ganado zebu (*Bos taurus indicus*) belonging to the Girardot population in Colombia were genetically analysed. The levels of inbreeding and genetic structure were estimated using the Wright (1951) statistics using the methodology of Nei (1987). Studies of cebuine races in the tropics are very scarce, and considering that more studies on population genetics in cebuine races, especially Brahman, are needed in Colombia, this study provides new information on genetic relationships in Colombia, South America and compares the data of this work with the previous ones (Novoa & Usaquen, 2010) realised, also were realised analyzes of spatial autocorrelation and analysis of Mantel with Imbalance of ligament for individuals of this population. It is sought to genetically characterise this breed by comparing molecular data and genealogical data, based on the behaviour of population parameters, the inference of genetic structure and in turn on the genetic relationships of this population with other populations of the region

Key Words: genetic diversity, cattle breeds, genetics populations, spatial autocorrelation, bottleneck analysis

MT232 Genetic diversity among domestic goats (*Capra hircus*) and wild goats (*Capra aegagrus*) in Turkey. I. S. Yildirim[1], M. Nizamlioglu[1], M. D. Oncu[2], E. K. Bastanlar[2], and Z. Bulut*[1], [1]*Selcuk University, Faculty of Veterinary Medicine, Departments of Biochemistry, Konya, Turkey;* [2]*TUBITAK-MAM, Genetic Engineering and Biotechnology Institute, Gebze, Kocaeli, Turkey.*

Characterisation of populations at the molecular level makes it possible to define genetic distances among and between populations. For this purpose, microsatellites are frequently preferred for that they generally are not subject to natural selection and that they show variation among and between populations directly according to time and mutation rate. In this study, the genetic similarities and differences between Kilis, Shami, Honamli, Saanen, Hair, Angora and wild goats were investigated through the use of 320 individual goat samples. DNA isolation was conducted using a standard phenol/chloroform method. Twenty different microsatellite loci were identified and PCR-amplified. The Beckman Coulter CEQ-8000 Genetic Analysis System was used to fractionate and genotype individual microsatellite markers. The total number of alleles, observed heterozygosity and expected heterozygosity values under Hardy–Weinberg Equilibrium were estimated. It was observed that the number of total alleles varied between four and 25 for different loci. While observed mean heterozygosity values differed between 0.499 and 0.632, it was seen that expected mean heterozygosity ranged between 0.609 and 0.705. Using the Structure program and FCA graphs, it was observed that wild goats are more genetically homogenous than domestic goats and that they could be classified separately. Interestingly, wild goat heterozygosity was lower than domestic goats. Furthermore, it was found that there were no genetic differences between the Ankara goat Eskisehir and Lalahan populations. This study was supported by Selcuk University BAP (Project Number: 15202004) and TUBITAK-KAMAG (Project Number: 109 G 103).

Key Words: genetic diversity, wild goat, domestic goat

MT233 Comparative genome-wide characterisation of five rare British Isles cattle breeds. P. Flynn*[1,2], J. Carlsson[2], and D. Berry[3], [1]*Weatherbys DNA Laboratory, Johnstown, Naas, Co. Kildare, Ireland;* [2]*University College Dublin, School of Biology & Environmental Science, UCD, Belfield, Dublin, Ireland;* [3]*Teagasc, Moorepark, Fermoy, Co. Cork, Ireland.*

Several cattle breeds within the British Isles have been subjected to reduction in population numbers over recent centuries. Five such breeds are Kerry (KY), Dexter (DX), DroimFhionn (DF), Irish Moiled (IM) and White Park (WP). Comparative genome wide characterisation studies contribute towards conservation strategies, aiming to ensure future survival and progression of such bovine genetic resources. Using a 4,345 SNP subset from the International Dairy and Beef (IDB) SNP chip, this study established genetic parameters such as diversity, differentiation and population structure for these five breeds. Samples were collected for each breed (total $n = 225$), with selection based on pedigree knowledge to maximise within breed representation. Available datasets for Angus (AN) and Holstein Friesian (HF) breeds were also included for comparative purposes (total $n = 100$). Reduced within breed genetic diversity, relative genetic isolation and strong population structure (@ K = 7) was observed for both WP (He 0.36502 ± 0.14465) and IM (He 0.36712 ± 0.14396). Greatest genetic distance was also observed between WP and IM (Fst 0.21624, $P < 0.01$). KY (He 0.41602 ± 0.10763) and DX (He 0.43498 ± 0.08935) displayed comparable within breed genetic diversity, however Principle Component and Structure analysis (@ K = 7) generated distinct clusters for both breeds. The DF breed displayed genetic diversity (He 0.41854 ± 0.10712) similar to the overall mean (He 0.41214 ± 0.10882) with comparative genetic distances ranging from closest - HF (Fst 0.09263, $P < 0.01$) to furthest - WP (Fst 0.17342, $P < 0.01$). Key findings within this dataset include – reduced genetic diversity and genetic isolation for both IM and WP, along with evidence of distinct differentiation between KY and DX. Novel insights into DF genetic diversity and distinctiveness were revealed within this breed's first ever comparative genome wide analysis. Results provide a genome wide snapshot of current genetic status for each rare breed and a benchmark to monitor future breeding strategies or genetic shifts.

Key Words: cattle and related species, comparative genomics, single-nucleotide polymorphism (SNP), breed diversity, conservation

MT234 Genetic continuity of maternal lineages in Iberian cattle populations since Roman times. L. Simões[1], A. E. Pires[2,3], C. Detry[4], I. Ureña[2], E. Svensson[1], J. Matos[5], C. Rodriguez-Fernández[6], A. M. Arruda[4], I. Fernandes[7], S. Davis[3], A. Götherström[8], and C. Ginja*[2], [1]*Department of Organismal Biology, Uppsala University, Uppsala, Sweden;* [2]*CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, Vairão, Portugal;* [3]*Laboratório de Arqueociências-InBIO, DGCP, Lisboa, Portugal;* [4]*UNIARQ, Centro de Arqueologia da Universidade de Lisboa, Faculdade de Letras, Universidade de Lisboa, Lisboa, Portugal;* [5]*Grupo de Biologia Molecular, Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal;* [6]*Departamento de Historia, Facultad de Filosofía y Letras, Universidad de León, León, Spain;* [7]*Câmara Municipal de Palmela, Palmela, Portugal;* [8]*Archaeological Research Laboratory, Department of Archaeology and Ancient History, Stockholm University, Stockholm, Sweden.*

Cattle mitochondrial DNA is geographically structured. This enables the association of maternal lineages to specific regions. For

example, the T1 and T3 haplogroups predominate in Africa and Europe, respectively. Extant cattle from the Iberian Peninsula show high genetic diversity despite their considerable geographical distance from their presumed Near-Eastern centre of domestication. However, it is not clear if this pattern is recent or ancient. Our aim is to use a zooarchaeogenetics approach to characterise the genetic diversity and investigate phylogenetic relationships of past domestic cattle populations from the Iberian Peninsula. Sixty-two specimens dating between the 1st century BC and the 15th century AD were selected for ancient DNA analysis. Targeted high-throughput 454-JS Junior sequencing technology (Roche) was used to examine ~220 bp of the mitochondrial D-loop region. Consensus sequences were aligned against NCBI reference data from extant cattle representing major haplogroups and phylogenetic relationships were inferred using median-joining networks. We successfully extracted and analyzed DNA sequences from cattle remains preserved under suboptimal environmental conditions, i.e. a temperate climate. High-coverage allowed sequence validation and authentication. Overall, T3-haplotypes predominate in the Iberian Peninsula (~80% of the total) at least since the Roman occupation, but T1-lineages of putative African origin were also detected (~15%). The distinct Q-lineage, which is found in low frequency in extant Iberian cattle (~5%), was observed for the first time in one specimen from Roman Monte Molião (southern Portugal), but also in one specimen from Moslem Alcáçova de Santarém (near Lisbon, Portugal) and in one specimen from post-medieval Christian Beja (southern Portugal). The T2-lineage, which is predominant in Asia, was only found in one specimen from Beja. Our results corroborate observations from other studies of both ancient and extant domestic cattle that indicate a genetic continuity of maternal lineages over time between cattle populations from the same location, and suggest post-Medieval cattle were improved locally.

Key Words: Iberian cattle, ancient DNA, mitochondrial sequencing, biodiversity

MT235 An ancient genomic perspective on the horse domestication process. P. Librado[1], A. Fages[1,2], C. Gaunitz[1], N. Khan[1], K. Hanghøj[1,2], C. Gamba[1], C. Der Sarkissian[1], M. Leonardi[1], M. Schubert[1], and L. Orlando*[1,2], [1]*University of Copenhagen, Centre for GeoGenetics, Natural History Museum of Denmark, Copenhagen, Denmark;* [2]*Université de Toulouse, University Paul Sabatier (UPS), Laboratoire AMIS, CNRS UMR 5288, Toulouse, France.*

The domestication of the horse in the Pontic-Caspian steppes some 6,000 years ago represents one major turning point in human history. With horses, humans could travel for the first time well above their own speed and carry their germs, culture and genes across vast geographic areas. The development of horse-drawn chariots and cavalry also radically changed the history of warfare and was instrumental to the emergence of transcontinental empires. Additionally, beyond the battlefield, farm horses have massively impacted agricultural productivity. The biological changes that accompanied the process of horse domestication are, however, difficult to reconstruct from current patterns of genetic diversity both due to the development of intensively selected and extremely influential breeds during the last two centuries, and the almost extinction of wild horses. Recent developments in ancient DNA research have opened for the characterisation of complete genomes, epigenomes and microbiota over long time series. We have applied such approaches to a large panel of horse remains spread across Eurasia and dated to 44,000–200 years ago. This started revealing the genetic structure of horse populations before and during early domestication stages as well as the history of genetic changes that accompanied their further transformation in a range of cultural contexts. I will present our latest progress made on an extensive dataset of ancient horse genomes spanning the whole domestication temporal and geographical range.

Key Words: ancient DNA, horse, domestication

MT236 Genome-wide assessment of genetic diversity in the Synbreed Chicken Diversity Panel. S. Weigend*[1], A. Weigend[1], D. Malomane[2], and H. Simianer[2], [1]*Friedrich-Loeffler-Institut, Institute of Farm Animal Genetics, Neustadt-Mariensee, Höltystraße, Germany;* [2]*University of Göttingen, Animal Breeding and Genetics Group, Göttingen, Albrecht-Thaer-Weg 3, Germany.*

Genetic diversity within a given farm animal species refers to the variety of genetic variants accumulated during domestication. High-density SNP genotyping arrays allow genome-wide assessment of structural variation between genomes of individuals, families and populations. Within the framework of the SYNBREED project a wide range of chicken breeds were sampled in 34 countries across 4 continents. Sampling was supported by a world-wide collaborative effort including 21 partners from 17 countries (Argentina, Australia, Bangladesh, Chile, Egypt, Ethopia, Finnland, Germany, Hungary, Pakistan, Saudi Arabia, Sudan, Switzerland, Tanzania, Turkey, United Kingdom, and Vietnam). It was augmented by samples of two Red Junglefowl populations (*Gallus gallus gallus* and *Gallus gallus spadiceus*) as well as 12 commercial purebred chicken lines (brown layers, white layers, broilers) and 10 local chicken breeds taken from the previous EU project AVIANDIV. This 'Synbreed Chicken Diversity Panel (SCDP)', which encompasses more than 3200 individuals of 175 populations, was genotyped with the 580K SNP Affymetrix Chicken Genotyping array. First cluster analyses showed a continuous transition from Asian type breeds to European breeds as well as a separation of breeds according to body size, i.e. normal sized breeds and bantam breeds. Wild populations as well as commercial broiler lines cluster within this spectrum of diversity, whereas commercial white and brown egg layer lines formed distinct and rather opposite edges of it. Chicken populations from Africa and Asia showed a lower proportion of genomic regions in Runs of Homozygosity (ROH), while chicken populations sampled in Europe displayed a wide variation ranging from 4 to ~70 percent of the genome being included in ROH. Regarding the commercial lines, the genome of white layer lines was least polymorphic, while broiler lines clustered at the polymorphic end of the SCDP spectrum. Brown egg layers showed a medium degree of variability. Detailed analyses will evaluate the extent and distribution of variation, the extent of linkage disequilibrium and the distribution of ROH across chromosomes. The SCDP is an excellent resource to get insight into mechanisms underlying the diversification within the species.

Key Words: chickens, biodiversity, SNP markers

Microbiomes

MT237 16SrRNA amplicon sequencing of mock microbial populations to investigate DNA extraction methodology, primer selection and PCR cycles. E. McGovern*[1,2], M. S. McCabe[1], A. K. Kelly[2], D. A. Kenny[1], P. Cormican[1], and S. M. Waters[1], [1]*Teagasc, Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre, Grange, Dunsa-*

ny, County Meath, Ireland; [2]University College Dublin, School of Agriculture and Food Science, Belfield, Dublin, Ireland.

Elucidating the composition, adaptation, and function of the rumen microbiome is of international interest due to its implications in climatology and applied animal production. Despite extensive use of 16SrRNA amplicon sequencing for rumen microbial phylogenetic analysis, it may not be generating an accurate representation of constituent microbial communities in samples due to inefficiencies/biases in DNA extraction method, primer selection and/or PCR amplification. The objective of this study was to assess these factors in relation to selected currently established methods for 16S phylogenetic community analysis on a microbial community standard (MS) and a DNA standard (DS) (ZymoBIOMICS). DNA was extracted from MS, and rumen solid digesta sample as a positive control (PC), using the repeated bead beating and column (RBB+C) method. 16S rRNA amplicon libraries were generated for MS, PC, DS and a negative control, using both 515F/806R and Pro341F/Pro805R designed by Caparaso *et al*. and Takahashi *et al*. respectively and subjected to both 20 and 28 PCR cycles under identical cycle conditions. Sequencing was conducted using the Illumina MiSeq platform. A high-throughput BLAST search against a NCBI 16S database was performed. Linear regression analysis of the 8 bacterial species present in MS amplified using 515F/806R and Pro341F/Pro805R showed the relative abundance tended towards the theoretical composition of MS ($P > 0.1$), indicating that the protocol is suitable for DNA extraction gram positive bacteria. The relative bacterial abundances from 515F/806R and Pro341F/Pro805R were comparable across each sample type ($r^2 = 0.9$). The relative abundance of bacteria amplified from DS with 515F/806R at 20 PCR cycles, showed a correlation with its theoretical composition ($P > 0.01$), indicating that 515F/806R are sufficient for accurate determination of microbial communities. Communities amplified with 20 PCR cycles resulted in a higher correlation to expected mock community composition than samples generated with 28 PCR cycles. In conclusion, using the RBB+C method for DNA extraction, 515F/806R primers to target the 16SrRNA gene using 20 PCR cycles was sufficient for amplicon sequencing to generate relatively accurate depiction of the bacterial communities present in rumen samples.

Key Words: next-generation sequencing, mock communities, 16SrRNA, DNA extraction, microbiota

MT238 Characterization of the gut microbiome along the digestive tract of Iberian pigs. D. Crespo-Piazuelo*[1,2], J. Estellé[3], M. Revilla[1,2], L. Criado-Mesas[1,2], Y. Ramayo-Caldas[3], C. Óvilo[4], A. I. Fernández[4], M. Ballester[5], and J. M. Folch[1,2], [1]*Plant and Animal Genomics, Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB Consortium, Bellaterra, Barcelona, Spain;* [2]*Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain;* [3]*Génétique Animale et Biologie Intégrative (GABI), Institut National de la Recherche Agronomique (INRA), AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [4]*Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain;* [5]*Departament de Genètica i Millora Animal, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Caldes de Montbui, Barcelona, Spain.*

The Iberian pig is a rustic animal with high intramuscular fat content which is relevant for the production of cured products like ham. Recent studies have found that pig lipid metabolism may be modified by the gut microbiome through gene expression regulation. Therefore, the aim of this study was to describe the differences of the microbiome found along the Iberian pig gut and evaluate their possible role in the whole-body energetic homeostasis. DNA was extracted from luminal content of five gut sections (duodenum, jejunum, ileum, and proximal and distal colon) of 13 120-day-old Iberian pigs with *PowerFecal* (*MoBio*) kit and the region V3-V4 of the 16S rRNA gene was sequenced in a *MiSeq* (*Illumina*) instrument. With *QIIME* pipeline, a total of 1,669 operational taxonomic units (OTUs) distributed in 179 genera were found, from which 643 were new regarding the *GreenGenes* 13.8 database. *Lactobacillus* and *Clostridium* spp. were the two most abundant genera in the small intestine, while *Prevotella* spp. was predominant in colon. Diversity studies were made with *vegan* R package showing that the α diversity was increasing whereas the β diversity was decreasing while advancing through the gut. The OTUs presence/absence analysis was carried out with *metagenomeSeq* R package using a model where the animal was included as co-factor and with a FDR ≤ 0.01 cut-off. From the total 1,669 OTUs, 946 were absent in the small intestine sections while 325 were not present in the large intestine. Metagenome KEGG Orthologies (KOs) were predicted with *PICRUSt* software. The differences in abundance of these KOs were pointed out by *DESEqn 2* R package. Due to the abundance of the previous genera, one of the most relevant pathways found in the small intestine was the phosphotransferase system while the dicarboxylate/4-hydroxybutyrate was most important in the large intestine. In summary, this study confirms that the energy pathways of the gut microbiome are different along its sections, and besides, these results represent, to our knowledge, the first description of the gut microbiota composition along the intestine in Iberian pigs.

Key Words: Iberian pig, gut microbiome, 16S rRNA, metagenome prediction, OTUs

MT239 Host genetics influences gut microbiota composition in pigs. J. Estellé*[1], N. Mach[1,2], Y. Ramayo-Caldas[1], F. Levenez[2], G. Lemonnier[1], C. Denis[1], M. Berri[3], M.-J. Mercat[4], Y. Billon[5], J. Doré[2], C. Larzul[1,6], P. Lepage[2], and C. Rogel-Gaillard[1], [1]*GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [2]*MICALIS, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [3]*ISP, INRA, Université de Tours, Nouzilly, France;* [4]*IFIP-BIOPORC, Pôle génétique, Le Rheu, France;* [5]*GENESI, INRA, Surgères, France;* [6]*GenPhySe, INRA, INP, ENSAT, Université de Toulouse, Castanet-Tolosan, France.*

Microbiomes and their effects on hosts have emerged as outstanding factors to take into account in livestock production. Despite the well acknowledged impact of maternal colonization and environmental factors for driving the gut microbiota composition, the genetics of the host is also likely to play a role. In this study, we aimed at studying the interplay between host genetics and variations of gut microbiota composition in pigs. A cohort of 518French Large White 60-day-old piglets was scored for faecal microbiota composition by sequencing the 16S rRNA bacterial gene, and genotyped with the Illumina PorcineSNP60 DNA chip. The relative abundances of operational taxonomic units (OTUs) and bacterial genera were obtained by using the Qiime package. Genetic parameters were estimated for a set of 63 bacterial genera present in the gut microbiota of pigs included in the study. Results showed that heritability was low ($0.1 < h^2 < 0.2$) for seven genera, medium ($0.2 < h^2 < 0.4$) for 15 genera, and high ($h^2 > 0.4$) for eight genera. Positive and negative genetic correlations were found between the relative abundances of various bacterial genera, with *Prevotella*, *Oribacterium*, *Selenomonas*, *Dialister* and *Megasphaera* genera being positively correlated. Genome-wide association studies (GWAS) revealed significant associations between genomic regions and relative abundances of *Flexispira*, *Megasphaera*, *Mitsuokella* or *Streptococcus* genera. GWAS uncovered also additional genomic regions associated with variations in OTU abundances. In conclusion, our results provide new evidences that the gut microbiota composition is influenced by host genetics. We anticipate that genetic approaches will provide complementary strategies to nutrition solutions able to modulate gut

microbiota composition for shaping production and health phenotypes relevant for promoting sustainable farm systems.

Key Words: pigs and related species, microbiomics, heritability, genome-wide association

MT240 Advanced bioinformatics and molecular analysis of whole-genome-shotgun metagenomics data from rumen microbiomes reveals remarkable diversity, structure and function. M. Watson*[1], R. Stewart[1], A. Warr[1], T. Snelling[3], M. Auffret[2], A. Walker[3], R. Wallace[3], and R. Roehe[2], [1]*The Roslin Institute, University of Edinburgh, Easter Bush, Scotland;* [2]*SRUC, Easter Bush, Scotland;* [3]*The Rowett Institute, University of Aberdeen, Aberdeen, Scotland.*

Rumens contain highly complex microbial communities, which are crucially important for the growth, development and health of the host animals. Despite huge numbers of microbial genomes becoming publicly available, they are often from model organisms and are not relevant for the interpretation of rumen microbiomes. As a result, metagenomics software tools based on public data can be misleading or simply do not work when applied to ruminants. We have applied several standard and custom molecular and bioinformatics techniques to help better understand whole-genome-shotgun sequencing data from rumen microbiomes, including (but not limited to) deep Illumina sequencing, long-read nanopore sequencing and rumen-specific classification databases. The results of our work show remarkable diversity, structure and function within rumen microbiomes and demonstrate that we have much to discover from these rich and diverse microbial communities

Key Words: rumen, microbiome, genomics, bioinformatics

MT241 The MetaPig project: Leveraging potentials in pig genomics and metagenomics to boost feed efficiency and gut health in modern pig production. P. Karlskov-Mortensen*[1], A. Ø. Pedersen[1], N. Canibe[2], P. Kiilerich[3], K. Kristiansen[3], and M. Fredholm[1], [1]*Department of Veterinary and Animal Science, Faculty of Health & Medical Sciences, University of Copenhagen, Frederiksberg, Denmark;* [2]*Department of Animal Science, Aarhus University, Tjele, Denmark;* [3]*Department of Biology, Faculty of Science, University of Copenhagen, Copenhagen, Denmark.*

Feed efficiency is a trait of great importance for profitability and environmental sustainability in modern pig production. Hence, it is of utmost importance to elucidate and understand all the complex factors and mechanisms involved in nutrient absorption and growth. In this project we take a holistic approach with the aim to characterise individual and combined effects of the host genome, metagenome, foodstuff composition and feed additives on feed efficiency and gut health. The project is divided into a discovery and a verification phase. In the first phase, the direct effect of feed formulation, feed additives and host genome on microbiota composition and subsequent effect on feed efficiency and gut health will be characterised in a total of 900 pigs. This phase includes genotyping of 700K SNP in 400 pigs and collection of gut epithelium for RNA sequencing and/or Fluidigm qPCR. Additionally, individual gut microbiota composition will be characterised using metagenome sequencing and/or 16S RNA gene sequencing. Together, the assembled data allow for an integrated analysis of the interplay between host genome, host transcriptome, microbiome, feed formulations and additives on gut health and feed efficiency. The analyses include QTL and eQTL mapping, transcriptome profiling and gene co-expression network analyses to identify major signalling pathways and host genes of key importance for microbiota composition, feed efficiency and gut health. Based on discoveries in phase one, up to four different feeding regimes including pre- and/or probiotic feed additives will be designed for the verification phase. In this phase, each of four diets will be tested in 1000 pigs to evaluate and verify host response and confirm positive effects on feed efficiency and gut health. We here present some preliminary results and outline perspectives for this line of investigations regarding feed efficiency and gut health for future pig production.

Key Words: pig, GWAS, RNAseq, metagenomics, systems biology

MT242 Bovine genes regulate the rumen microbial composition. O. Gonzalez-Recio*[1], I. Zubiria[2], A. García-Rodriguez[2], A. Hurtado[3], and R. Atxaerandio[2], [1]*Departamento de Mejora Genética Animal. Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain;* [2]*Departamento de Producción Animal. NEIKER-Tecnalia. Granja Modelo de Arkaute, Vitoria-Gasteiz, Alava, Spain;* [3]*Departmento de Salud Animal. NEIKER-Tecnalia, Derio, Bilbao, Spain.*

The rumen microbiota plays an important role during feed digestion, and influences relevant traits like feed efficiency and methane yield. The microbiota composition is partially regulated by the host, and is associated to rumen conformotion and phisiology, feed transit speed and eating behaviour. All this characteristics are also regulated at the host genetic level. Hence, we hypothesise that the rumen microbiome is also regulated at the host genetic level. Sequencing technoligies allow to determine the taxonomy of the microbiome with a rather satisfactory depth. This study reveals certain genetic control on the microbiome composition of the rumen in cattle. In total, 13 genera were analysed for bacteria (5), archaea (1), and ciliates (7) in cattle rumen from 16S and 18S rRNA gene-based analyses. All these bacteria and archaea genera showed association to the host genetic background both for breed and SNP markers. *Butyrivibrio* and *Ruminococcus* genus showed association with the SNP markers but not with the breed composition. The breed composition had a significant effect on *Isotricha*, *Ophryoscolex* and *Polyplastron* genus, and the SNP markers on *Entodinium*, *Ophryoscolex* and *Polyplastron*. In total, 77% (10/13) of microbes analysed showed to be associated to the host genetic background. This study also evaluated statistical association between candidate genomic regions and the relative abundance of these microbes. Then, DGAT1, ACSF3, AGPAT3, STC2 genes showed to be associated to the relative abundance of *Prevotella* genus with a false discovery rate lower than 15%. We showed some evidences for a host genetic control of the microbiome in cattle.

Key Words: genomic, breed, microbiome, NGS

Pig Genetics and Genomics

MT243 Quantitative trait loci (QTL) mapping of growth and conformation traits in F_2 intercross progenies of Nigerian indigenous and Large white pigs. V. M. O. Okoro*, C. L. Okoro, and C. A. Mbajiorgu, *University of South Africa, Johannesburg, Gauteng, South Africa.*

Microsatellite genetic markers were used to map the chromosomal quantitative trait loci (QTL) regions for growth and conformation traits in F_2 progenies of Nigerian indigenous and Large white pig crosses. Three growth traits of birthweight (BW, n = 172), weaning weight at 5 weeks (WWT5, n = 164) and bodyweight at 20 weeks (BWT20, n = 156), as well as 12 conformation traits of body length (BL, n = 164, 160 and 156), height at withers (HW, n = 164, 160 and 156), ear length (EL, n = 164, 160 and 156) and chest circumference (CC, n = 164, 160 and 156) at 5, 10 and 20 weeks of

age respectively of the Grandparents (GP) (n = 12), parents (P) (n = 48) and F_2 progenies were taken. The GP, P and F_2 individuals were genotyped using 29 Microsatellite markers across chromosome 1 to 10 of pigs. A Mendelian and multiple QTL models were fitted to analyse and identify QTLs. Haley-Knot regression standard interval mapping for Mendelian QTL identification was used to identify single QTLs, while multiple chromosome scan with interacting covariates was used to identify multiple QTLs. Using permutation tests at 1000 permutations, significant thresholds were set. QTLs were detected at $\alpha = 0.05$, and 0.10 thresholds on chromosomes 1 to 10. Using Mendelian scan, two significant QTLs were detected for the BW around SW2410, S0005 and IGF1 while at BW20; three significant QTLs were found around IGF1, SW1067 and S0228 respectively. Also on conformation traits, significant QTLs were detected only on the body length at birth and ear length at birth traits respectively. Significant QTLs were detected around SSC 2 and 8 respectively, and precisely around markers SW1828, SW240, S0226 and SW72 on SSC 2 and S0101, SW2410, and S0178 on SSC 8. However, using multiple genome wide scan, significant QTLs were detected for bodyweight traits around SSCs 2, 5, and 8 respectively. However, significant QTLs were detected for 6 conformation traits when multiple genome wide scan was used. Generally, at 5% significance of chromosome wide scan, 4 QTLs were detected for growth traits at SSCs 2, 5, 6, and 8, while 5 QTLs were detected for conformation traits at SSCs 2, 3, 5, 6, and 8.

Key Words: microsatellite markers, QTL, Mendelian model, chromosome wide scan, regression

MT244 Convergent and divergent genetic changes in Chinese and European pig domestication. J. Wang*, H. Zou, N. Li, X. Hu, Y. Zhao, and Y. Zhao, *China Agricultural University, Beijing, China.*

Since 10,000 BC, continuous human selection has led to intense genetic and phenotypic changes in pig (*Sus scrofa*) domestication. Through whole genome analysis of 263 individuals, we demonstrated artificial unidirectional and bidirectional selection as the primary force to shape the convergent and divergent changes between Chinese domestic pigs (CHD) and European domestic pigs (EUD). We identified 42 genes in unidirectional selection regions that might be related to fundamental domestication requirements in pigs. And these genes belong predominantly to categories related to the nervous system, muscle development, and especially to metabolic diseases. In addition, 48 genes, representing different breeding preference, were found under bidirectional selection for the distinct leanness and reproduction traits between CHD and EUD. The convergent genetic changes, contributing physical and morphological adaption, represent the common concerns on pig domestication. And the divergent genetic changes reflect distinct breeding goals between Chinese and European pigs. Using *ITPR3, AHR* and *NMU* as examples, we explored and validated how the genetic variations contribute to the phenotype changes.

Key Words: pig domestication, convergent genetic changes, divergent genetic changes

MT245 Identification and prioritization of SNPs potentially involved in transcriptomic and phenotypic differences between pure and crossbred Iberian pigs. M. Ayuso*[1,2], J. Garrayo[3], A. Fernandez[3], Y. Nuñez[3], R. Benitez[3], C. Garcia-Contreras[3], M. Vazquez-Gomez[1], B. Isabel[1], A. Fernandez[3], A. Rey[1], A. Golzalez-Bulnes[4], C. Lopez-Bote[1], and C. Ovilo[3], [1]*Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain;* [2]*Applied Veterinary Morphology, Department of Veterinary Sciences, University of Antwerp, Wilrijk, Antwerp, Belgium;* [3]*Departamento de Mejora Genética Animal, INIA, Madrid, Spain;* [4]*Comparative Physiology Lab SGIT-INIA, Madrid, Spain.*

Iberian pig production is based on both purebred Iberian (IB) and crossbred Duroc X Iberian (DUxIB) pigs. These two genetic types show important differences in growth, fattening and tissue composition. This study was conducted to identify structural genetic variants in IB and DUxIB using muscle RNA-Seq data from 24 pigs (12 IB and 12 DUxIB). Two methodologies were used: a commercial (CLC genomics) and a freely available (SAMtools) software. The analysis was performed in a set of 49 selected candidate genes and transcription factors of interest, which were prioritized from a previous study that assessed *Biceps femoris* and *Longissimus dorsi* muscles transcriptome, based on differential expression and functional criteria. The comparison between software revealed a similar number of detected variants: 548 and 527 variants were identified in IB and DUxIB after the CLC software analysis, whereas SAMtools identified 480 and 531 variants in IB and DUxIB, respectively. However, the number of variants jointly identified by both software is remarkably low: only four in the IB and three in the DUxIB group. We prioritized the most interesting variants of the whole set of results from a functional point of view (i.e. those with higher probability to be involved in the phenotypic differences between IB and DUxIB). For this end, a series of filters were applied to the identified variants. Only single nucleotide polymorphisms (SNPs) that were segregating (frequency 20–80%) in the DUxIB group, produced a non-synonymous mutation and were far enough to be considered as non-co-segregating SNPs were chosen. After filtering, a total of 100 SNPs remained. Those SNPs were found in genes related to adipose and muscle tissue development, such as *ADAMTS8*, *CREBBP* or *MYOD1*. To further investigate these SNPs, a genotyping chip has been constructed to perform an association study in a commercial DUxIB population. The screening strategies reported in the present study, allowed the selection of a reduced subset of potentially relevant SNPs. We believe this is a suitable approach to extract the most relevant structural information in RNA-Seq studies.

Key Words: pig, RNA-Seq, bioinformatics, polymorphism, meat production

MT246 Investigating the genomic basis of pigs that have died in transit. F. Bertolini*[1], K. Zurbrigg[2], T. van Dreumel[2], T. O'Sullivan[2], and M. F. Rothschild[1], [1]*Department of Animal Science, Iowa State University, Ames, IA, USA;* [2]*University of Guelph, Guelph, Ontario, Canada.*

The death of market pigs during transportation is both a welfare and economic concern for the pig industry. One hypothesis suggested is that these deaths result from pre-existing cardiac problems. A total of 510 commercial pigs were sampled during 2013–2014. Several phenotypes were collected, including dead/survived during the transport (ITLYN), presence/absence of dilation of one or both of the atrial chambers (ATRIALYN) and presence/absence of a dilated aorta and pulmonary artery (AORTAYN). For each of these phenotypes two independent analyses were performed: 1) Fst analyses considering single SNPs and 500Kb overlapping windows. The top 15 SNPs/windows of each analysis were investigated by examining annotated genes within the selected windows or nearby the single SNPs (± 100Kb) and the genes that could have a direct or indirect connection with heart development/disease and 2) RNA-seq differential expression analysis from left ventricle of 15 random samples (ITLYN = 9 *v.* 6 animals, ATRIALYN = 5 *v.* 10 animals, AORTAYN = 6 *v.* 9 animals). The reads of each animal were aligned using Tophat2/bowtie software. For each animal, the raw count of aligned reads in all the genes was performed through HTseq software and differential expression of each group of animals was performed with the DesEqn 2 R-package, where adjusted P values were calculated. Then, only the 20 genes identified with Fst were considered using significant (< 0.05) or moderate (< 0.1) differences in

expression. For the ITLYN comparison, 4 genes showed significant difference while 6 showed moderate difference; for the AORTAYN, 5 genes showed significant difference while 4 showed moderate differences; for the ATRIALYN, 0 of the 20 genes were detected as differentially expressed. Among the differentially expressed genes in ITLYN and AORTAYN, six were common between the two comparisons. Four of these six genes are up-regulated during cardiomyocyte stresses or pressure overload and during incidents of acute stress. The comparison identified some genes that are involved with vascular pathogenesis or activated during cellular stress. Analysis of pig sudden death combined with comparative research may provide further understanding of transit losses.

Key Words: cardiac defects, transit loss, pig

MT247 GWAS on coat color in wuzhishan pig using the Porcine SNP80 BeadChip. X. Qiao* and F. Meiying, *China Agricultural University, Beijing, Beijing, China.*

Long-term selection breeding has resulted in diverse coat colour phenotypes in Chinese indigenous breeds, including solid black, white, two-end black, belted and spotted several different types. Normally, all pigs share the same coat colour as breed character in one breed. However, variable colours are allowed within one pig breed for different selection purpose by artificial selection, for example, in Chinese Wuzhishan pig breed, which segregates into white, black with white belly, and black coat colour three different coat colours for specific aims. Therefore, the objective of this study was to investigate the candidate genes that affect coat colour variant in Wuzhishan pig breed by GWAS using Illumina Porcine SNP80 BeadChip. A total of 215 Wuzhishan pig samples were sampled from Hainan province of China, including white (n = 61), black with white belly (n = 96) and black (n = 58) coat colour Wuzhishan pig. Genotyping was conducted using the Illumina Porcine SNP80 BeadChip. After filtering, alignment and SNPs calling, we obtained 40345 high quality SNPs for GWAS. Our results showed that KIT, RAPGEF2 and PDGFRA as the candidate genes, might be responsible for the coat colour phenotypes in Wuzhishan population through the haplotype analysis, gene ontology analysis and literature research. The verification results revealed that white coat colour caused by the KIT mutations in the Wuzhishan pig popular, raising the possibility that there is blood relationship between white coat colour Wuzhishan pig and overseas white coat colour breeds (Yorkshire or Landrace). The expression levels of RAPGEF2 and PDGFRA were significantly higher in black skin than in white skin from three black with white belly Wuzhishan pigs indicated that the regulation of melanogenesis might be caused by the RAPGEF2 and PDGFRA, result in coat colour phenotypes in black with white belly Wuzhishan popular. The development of hair follicles and melanocytes is a complex event involving numerous genes and pathways that interact and show cross-talk. Our GWAS and validation results revealed that the KIT mutations lead to white coat colour phenotypes of Wuzhishan popular, RAPGEF2 and PDGFRA might be new candidate genes responsible for white coat colour loci of Chinese indigenous pig breeds.

Key Words: GWAS, coat color, Wuzhishan pig, SNPs

MT248 Genetic polymorphism of candidate genes in SSC13q41 region affecting Indian pigs differentially adhesive to diarrhoeagenic *E.coli*. N. R. Sahoo*[1], R. Sinha[1,2], C. Rawat[1], W. SS[1], P. Kumar[1], S. Qureshi[1], A. Kumar[1], S. Kumar[1], and B. Bhushan[1], [1]*ICAR-Indian Veterinary Research Institute, Izatnagar, UP, India;* [2]*ICAR-National Dairy Research Institute, Karnal, Haryana, India.*

We isolated the diarrhoeagenic *E. coli* from diarrheic piglets, identified using biochemical and molecular means by amplification with sequencing of 16s rRNA. A total of 450 fecal samples of diarrheic piglets were screened and 60 isolates of *E. coli* were subjected to PCR based typing of F4 fimbria which revealed absence of F_4 (K^{88}) fimbria. Phenotyping of 150 local pigs was done by Microscopic Adhesion Assay using this isolated strain which revealed that 4% (6) strongly adhesive, 58% (87) adhesive, 15.33% (23) weakly adhesive and 22.66% (34) were non-adhesive. Nonsignificant effect of non-genetic factors was observed on adhesion pattern. To study effect of genetic factors, a total of 25 SNPs located on SSC13q41 genomic region were targeted on the basis of earlier reports on ETEC-F4ab/ac association covering 5 genes (MUC4, MUC13, MUC20, TFRC and ACK1). The PCR-RFLP of native pigs revealed that only 8 of 25 SNPs were under HWE. The association study by logistic regression model of SAS 9.3 software revealed that AG genotype of SNP g.22304 MUC 13, TT genotype of SNP g.191 MUC 20, CC genotype of SNP g.8227 MUC 4 and CA genotype of g.107371 ACK1 were found to be associated with non adhesive pattern of *E. coli.* However, while studying the effect of alleles on adhesion pattern it was found that proportion of C allele of SNP g.8227 MUC 4, C allele of SNP g.13383 MUC 4 and T allele of SNP g.191MUC 20 were more in non-adhesive phenotypes. The linkage analysis revealed that, the significantly associated loci were linked with 6 other loci (g.22124T>C, rs81218930 C>T, g.101030382 T>C, g.93222 C>A, g.101030382 T>C and g. 291 C >T) which were otherwise not associated with adhesion pattern. The presence of non-adhesive phenotypes along with significantly associated SNPs in SSC13q41 genomic region speculates the scope of breeding programme for *E. coli* mediated diarrhoea resistance in native pigs. The SNPs (g.8227 G>C MUC 4 and g.191 *MUC 20*) previously reported to be associated with the susceptibility to ETEC F4ab/ac, however, in native pigs was also found to be associated with the diarrhoeagenic *E. coli* adhesion pattern which was not ETECF4.

Key Words: native pigs, *E. coli* mediated diarrhea, MAT, polymorphism, association

MT249 Genome-wide analysis reveals important contribution of structure variants to environmental adaptations and artificial selections in Chinese pigs. R. Yang*[1,2], S. Fang[2], J. Wang[2], C. Zhang[2], R. Zhang[2], D. Liu[4], Y. Zhao[1,2], X. Hu[2,3], and N. Li[2], [1]*Beijing Advanced Innovation Center for Food Nutrition and Human Health, China Agricultural University, Beijing, China;* [2]*State Key Laboratory of Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing, China;* [3]*National Engineering Laboratory for Animal Breeding, China Agricultural University, Beijing, China;* [4]*Institute of Animal Industry, Heilongjiang Academy of Agricultural Sciences, Harbin, Heilongjiang, China.*

Domestic pigs have experienced long-term selections during domestication, resulting in dramatic phenotypic changes. Structure variants (SVs) are reported to exert extensive impacts on phenotypic changes. Here we built a high resolution and informative SV map based on high-depth sequencing data from 66 Chinese domestic and wild pigs. We inferred the SV formation mechanisms in the pig genome and used SVs as materials to perform a population-level analysis. We detected the selection signals on chromosome X for northern Chinese domestic pigs, as well as the differentiated loci across the whole genome. Analysis showed that these loci are associated with environmental adaptations or artificial selections between southern and northern Chinese domestic pigs. Our study reveals important contribution of SVs to the domestication of Chinese pigs, especially for the deleted discrepancy of potentially regulatory elements, which has not been extensively addressed before.

Key Words: pigs and related species, animal breeding, animal domestication

MT250 Transcriptome analysis reveals long intergentic noncoding RNAs involved in skeletal muscle growth and development in pig. C. Zou*, J. Li, W. Luo, L. Li, A. Hu, Y. Fu, Y.

Hou, and C. Li, *College of Animal Science, Huazhong Agricultural University, Wuhan, Hubei province, China.*

Long intergenic noncoding RNAs (lincRNAs) play essential roles in numerous biological processes and have been widely studied in recent years. Skeletal muscle is an important tissue which plays an essential role in individual movement ability. While lincRNAs in pig skeletal muscles are largely undiscovered and their biological functions remain elusive. In this study, we assembled the transcriptomes using published RNA-seq data in previous studies of our laboratory, and identified 323 lincRNAs in porcine leg muscle. We found these lincRNAs have shorter transcript length, fewer exons and lower expression level compared with protein-coding genes. Gene ontology and pathway analysis indicated that many potential target genes (PTGs) of lincRNAs were involved in skeletal muscle related processes such as muscle contraction and muscle system process. Combined our previous studies, we found a potential regulatory mechanism that promoter methylation of lincRNAs could negatively regulate its expression and then positively regulate PTGs expression, which could finally result in the abnormal phenotypes of cloned piglets by a certain unknown way. This work characters several lincRNAs and their target genes that are involved in skeletal muscle growth and development and facilitates future studies on their roles in skeletal muscle growth and development.

Key Words: pigs, epigenomics, RNA-seq, muscle, genomic prediction

MT251 pigFit—Genetic analysis of immunological competence, survivability and postnatal growth of piglets. M. J. Pröll*[1], E. Heuß[1], C. Große-Brinkhaus[1], H. Henne[2], A. K. Appel[2], K. Schellander[1], and E. Tholen[1], [1]*Institute of Animal Science, University of Bonn, Bonn, Germany;* [2]*BHZP GmbH, Dahlenburg-Ellringen, Germany.*

Piglet mortality has a negative impact on animal welfare, public acceptance and decreases the subsequent viability of pig performance. Moreover, the economic viability of piglet producers is determined by the piglet survival rate. This situation is intensified by an increasing litter size which influences negatively birthweight and piglet survival. In this context, the 'pigFit'-project aims to improve the survivability, health and immune status of piglets and of growing pigs in the maternal lines Landrace (LR) and Large White (LW). The objectives of this study were to evaluate a breeding-based improvement of health traits and survival of piglets and growing pigs through immune profiling and genomic selection. Datasets of LR and LW populations were provided by a German breeding organisation. All piglets born dead or alive were individually identified and weighted immediately after birth. Blood samples of sows (N = 500) and piglets born alive (N = 1000) were collected after birth and post weaning, respectively. Complete blood count (CBC) was performed with a hematology analyzer and serum haptoglobin was measured by classic photometric method. In addition, cytokine levels (IL-1β, IL-6, IL-4, IL-8, IL-12, IL-10, TNF-α, and IFN-γ) of sows and piglets were quantified by using 'Porcine Cytokine/Chemokine Multiplex Magnetic Bead 8-plex Panel'. In a first step heritabilities of all immune traits were estimated in dams and their piglets by using an animal model comprising relevant fixed effects. Estimates of heritabilities were weakly/moderately for lymphocytes and neutrophils for piglets and moderately for sows. Several cytokines showed weak to moderate heritability, including pro-inflammatory cytokines (IL-1β, TNF-α). Based on these insights, genomic methods will be applied, aiming to improve the vitality and the robustness of piglets by breeding.

Key Words: piglet mortality, immune profiling, heritability, lymphocytes, pro-inflammatory cytokines

MT252 An integrative gene network analysis of the genetic determination of pig fatty-acid composition based on adipose tissue RNA sequencing. M. Revilla*[1,2], D. Crespo-Piazuelo[1,2], A. Rau[3], Y. Ramayo-Caldas[3], J. Estellé[3], A. Castelló[1,2], A. I. Fernández[4], M. Ballester[5], and J. M. Folch[1,2], [1]*Plant and Animal Genomics, Centre de Recerca en Agrigenòmica (CRAG), Consorci CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, Barcelona, Spain;* [2]*Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain;* [3]*Génétique Animale et Biologie Intégrative (GABI), Institut National de la Recherche Agronomique (INRA), AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [4]*Departamento de Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain;* [5]*Departament de Genètica i Millora Animal, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Torre Marimon, Caldes de Montbui, Barcelona, Spain.*

An increasing number of studies in swine now focus on fat content and fatty-acid composition due to their effect on the nutritional quality of meat. Meat-quality traits are essential for end-consumer acceptance, and as a result, these traits have been the focus of many studies. High-throughput assays of gene expression in relevant tissues for lipid metabolism provide valuable information to better understand the regulation of lipid metabolism and increase our knowledge of the molecular mechanisms that play a role in meat-quality traits. The aim of this work is to identify porcine adipose tissue gene co-expression networks, pathways, and transcriptional regulators associated with fatty-acid composition using RNA-Seq data. Thirty-six animals from three different backgrounds were selected for adipose tissue RNA-Seq analysis. Samples were sequenced using a *Hi-SEqn 2000* platform (Illumina). *STAR* program was used to align the reads and *HTSeq* to quantify them. A Weighted Gene Co-expression Network Analysis (WGCNA) was applied to detect clusters of highly co-expressed genes (modules). Functional annotation and enrichment analyses of the identified modules were performed with *GeneTrail2*, and *Cytoscape* was used to perform network visualisation. The WGCNA analysis revealed six modules that were strongly correlated with at least one fatty-acid measured in adipose tissue (correlations ranging from -0.71 to 0.70, $P < 0.05$). Functional annotation identified one of these modules as being strongly associated with lipid metabolism pathways. This module was also characterised by a close association with stearic (C18:0) and arachidonic (C20:4(n-6)) fatty acids. The network visualisation revealed several potential regulatory genes (*ABHD5, ACAD8, ACOX1, ACSL1, FITM2, GPAT4*, and *HADHB*) that were highly correlated hubs within their modules, providing a better understanding of the complex transcriptional regulation of lipid metabolism. This study revealed biologically relevant networks and candidate regulatory genes of fatty-acid composition through a systems biology approach, confirming the complexity of the regulation of meat-quality traits.

Key Words: pigs, systems biology, network analysis, fat/lipid, gene expression

MT253 Identification of key expression regulators of candidate genes for fatty acid composition in pig muscle. L. Criado-Mesas*[1,2], M. Revilla[1,2], D. Crespo-Piazuelo[1,2], A. Castelló[1,2], A. I. Fernández[3], M. Ballester[4], and J. M. Folch[1,2], [1]*Plant and Animal Genomics, Centre de Recerca en Agrigenòmica (CRAG), Consorci CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, Barcelona, Spain;* [2]*Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain;* [3]*Departamento de Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain;* [4]*Departament de Genètica i Millora*

Animal, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Torre Marimon, Caldes de Montbui, Barcelona, Spain.

Intramuscular fat content and its fatty acid composition are involved in porcine meat quality and differ according to the genetic background. The detection of quantitative trait loci associated with gene expression levels (eQTLs) has been proposed as a suitable approach to identify candidate genes affecting quantitative complex traits. The objective of this work was to study and validate the genetic basis of the expression of genes involved in lipid metabolism in the porcine muscle *(Longissimus dorsi)* in three different experimental backcrosses: BC1_LD (F1 (Iberian males × Landrace females) × Landrace females), BC1_DU (F1 (Iberian males × Duroc females) × Duroc females) and BC1_PI (F1 (Iberian males × Pietrain females) × Pietrain females). The expression of 45 genes was analysed in a total of 364 animals: 114 from BC1_LD, 126 from BC1_DU and 124 from BC1_PI. An expression genome-wide association study (eGWAS) was performed with the muscle gene expression values, measured by real-time quantitative PCR, and the genotypes of 34,316 SNPs distributed along all chromosomes. These SNPs were obtained from the PorcineSNP60K BeadChip (Illumina) in BC1_LD and BC1_PI and from the Axiom Porcine Genotyping Array (Affymetrix) in BC1_DU. The eGWAS identified 86 SNPs located in four chromosomal regions on pig chromosomes SSC2, SSC3, SSC10, and SSC11 and were associated with the expression of the *IGF2*, *ACSM5*, *AQP7,* and *FOS* genes (FDR < 0.05). Two eQTLs for *IGF2* and *ACSM5* were classified as *cis*-acting eQTLs, suggesting a mutation in the same gene affecting its expression, meanwhile three eQTLs had *trans* regulatory effects on the gene expression traits. The *IGF2*g.3072G>A polymorphism was the most significant SNP associated with its expression. For *ACSM5* gene expression, a polymorphism located in the promoter region showed the strongest association. The obtained results suggest that proximal genetic variants regulate the expression in muscle of *IGF2* and *ACSM5* genes in different genetic backgrounds and may be involved in the determination of intramuscular fat content and fatty acid composition.

Key Words: pig, muscle, gene expression, eQTLs, candidate genes

MT254 SNP association analyses for meat tenderness and thaw and cooking losses in Iberian pigs. M. A. Fernández-Barroso*[1,2], E. Alves[2], L. Silió[2], C. Rodriguez[2], F. Sánchez-Esquiliche[3], J. M. García-Casco[1,2], and M. Muñoz[1,2], [1]*Centro de I+D en Cerdo Ibérico, INIA, Zafra, Badajoz, Spain;* [2]*Departamento de Mejora Genética Animal, INIA, Madrid, Spain;* [3]*Sánchez-Romero Carvajal, Jabugo, Huelva, Spain.*

Iberian fresh meat and dry-cured products are increasingly valued in the Spanish and international market. Previous genetic studies on meat tenderness and cooking losses in Iberian pigs have been limited to polymorphisms of *CAST*, *CTSD* and *CTSF* genes. The objective of the current work was to search polymorphisms on candidate genes for these traits which segregate in Iberian pigs and analyse their effects in an Iberian pig line. Twelve genes (*CAPN1*, *CAPN3*, *CAPNS1*, *CAPNS2*, *CASP3*, *CASP9*, *CTSB*, *CTSL*, *MYH1*, *MYH3*, *MYOD* and *PRKAG3*) were selected as candidate genes for Warner-Blatzer shear force and thaw and cooking losses. Priority was given to the genes that code for a protein with a function related with these traits and/or that display polymorphisms with reported effects in other porcine breeds. A survey of polymorphisms on the selected genes was performed over the genomic sequence of 15 Iberian individuals using CLC Genomics Workbench Software. The 32 SNPs selected were genotyped on 475 purebred Iberian individuals using TaqMan OpenArray Genotyping Plates and additive effects were estimated using univariate animal models with QXPAK software. Four out of the 32 SNPs were fixed in the typed individuals and one showed genotyping problems. The other 27 had a MAF >0.05, and nine of them showed intermediate frequencies. Nineteen SNPs were used for association analyses since the remaining eight co-segregate. Significant associations were found between: 1) *CAPN1* and *CASP3* SNPs on shear force of loins, 2) *CASP3*, *CTSL* and *PRKAG3* SNPs on thaw losses and 3) *CAPNS2*, *CASP3*, *CTSB*, *MYH3*, *PRKAG3* SNPs on cooking losses. The most relevant effects were observed for *PRKAG3_rs343733804* on thaw (a = -0.91 ± 0.20) and cooking losses (a = -1.00 ± 0.20). Although effects of other *PRKAG3* SNPs on water losses have already been reported in other breeds, this is the first time that these effects are observed in the Iberian breed. This and other SNPs with relevant effects may be included in Iberian breeding programs.

Key Words: meat quality, Iberian pig, SNP chip, candidate genes

MT255 Genome-wide identification and functional analysis of Long Noncoding RNA in the pig multi-tissue transcriptome. P. Zhao*, W. Feng, X. Zheng, and J.-F. Liu, *China Agricultural University, Beijing, China.*

Long noncoding RNAs had a large quantities and played an important roles in regulating biological function of organisms. To date, genome-wide studies in humans, mouse and zebrafish have annotated lncRNAs expressed in multiple cell lines and tissues, but a short of systematic research on the domesticated animals especially pig. In our study, we identified 32,212 non-redundant lncRNAs isoforms from 18,676 lncRNAs loci across 34 normal tissues using high-throughput sequencing and deposited these LncRNAs with high-confidence in the publicly available NONCODE database (http://www.noncode.org/). Besides, we found these LncRNAs in pigs appeared similar pattern with other mammalian: the shorter length, fewer number, lower expression, higher conservation levels in exons and highly enriched in chromatin status. We also found 17.63% of lncRNAs demonstrated the specific expression pattern for per tissue on average, which used to better realise the functional role of lncRNAs in tissue differentiation. In addition, the potential function of LncRNAs were speculated by their location, sequence homology and expression pattern. At last, our study identifed the conserved lncRNA cross multiple species and investigated the genome collinearity between human and pig at the lncRNA levels.

Key Words: lncRNA, pig, NONCODE, tissue specificity

MT256 Small non-coding RNAs (sncRNA) regulate gene silencing and modify homeostatic status in animals faced with porcine reproductive and respiratory syndrome virus (PRRSV). D. S. Fleming*[1,2] and L. Miller[1], [1]*USDA-ARS-NADC, Ames, IA, USA;* [2]*ORISE-ORAU, Oak Ridge, TN, USA.*

It has been established that reduced susceptibility to porcine reproductive and respiratory syndrome virus (PRRSV) has a genetic component. This genetic component may take the form of small non-coding RNAs (sncRNA), which are molecules that function as regulators of gene expression. Various sncRNAs have emerged as having an important role in the immune system in humans. Among them are microRNAs (miRNA) and tRNA-derived RNA fragments (tRFs). Establishing the difference in type and quantity of sncRNAs between healthy and PRRSV-infected pigs will produce information needed to understand how gene function in the pig can become dysregulated by PRRSV in conjunction with how the pig's immune system responds to the virus. The objectives are to 1) identify differences in sncRNA expression in pigs, with emphasis on miRNA and tRF expression between healthy pigs and PRRSV challenged pigs; 2) establish if differences are due to changes in type and/or quality of sncRNA; and 3) establish if differences are associated with gene targets of observed sncRNAs. Transcriptomic analysis of whole blood samples from PRRSV experiments will be used to prepare sequencing libraries on a HiSEqn 3000 (Illumina). Sequences will be mapped to the Sus scrofa genome and then to a database containing different annotated sncRNA features to determine their origin. The main expected result will be the identification of any changes

in type (i.e. profile) and/or expression of sncRNA associated with PRRSV-infection in pigs.

Key Words: PRRSV, small RNA, immunity, transcriptome analysis

MT257 Molecular cloning and characterization of the promoter region of the porcine stearoyl-CoA desaturase gene. S. Gol*, M. Tor, J. Estany, and R. Pena, *University of Lleida, Lleida, Spain.*

Recently, our group identified a haplotype of three polymorphisms in the porcine stearoyl-coA desaturase (*SCD*) promoter that is strongly linked to the desaturation index of intramuscular fat, and therefore, to nutritional, sensory and technological quality of pork (Estany *et al*. 2014 PlosOne 20;9(1):e86177). In particular, the H1 haplotype (composed by nucleotides C-T-A) is considered beneficial as it promotes deposition of less saturated fat and increases the unsaturation index of pork. In Duroc pigs, the most common variant is H2 (nucleotides T-C-G). Animals of H1H1 diplotype express higher levels of *SCD* compared to H2H2 pigs, being the effect clearly additive. We have conducted a functional study in *in vitro* cultured cells in order to investigate (i) whether the haplotype H1 is responsible for the increased gene expression and (ii) which one of the three SNPs is the causal mutation. For this propose, 750 bp of the proximal *SCD* promoter were amplified by PCR from pigs with diplotype H1H1 and H2H2. These fragments were cloned into the pGL3 vector that directs the expression of firefly luciferase reporter, generating the clones pH1 and pH2. Clones were transfected into immortalized human liver cells (HepG2 line) with the pRL-TK vector which promotes the expression of Renilla luciferase. After transfection cells were incubated for 48h with basal medium or two reagents that promote (insulin, retinol) or inhibit (oleic acid, linoleic acid) *SCD* expression, each at two different concentrations. After 48h, cells were lysed and Firefly and Renilla luciferase measured by a dual luciferase assay. Basal expression of plasmid pH1 was 1.7-fold higher than pH2, agreeing with previous results in pig muscle and adipose. Moreover, plasmids pH1 and pH2 clearly showed distinct activation profiles, confirming that the mutations in H1 and H2 haplotypes respond differently. Next, a pH3 construct was prepared which combines the two haplotypes resulting in the T-T-G SNP. This plasmid will allow us to explore the role of the 2nd SNP in the transcriptional activation of this gene. In the near future, we plan to replicate the experiment in mouse myoblast cells (line C2C12) to contrast the expression of the *SCD* haplotype in these two cell lines.

Key Words: Duroc, HepG2, luciferase, *SCD*

MT258 Expression QTL for longissimus dorsi muscle gene transcripts co-localized with phenotypic QTL for meat quality traits in an F2 Duroc × Pietrain resource population. D. Velez-Irizarry*[1], S. Casiró[1], Y. B. Rubio[2], R. Bates[1], N. Raney[1], J. Steibel[1,3], and C. Ernst[1], [1]*Department of Animal Science, Michigan State University, East Lansing, MI, USA;* [2]*Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI, USA;* [3]*Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, USA.*

Meat quality phenotypes result from a cascade of molecular events starting before exsanguination and continuing throughout the conversion of muscle to meat. Evaluating transcriptomic profiles of skeletal muscle during the initial steps leading to the conversion of muscle to meat can identify key regulators of polygenic meat quality traits. In this study, we aim to identify potential candidate genes and molecular markers regulating meat quality traits in an F_2 Duroc × Pietrain pig population. Genes with transcript abundance subject to genetic control were identified through expression QTL (eQTL) mapping using gene transcripts obtained with RNAseq of longissimus dorsi muscle from 168 F_2 animals. We used a GBLUP-based-GWA model to test for association of gene expression with SNP genotypes between 18,839 gene transcripts and 20,583 SNP markers. A total of 242 eQTL were mapped for 229 genes (FDR ≤ 0.01) with 60% identified as local acting regulators (i.e. eQTL regions that overlap the associated gene transcripts). A genome-wide association (GWA) analysis of 67 traits for 960 animals from the same F_2 population identified 58 phenotypic QTL (pQTL) (FDR ≤ 0.05), with 21 of these related to meat quality traits. Co-localization of the meat quality pQTL with eQTL identified 10 eQTL co-localised with 11 pQTL (p-value ≤ 0.05), 70% of which were local regulators. Meat quality traits with pQTL, including pH 24 h postmortem, drip loss, cook yield and Warner-Bratzler shear force, were localised to three genomic regions containing 4 eQTL on SSC2 (8.8 Mb region), 1 eQTL on SSC5 (35 Mb region) and 5 eQTL on SSC15 (121 Mb region). Molecular mechanisms identified for co-localised eQTL genes include calcium homeostasis on SSC15 (*CHRNA9*) and apoptotic mitochondrial changes on SSC2 and SSC15 (*KCNK4* and *SPEG*). The rate of calcium release and apoptotic processes play a major role in the quality attributes of pork. This study highlights plausible candidate genes for meat quality traits and molecular markers regulating their expression.

Key Words: expression QTL, RNAseq, skeletal muscle, pig

MT259 Mitochondrial DNA, Y-chromosome, and *MC1R* data shed light on ancestry of Nigerian indigenous pigs. A. C. Adeola*[1,2], O. O. Oluwole[3], M. O. Oladele-Bukola[3], Olorungbounmi[3], B. A. Boladuro[3], S. C. Olaogun[4], L. M. Nneji[1], O. J. Sanke[5], P. M. Dawuda[6], O. G. Omitogun[7], L. Frantz[8], R. W. Murphy[1,9], H.-B. Xie[1,2], M.-S. Peng[1,10], Y.-P. Zhang[1,11], [1]*State Key Laboratory of Genetic Resources and Evolution & Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China;* [2]*Sino-Africa Joint Research Center, Chinese Academy of Sciences, Kunming, China;* [3]*Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan, Nigeria;* [4]*Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria;* [5]*Taraba State Ministry of Agriculture and Natural Resources, Jalingo, Taraba State, Nigeria;* [6]*Department of Veterinary Surgery and Theriogenology, College of Veterinary Medicine, University of Agriculture Makurdi, Makurdi, Benue State, Nigeria;* [7]*Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria;* [8]*The Palaeogenomics and Bio-Archaeology Research Network, Research Laboratory for Archaeology, University of Oxford, Oxford, UK;* [9]*Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, Toronto, Canada;* [10]*Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, China;* [11]*State Key Laboratory for Conservation and Utilization of Bio-Resources, Yunnan University, Kunming, China.*

Pig history in Africa still remains controversial due to insufficient evidence from archaeological and genetic data. A Western ancestry for West African pigs was reported previously based on of loci encoding coat color. We investigated the genetic diversity of Nigerian indigenous pigs (NIP) by simultaneously analysing variation in mitochondrial DNA (mtDNA), Y-chromosomes and melanocortin receptor 1 (*MC1R*). Median-joining network analysis of mtDNA D-loop sequences from 201 NIP and previously characterised loci clustered NIP with populations from the West (Europe/North Africa) and East/South-east Asia. Analysis of partial sequences of Y-chromosome in 57 Nigerian boars clustered NIP into lineage HY1. Finally, *MC1R* of 90 NIP formed seven haplotypes of which one individual carried the European wild boar haplotype and majority of other NIP (93%) the European dominant black. The remaining five unique haplotypes differed by a single synonymous substitution from European wild type, European dominant black and Asian

dominant black haplotypes. Our results demonstrate a European and East/South-east Asian ancestry for NIP. They provide further evidence from *MC1R*. More genetic analyses and archaeological studies may provide further insights into the history of African pig breeds.

Key Words: Nigerian indigenous pigs, *MC1R*, mitochondrial DNA, Y-chromosome

MT260 Functional analysis on microRNAs by comparing the expression of milk and exosome in porcine. Y. Xie[1,2], M. Zhao[1,3], Z. Wang[1,3], R. Nai[1,3], L. Mi[1,2], L. Ma[1,4], Y. Zhao[1,4], J. Li[1,2], H. Xiao[1,3], and Z. Liu*[1,2], [1]*Inner Mongolia Agricultral University, Hohhot, Inner Mongolia, China;* [2]*The Ministry of Agriculture Key Laboratory of animal genetics and breeding of sheep, Hohhot, Inner Mongolia, China;* [3]*Inner Mongolia Key Laboratory of animal genetics, breeding and reproduction, Hohhot, Inner Mongolia, China;* [4]*Inner Mongolia Engineering Center of goat genetics and breeding, Hohhot, Inner Mongolia, China.*

MicroRNAs are found in most body liquids, such as milk, serum, blood plasma and saliva.In swine milk, microRNAs exist in the exosomes.According to some previous studies, microRNA plays a vital role in regulating genes expression during lactation and the growth of breast. Therefore, studying exosomal microRNAs in milk is a key to improving the milk quality and survival rate of piglets. In our study, exosomalmicroRNAs and whey microRNAs from swine milk were collected at day 31 during lactation, samples were sequenced and analysed to find out up-regulated differential expressed microRNAs.Functional enrichment analysis on targets of these microRNAs were conducted with gene clusters and KEGG pathways. We found that(1)the rate of tiny fragment of microRNA in exosome rose, which means that exosome might selectively wrap small microRNA.(2)280 pre-miRNAs and 326 mature microRNAs were detected in swine milk. (3)37 microRNAs were found to be 2-fold higher expressed in milk exosome than in whey, 6 of them were expressed up to 10-fold higher in exosomes compared with in whey.(4) By analysing gene family of the 37 up-regulated microRNA.With chromosomal localization of 41 pre-miRNA, there were some gene clusters on chromosome 9 and X chromosome.(5)9262 genes were targeted by the 37 up-regulated exosomal microRNAs. Functional enrichment analysis indicated that these genes are mainly involved in three pathways, metabolic pathway, pathways in cancer and PI3K-Akt signalling pathway.After analysing the localization, distribution and function of the 37 up-regulated exosomal microRNAs, we are able to have profound insights about exosomal microRNAs in swine milk.

Key Words: porcine, microRNAs, milk, exosome

MT261 Identification of a stop mutation in the porcine BMP15 gene causing female infertility. G. Flossmann*[1], H. Pausch[1,4], C. Wurmser[1], G. Dahinten[2], K.-U. Götz[2], D. Seichter[3], and I. Russ[3], [1]*Lehrstuhl für Tierzucht, TU München, Freising, Germany;* [2]*Institut für Tierzucht, Bayerische Landesanstalt für Landwirtschaft, Poing, Germany;* [3]*Tierzuchtforschung e.V. München, Poing, Germany;* [4]*Animal Genomics, Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland.*

The current study shows the detection of a mutation within the porcine *BMP15* gene (Bone morphogenetic protein 15) that most likely causes for female infertility in a German Landrace population based on a Genome-wide association study and genome-wide re-sequencing. At the outset of the study were reports of sows with atrophied vulvae that turned out to be infertile. A case-control study was performed with 15 animals with atrophied vulvae and two infertile sows for which dissection revealed atrophied uteri in the case group and 1.801 inconspicuous sows in the control group. After correcting for stratification and multiple testing, significant association signals were detected on the X-chromosome. Eighteen SNPs with p values less than $1{,}12 \times 10^{-6}$ were located between 41 and 124 Mb on the X-chromosome. To identify putative causal mutations genome-wide resequencing data of 42 pigs (including the sire of the two dissected sows) were analysed. A total of 1.090 coding variants including six nonsense mutations were found in the range of the associated SNPs. One out of these nonsense mutations was carried by the sire of affected sows and thus likely to be causal. The candidate variant is a C/T polymorphism in the second exon of the *BMP15* gene (p.R204*). Variants in *BMP15* influence the function of ovaries and can cause fertility disorders in human and animals. The p.R204* variant was verified by Sanger sequencing. We also genotyped the mutation in 1.380 German Landrace pigs (43 suspected cases, 29 inconspicuous full siblings of possibly affected animals, 1.005 fertile sows and 303 boars). None of the fertile sows was alternatively homozygous. Of the 43 suspected cases, 36 (including the two sows with atrophied uteri) were homozygous for the mutation, three were heterozygous and four were homozygous for the wild type allele. This apparent discrepancy between phenotype and genotype is most likely due to difficulties in classifying animals based on the appearance of the vulva. In order to study the detailed effects of the mutation in depth, investigations of the reproductive organs of female pigs, that are homozygous to the p.R204* mutation, are presently underway.

Key Words: pigs, genome sequencing, genome-wide association, fertility

MT262 Genetic dissection of mechanisms underlying heat adaptation in pigs. J. Riquet*[1], H. Gilbert[1], K. Fève[1], Y. Labrune[1], R. Rose[2], Y. Billon[3], M. Giorgi[2], T. Loyau[2], J. Gourdine[2], and D. Renaudeau[4], [1]*INRA-GenPhySE, Castanet-Tolosan, France;* [2]*INRA-URZ, Petit-Bourg, France;* [3]*INRA-GenESI, Surgères, France;* [4]*INRA-PEGASE, Saint-Gilles, France.*

Heat stress has a major impact on pig production in tropical and sub-tropical areas, but also during summer heat waves in temperate regions. With climatic change, heat stress-related problems will increase in the future and a major research effort is currently underway to develop strategies for reducing the impacts of heat challenge on pig performance. An original experimental backcross population based on Large White and Créole pigs (tropical breed) has been set up at INRA to test 2 × 560 backcross progeny from the same 10 F1 sires in a tropical and in a temperate environment from 11 to 23 weeks of age. In addition, pigs raised in temperate environment were submitted to an experimental heat challenge (30°C for 3 consecutive weeks) at 23 weeks of age to mimic the effects of summer heat waves. Animals were recorded for main production traits (growth rate, feed intake and efficiency, backfat thickness) and thermoregulation traits (skin and rectal temperatures). At 23 weeks of ages in both environments and during the simulated heat wave challenge, blood samples were collected for a ^{1}H NMR-based metabolomic and a whole blood transcriptomic analysis. This pig genetic material represents a unique genetic resource to better understand the genetic and physiological bases of heat tolerance, to estimate its genetic relationships with other traits, and to better understand the genotype × environment interactions. Most production traits showed positive genetic correlations between environments differing from 1 (from 0.31+/−0.04 (backfat thickness) to 0.82 +/−0.02 (feed conversion ratio). Thermoregulatory traits had small to negative genetic correlations between environments (from 0.08 +/− 0.02 to -0.72 +/− 0.08). Association studies were performed for quantitative traits and biological phenotypes (metabolites from plasma) to dissect the genetic basis of these interactions using a 60K SNP chip. Significant genomic regions were essentially detected in one or the other environment, confirming the strong GxE underlying these traits. Some of these regions were detected also on metab-

olomic data, suggesting first physiological mechanisms involved in the responses to heat stress in growing pigs.

Key Words: pigs and related species, genome-wide association, genotyping, adaptation, environment

MT263 Can targeted-enrichment next-generation sequencing be a potential screening tool for searching for molecular markers of growth traits in pigs? K. Piórkowska*[1], A. Stuczynska[2], and K. Zukowski[1], [1]*National Research Institute of Animal Production, Cracow, Poland;* [2]*University of Agriculture in Cracow, Cracow, Poland.*

Targeted enrichment of genomic DNA regions for next-generation sequencing (TEDNAseq) is a novel technique that enables to achieve deep sequencing of precise chromosome region. This new approach was used to screen QTL-rich region on chromosome 15 in pigs located between microsatellites *SW1683* and *SW906* (127–135 Mbp). As the subject of the study 16 pigs were chosen from two breeds: Polish Landrace (n = 8) and Pulawska (n = 8) that differ significantly in traits. The experiment was performed by using 1x tiling RNA hybridization probes designed based on sequence NC_010457.4 (NCBI) to prepare DNA libraries for the region of interest. The samples were sequenced in 75 pair-end cycles on HiScan SQ (Illumina). The general linear model (R) and Panther were used to indicate the interesting genes with significant variants associated with growth traits after performing TEDNAseq. Four *PLCD4*, *PECR*, *FN1* and *PNKD* genes were validated on more numerous Polish pig populations included approx. 100 pigs from 5 each breed: Polish Landrace, Pulawska, Pietrain, Duroc and Polish Large White. The rs324680963 *PLCD4* variant was successfully detected with PCR-RFLP-ACRS technique, the SNPs rs792423408 *FN1* and rs343851532 *PECR* using the HRM technique with KAPA HRM FAST PCR Kit (Kapa Biosystems) on QuantStudio 7 Flex Real-Time PCR System (ThermoScientific) and frameshift rs792243103 and missense rs329501722 of *PNKD* gene using the Sanger sequencing technique on 3500 XL Genetic Analyzer (Applied Biosystems). After analysing variants in *PLCD4*, *FN1*, *PECR* and *PNKD* genes on Polish pig populations, there is significant difference between Polish Landrace and other breeds. Polish Landrace vary in genotype within the population, but the other breeds are mostly homozygous. Further, there will be performed the associated study to find out if the TEDNAseq is an applicable screening technique for identification the molecular markers in QTL-rich regions. The study was supported by the Polish Ministry of Science and Higher Education (2013/09/D/NZ9/02452).

Key Words: targeted enrichment of genomic DNA, pig, QTL-rich region, SSC15

MT264 Allele Specific Expression and imprinting analysis of selected genes in the brain of pigs. M. Oczkowicz*, T. Szmatola, K. Piórkowska, and K. Ropka-Molik, *National Research Institute of Animal Production.*

Variant calling using RNA-seq technology has recently revealed that there is a plenty of genes which are expressed in Allele Specific Expression (ASE) manner in the mammalian genome. RNA-seq also improved our knowledge about imprinting - phenomenon in which only one parental allele is expressed in the offspring. In our experiment, we have performed RNA-seq of brain tissues from eleven adult pigs. RNA from the brain was isolated using Purelink RNA Isolation Kit (Thermoscientific). Complementary DNA libraries were synthesised using 300 ng of total RNA with the use of TruSeq RNA Sample Preparation Kit v2 (Illumina, San Diego, CA) according to the protocol for each sample. The clustering of the flowcell was performed using TruSeq SR Cluster Kit v3-cBot-HS (Illumina) on cBot Instrument (Illumina). Sequencing by synthesis was performed using TruSeq SBS Kit v3- HS chemistry (50 single-end cycles)(Illumina). At the start 13361 polymorphic variants were identified. After applying filter for eliminating SNP clusters the number of variants diminished to 10966, among which 7277 were Single Nucleotide Polymorphisms (SNPs). Calculation of the allelic ratio for identified SNPs revealed that 54% of genes in porcine brain are subjected to Allele Specific Expression (ASE) – phenomenon in which one of the allele is preferentially expressed. Finally, results of RNA-seq experiment were used the for the identification of SNPs in a putatively imprinted genes. We have used this SNPs for the verification of imprinted status of *INPP5f var. 2, LRRTM1* and *HM13* genes in pigs by Sanger sequencing. *INPP5f var. 2* was paternally expressed, while *HM13* and *LRRTM1* were bialleleically expressed in porcine brain. We have also confirmed maternal expression of *MEG3* gene in pigs. Our results present how RNA-seq data may be used for imprinting studies without sequencing of parental genomes.

Key Words: RNA-seq, allele specific expression, imprinting, pigs, variant calling

MT265 Expression QTL regulating mRNA levels in the porcine skeletal muscle and liver mostly act in a tissue-specific manner. R. Gonzalez-Prendes[1], R. Quintanilla[2], A. Zidi[1], Y. R. Caldas[2], T. F. Cardoso[1,4], A. Manunza[1], A. Canovas[3], A. Castello[1], and M. Amills*[1], [1]*Center for Research in Agricultural Genomics, Bellaterra, Spain;* [2]*IRTA, Caldes de Montbui, Spain;* [3]*University of Guelph, Guelph, Ontario, Canada;* [4]*CAPES Foundation, Brasilia, Brazil.*

The main goal of this study was to compare the regulation of gene expression in the porcine skeletal muscle and liver, two tissues with different embryonic origins and highly differentiated transcriptomic profiles. We have genotyped 104 pigs with the Porcine SNP60K BeadChip and mRNA expression in the gluteus medius (GM) muscle and hepatic tissues has been measured with microarrays. Performance of a genome-wide association analysis of gene expression has made possible to identify 620 *cis*-eQTLs and 607 *trans*-eQTLs in the GM muscle and 630 *cis*-eQTLs and 3,312 *trans*-eQTLs in the liver. The high number of hepatic trans-eQTL might reflect the functional complexity of this organ, that is involved in many unrelated biological functions such as lipoprotein and glucose metabolism, detoxification, bile production and red blood cell destruction. The level of positional concordance between eQTL detected in the GM muscle and liver was limited, suggesting that regulation of gene expression is essentially tissue-specific in pigs. These results contrast with those obtained in humans where the majority of eQTL identified so far act *in cis* and there is a 50% of eQTL sharing among tissues

Key Words: expression QTL, pig, genetic regulation

MT266 Development of a simple *SLA-1* copy-number variation typing and the comparison of typing accuracy between real-time quantitative PCR and droplet digital PCR. J. Lee, M. T. Le, M.-K. Choi, C. Q. Le, H. Lee, and C. Park*, *Department of Animal Biotechnology, Konkuk University, Seoul, South Korea.*

Characterisation of the major histocompatibility complex (MHC) genetic diversity is important for understanding its effects on host immune responses during pathogenic infections. However, this information has been difficult to obtain in domestic animals, such as pigs, because of extreme diversity and inter-locus similarity among MHC gene sequences. Swine leukocyte antigen 1 (*SLA-1*) is duplicated in some haplotypes and may, therefore, have two to four protein-encoding alleles. Hence, accurate typing, even for existing alleles, has proved difficult without supporting evidence from molecular cloning. Therefore, we developed a simple method to accurately determine the copy numbers of *SLA-1* from genomic DNA applicable to both real-time quantitative PCR(RT–PCR) and droplet

digital PCR (ddPCR). Our systematic analysis resulted in designing a pair of primers to universally amplify SLA-1 alleles from both RT- and ddPCR. For a single copy control, the *GCG* gene was used. Our typing results against a panel of DNA (n = 29) with known SLA-1 types with 2 to 4 alleles were completely consistent with the previous results. Although the copy number determination of *SLA-1* using ddPCR showed more clear separation among samples with different copy numbers of *SLA-1* than that of using real-time PCR, the results were consistent between the two different methods. This new method should help to clarify previous confusion in *SLA-1* typing results in pigs.

Key Words: copy number variation, MHC, pig, SLA-1, Immunogenomics

MT267 Food intake promotes changes in microRNA muscle expression profile in pigs. E. Mármol-Sánchez*[1], R. Quintanilla[2], T. F. Cardoso[1,3], J. Tibau[4], O. González-Rodríguez[2], R. González-Prendes[1], M. Ballester[2], and M. Amills[1], [1]*Center for Research in Agricultural Genomics, Bellaterra, Spain;* [2]*IRTA, Caldes de Montbui, Spain;* [3]*CAPES Foundation, Brasilia, Brazil;* [4]*IRTA-Monells, Monells, Spain.*

The aim of this work was to evaluate the impact of food intake on porcine microRNA (miRNA) expression profile in muscle by miRNA-seq. Forty eight Duroc pigs were divided in 4 groups i.e. pigs fed *ad libitum* and slaughtered under fasting conditions (AL-T0), pigs fed *ad libitum* and slaughtered 5 and 7 h after last food intake (AL-T1 and AL-T2 respectively), and pigs fed with restricted feeding during the first fattening phase and slaughtered under fasting conditions (AR-T0). *Gluteus medius* samples were collected and the small RNA fraction was sequenced with a HiSEqn 2500 platform. Bowtie1 was used to align reads against the pig reference genome (Sscrofa10.2) and FeatureCounts software was used to count aligned reads. A comparative bioinformatics differential expression pipeline was used, combining the R software packages EdgeR and DESEqn 2. By doing so, we detected seven differentially expressed miRNAs (FDR < 0.05, $|\log_2 FC| > 0.6$) between AL-T0 *v.* AL-T2 (ssc-miR-1285, ssc-miR-339, ssc-miR-421–5p, ssc-miR-374a-3p, ssc-miR-129a-3p, ssc-miR-296–5p and ssc-miR-7). Currently, we are analysing the correlations between the expressions of DE miRNAs and previously obtained DE mRNAs from same population, by carrying out Pearson correlation tests with multiple testing correction (FDR). Besides, the miRgate target prediction tool was used to evaluate the potential biological significance of the correlations found. Our preliminary results indicate a strong negative correlation between the mRNA levels of TMEM120B, a gene with an important role in glucose uptake and adipocyte differentiation, and the expression of sc-miR-339 and ssc-miR-296–5p miRNAs.

Key Words: microRNA, RNA-seq, bioinformatics, animal nutrition

MT268 Nutrient supply drives changes in the muscular expression of protein-encoding and non-coding RNA genes. T. F. Cardoso*[1,2], R. Quintanilla[3], J. Tibau[4], M. Gil[4], E. Mármol-Sánchez[1], O. González-Rodríguez[3], R. González-Prendes[1], and M. Amills[1], [1]*Center for Research in Agricultural Genomics, Bellaterra, Spain;* [2]*CAPES Foundation, Brasilia, Brazil;* [3]*IRTA, Caldes de Montbui, Spain;* [4]*IRTA-Monells, Monells, Spain.*

We have investigated how the expression of mRNAs and non-coding RNAs (ncRNAS) changes in response to food ingestion. This goal has been achieved by comparing the *gluteus medius* muscle transcriptomes of Duroc pigs before (T0) and 5 h (T1) and 7 h (T2) after feeding. By using an RNA-seq approach, we have measured gene expression in 36 sows (12 individuals per treatment). The *STAR* alignment algorithm was used to map raw reads, while the *FeatureCounts* software was used for counting reads to genomic features. Differential expression (DE) analysis was performed by using the *DESEqn 2* and *EdgeR* software packages. By doing so, we have identified 199 mRNAs and 27 long non-coding RNAs that happen to be DE before and after feeding. Several of these DE mRNA genes do not have an obvious relationship with metabolism (*e.g. SDC4, FAM73B and TMEM169*). Interestingly, we have also observed DE for many transcription factors, and several of them (*PER1, PER2* and *ARNTL)* are known to regulate the circadian rhythms. This result is consistent with the fact that feeding is one of the main *zeitgebers* of the peripheral clocks modulating gene expression in tissues. The number of DE lncRNAs was lower than that of mRNAs, possibly because of the intrinsic difficulties in annotating them. Several of these DE lncRNAs were located close to DE mRNA genes, a result that is consistent with the cis-regulatory role of such molecules. These results suggest that nutrient intake elicits changes in gene expression aimed to maintain energy homeostasis in the porcine muscle.

Key Words: RNA-seq, gene expression, non-coding RNA, animal nutrition

MT269 Influence of high fat diet on lipid metabolism in ham muscle of finishing Iberian pigs. Y. Nuñez*[1], A. Fernández[1], J. Segura[2], R. Benítez[1], J. Olivas[3], J. Viguera[4], C. López-Bote[2], L. Calvo[3], and C. Óvilo[1], [1]*INIA, Madrid, Spain;* [2]*UCM, Madrid, Spain;* [3]*Incarlopsa SA, Toledo, Spain;* [4]*IMASDE, Madrid, Spain.*

The modification of energy and nutrient composition of diets for growing animals may be a tool to influence meat fat quantity and quality. The metabolic response to different diets depends on many factors as genetic background, age or sex. This study aimed to analyse the effects of finishing diets differing in fat content on ham muscle phenotypic traits and gene expression of enzymes involved in lipogenesis. Eighty animals of both sexes (45 females and 35 castrated males) were subjected to a high fat diet (HF with 7% fat, n = 34) or an isocaloric and isoproteic low fat diet (LF with 3% fat, n = 46) during the finishing period. Animals were sacrificed with a mean live weight of 153 ± 6 kg. Diet influenced intramuscular fat content and composition, which was measured in different locations (loin and ham), with LF animals showing higher fatness (P < 0.05). Also, higher MUFA (P < 0.005) and lower PUFA content (P < 0.05) was observed in *Biceps femoris* of LF animals. Expression of *SREBP1, FASN, ME1, ACACA* and *SCD* genes was assessed by RT-qPCR in *Biceps femoris* muscle samples obtained from 30 animals (14 HF and 16 LF). *SREBP1, FASN* and *ME1* genes were differentially expressed according to dietary group (P < 0.001, P < 0.01 and P < 0.001, respectively), with a higher expression for all enzymes found in LF group, in agreement with phenotypic results. The differences found for phenotype as well as for gene expression were clearer in males, with a significant interaction diet*sex on the expression of *SREBP1* and *ME1* genes (P < 0.05 and P < 0.01 respectively). The *FASN* and *SCD* genes were regulated by gender, as reported in other species, with a higher expression in castrated males (P < 0.05 for both). In conclusion, a high fat diet depressed *de novo* lipogenesis, counteracting adipose tissue expansion in muscular tissues. Our work provides novel insights in muscle metabolism regulation by diet, because although the inhibition of lipogenesis by HF has been reported in fat and liver, muscle is supposed to be less responsive to diet. Interesting gender and interaction effects were found which may influence the usefulness of the tested diets.

Key Words: nutrigenomics, dietary fat, iberian pig, fatness, meat quality

MT270 RNA-sequencing of liver in pigs divergent for residual feed intake and meat quality. J. Horodyska*[1,2], R. M. Hamill[1], H. Reyer[2], N. Trakooljul[2], P. G. Lawlor[3], and K. Wimmers[2], [1]*Teagasc, Food Research Centre, Ashtown, Dublin, Ireland;* [2]*Research Institute for the Biology of Farm Animals (FBN), Dum-*

merstorf, Germany; [3]*Teagasc, Pig Production Development Unit, Moorepark Research Centre, Fermoy, Co. Cork, Ireland.*

Hepatic nutrient partitioning has a direct influence on efficiency of energy utilisation and also plays a systemic role, influencing metabolism in tissues such as muscle and adipose and potentially contributing to regulation of feed efficiency, carcass composition and meat eating quality. The objective of this study was to investigate transcriptomic changes in liver of pigs divergent for residual feed intake (RFI) and meat quality. RNA-Seq analysis was carried out in the liver of Maxgro × (Landrace × Large White) pigs from low (n = 8) and high (n = 8) RFI groups using the TruSeq Stranded mRNA protocol. An average of 105.6 million high-quality paired-end reads per sample was mapped to the reference with a mean of 89.2% mapping efficiency using TopHat (2.1.0). Count reads were assigned to the gene features using HTSEqn (0.6.1) and differential gene expression analysis was done with edgeR package. 191 genes were differentially expressed between the RFI groups (P < 0.05) and qPCR of all four genes (*CXCL10*, *KIT*, *PON3* and *SAA3)* agreed well with the RNA-Seq (R^2 0.56–0.98). Annotation analysis revealed that the most significant functions were cellular growth, organismal development and cellular movement. Directionality of differentially expressed genes predicted inhibition of endothelial cell migration in liver of more efficient pigs. Proliferation and migration of endothelial cells play a role in liver regeneration and angiogenesis, thus potentially affecting hepatic development and health status of RFI divergent pigs. Canonical pathway analysis revealed FXR/RXR & LXR/RXR activation and AHR signalling were significantly enriched in the dataset. Up-regulation of *PON3*, *CYP1A1* and *CYP1A2* genes suggests that low RFI pigs may exhibit increased cholesterol and bile acid synthesis which might contribute to divergence in lipid metabolism. In conclusion, RNA-sequencing of liver suggests cellular development and lipid metabolism are modulated in pigs divergent for RFI and eating quality.

Key Words: feed efficiency, transciptomics porcine, intramuscular fat

MT271 Transcriptomic analysis of adipose tissue from pigs divergent in residual feed intake and muscle adiposity. J. Horodyska[1,2], H. Reyer[2], K. Wimmers[2], N. Trakooljul[2], P. G. Lawlor[3], and R. M. Hamill*[1], [1]*Teagasc, Food Research Centre, Ashtown, Dublin, Ireland;* [2]*Research Institute for the Biology of Farm Animals (FBN), Dummerstorf, Germany;* [3]*Teagasc, Pig Production Development Unit, Moorepark Research Centre, Fermoy, Co. Cork, Ireland.*

Adipose tissue (AT) is an active endocrine organ that communicates with skeletal muscle to influence energy partitioning and metabolism with potential consequences for feed efficiency, muscle composition and meat quality. Residual feed intake (RFI) is the difference between actual feed intake and predicted feed requirements. The objective of this study was to examine the transcriptomic profiles of subcutaneous AT of pigs divergent for RFI and muscle adiposity. RNA-Seq analysis was carried out in the subcutaneous AT of Maxgro × (Landrace × Large White) pigs from low (n = 8) and high (n = 8) RFI groups using the TruSeq Stranded mRNA protocol. An average of 105.5 million high-quality paired-end reads per sample was mapped to the reference with a mean of 87.5% mapping efficiency using TopHat (2.1.0). Count reads were assigned to the gene features with HTSEqn (0.6.1) and differential gene expression analysis was done using edgeR package. 373 genes were differentially expressed in relation to RFI (P < 0.05) and qPCR of all four genes (*COL8A1*, *MMP16*, *PLCE1* and *SGSM1)* agreed well with the RNA-Seq (R^2 0.74–0.99). Functional annotation analysis suggested that the differentially expressed genes were enriched for functions such as cellular movement, growth & proliferation, hematological system development, immune cell trafficking and cell death & survival. Endothelial growth was repressed suggesting an impact on AT development and extracellular matrix formation in more efficient pigs. Cellular growth and proliferation, including muscle cell formation, was enhanced in low RFI pigs displaying reduced muscle adiposity. The most significantly enriched pathways were complement system & acute phase response signalling. Low RFI pigs may display a more rapid and efficient inflammatory response. Overall findings are in accordance with phenotypic observations of reduced development and formation of AT in more efficient pigs and depict putative links between strategies to cope with inflammation and RFI.

Key Words: feed efficiency, meat quality, gene expression, RNA-sequencing

MT272 Genome wide association studies for haematological traits in Italian Large White pigs. S. Bovo[1,2], G. Mazzoni[1,3], G. Schiavo[1], F. Bertolini[1,4], G. Galimberti[5], A. B. Samorè[1], S. Dall'Olio[1], and L. Fontanesi*[1], [1]*Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy;* [2]*Biocomputing Group, Department of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy;* [3]*Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Copenhagen, Denmark;* [4]*Department of Animal Science, Iowa State University, Ames, IA, USA;* [5]*Department of Statistical Sciences 'Paolo Fortunati', University of Bologna, Bologna, Italy.*

Genetic improvement for disease resistance and resilience is becoming an essential issue in pig breeding. Haematological traits reflect, at least in part, the immune capacity and the basic physiological states of the animals that can be considered as potential indicators of the capacity of the animals to cope with infections. We recently demonstrated that several of these parameters are highly heritable in pigs and that, for this reason, it could be possible to use them as proxies of disease resistance-related traits in breeding programmes. With the aim to identify genetic factors that might indirectly explain part of the genetic variability of these traits, we carried out genome wide association studies for 15 haematological parameters measured in ~900 performance tested Italian Large White pigs. All animals were genotyped with the Illumina PorcineSNP60 BeadChip genotyping tool. Association analysis was carried out using GEMMA. The most significant QTLs were identified for the number of basophils on porcine chromosome (SSC) 14, the number of eosinophils on SSC3, SSC7 and SSC10, the number of monocytes on SSC15, mean corpuscular hemoglobin level on SSC14 and hematocrit on SSC18. These results represent the first step for the identification of the causative mutations of these phenotypes that could be important to dissect disease resistance and resilience related traits in pigs.

Key Words: GWAS, hematology, disease resistance, basophil

MT273 *In silico* analysis of non-synonymous SNPs in the selective sweeps of Landrace genome. K.-H. Won*, D. Shin, and K.-D. Song, *Department of Animal Biotechnology, Chonbuk National University, Jeonju-si, Jeollabuk-do, Republic of Korea.*

Since Landrace is a major line used as a mother line, our research team has conducted a whole-genome resequencing study in 2015 to find out a selective sweep region within a Landrace by comparative study with other breeds (Moon *et al*., 2015). In addition to searching for evolutionary evidence of Landrace, we have conducted in-depth studies on non-synonymous SNPs (nsSNPs) that affect the biological mechanism by actually causing amino acid sequence changes. Using the Illumina HisEqn 2000 platform, 110 whole genome data in pigs were obtained. 67,329 nsSNPs were identified in the mapped region with 110 porcine genomes containing 14 Land races. In the selective sweep region of Landrace genome, we identified 1,579 nsSNPs and 405 genes associated with these mutations. We also identified the Jak-STAT signalling pathway and

the cytokine recpetor activity in various categories of biological fuction using gene ontology analysis. CX3CR1, GHR, IL11RA, IL12RB2, IL22RA2, IL4R, IL6ST, OSMR and IFN-KAPPA genes could be predicted to be related to reproduction, lactation ability and immunity. We calculated the ranking based p-value using nsSNPs for 12,575 genes containing at least 1 SNP in 110 porcine NGS data, and found that p-value less than 0.01 had significant nsSNPs. Among 405 genes in the selective sweep region of the Landrace, 11 genes were found to have a p-value of less than 0.01. In order to confirm the influence of nsSNPs on protein function and structure, SIFT and Polyphen-2 analyzes were carried out using nsSNPs in the selective sweep regions. As a result, 105 nsSNPs which is predicted to have a biologically strong influence. Changes in protein structure can cause phenotypic changes. Therefore, Protein 3D structure was predicted by homology analysis to confirm the change of protein structure due to non-synonymous SNPs. Unlike other studies, we focused on nsSNPs for depth analysis of Landrace genome. Through this study, we were able to identify novel biologic meaning and deeply understand of Landrace domestication. These results will help to support previous studies and provide the basis for future research on properties of Landrace genome.

Key Words: Landrace, non-synonymous SNP, selective sweep, protein modeling, NGS

MT274 *In silico* approaches to identify functional impact of non-synonymous SNPs in the Yorkshire selective sweep regions. S. Son, D. Shin*, and K.-D. Song, *Department of Animal Biotechnology, Chonbuk National University, Jeonju-si, Jeollabuk-do, Korea.*

Domestication including natural and artificial selection has led a remarkable change in phenotypic variation in pigs. Comparison of the genomes of native and domesticated pigs provides a unique opportunity for exploring the history of domestication through identification of signatures of artificial selection. Yorkshire is known for its fast growth and excellent breeding compared to other pig breeds. We examined of non-synonymous single nucleotide polymorphisms (nsSNPs) to identify the variations that could affect economic trait of Yorkshire. We used whole genome re-sequencing data of 110 pigs including 16 Yorkshires using the Illumina HisEqn 2000 platform. As a result, we identified 31,846 non-synonymous SNPs in the mapped regions and among them, 437 nsSNPs were found in Yorkshire selective sweep regions that were reported in a previous study. The genes encompassing these nsSNPs fall into growth traits of Yorkshire. Impacts of nsSNPs on protein function were predicted with SIFT and Polyphen-2 (polymorphism phenotyping V2) to reveal the potential roles in biological processes which might be associated growth traits of Yorkshire. Our novel findings were very helpful to better understand Yorkshire domestication and common agreed with previous studies. These discoveries confirmed Yorkshire history by nsSNPs and illustrate how domestication has made the patterns of generic variation. In summary, we investigated deeply the relationship between genomic composition and this phenotypic trait of Yorkshire by scanning for nsSNPs in large scale whole-genome sequencing data.

Key Words: Yorkshire, non-synonymous SNP, selective sweep, NGS

MT275 Rewiring of porcine mRNA and miRNA networks in response to selection for residual feed intake. H. Beiki[1], M. Schroyen*[1,2], A. Rakhshandeh[3], N. Gabler[1], J. Dekkers[1], and C. Tuggle[1], [1]*Department of Animal Science, Iowa State University, Ames, IA, USA;* [2]*Département AgroBioChem, Université de Liège, Gembloux, Belgium;* [3]*Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA.*

A major production costs in the pig industry is feed and as such improving feed efficiency is a major goal. We are evaluating the performance of two pig lines selected for residual feed intake (RFI), to see if RFI represents a useful trait to use in selection. In this study, we aimed to identify gene pathways and networks in a given tissue that differ between high and low RFI pigs as well as between lipopolysaccharide (LPS) treated and nontreated pigs so that more sensitive analysis of biomarkers for line differences in healthy pigs or those under an inflammatory stress like LPS can be identified. Twelve 18-week-old Yorkshire gilts from the HRFI and 12 from the LRFI selection lines were randomly selected. On 0, 48, 96 and 144 h, 6 pigs of each group were injected with LPS, the others were injected with saline. Animals were sacrificed at 168 h. The transcriptomes of ileum, liver, longissimus dorsi (LD) and spleen were measured, using both mRNA-seq and miRNA-seq. Differential expression (DE) analysis revealed very few DE mRNAs/miRNAs in any tissue due to line or LPS treatment. To expand from an individual gene level to a network analysis, network structures within line and treatment datasets were compared using different network statistics to find significantly 'rewired' modules (SR) between line or treatment. Correlation analysis between SR mRNA and miRNA module eigengenes revealed both significant positive and negative correlations. For instance, for line effect in LD, five SR mRNA and four SR miRNA modules were found. One of the SR miRNA modules was positively correlated with three of the mRNA modules and negatively correlated with a fourth one. Three out of five SR mRNA modules were highly enriched for different GO terms. The biological function of these modules was related to the regulation of calcium ion transport into cytosol, regulation of transferase activity and neural tube formation. These results suggest significant mRNA and miRNA network changes between lines in muscle tissue and could help to find a causal link between miRNAs and mRNAs. Funding acknowledgment: NIFA-AFRI-2011–68004–30336.

Key Words: pig, network analysis, feed efficiency, functional genomics, RNA-seq

MT276 Analyses of hypothalamic transcriptome to explore porcine growth and fatness regulation in Iberian genetic backgrounds. M. Muñoz*[1,2], A. Martínez-Montes[2], Y. Núñez[2], A. Fernández[2], J. Folch[3,4], and A. Fernández[2,5], [1]*Centro de I+D en Cerdo Ibérico INIA-Zafra, Zafra, Badajoz, Spain;* [2]*Departamento Mejora Genética (INIA), Madrid, Madrid, Spain;* [3]*CRAG, Plant and Animal Genomics, Bellaterra, Barcelona, Spain;* [4]*Universitat Autònoma de Barcelona, Departament de Ciència Animal i dels Aliments, Bellaterra, Barcelona, Spain;* [5]*Hospital Universitario Gregorio Marañón, Servicio Cardiología, Madrid, Madrid, Spain.*

The knowledge of the genetic mechanisms underlying growth and fatness in pigs is essential for pork production and biomedical applications. The Iberian pig breed has a metabolism different from many other porcine breeds since it is characterised for having a low growth rate, high adipogenic potential and high appetite. The hypothalamus gland is one of the main tissues involved in the regulation of feeding behaviour, growth and fat accumulation. The aim of the current study was to identify genes and gene networks involved in pig growth and fatness traits through independent analysis of the transcriptome of hypothalamus on two different Iberian backcrosses using RNA-seq technology. Divergent individuals for growth and fatness traits were selected from two different backcrosses: F1 (Iberian × Pietrain) × Pietrain (BC_PI) and F1 (Iberian × Landrace) × Landrace (BC_LD) using principal component analyses. Six males of BC_PI and five of BC_LD were selected from each divergent group. Hypothalamic RNA samples were sequenced with llumina Hi-SEqn 2000 equipment and analysed using Tuxedo pipeline. Raw data was trimmed according standard criteria and mapped against Sscrofa10.2 assembly. Two samples from BC_LD, one of each group, were discarded according to clustering analyses performed with CummeRbund Bioconductor R package probably due to sampling or RNA processing problems. More than 17,500 genes of the 25,322 annotated were expressed in the hypothalamic tissue.

In addition, around 55,000 new isoforms were identified. A total of 159 and 281 differentially expressed (DE) genes and novel isoforms (FDR < 5%) were detected on BC_PI and BC_LD, respectively. Also, 12 out of the DE genes on BC_PI and 5 on BC_LD mapped on QTL regions detected in a parallel study. All the DE genes detected were different in the two backcrosses except *OLR1*. DE genes observed on BC_PI affected mainly biological process as feeding behaviour and skeletal muscle, while those observed on BC_LD were related with fat deposition and fatty acid metabolism. Strong candidate genes for growth and fatness such as the *OLR1, OPRM1*, *MYLPF*, *ANO3*, *IBSP2* and *GAL2R* can be proposed from this study.

Key Words: growth, fatness, transcriptome, hypothalamus, Iberian backrosses

MT277 Effect of *SCD* and *LEPR* mutations on blood free fatty acid profiling and milk fat composition. R. Pena*[1], M. Tor[1], F. Vilaró[2], R. Ros-Freixedes[1], J. Álvarez-Rodríguez[1], L. Bosch[3], J. Reixach[4], and J. Estany[1], [1]*Department of Animal Science, University of Lleida - Agrotecnio Center, Lleida, Spain;* [2]*Scientific- Technical Services, DATCEM, University of Lleida - Agrotecnio Center, Lleida, Spain;* [3]*Departament d'Enginyeria Química, Agrària i Tecnologia Agroalimentària, University of Girona, Girona, Spain;* [4]*Selección Batallé S.A, Riudarenes, Spain.*

Two mutations in the exon 14 of the leptin receptor (*LEPR*; g1987C>T) and in the proximal promoter of the stearoyl-coA desaturase (*SCD*; g2228T>C) genes have been associated to changes in muscle fat content and composition, respectively. The aim of our study is to analyse if blood free fatty acids and milk fat composition are affected by these two polymorphisms. Plasmatic non-esterified fatty acids (NEFA) profiles can provide valuable information on the metabolic state of the organism because they are the substrate for the triacylglycerol synthesis in adipocytes, myocytes and mammary epithelial cells at lactation. NEFA composition could be a useful surrogate marker of adipose tissue composition and an indicator of pork quality, which in its own turn can be affected by the action of DNA polymorphisms in key lipogenic genes. Blood was collected from 150 castrated Duroc pigs at 180 and 210 days of age and NEFA profile was analysed by UPLC-TQ-MS. Fatty acids 16:0, 18:0, 18:1, n-9 and 18:2 constituted >90% of total NEFA. The content of individual NEFAs remained constant at both ages, except for 20:2 and 20:4. The *SCD* genotype had no effect on NEFA profile. In contrast, pigs of *LEPR* TT genotype had significantly less plasmatic NEFAs ($P < 0.05$). Plasmatic levels of 14:0, 16:0, 16:1, 18:1, 18:2 and 18:3 NEFAs were 15–28% lower in TT pigs compare to CC and CT. The opposite situation was observed regarding milk fat composition. For this analysis, 60 samples of milk from primiparous sows were collected between days 1 to 13 of lactation. A compositional analysis of milk fat revealed that sows milk is composed of 28 fatty acids, being 16:0, 18:0, 18:1, n-9 and 18:2 the most predominant (as in blood). There were compositional changes along lactation, particularly regarding the relative content of total MUFA and SFA. In addition, the *SCD* gene polymorphism had a significant effect on the fatty acid composition of the sow's milk, with allele T increasing the proportions of MUFA and 18:1, n-9. In contrast, the *LEPR* polymorphism did not influence milk's fat composition. In summary, the *LEPR* and *SCD* polymorphisms affect fat content and/or composition of body fluids such as blood and milk.

Key Words: pigs, animal breeding, biomarker, genomic prediction, milk

MT278 Genetic architecture of a QTL for teat number on porcine chromosome 7. M. van Son[1], M. S. Lopes[2], J. Kongsro[1], L. E. Gangsei[3], E. Grindflek[1], E. F. Knol[2], and B. Harlizius*[2], [1]*Norsvin SA, Hamar, Norway;* [2]*TopigsNorsvinResearch Center, Beuningen, the Netherlands;* [3]*Animalia, Oslo, Norway.*

To identify the causative mutation and clarify the genetic mechanism causing a quantitative trait locus on SSC7 with a major effect on number of teats, the variation was further investigated in 2 commercial sow lines (Landrace, Large White-based) and one boar line (Duroc). At the sequence level, the PRE1 element insertion and a SNP in the promotor region were genotyped and imputed to 660K data for a total of 29 510 animals to test the effect on number of teats. At the phenotypic level, 602 Landrace (LR) animals and 646 Duroc animals were counted for number of vertebrae derived from computer tomography images. The GWAS analysis resulted in a clear peak around the position 103,22 Mb (LR) and 103,41 Mb (Duroc) around 200 kb apart as reported earlier for number of teats (103,22 Mb in LR and 103,37 Mb in Duroc). The size of the effect estimated on number of teats (0.29 and 0.36) increases up to 0.48 and 0.63 for the pleiotropic effect on number of vertebrae in LR and Duroc, respectively. The effect of a single SNP explains all genetic variation observed for number of vertebrae in this region. The results from the annotation of the new reference genome build 11 will be incorporated as soon as they become available. The results show how trait definition can disentangle a complex quantitative trait into underlying single monogenic compounds. The downstream loci modulating the number of teats resulting from two additional ribs will be investigated further.

Key Words: pigs, reproduction, quantitative trait locus, pleiotropy, genome sequencing

MT279 Genome wide association of changes in feeding behavior due to heat stress in pigs. A. J. Cross[1], B. N. Keel[2], T. M. Brown-Brandl[2], and G. A. Rohrer*[2], [1]*South Dakota State University, Brookings, SD, USA;* [2]*US Meat Animal Research Center, USDA Agriicultural Research Service, Clay Center, NE, USA.*

Heat stress negatively impacts pork production, losses include decreased growth, reduced feed intake and mortality. Therefore, the objective of this study was to identify genetic markers associated with changes in feeding behaviour due to heat stress in grow-finish pigs. Data were collected on grow-finish barrows and gilts (n = 1345) from July 2011 to March 2016 in groups of 240 (6 pens with 40 pigs per pen). Pigs were from Landrace or Yorkshire sire lines. Animals were tagged with an electronic identification tag upon entry into the barn fitted with a feed system that monitored feeding behaviour. Temperature and relative humidity were obtained and temperature humidity indices (THI) calculated. THIs were divided into groups, where less than 23.33°C was considered 'normal', between 23.33°C and 26.11°C considered an 'alert', between 26.11°C and 28.88°C considered a 'danger', and greater than 28.88°C considered an 'emergency'. Standardized differences between a pig's feeder activity and the average feeder visit activity for an animal of that sex, breed of sire and THI category were calculated. DNA was extracted and genotyping conducted using Illumina BeadChip products, all animals (n = 853) were imputed to the NeoGen Porcine GGPHD chip. A genome-wide association study (GWAS) for an animal's change in feeding behaviour between different THI categories was conducted using Bayesian analyses in GenSel. Candidate genes were identified using a 200-kb region around significant single nucleotide polymorphisms (SNP). Heritabilities for difference of a pig's ranking between each of the THI categories from GenSel were moderate to high (0.146 to 0.478). Greater than 71% of genetic variation was explained by regions within eight chromosomes when comparing feeder visit activity differences between danger and emergency THI. Gene ontology (GO) enrichment analysis showed that biological processes related to sensory perception and detection of chemical stimuli over-represented in genes in the identified regions. These genetic markers may facilitate genetic selection for

improved grow-finish performance during elevated ambient temperatures.

Key Words: pig, GWAS, heat stress

MT280 Identification of SNPs associated with meat pHu in Italian Duroc pigs. R. Davoli[1], P. Di Battista[2], M. Zappaterra[1], and P. Zambonelli*[1], [1]*Department of Agricultural and-Food Sciences (DISTAL), Bologna University, Bologna, Italy;* [2]*Centro Interdipartimentale di Ricerca Industriale Agroalimentare (CIRI Agroalimentare), Cesena, Italy.*

Muscle pH, meat colour, and water holding capacity are the main features influencing the technological and nutritional quality of meat products and are very important for the pig processing industry. In particular, the values of meat pH measured at 45 min (pH1) and at 24 h (pHu) after slaughter influence the biochemical changes during conversion of muscle to meat. These traits have been extensively studied for many years to identify the associated genes. Recently, genome wide association studies (GWAS) have been carried out and many markers linked to these traits have been found. A population of 280 sib tested Italian Duroc (IDU) pigs was genotyped with the Porcine SNP60 v2 Beadchip and was used to perform a GWAS for pH, colour and water holding capacity recorded on Semimembranosus muscle. The pigs were provided by the Italian National Association of Pig Breeders and slaughtered at a live weight of at least 155 kg in the same abattoir in 20 batches. Muscle samples were collected after slaughter and immediately frozen. Quality control and GWAS were carried out using GenABEL package in R. The association analysis was carried out through a polygenic model with a kinship matrix and the mmscore function. Genome wide significant SNPs with a Bonferroni-corrected p-value below 1.47e-06 were considered. We detected new markers associated with muscle pHu mapping in a region of ~0.9 Mb on chromosome 3. Investigating this region on 10.2 porcine genome assembly, we observed that three of the most significant markers are located in an intronic region of Hydroxy-delta-5-steroid dehydrogenase, 3 β- and steroid delta-isomerase 7 (HSD3B7) gene and in two exons of Sulfatase modifying factor 2 (SUMF2) gene. Furthermore, 3 QTL for pHu mapping in the same region are reported in pigQTLdB in 5 pig breeds. On the whole, the results suggest a significant relationship between meat pHu and this genomic region. Further studies considering the functional role of the genes located on this portion of SSC3 may contribute to elucidate the molecular mechanisms influencing the pork pHu levels. This work was supported by AGER–Hepiget, grant N. 2011- 0279

Key Words: pigs, Duroc, GWAS, meat pH

MT281 Alentejano pig: A puzzle piece to uncover the genetic basis of lipogenesis. A. Amaral*[1,2], M. Bressan[3], C. Bettencourt[3], J. Almeida[3], J. Sá[2], M. Gama-Carvalho[2], J. Santos-Silva[3], A. Belo[3], H. Bovenhuis[4], O. Moreira[3], R. Bessa[5], and L. Gama[5], [1]*Instituto de Medicina Molecular, Lisbon, Portugal;* [2]*BioISI- Biosystems and Integrative Sciences Institute, Lisbon, Portugal;* [3]*Instituto Nacional de Investigação Agrária e Veterinária, Santarém, Portugal;* [4]*Animal Breeding and Genomics Centre, Wageningen, Netherlands;* [5]*Faculdade de Medicina Veterinária, Lisbon, Portugal.*

In contrast to commercial pig breeds, Iberian pigs (of which Alentejano is a branch raised in southern Portugal) are characterised by producing high-quality meat, and consequently pork products, which are highly valued by consumers. This is largely due to the inherent ability of Iberian pigs to deposit intra-muscular and subcutaneous fat, especially when they are finished on pasture and acorn, producing carcasses with higher proportion of oleic fatty acid (>53%), which is of higher nutritional value for the human diet. This inherent ability must have a genetic background, which is not well understood, and is highly dependent on an environmental factor, the availability of acorns in the finishing diet. To our knowledge, no specific mutations have been associated with fatty acid profiles, even though previous research using a QTL approach indicates that genetic variability, especially in some regions of chromosome 4, may be related with lipid metabolism. We have conducted a GWAS study that aimed to compare Alentejano (N = 60) and commercial (N = 60) pigs, which were finished on pasture and acorn. All animals were genotyped using the 60K SNP chip and ~40 parameters related with performance and meat quality, including protein and fatty acid profiles have been measured. This work will contribute to unravel the genetics underlying the Alentejano ability to produce pork of higher nutritional value.

MT282 Heterospermic fertility index applied to frozen and refrigerated swine semen. C. Previtali[1], G. Bongioni*[1], and A. Galli[2], [1]*Istituto Sperimentale Italiano Lazzaro Spallanzani, Rivolta d'Adda, Cremona, Italy;* [2]*Centro di ricerca per le produzioni foraggere e lattiero-casearie CREA, Lodi, Italy.*

In swine the artificial insemination is practiced using refrigerated semen while the frozen semen is not used routinely. Aim of the project was to develop an heterospermic fertility index useful to measure and compare the fertility obtained with frozen or refrigerated semen. Seven boars were used. The heterospermic doses, frozen and refrigerated, were prepared with the same spermatozoa numbers from two boars. Twenty one different combinations were used to inseminate 112 healthy sows. As first, semen doses were analysed to verify the 1:1 ratio of the two boars with Real Time PCR using Single Nucleotide Polymorphism, then were done qualitative valuation of semen: motility, concentration, semen morphology and membrane integrity. The frozen semen doses were analysed with flow citometry using the Sperm Chromatin Structure Assay to have information of the DNA structure (DFI). The DNA from each piglet born was extracted and amplified with 12 microsatellites, the genotypes, obtained from the GeneMapper Software (*v.*4.0) were analysed using a Windows-based procedure to assign the paternity through the exclusion method, then was calculated the Competitive Fertility Index (CFI) as relative frequency of piglets born. The qualitative parameters of semen were good, the percentage of not return for the two typologies of semen were similar (70.0% refrigerated *v.* 72.0% frozen) and the DFI values of frozen semen were low (1.88 - 2.95), indicating a high fertilization capacity of semen. Fertility, considered as not return percentage, was related with DFI and CFI, confirming that the frozen semen was of good quality as the refrigerated. Only the FCI parameter showed some differences. The boars were classified in ranks of fertility and for refrigerated semen they were distributed in 6 different ranks, while for frozen semen they were distributed in 3 ranks, showing a lowering of fertility towards the medium rank. Only a boar with low fertility remained minus-variant, confirming its low fertility with both treatments. This last result confirmed that the fertility isn't associated to semen quality, but is an intrinsic characteristic of the animal.

Key Words: pig, genetic identification, microsatellite, fertility, parentage

MT283 The genetics of phosphorus and calcium levels in serum towards improved phosphorus efficiency in pigs. H. Reyer*[1], M. Oster[1,2], F. Just[1,2], S. Ponsuksili[1], and K. Wimmers[1,2], [1]*Institute for Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany;* [2]*Leibniz Science Campus Phosphorus Research, Rostock, Germany.*

Sufficient dietary phosphorus (P) and calcium levels are required to ensure appropriate bone mineralization and normal immune cell function. To maintain an adequate blood P concentration, several known and yet to be elucidated regulators, transporters, hormones, and paracrine signals are involved. The plasticity of pigs regarding their serum P levels (mean ± s.d.: 9.2 ± 1.1 mg/dl; min:

6.0 mg/dl; max: 12.7 mg/dl in German Landrace) provides a huge potential for selective breeding towards improved P efficiency. Similarly, serum calcium values also vary considerably (mean ± s.d.: 9.9 ± 0.8 mg/dl; min: 6.8 mg/dl; max: 11.9 mg/dl). To elucidate the genetics of the calcium-P balance and the P utilisation in pigs, both candidate gene approaches (including *VDR*, *CALCR*, *PTH1R*, *RANK*, and *OPG*) and genome-wide association studies (GWAS) were employed. Analyses were conducted based on a German Landrace population of 590 pigs which were genotyped using 60K-SNP arrays. The analysis revealed a moderate genetic contribution to the phenotypic variance of serum P levels. Most promising quantitative trait loci were detected on chromosomes 4 and 14 ($P < 10^{-6}$) covering each a 2 Mb region. Positional candidates are involved in osteogenic differentiation and osteoclast activity. For a single nucleotide polymorphism (SNP) located in *TRAFD1* (TRAF-Type Zinc Finger Domain Containing 1) significant associations with the serum P level were found. At this locus, homozygous carriers of the alternative allele showed an increased serum P level. Selected SNPs of functional candidate genes of calcium/P regulating and signalling pathways such as *CALCR* (calcitonin receptor) and *CASR* (calcium sensing receptor) showed no consistent association with serum calcium levels. This suggests for a complex genetic contribution beyond major regulators of the calcium-P balance in pigs. The identification of genetic variation in functional and positional candidate genes relevant for P efficiency will contribute to establish novel approaches of P management to balance economic and environmental sustainability. Consequently, associated positional and functional candidate genes will be further investigated.

Key Words: pig, genome-wide association, phosphorus efficiency, candidate gene

MT284 RNA-Seq analysis of gut-associated lymphoid tissue in pigs revealed few differences in transcription profiles of ileal and jejunal Peyer's patches. T. Maroilley[1], M. Berri[2], D. Esquerré[3], C. Chevaleyre[4], G. Lemonnier[1], J. J. Leplat[1,4], S. Vincent-Naulleau[1,4], M. J. Mercat[5], Y. Billon[6], P. Lepage[7], C. Rogel-Gaillard[1], and J. Estellé*[1], [1]*GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [2]*ISP, INRA, Université de Tours, Nouzilly, France;* [3]*GenPhySe, INRA, INP, ENSAT, Université de Toulouse, Castanet-Tolosan, France;* [4]*CEA, DSV, IRCM, Laboratoire de Radiobiologie et Etude du Génome, Jouy-en-Josas, France;* [5]*IFIP-BIOPORC, Pôle génétique, Le Rheu, France;* [6]*GENESI, INRA, Surgères, France;* [7]*MICALIS, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France.*

The gut-associated lymphoid tissue has an essential role on local immune response, but very little is known about gene expression differences between porcine jejunal and ileal Peyer's patches. The aim of our study was to compare the transcriptome profiles of jejunal and ileal Peyer's patches, mesenteric lymph nodes and also peripheral blood. Four 60-day-old male piglets were sampled and the transcriptome of each of the four target tissues was characterised by sequencing with an RNAseq stranded paired-end protocol. After standard bioinformatics analyses with TopHat and HTSeq-count, we performed a differential expression analysis with the edgeR R package. Beyond expected major expression differences between blood and gut associated lymphoid tissues, we identified 993 and 773 differentially expressed (DE) genes between mesenteric lymph nodes and ileal and jejunal Peyer's patches, respectively. Conversely, we detected only 66 DE genes between ileal and jejunal Peyer's patches, with 40 genes being down-regulated in ileal Peyer's patches. At the functional level, Ingenuity Pathway Analysis (IPA) showed that the set of 66 DE genes was enriched in pathways such as VDR/RXR and FXR/RXR activation, and actin cytoskeleton signalling. In addition, 51 of these genes were assembled in an IPA-constructed network associated to 'Cellular development', 'Connective Tissue Development and Function' and 'Tissue Development' functions. In previous results on transcriptome variations along the porcine small intestine, we observed also several of these functions and pathways as differentially enriched in DE genes between ileal and jejunal segments. Thus, the present results suggest that the limited differences between jejunal and ileal Peyer's patches would not be related to specific immune functions.

Key Words: pig, gut, transcriptome, RNA-seq, Peyer's patches

MT285 Assessment of the boar sperm microRNAome by RNA-seq: comparison of two protocols and characterization of the transcriptome profile. M. Gòdia*[1], A. Castelló[1], M. Montfort[2], J. Hecht[2], J.-E. Rodríguez-Gil[3], A. Sánchez[1], and À. Clop[1], [1]*Center for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Bellaterra, Spain;* [2]*Center for Genomic Regulation (CRG), Barcelona Biomedical Research Park (PRBB), Barcelona, Spain;* [3]*Unit of Animal Reproduction, Department of Animal Medicine and Surgery, Universitat Autònoma de Barcelona, Bellaterra, Spain.*

Boars used for artificial insemination are mainly selected based on genetic merit for meat or carcass quality traits. Yet, a proportion of these animals fail to match the required semen quality parameters and end up being rejected. Hence, a noninvasive prediction test would help selecting these boars. We have previously described the boar sperm transcriptome, which contains small amounts of highly degraded RNA which could both be remnants from spermatogenesis and play key roles in fertilization ability. microRNAs have emerged as key regulatory RNAs with strong impact on a vast plethora of phenotypes including sperm quality and fertility in animals. The objective of this study was to evaluate the efficiency of two different microRNA library prep kits and characterise the microRNA population of the boar sperm using RNA from highly purified sperm samples. Ejaculated sperm from 3 pigs was purified from somatic cells and subjected to total RNA extraction. miRNA libraries were then prepared, in parallel, with the NEBNext Small RNA Library Prep Set (New England Biolabs) or the TailorMix miRNA Sample Preparation v2 kit (SeqMatic). Libraries were sequenced on an Illumina HiSEqn 2500 platform to obtain 50bp single end reads. The reads were mapped onto the swine genome (Ssc10.2) with Bowtie1. Annotation was made with Bedtools intersect and reads were divided into gene biotypes (e.g. miRNA, snoRNA, Mt tRNA, etc). In average, each sample produced 1,194,609 reads. 77% of the reads were mapped into the pig genome, 71% of which mapped to gene annotations. Most of these annotations corresponded to microRNAs (33%), mitochondrial tRNAs (27%) and protein coding RNAs (18%). The top 10% most expressed microRNAs included among many others, mir-34c required for oocyte activation, and the mir-29 family, global regulators during germ line meiosis. In the near future, we will compare the sperm microRNAomes of pigs with divergent sperm qualities to identify particular profiles associated to these phenotypes. To the best of our knowledge, this is the first RNA-seq experiment to describe the microRNAome of the boar's ejaculated sperm carried to date.

Key Words: pig, microRNA, reproduction

MT286 Expression of identical genetic mutations across Oncopig cell types results in distinct expression profiles recapitulating transcriptional hallmarks of human tumors. K. M. Schachtschneider*[1], R. M. Schwind[1], K. A. Darfour-Oduro[2], Y. Liu[2,3], S. Mäkeläinen[4,5], A. K. De[2], L. A. Rund[2], O. Madsen[4], M. A. M. Groenen[4], R. C. Gaba[1], and L. B. Schook[1,2], [1]*Department of Radiology, University of Illinois at Chicago, Chicago, IL, USA;* [2]*Department of Animal Sciences, University of Illinois, Champaign-Urbana, IL, USA;* [3]*Department of Animal Genetics and Breeding, Sichuan Agricultural University, Chengdu, China;* [4]*Wageningen University & Research, Animal Breeding and Genomics, Wageningen, The Netherlands;* [5]*Department of Animal*

Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Difficult questions confront clinicians attempting to improve patient outcomes for a wide range of cancer types. A large animal model with genetic, anatomic, and physiologic similarities to humans is required for transitioning between preclinical mouse models and human clinical trials in order to address unmet clinical needs. We previously reported the production of an inducible porcine cancer model (Oncopig) encoding Cre recombinase inducible porcine transgenes encoding *KRAS*^{G12D} and *TP53*^{R167H}, which represent a commonly mutated oncogene and tumour suppressor in human cancers, respectively. To validate the ability of the Oncopig cancer model to mimic human cancers on a transcriptomic level, gene expression profiles were produced for Oncopig primary and transformed cell lines (fibroblasts and hepatocytes), as well as *in vivo* tumours (leiomyosarcomas and hepatocellular carcinomas (HCC)) using RNA-seq. Results based on the relative expression of 11,041 known genes for which expression information was available for each sample resulted in samples clustering by group. In addition, both sarcoma and HCC cell lines and tumours recapitulated key transcriptional hallmarks observed in their respective human malignancies. These included *TERT* reactivation, apoptosis evasion, angiogenesis activation, altered cell cycle regulation, and Wnt signalling activation in HCC samples, and altered *TP53* signalling, Wnt signalling activation, and epigenetic reprogramming in sarcoma samples. Master regulators of Oncopig gene expression previously implicated in human sarcoma (*FOSL1*, *SPI1*, *MEF2C,* and *ETV4*) and HCC (*HDAC2*, *HNF4A*, *FOXA2*, and *EP300*) development were also identified. Direct comparisons also identified conservation of 8 master regulators across Oncopig and 18 human HCC cell lines. Collectively, these results demonstrate expression of identical genetic mutations (*KRAS*^{G12D} and *TP53*^{R167H}) across Oncopig cell types results in distinct expression profiles recapitulating transcriptional hallmarks of human tumour types, demonstrating the value of the model across distinct human cancer subtypes.

Key Words: gene expression, biomedical model, pig, sarcoma, hepatocellular carcinoma

MT287 Updated pig genome resources in Ensembl. T. Hourlier*[1], L. Eory[2], K. Billis[1], C. García Girón[1], L. Haggerty[1], O. Izuogu[1], D. N. Murphy[1], R. Nag[1], F. J. Martin[1], A. L. Archibald[2], B. Aken[1], and P. Flicek[1], [1]*European Molecular Biology Laboratory, European Bioinformatics Institute, Hinxton, Cambridgeshire, UK;* [2]*The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, Edinburgh, UK.*

Ensembl provides high-quality, reference annotation resources for publicly available genome assemblies, including domestic pig (*Sus scrofa*). Pig is an important model for cardiovascular disorders, infectious diseases, and xenotransplantation, and also an economically important species for meat production. We have fully updated all pig genome resources in Ensembl based on the recently improved reference assembly Sscrofa11.1 (GCA_000003025.6) produced by the Swine Genome Sequencing Consortium. These updates include a curated gene annotation using new Illumina and PacBio transcriptome data that comply with the FAANG meta data submission guidelines, which we will describe in detail. Also updated to the new assembly are our pig genome variation resources, gene names, gene-trees and orthologues, cross-references to external databases including UniProt and RefSeq, vertebrate multiple whole genome alignments, and conserved and constrained elements. These updates will be released as part of Ensembl version 90, which is expected in July. All data are freely available through our website at http://www.ensembl.org, REST API (http://rest.ensembl.org) and our public MySQL server (ensembldb.ensembl.org) as well as tools such as the Ensembl Variant Effect Predictor (www.ensembl.org/Tools/VEP) and BioMart (http://www.ensembl.org/biomart). Ensembl also supports the upload and visualisation of data in multiple file formats such as BAM or GFF3. We have developed the Track Hub Registry (http://trackhubregistry.org/) to facilitate data sharing through discovery of publicly accessible track data hubs and viewing them alongside Ensembl data. Additionally, data can now be viewed in Ensembl even if the files are not hosted locally by submitting them in CRAM format to the ENA and registering a track hub with the ENA accession numbers.

Key Words: pigs and related species, genome annotation, Functional Annotation of Animal Genomes (FAANG), bioinformatics tools, databases/repositories

MT288 Genome-scale sgRNA library construction and use for CRISPR/Cas9 based genetic screens in the pig. C. Zhao*[1], G. Yang[1], X. Han[1], Y. Gao[1], X. Li[1,2], S. Xie[1,2], and S. Zhao[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding, and Reproduction of the Ministry of Education & Key Laboratory of Swine Genetics and Breeding of the Ministry of Agriculture, Huazhong Agricultural University, Wuhan, Chin;* [2]*The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan, China.*

The CRISPR-Cas9 system enables efficient targeting to large numbers of genes through the use of single guide RNA (sgRNA) libraries. The ability to systematically disrupt porcine genes serves as a powerful tool for understanding their function. One key challenge is the design and production of high-quality oligonucleotides. In this study, a Pig Genome-scale CRISPR Knockout library (PigGeCKO) was designed and constructed. First, we designed a CRISPR library (CRISPR-lib) design software, which implements an end-to-end design of custom sgRNA libraries targeting the genomes of many different species. CRISPR-lib automates all tasks for the generation of sgRNA libraries. It can design libraries of variable size ranging from a few hundred genes to genome-scale for any genomes. Then, we used CRISPR-lib to design a PigGeCKO consisting of more than 85,000 sgRNAs, of which these sgRNAs were directed against the whole genome-wide porcine genes, lincRNAs, miRNAs and 1000 scrambled-sequence sgRNAs serving as negative controls. All genes were covered with 3 sgRNAs per gene. For library construction, the PCR- and Gibson assembly based approaches were employed to create large polyclonal pools of lentiviral sgRNA vectors. Subsequently, library coverage was determined by Illumina sequencing of plasmid libraries, the result showed that up to 96.17% designed sgRNAs were detected in the plasmid pool. In addition, a stable Cas9 expression PK-15 cell line was constructed for genetic screening using PigGeCKO. The PigGeCKO plasmid pool was packaged into lentiviral particles and used to generate knockouts in the PK-15-Cas9 cell lines. In summary, CRISPR-lib software is suitable for the design of libraries using CRISPR/cas9 and targeting any species. And the PigGeCKO is also suitable for High-throughput screening of a CRISPR/Cas9 library for functional genomics in the pig. This work was supported by the National High Technology Research and Development Program of China (863 Program, 2013AA102502) and the National Transgenic Project of China (2016ZX08006003–004).

MT289 Characterization of 3D genomic interactions in fetal pig muscle. M. Marti-Marimon*[1], H. Acloque[1], M. Zytnicki[2], D. Robelin[1], S. Djebali[1], N. Villa-Vialaneix[2], O. Madsen[3], Y. Lahbib-Mansais[1], D. Esquerré[1], F. Mompart[1], L. Liaubet[1], M. Groenen[3], M. Yerle-Bouissou[1], and S. Foissac[1], [1]*GenPhySE, University of Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France;* [2]*MIAT, University of Toulouse, INRA, Castanet Tolosan, France;* [3]*Animal Breeding and Genomics Centre (ABG), Wageningen University, Wageningen, Netherlands.*

Genome sequence alone is not sufficient to explain the overall coordination of nuclear activity in a particular tissue. The nuclear organisation and genomic long-range intra- and inter-chromosomal interactions play an important role in the regulation of gene ex-

pression and the activation of tissue- specific gene networks. Here we present an overview of the pig genome architecture in muscle at two late developmental stages. The muscle maturation process occurs between the 90th day and the end of gestation (114 days), a key period for survival at birth. To characterise this period we profiled chromatin interactions genome-wide with *in situ* Hi-C (High Throughput Chromosome Conformation Capture) in muscle samples collected at 90 and 110 days of gestation, specific moments where a drastic change in gene expression has been reported. About 200 million read pairs per library were generated (3 replicates per condition). This allowed: (a) the design of an experimental Hi-C protocol optimized for frozen fetal tissues, (b) the first Hi-C contact heatmaps in fetal porcine muscle cells, and (c) to profile Topologically Associated Domains (TADs) defined as genomic domains with high levels of chromatin interactions. Using the new assembly version *Sus scrofa* v11, we could map 82% of the Hi-C reads on the reference genome. After filtering, 49% of valid read pairs were used to infer the genomic interactions in both developmental stages. In addition, ChIP-seq experiments were performed to map the binding of the structural protein CTCF, known to regulate genome structure by promoting interactions between genes and distal enhancers. The Hi-C and ChIP-seq data were analysed in combination with the results of a previous transcriptome analysis, focusing on the hundreds of genes that were reported as differentially expressed during muscle maturation. We will report the observed general differences between both developmental stages in terms of transcription and structure.

Key Words: nuclear architecture, fetal development, Hi-C (high-throughput chromosome conformationcapture), pig muscle, CTCF

MT290 Genome-wide scanning of the *cis*-effects of sequence variations on enhancer activity in the F6 swine heterogeneous stock. Z. Zhang*, Y. Zhu, Z. Zhou, W. Li, and L. Huang, *State Key Laboratory of Pig Genetic Improvement and Production Technology, Jiangxi Agricultural University, NanChang, JiangXi Province, China.*

Genome-wide association studies (GWAS) have highlighted thousands of variants associated with numerous traits in human and farm animals. And the majority of these variants located in non-coding regions, leading to difficulty in functional annotation. To meet the significant challenges in post-genomic era, working together with FANNG, a two phase research project has been carried out to identify and annotate the functional elements of the pig genome at different developmental stages, different tissues in both male and female individuals of Asian and western pig breeds (Phase I) and then to investigate the sequence variations of these genomic elements with the corresponding transcriptome, proteome, metaboliome and phenome change of the swine complex traits for the benefit of effective meat production as well as disease models for human beings (Phase II). Here, we report our effort to systematically archive enhancers in liver tissues in an experimental F6 heterogeneous pig population (multigenerational outbred comprising of 8 pig breeds) and decipher the *cis*-effects of genome-wide sequence variants on the activity of these enhancers and expression of related genes. We established a stable protocol to define active enhancers by ChIP-seq with H3K27Ac antibody in liver, and ~89% enhancer peaks we detected are nicely overlapped with that from previous report (Villar et al., 2015). We will characterize the active enhancers in livers of ~150 F6 mosaic pigs. Integrating of DNA re-sequencing, liver transcriptome as well as detailed phenotyping of these pigs with ~220 traits, we will create a resource to understand how sequencing variants perturb enhancers and further affect gene expression patterns in liver in pigs. This resource will be valuable not only for functional interpretation of the genome wide active enhancer variants but also their potential effects on the related complex traits in this important farm animal.

Key Words: swine heterogeneous stock, ChIP-seq, enhancer, integrative genomics, complex trait

MT291 A metagenomics study on a non-metagenomics experiment: mining next-generation sequencing datasets from porcine DNA identified unexpected viral sequences. S. Bovo[1,2], G. Mazzoni[1,3], A. Ribani[1], V. J. Utzeri[1], F. Bertolini[1,4], G. Schiavo[1], and L. Fontanesi*[1], [1]*Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy;* [2]*Biocomputing Group, Department of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy;* [3]*Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Copenhagen, Denmark;* [4]*Department of Animal Science, Iowa State University, Ames, IA, USA.*

Shot-gun next-generation sequencing (NGS) on whole DNA extracted from specimens collected from mammals often produces reads that are not mapped (i.e. unmapped reads) on the host reference genome and that are usually discarded as by-products of the experiments. In this study, we mined Ion Torrent reads obtained by sequencing DNA isolated from archived blood samples collected from 100 performance tested Italian Large White pigs. Two reduced representation libraries were prepared from two DNA pools constructed each from 50 equimolar DNA samples. A specific bioinformatics pipeline was designed to mine unmapped reads on the reference pig genome that were obtained from the two NGS datasets. *In silico* analyses included read mapping and sequence assembly approaches for a viral metagenomics analysis using the NCBI Viral Genome Resource. Our approach identified sequences matching several viruses of the *Parvoviridae* family: porcine *Parvovirus* 2 (PPV2), PPV4, PPV5 and PPV6 and porcine *Bocavirus* 1-H18 isolate (PBoV1-H18). The presence of these viruses was confirmed by PCR and Sanger sequencing of individual DNA samples. PPV2, PPV4, PPV5, PPV6 and PBoV1-H18 were all identified in samples collected in 1998–2007, 1998–2000, 1997–2000, 1998–2004 and 2003, respectively. Our study provided a retrospective evaluation of apparently asymptomatic parvovirus infected pigs providing information that could be important to define occurrence and prevalence of different parvoviruses in South Europe and to evaluate resistance of these animals to viral infections. This study demonstrated the potential of mining NGS datasets non-originally derived by metagenomics experiments for viral metagenomics analyses in a livestock species.

Key Words: metagenomics, unmapped reads, parvovirus, disease resistance, NGS

MT292 Dietary vitamin A differentially affects fat desaturation in porcine stearoyl-CoA desaturase genotypes. J. Estany*[1], S. Gol[1], M. Tor[1], L. Bosch[2], J. Reixach[3], and R. Pena[1], [1]*Departament of Animal Science, University of Lleida, Agrotecnio Center, Lleida, Spain;* [2]*Departament of Agriculture Engineering and Food Technology. University of Girona, Girona, Spain;* [3]*Selección Batallé, S.A, Riudarenes, Spain.*

The single nucleotide polymorphism (g.2228T>C) in the promoter region of the porcine stearoyl-coA desaturase (*SCD*) gene is associated to fatty acid desaturation in Duroc pigs (Estany *et al*. 2014 PlosOne 20;9(1):e86177). This mutation is positioned in the core sequence of two putative transcription binding motifs for retinoid and retinoic acid receptor response elements. Thus, it could be hypothesised that the effect of the *SCD* genotypes on fat desaturation may differ according to dietary vitamin A. To test this hypothesis, we conducted an experiment in which two batches of Duroc pigs of the three S*CD* genotypes (n = 142) were subjected to two identical growing-finishing diets differing in the vitamin A content.

The first diet was not supplemented with vitamin A while the second diet was supplemented with retinyl acetate at commercial levels. At slaughter, a sample of m. gluteus medius and m. longissimus thoracis were taken to determine the intramuscular fat content and fatty acid composition. As expected, the *SCD* genotype modified the desaturation ratio, with allele T increasing C16:1/C16:0 and C18:1/C18:0 ratios ($P < 0.01$). The diet had no effect on fatty acid composition, but an interaction between the diet and the *SCD* genotype was revealed for C16:1/C16:0 and C18:1/C18:0, both in m. gluteus medius ($P < 0.01$) and in m. longissimus thoracis ($P < 0.05$). In both muscles, the allele substitution effect of allele T for allele C at *SCD* g.2228T>C for C16:1/C16:0 and C18:1/C18:0 was, respectively, around 3-fold and 2-fold higher when vitamin A was not added to the diet. The results obtained indicate that supplementation of vitamin A at commercial amounts promotes the activity of allele C but depresses that of allele T, thereby giving rise to a good example of how genetic variation influences nutrient response.

Key Words: pig, vitamin A, intramuscular fat, oleic acid, MUFA

MT294 Distribution of polymorphisms in major and candidate genes for productive and domestication-related traits in European local pig breeds. A. Fernández[1], M. Muñoz[1], F. García[1], Y. Núñez[1], C. Geraci[2], A. Crovetti[3], J. García-Casco[1], E. Alves[1], M. Skrlep[4], J. Riquet[5], M. Mercat[6], R. Bozzi[3], M. Candek-Potokar[4], L. Fontanesi[2], C. Óvilo*[1], [1]*INIA, Madrid, Spain;* [2]*UNIBO, Bologna, Italy;* [3]*UNIFI, Firenze, Italy;* [4]*KIS, Ljubljana, Slovenia;* [5]*INRA, Toulouse, France;* [6]*IFIP, Paris, France.*

TREASURE is a multidisciplinary European project focused on the development of activities for the benefit of sustainable pork chains based on European local pigs. One of its main objectives is the genetic characterisation of local pig breeds participating in the project, by using genetic and genomic tools. The most relevant genes and mutations associated with pig productive, meat quality, reproductive and disease resistance traits were prioritized and analysed in order to identify useful markers for authentication, traceability, conservation and breeding programmes. A panel of 32 SNPs were selected and a genotyping chip was designed and employed to genotype 48 animals from each one of 20 breeds included in the project (Schwäbisch Hällisches, Iberian, Black Majorcan, Basque, Gascon, Black Slavonian, Turopolje, Apulo Calabrese, Casertana, Cinta Senese, Mora Romagnola, Black Sicilian, Sarda, Lithuanian indigenous wattle, Old Lithuanian White, Alentejano, Bisaro, Mangalitsa, Moravka, Krskopolje). Twenty seven SNPs located in 24 genes were succesfully genotyped (*MC1R, TYRP1, NR6A, PCK1, RYR1, IGF2, MC4R, PHKG1, SCD, GBP5, TAS2R39, TAS2R4, MUC4, ESR1, CYP2E1, LEP, CAST, MTTP, CYB5A, FTO, PPARGC1A, CAPN1, PPARD, CTSL*). Results show very interesting findings, as lack or scarce segregation of markers in genes involved in coat colour or productive and reproductive traits, such as *MC1R*, *ESR1* or *CTSL* in all the analysed breeds, with useful implications for traceability. On the other hand, major gene alleles with contrasted effects on production and fatness (such as *RYR1*, *IGF2*, *MC4R*, *LEP*), meat quality (*SCD*, *CAST*, *MTTP*) or disease resistance (*MUC4*, *GBP5*) segregate in most breeds, in some cases with intermediate frequencies, opening selection possibilities. These results joint with ongoing genomic, transcriptomic and metagenomic assays, will provide essential information regarding genetic diversity, structure, selective signatures and population-specific biological processes responsible for specific production and quality traits. TREASURE project is funded under European Union's Horizon 2020 research and innovation programme, grant no. 634476

Key Words: pig, local breed, SNP, major gene, allele frequency

Ruminant Genetics and Genomics

MT295 Genetic correlations of female fertility and calf survival with VIA carcass traits in UK beef cattle. A. Moran*, M. Coffey, and K. Moore, *SRUC, Edinburgh, Scotland, UK.*

Fertile suckler beef cows and low calf mortality are essential for a profitable beef production system. Little genetic progress has been made for female fertility or calf survival. The aim of this study was to estimate genetic parameters for female fertility and calf survival traits and their genetic correlation with video image analysis (VIA) carcass traits in UK beef cattle. The genetic parameters from this study can subsequently be used for genomic evaluation of female fertility and calf survival to provide accurate breeding values from early in life for these late in life traits. Traditionally genetic evaluations have been based on voluntary recording in pedigree animals but this study utilised commercial data to reflect industry practices. The dataset contained 12.5 million commercial records from animals which were greater than 45% Limousin. The three female fertility traits investigated were age at first calf (AFC), calving interval (CI) and lifespan (LS). Parameter estimation was carried out in ASREML using a reduced dataset ($N = 58{,}148$) resulting in heritabilities of 0.13 (± 0.014), 0.045 (± 0.015), 0.049 (± 0.012) for AFC, CI and LS respectively. The heritability of calf survival was found to be 0.13 (± 0.02). Preliminary work has found fertility traits are well correlated with VIA carcass traits. Understanding the interactions between fertility and carcass traits will enable us to build a detailed picture of the impact of selection on carcass traits. Thanks to Innovate UK, BBSRC and the British Limousin Cattle Society.

Key Words: cattle and related species, animal breeding, genetic improvement

MT296 A comparative study on reproductive performance of zebu and *taurus* genotypes. M. Khan* and M. Siddiki, *Department of Dairy Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.*

Poor reproductive performance of crossbred cattle in Bangladesh is due to inadequate nutrition is a major problem. To improve cattle productivity, temperate dairy breeds and improved zebu dairy breeds have been introduced for years. There is no specific study related to reproductive performance of zebu and taurus genotype in our tropical environment. So, the present study has been taken into consideration to investigate the suitable genotype in tropical environment having better reproductive performance. A total of 80 crossbred cows were selected of which 40 cows were Holstein cross (HC) (local zebu × Holstein) and 40 cows were Sahiwal cross (SC) (local zebu × Sahiwal). From each genotype, 20 cows were supplemented with Urea Molasses Block (+UMB) and 20 cows remained as control (-UMB). The animals were < 2nd lactation and average bodyweight was 286 kg. All the cows under same management condition and were reared on straw based diet. All animals were fed rice straw *ad libitum* and limited amount of seasonal cut and carry grass (3 kg/d). Reproductive intervals were reduced by supplementation in both genotypes. Reduction of calving to 1st Progesterone rise was 14 and 39 days in HC and SC cows respectively which differs significantly ($P > 0.05$). The interval from calving to conception was reduced by 54 and 81 days in HC and SC cows ($P > 0.05$). The result suggests that between the two genetic group of cows, SC performed better related to reproductive parameters than that of HC cows.

Key Words: cows, reproductive performance, genetic group

MT297 Co-expression network analysis identifies genes associated with meat tenderness. W. Diniz*[1], A. Cesar[2], L. Geistlinger[3], P. Tizioto[2], P. Oliveira[3], J. Afonso[1], M. Rocha[1], A. Lima[1], C. Buss[1], L. Coutinho[2], and L. Regitano[3], [1]*Departament of Genetic and Evolution, Federal University of São Carlos, São Carlos, São Paulo, Brazil;* [2]*Department of Animal Science, University of São Paulo/ESALQ, Piracicaba, São Paulo, Brazil;* [3]*Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil.*

Tenderness is an important trait of meat quality and is known to be influenced by several factors including genetic composition. Although various studies have reported gene expression differences between tough and tender meat, it has been rarely analysed to which extent this can be attributed to interaction among genes and coordinated activation or repression of specific pathways. To identify candidate gene networks associated with meat tenderness, we performed weighted gene co-expression network analysis (WGCNA) in a Nelore cattle population of 129 steers. Gene expression was measured with RNA-seq in *Longissimus dorsi* muscle samples and genomic estimated breeding values for shear force (GEBV-SF) were used as a quantitative trait for meat tenderness. From the co-expression modules detected by WGCNA, we found two modules to be significantly negatively correlated with GEBV-SF. Genes in these modules are involved in muscle fibre composition, muscle contraction, and cell morphology. Proteolytic processes, known to play a key role in meat tenderness variation, were found significantly enriched for the genes presented in the associated modules. We also found tenderness markers such as *CAPNS1*, *MAP2K5*, *MYOZ1*, *TNNT3*, and *HMGA1* contained in the detected modules. We further observed *EIF3L* as a hub gene, which has been previously reported as involved in formation of primary myoblasts and differentiation of muscle fibres but not described as related to shear force. The detected co-expression modules in this study provide evidence for substantial interplay between genes in processes influencing meat tenderness. Further studies will be required to elucidate the underlying regulatory mechanisms of the observed co-expression networks. This project was supported by FAPESP (12/23638–8, 15/09158–1).

Key Words: cattle and related species, gene expression, network analysis, RNA-seq, systems biology

MT298 Comparative genome-wide methylation analysis of longissimus dorsi muscles between Japanese Wagyu and Chinese Red Steppes cattle. X. Fang[1], Z. Zhao[1], G. Li[2], X. Yu[3], Z. Wei[2], H. Yu[1], and R. Yang*[1], [1]*College of Animal Science, Jilin University, Changchun, Jilin Province, China;* [2]*The Key Laboratory of National Education Ministry for Mammalian Reproductive Biology and Biotechnology, Inner Mongolia University, Hohhot, Inner Mongolia Autonomous Region, China;* [3]*Department of Biological Sciences, Clemson University, Clemson, SC, USA.*

DNA methylation is an important epigenetic mechanism involved in many biological processes including muscle development and lipometabolism. DNA methylation can affect meat quality traits and fat deposition traits by regulating the expression of genes important for myoblast proliferation and adipocyte differentiation. Significant differences in meat quality traits have been reported between Japanese Wagyu and Chinese Red Steppes cattle, which presented a unique model for analysing the effects of DNA methylation on these economically important meat quality traits. In the present study, we sequenced the whole genome DNA methylation in the longissimus muscle of these two cattle breeds to detect whether DNA methylation plays a role in determining the fat deposition and meat quality traits of beef battle by whole genome bisulfite sequencing (WGBS) method. A high quality methylation map of two cattle breeds was obtained in this study. 23150 differentially methylated regions (DMRs) were identified which were located in 8596 genes enriched in 9922 GO terms, of which 1046 GO terms were significantly enriched ($P < 0.05$) including lipid translocation (GO:0034204) and lipid transport (GO:0015914). KEGG analysis showed that the DMR related genes were distributed among 276 pathways. Correlation analysis found that 331 DMRs were negatively correlated with expression levels of differentially expressed genes (DEGs) with 21 DMRs located in promoter regions. Interestingly, DNA methylation of ZBED-6, IGF-2R, IGFBP-5 and GJC1 showed significant difference across breeds, and five CpG were significantly correlated with RNA expression. This study identified novel candidate DMRs and DEGs correlated with muscle development and lipometabolism, which will be provides a thorough understanding of meat quality traits variation in beef cattle from an epigenetic perspective.

Key Words: cattle, epigenomics, genome sequencing, candidate gene, meat production

MT299 Scanning of selection signature provides a glimpse into important economic traits in goats (*Capra hircus*). D. Guan*, N. Luo, X. Tan, Z. Zhao, Y. Huang, R. Na, J. Zhang, and Y. Zhao, *College of Animal Science and Technology, Southwest University, Chongqing, China.*

Goats (*Capra hircus*) are one of the oldest domesticated species, which are distributed over all types of ecological areas with more concentrated in the tropics, dry zones and developing countries and have been used for their milk, meat, hair and skins over much of the world. However, the genetic components underlying these phenotypic traits remain largely unknown. We collected a total of 12 blood samples of unrelated individuals from Dazu black female goats (DBG, $n = 6$) and Inner Mongolia cashmere female goats (IMCG, $n = 6$). Genomic DNA was extracted and sequenced. We performed SNP calling on a population scale for two groups. Selective sweep analyses were performed by calculating heterozygosity (Hp) and population differentiation (Fst). The study presented here has generated 192.747G raw data and identified more than 5.03 million SNPs and 334,151 Indels. In addition, we identified 155 and 294 candidate regions harboring 86 and 97 genes based on allele frequency differences in DBG and IMCG, respectively. Populations differentiation reflected by Fst values detected 368 putative selective sweep regions including 164 genes. The top 1% regions of both low heterozygosity and high genetic differentiation contained 239 (135 genes) and 176 (106 genes) candidate regions in DBG and IMCG, respectively. These genes were related to reproduction and production traits, such as 'neurohypophyseal hormone activity' and 'adipocytokine signaling pathway'. The works performed here provided an important resource for future goat breeding.

Key Words: *Capra hircus*, whole genome sequencing, selective signature, heterozygosity (Hp), population differentiation (Fst)

MT300 Transcriptome analyses reveal reduced hepatic lipid synthesis and accumulation in more efficient beef cattle. R. Mukiibi*[1], M. Vinsky[2], C. Fitzsimmons[1,2], S. M. Waters[3], P. Stothard[1], and C. LI[1,2], [1]*Department of Agriculture, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada;* [2]*Agriculture and Agri-Food Canada, Lacombe Research and Development Centre, Lacombe, Alberta, Canada;* [3]*Animal and Bioscience Research Department, Teagasc, Grange, Dunsany, County Meath, Ireland.*

The specific genes and variants underlying the genetic control of residual feed intake (RFI) in beef cattle are largely unknown. In the present study, we aimed to identify genes associated with RFI using whole transcriptome analyses in three Canadian beef cattle breeds raised under similar environments. Liver samples were collected at slaughter from high ($n = 6$) and low ($n = 6$) RFI phenotype steers of Angus, Charolais, and Kinsella composite (KC) breeds. RNA was extracted and gene expression profiles obtained by mRNA sequencing (RNA-seq) using Illumina HiSeq sequencing technology yielding an average of 36 million reads with a mean

Phred quality score of 36 per individual animal. On average, 89.7% of the sequence reads aligned and mapped to the bovine reference genome UMD_3.1, and differential gene expression analyses between high and low RFI groups were performed within breed using a generalized linear mixed model as implemented in edgeR. At a false discovery rate (FDR) < 0.05 and fold change >2, 72 genes were differentially expressed (DE) in Angus (46 down-regulated and 26 up-regulated), 41 in Charolais (19 down-regulated and 22 up-regulated), and 175 in KC (108 down-regulated and 67 up-regulated). Five DE genes were common across the three breed populations, four of which (*LPIN1*, *SCD*, *TP53INP1*, *LURAP1L*) were down-regulated in steers with low RFI in all of the three populations. Angus and KC had the greatest number of shared DE genes (n = 15), while Angus and Charolais had the fewest (n = 3). Functional analyses with Ingenuity Pathway Analysis (IPA) showed that the DE genes from the three breeds were mainly involved in lipid metabolism, molecular transport, small molecular biochemistry, carbohydrate metabolism, cellular development, energy production, amino acid metabolism, and cell death and survival. Furthermore, results from the IPA analyses predicted decreased lipid synthesis and accumulation in low RFI (more efficient steers) from all the three breeds. These results provide an insight into the molecular architecture of RFI and may help to identify causative gene variants for feed efficiency traits in beef cattle.

Key Words: feed efficiency, RFI, RNA-seq, fat metabolism, beef cattle

MT301 Assessment of the pedigree quality on genetic evaluation in Tunisian Holstein cows. Y. Ressaissi*, *INAT, Tunis, Mahrajène, Tunisia.*

The impact of genealogical information availability on the accuracy of the genetic evaluation process in Holstein cows was tested by using 3 pedigree files. The first was the original heterogeneous file which included the whole cows from which two homogeneous files were generated: one of which included only data of the knowing mothers cows and the other contained only data from cows whose both parents were known. A uni-trait animal model with permanent environment effect was fitted to 305-days (MY305) and to Test-day (TDMY) milk records. Based on the restricted maximum likelihood procedure, the matrix relationship between cows and their ancestors was constructed. Contemporary groups were defined as herd*calving year for MY305 and as herd*control year for TDMY. Pearson correlations and genetic variability distributions were established by group of herd sizes to evaluate the effect of balanced pedigree on the prediction of breeding values and estimation of genetic diversity between herds. Heritabilities have respectively varied between 0.02 and 0.30 for TDMY and between 0.02 and 0.24 for MY305 while repeatabilities have respectively ranged from 0.18 and 0.46 and from 0.18 and 0.39. Genetic ranking correlations were positive and have oscillated between 0.76 and 0.97 within group of herds while higher correlations were observed between the breeding values which were predicted from the balanced pedigrees. Furthermore, estimation of genetic parameters was ameliorated and the distribution of genetic standard deviation between herds was proved to be more diversified and better illustrated within groups. Lack in animal identification generates unbalanced pedigrees of poor quality which has repercussions on the appropriate combination of the performances with the genealogical information and consequently affects the relevance of genetic evaluation and the estimation of genetic variability.

Key Words: quantitative genetics, statistical genetics, selection, milk production

MT302 Gene networks contributing to skeletal muscle compensatory growth in cattle. K. Keogh*, D. A. Kenny, and S. M. Waters, *Animal and Bioscience Research Department, Teagasc, Grange, Dunsany Co. Meath, Ireland.*

Compensatory growth (CG) is an accelerated growth phenomenon observed in animals upon re-alimentation following a period of dietary restriction and is utilised worldwide in animal production systems as a method to lower feed costs. However, the biochemical mechanisms controlling this phenomenon are yet to be elucidated fully. The objective of this study was to identify genes significantly associated with the expression of CG through network analysis. Thirty Holstein Friesian bulls were fed either a restricted diet for 125 days (Period 1), following which they were allowed feed *ad libitum* for a further 55 days (Period 2) or fed *ad libitum* for the entirety of the trial. *M. longissimus dorsi* biopsies were harvested from all bulls on day 15 of Period 2 and RNAseq analysis was performed. During re-alimentation, previously restricted animals displayed CG, growing at 1.8 times the rate of the *ad libitum* control animals. The weighted gene co-expression network analysis (WGCNA) software package was used to identify modules containing genes that were co-expressed and also correlated with the accelerated growth of CG (average daily gain, (ADG)). Gene expression data were filtered by removal of lowly expressed genes, then normalised and subsequently Log2 transformed. Unsigned, weighted correlation network construction and module detection was performed using the automatic network construction and module detection. Module-Trait relationships were calculated by Pearson correlation. Modules with statistically significant correlations were selected for further analysis as potentially interesting modules associated with the expression of CG. WGCNA identified a significant gene module containing 1089 genes that were positively correlated with accelerated growth (ADG; r = 0.71, p = 0.03). Functional analysis of this module revealed significant enrichment of genes involved in cellular organisation and interaction, the cytoskeleton, signal transduction and metabolism. Our results suggest that the skeletal muscle of cattle undergoing CG may have increased cellular activity as a consequence of re-alimentation and that this may be contributing to CG in skeletal muscle in cattle.

Key Words: gene network, compensatory growth, cattle

MT303 Genome analysis of Sudanese goat breeds identifies regions associated with growth. S. A. Rahmatalla*[1,2], D. Arends[1], M. Reissmann[1], A. S. Ahmed[1], K. Wimmers[3], H. Reyer[3], and G. A. Brockmann[1], [1]*Albrecht Daniel Thaer-Institut für Agrar- und Gartenbauwissenschaften, Humboldt-Universität zu Berlin, Berlin, Germany;* [2]*Department of Dairy Production, Faculty of Animal Production, University of Khartoum, Khartoum North, Sudan;* [3]*Leibniz-Institut für Nutztierbiologie (FBN), Institut für Genombiologie, Dummerstorf, Germany.*

Sudan is the largest producer of goat milk in Africa and worldwide the 3rd largest. Indigenous goat breeds in Sudan are adapted to local environment conditions and have an important contribution to food security. Using the high marker density of the Goat 50K SNP chip we assessed the genetic variation within and among the four major Sudanese breeds: Desert, Nilotic, Nubian, and Taggar. Genetic diversity was evaluated by comparing minor allele frequency (MAF), proportion of polymorphic SNPs, heterozygosity, inbreeding coefficients and principal component analysis (PCA). The average MAF in all four populations was around 0.3 (± 0.13). The proportion of polymorphic SNPs ranged from 96.9% for Taggar to 98.2% for Desert goats. In all breeds, no significant difference between the observed and expected heterozygosity (0.4) was found. Average inbreeding coefficient (F_{IS}) did not differ from zero, providing no evidence for inbreeding in the studied populations. Principle component analysis (PCA) was applied to classify the four breeds using the first and second principal components, accounting for 2.5 and 1.9% of the genetic variance, respectively. The first principal component distinguished three populations: Taggar, Nilotic and a mixture of Nubian and Desert goats, while the second

principal component separated Nubian from Dessert goats. Investigation of the SNPs which contributed highly to the first principal component allowed us to identify genomic regions containing interesting candidate genes known to influence animal growth. One of the species studied (Tagger) is a species of dwarf goat, as such it is not unsurprising that our follow up PCA analysis identified regions with known growth genes. The results pertaining to genetic variability and diversity can be used for classification of local breeds. The genomic regions identified using PCA provide interesting targets for genomic selection to improve local breed competitiveness compared to imported goat breeds.

Key Words: goats and related species, comparative genomics, single nucleotide polymorphism (SNP), breed diversity, breed/population identification

MT304 Effects of DNA markers associated with carcass traits and fatty acid composition on fertility traits in Japanese Black cows. M. Tsuchimura*[1], K. Fukazawa[1], F. Kawaguchi[1], T. Matsuhashi[2], S. Maruyama[3], K. Oyama[4], S. Sasazaki[1], and H. Mannen[1], [1]*Graduate School of Agricultural Science, Kobe University, Kobe, Japan;* [2]*Institute of Advanced Technology Kindai University, Wakayama, Japan;* [3]*Gifu Prefectural Livestock Research Institute, Takayama, Japan;* [4]*Food Resources Education & Research Center, Kobe University, Kasai, Japan.*

Recently, several DNA markers associated with carcass traits and fatty acid composition have been developed for marker-assisted selection in Japanese Black cattle. In the present study, we investigated effects of these markers on fertility traits to evaluate effectiveness as selective markers. We genotyped 6 DNA markers, SCD V293A and FASN g.841G>C, which are associated with fatty acid composition, PLAG1 ss319607405 and NCAPG I442M, which are associated with carcass weight, DGAT1 K232A and EDG1 g.1471620G>T, which are associated with subcutaneous fat thickness and beef marbling standard, respectively, in 516 Japanese Black cows in Gifu prefecture, Japan. Then, analysis of variance was performed to investigate the association with the estimated breeding value of 3 fertility traits (age at first calving, calving interval and the number of calves produced at 4 years of age) and 6 carcass traits (carcass weight, rib eye area, rib thickness, subcutaneous fat thickness, yield estimate and beef marbling standard), and Tukey-Kramer HSD test was also conducted to investigate significant differences between genotypes. Genotyping results showed that minor allele frequencies were 0.37(SCD), 0.08(FASN), 0.23(DGAT1), 0.27(PLAG1), 0.49(EDG1) and 0.20(NCAPG). As a result of analysis of variance, SCD and NCAPG had significant effects on age at first calving ($P < 0.001$) and the number of calves produced at 4 years of age ($P < 0.05$, $P < 0.001$), and EDG1 had significant effects on calving interval ($P < 0.05$) and the number of calves produced at 4 years of age ($P < 0.05$), while FASN, DGAT1 and PLAG1 showed no significant effects on fertility traits. These results suggested that we should consider the influence of SCD, NCAPG and EDG1 on fertility traits when these markers are applied in marker-assisted selection. These findings would be useful information for the future breeding in this population.

Key Words: carcass traits, fatty acid composition, fertility, DNA marker, cattle

MT305 Association of VNN1 gene polymorphism with fatty acid composition in Japanese Black cattle. H. Kigoshi*[1], Y. Matsumoto[1], F. Kawaguchi[1], Y. Uemoto[2], E. Kobayashi[3], M. Fukushima[4], E. Iwamoto[5], E. Yoshida[5], T. Akiyama[4], N. Kohama[4], K. Oyama[6], T. Honda[6], H. Mannen[1], and S. Sasazaki[1], [1]*Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Japan;* [2]*National Livestock Breeding Center, Fukushima, Japan;* [3]*Animal Breeding and Reproduction Research Division, NARO Institute of Livestock and Grassland Science, Tsukuba, Japan;* [4]*Northern Center of Agricultural Technology, General Technological Center of Hyogo Prefecture for Agriculture, Forest and Fishery, Asago, Japan;* [5]*Hyogo Prefectural Technology Center of Agriculture, Forestry and Fisheries, Kasai, Japan;* [6]*Food Resources Education & Research Center, Kobe University, Kasai, Japan.*

Fatty acid composition of adipose tissue is an important trait in beef industry. In our previous study, we performed a genome wide association study for oleic acid percentage and identified QTL (BTA9: 65–75Mbp) in Japanese Black cattle population. The objective of the current study is to search for a responsible mutation and develop DNA marker for fatty acid composition. There are 43 genes in the region and we selected genes that would be involved in fatty acid metabolism. In the current study, we focused on VNN1 gene participating in Pantothenate and CoA biosynthesis pathway as a candidate gene. We searched polymorphisms in full length CDS of VNN1 and investigated the associations with fatty acid composition (C14:0, C14:1, C16:0, C16:1, C18:0 C18:1 C18:2, MUFA, PUFA, SFA). Six SNPs (c.34G>A, c.197C>T, c.831G C, c.906T>C, c.1370A>G, c.1545T>C) were identified by sequence comparison among eight animals (four from high and four from low oleic acid percentage). Three of them (c.34G>A, c.197C>T, c.1370A>G) were predicted to cause amino acid substitutions (V12I, T66M, N457S, respectively) and we selected T66M for further analysis because it showed significant difference of allele frequency between high and low group. We investigated associations between these genotypes and fatty acid composition in the Japanese Black population ($N = 885$). Significant associations with C14:0, C16:0, C18:1, MUFA and SFA ($P < 0.01$) were observed by using analysis of variance test. Tukey-Kramer's honestly significant difference test revealed that C/C genotype in c.197C>T indicated higher percentage of C18:1 and MUFA (2.03 and 1.93, respectively) and lower percentage of C14:0, C16:0 and SFA (0.25, 1.31 and 1.92, respectively) than T/T genotype. These results suggested that VNN1 gene genotype could be used as DNA marker for fatty acid composition in beef cattle.

Key Words: cattle, fatty acid composition, VNN1, responsible mutation, DNA marker

MT306 Population genetic structure, inbreeding levels and runs of homozygosity in Swakara pelt producing sheep: Implications on sub-vital performance. F. Muchadeyi[1], M. Malesa[1,2], P. Soma*[3], and E. Dzomba[2], [1]*Agricultural Research Council, Biotechnology Platform, Onderstepoort, South Africa;* [2]*Discipline of Genetics, School of Life Sciences, University of KwaZulu-Natal, Scottsville, South Africa;* [3]*Agricultural Research Council, Animal Production Institute, Irene, South Africa.*

Sub-vital performance is a phenotype whereby some pure white Swakara sheep die within 48 h of birth. It is hypothesised due to high levels of inbreeding, Swakara sheep carry a recessive mutation affecting white fleece colour subpopulation resulting in sub-vital production performance. The aim of this study was to use Ovine SNP50K data to investigate population genetic structure, inbreeding levels and occurrence of ROH in Swakara sheep genome to gain insight on the genetic basis of sub-vital performance. Ninety-four Swakara individuals comprising grey ($n = 22$), black ($n = 15$), white vital ($n = 41$) and white sub-vital ($n = 16$) were genotyped. Principal component analysis revealed presence of population sub-structuring while an admixture of pelt colour based subpopulations was observed. The sub-vital and black sheep grouped together while grey subpopulation revealed genetic similarity to white vital. The ADMIXTURE cross validation error rate (CV = 0.669) was for $K = 4$ and determined the best number of ancestral population of the Swakara sheep. The grey and white sub-vital subpopulations had the highest gene diversities $H_E = 0.342$ and $H_E = 0.340$, respectively. The least inbreeding was observed in the grey sub-population $F_{IS} = 0.009$. The highest genetic diversity was between the black and

white subpopulations with F_{ST} of 0.038. The white sub-vital differed from the grey subpopulation (F_{ST} = 0.032) followed by black (F_{ST} = 0.028) and least with white vital subpopulation (F_{ST} = 0.027). Highest per marker F_{ST} diversity was between white sub-vital *v.* grey subpopulations with genetic differentiation F_{ST} of 0.685 for a SNP on chromosome 5 in region of close proximity to PLAC8L1, POU453, PPP2R2B and GPR151 genes. Four hundred and thirty six unique ROH regions spanning between 1001 to 6594 Kb were observed on 25 chromosomes in 94 individuals of the four-colour subpopulations. Three consensus ROH (cROH) were prevalent in sub-vital Swakara sheep. Results suggested alternative genetic mechanisms to sub-vital performance other than was initially hypothesised that sub-vital performance was due to recessive mutations prevalent in inbred white Swakara sheep.

Key Words: Swakara, ovine SNP50K, pelt color, mutation

MT307 Metabolomics and transcriptomics profiling of mammary gland in cows fed different forage source diets. H. Sun*[1,2], O. Wang[2], D. Wang[1], H. Liu[1], J. Liu[1], and L. L. Guan[2], [1]*Institute of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China;* [2]*Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.*

The mammary gland (MG) of lactation dairy cow plays a vital role in regulating synthesis and secretion of milk, but its metabolism and gene function under different forage source diets have not been well characterized. This study aimed to investigate the metabolic and molecular functional changes in the MG tissues when cows fed alfalfa hay (AH, n = 6) and corn stover (CS, n = 6) using metabolomics and transcriptomics. Totally 273 metabolites were found in MG with 9 metabolites significantly different between two diets (VIP >1 and $P < 0.05$). Uptake of amino acids, carbohydrate metabolism, oxidation of glucose-6-phosphate, oxidation of monosaccharide, amino acid metabolism were identified as the key down-regulated pathway functions ($\log_2$(z-score) < -1& $P <$ 0.05), whereas pathway functions of organismal survival, cell death and survival, cellular growth and proliferation were up-regulated ($\log_2$(z-score) >1 and $P < 0.05$) in MG of cows fed with CS. Maleic acid (fold change (FC) = 2.375), pyrogallol (FC < 0.001), and succinic acid (FC = 1.531) were identified as biomarkers in the MG which could explain 99.6% of the discriminations between 2 diets. Moreover, the expression of 11,193 genes was detected (with counts per million >1 in more than 50% of samples) in MG and 114 of them were differentially expressed ($P < 0.001$, FDR < 0.1) between AH and CS fed cows. Further weighted correlation network analysis revealed the co-expression of 107 and 912 genes had significant negative correlations with maleic acid (R = -0.81, $P = 0.002$) and succinic acid (R = -0.83, R = 0.002), respectively, with gene functions involved in acetylation, organelle membrane, mitochondrial membrane identified. Moreover, the co-expression 421 genes had a significant positive correlation with pyrogallol (R = 0.71, $P = 0.01$) with functions involved in disulfide bond and secretion. Based on integrated analysis of metabolomics and transcriptomics, we could conclude that the CS limited the various metabolic and molecular functions in the MG. The findings from current study provide a fundamental understanding of metabolic function and the mechanism behind MG when fed with CS which is vital for the future strategies to utilize the low quality forage.

Key Words: dairy cow, mammary gland, metabolomics, RNA-Seq

MT308 Genome-wide association study revealed a candidate region for beef marbling on BTA7 in Japanese Black cattle. S. Sasazaki*[1], T. Nakajima[1], Y. Uemoto[2], M. Fukushima[3], E. Yoshida[4], E. Iwamoto[4], T. Akiyama[3], N. Kohama[3], E. Kobayashi[5], K. Oyama[6], and H. Mannen[1], [1]*Graduate School of Agricultural Science, Kobe University, and Kobe, Hyogo, Japan;* [2]*National Livestock Breeding Center, and Nishigo, Fukushima, Japan;* [3]*Northern Center of Agricultural Technology, General Technological Center of Hyogo Prefecture for Agriculture, Forest and Fishery, and Asago, Hyogo, Japan;* [4]*Hyogo Prefectural Technology Center of Agriculture, Forestry and Fisheries, and Kasai, Hyogo, Japan;* [5]*Animal Breeding and Reproduction Research Division, NARO Institute of Livestock and Grassland Science, and Tsukuba, Ibaraki, Japan;* [6]*Food Resources Education & Research Center, Kobe University, and Kasai, Hyogo, Japan.*

Fat percentage in rib-eye area (FPR), which is measured by image analysis of carcass cross-section, would be an attractive alternate for evaluation of beef marbling. In our previous study, we performed pool-based GWAS using illumine bovineSNP50 chip to identify the genomic region associated with FPR and detected two candidate regions in BTA7 and BTA12 (34th ISAG). The objective of the present study was to validate these regions for identification of responsible mutation by confirming the effect of significant SNP detected by GWAS. We individually genotyped the most significant SNP in each candidate region (ARS-BFGL-NGS-35463 on BTA7 and ARS-BFGL-NGS-2915 on BTA12) using 200 animals of pooled sample to validate the result of GWAS. As a result, the SNP on BTA12 did not show the significant difference of allele frequency between the high and low group and therefore we removed the region from further analysis. ARS-BFGL-NGS-35463 on BTA7 was further evaluated using 704 animals randomly selected from 1836 animals and investigated the allelic effect on FPR. ANOVA analysis indicated the significant association between the SNP and FPR ($p<0.05$) and Tukey-Kramer's honestly significant difference test revealed that the animals with AA type (n=64) had 2.0% higher FPR than GG type (n=329). These results confirmed the QTL on BTA7 detected by pooling GWAS and would contribute to identification of responsible gene and mutation for beef marbling in future study.

Key Words: beef marbling, GWAS, Japanese Black cattle

MT309 *PLAG1* polymorphism (ss319607405) is associated with oleic acid percentage in Japanese Black cattle. F. Kawaguchi*[1], H. Kigoshi[1], A. Nakajima[1], Y. Matsumoto[1], Y. Uemoto[2], M. Fukushima[3], E. Yoshida[3], E. Iwamoto[3], T. Akiyama[3], N. Kohama[3], E. Kobayashi[4], T. Honda[5], K. Oyama[5], H. Mannen[1], S. Sasazaki[1], [1]*Graduate School of Agricultural Science, Kobe University, and Kobe, Japan;* [2]*National Livestock Breeding Center, and Fukushima, Japan;* [3]*Northern Center of Agricultural Technology, General Technological Center of Hyogo Prefecture for Agriculture, Forest and Fishery, and Asago, Japan;* [4]*Animal Breeding and Reproduction Research Division, NARO Institute of Livestock and Grassland Science, and Tsukuba, Japan;* [5]*Food Resources Education & Research Center, Kobe University, and Kasai, Japan.*

Oleic acid percentage (C18:1) is important for beef quality in term of the effect on beef palatability and our health. We previously performed GWAS in Japanese Black cattle and detected a significant SNP (BTB-00554873) for C18:1 on BTA14 (ISAG conference 2014). Around BTB-00554873, *PLAG1* gene is located. In the current study, we investigated the effect of *PLAG1* polymorphism (ss319607405), which is considered as a causative mutation affecting the bovine stature, on C18:1 in Japanese Black cattle. We genotyped ss319607405 in Japanese Black cattle population: 441 animals (JB1) randomly selected from 1836 animals used in previous GWAS. Then, we investigated the effect of ss319607405 on C18:1 and calculated the linkage disequilibrium coefficient (r^2) between ss319607405 and BTB-00554873 in JB1. As a result, in JB1, ss319607405 was significantly associated with C18:1 ($p = 0.010$) and showed high linkage disequilibrium with BTB-00554873 (r^2 = 0.74). In addition, Tukey- Kramer's honestly significant difference test revealed that 9/9 genotype indicated 1.39 higher percentage of C18:1 than 11/11 genotype ($p < 0.05$). These results suggested that

PLAG1 would have an effect on fatty acid composition as well as stature and ss319607405 might be a responsible mutation for the QTL detected by GWAS. Further investigation will be needed to elucidate the effect of ss319607405 on C18:1 in Japanese Black cattle.

Key Words: Japanese Black cattle, oleic acid percentage, PLAG1

MT310 Tapping the evolutionary potentials by sourcing novel livestock traits from the wild: Insights from the Giraffe genome. E. Ishengoma*[1,2] and M. Agaba[1], [1]*Nelson Mandela African Institution of Science and Technology, and Arusha, Tanzania;* [2]*Mkwawa University College of Education, and Iringa, Tanzania.*

Wild ruminants adaptation to diverse environment and conditions presents unique opportunities for domestic production. Giraffe has evolved many unique adaptations, some of which can be relevant for domestic ruminant production. For example, giraffe seems to possess an extraordinary capacity to digest tannin-rich diet as well as tolerance to aridity. Since ruminant species express variability in some production traits, we undertook a phylogenomic approach to decipher genetic mechanisms underlying differences in phenotypes, including those with domestic production potentials, between giraffe and other ruminants. To achieve this goal, we have sequenced the genomes of two very closely related antelopes: savannah-adapted giraffe (1200Kg) and the forest-adapted okapi (300Kg). A phylogenetic comparison of the genomes of the two antelopes with those of more than 50 vertebrate species reveals genes with unique alleles in giraffe that may explain the unique adaptations of the respective species. Through positive selection analysis and functional screening, we have identified novel, potentially function changing mutations in candidate adaptive giraffe genes involved in tannin and folate metabolism. Such genes include those encoding Proline rich 19 (PRR19) and Folate Receptor 1 (FOLR1)[1]. Furthermore, we have identified immune response genes that show remarkable evolutionary acceleration in giraffe. A detailed study reveals giraffe Toll-like receptor 3 (TLR3) to possess unique substitution at a nonsynonymous site known to affect receptor signalling activity[2]. Based on these findings, we propose a programme that systematically targets selected wild ruminants for comprehensive genome studies. Exploring the genomes of wild ruminants can discover, validate and, through revolutionary genome editing technologies, transfer desirable attributes for domestic ruminant production. Agaba *et al*. 2016 Nature Commun. 7; Ishengoma and Agaba 2017 BMC Evol. Biol. 17.1:54.

Key Words: wild ruminants, giraffe, domestic ruminants, evolutionary adapatation, production traits

MT311 Understanding the genetic diversity and demographic dynamics of indigenous goats in Cameroon through maternal DNA and SNP Chip array. G. M. Tarekegn*[1,2], F. Meutchieye[3], J. Mwacharo[4], A. Djikeng[2], M. Raphael[5], B. Liu[6], W. Zhang[7], O. A. Mwai[5], T. Dessie[8], C. Mutai[2], S. Osama[9], P. Wouobeng[3], K. S. Jaures[3], and J. Birungi[2], [1]*Department of Animal Production and Technology, Biotechnology Research Institute, Bahir Dar University, Bahir Dar, Ethiopia;* [2]*Biosciences eastern and central Africa-International Livestock Research Institute (BecA-ILRI) Hub, Nairobi, Kenya;* [3]*Faculty of Agronomy and Agriculture, University of Dschang, Dschang, Cameroon;* [4]*Small Ruminant Genetics and Genomics Group, International Centre for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia;* [5]*International Livestock Research Institute (ILRI), Nairobi, Kenya;* [6]*Nei Mongol BioNew Technology Co. Ltd, Hohhot, China;* [7]*College of Animal Science, Inner Mongolia Agricultural University, Hohhot, China;* [8]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia;* [9]*The University of Queensland, Queensland, Australia.*

A study was conducted to understand the genetic diversity and evaluate the demographic dynamics of indigenous goat populations in Cameroon by using mtDNA and SNP chip array datasets. Ninety-three animals from Djallonke and North-west Highland goats were used for mtDNA analysis, and 421 animals from Central Highland, North-west Highland and Djallonke goats for SNP CHIP array analysis. Abergelle, Afar, Keffa and Gumez goats from Ethiopia and Cashmere from China were included in the SNP chip analysis for comparison. The mtDNA analysis revealed 81 haplotypes from 121 segregating sites generated from 1219 bp of the *d*-loop. The average haplotype and nucleotide diversities were 0.994 ± 0.006 and 0.00991 ± 0.00058, respectively. A combined analysis involving reference haplotypes representing six globally defined haplogroups revealed only haplogroup A indicating that no other maternal origins arrived in Cameroon. However, 20 haplotypes were shared among Cameroonian, Mozambiquan, Namibian, Zimbabwean, Kenyan and Ethiopian goats. The Bayesian coalescent-based analysis showed an event of the goat population expansion in Cameroon, while the genomic analysis using the 52K SNP chip revealed lowest and highest observed heterozygosity (H_O = 0.168 ± 0.083) and (H_O = 0.335 ± 0.132) for Djallonke and North-west Highland goat populations in that order and 0.288 ± 0.132 and 0.368 ± 0.134 expected heterozygosity values for Djallonke and Central Highland goat populations, respectively. Both estimates of heterozygosity were lower than the values obtained for the Ethiopian and Cashmere goats. Unexpectedly very high values of F_{IS} were observed in Djallonke (F_{IS} = 0.388) and Central Highland (F_{IS} = 0.163) goats. These two goat populations accumulated highest number of markers which deviated from Hardy–Weinberg Equilibrium (HWE: P ≤ 0.05), 27207 SNPs for Djallonke and 13450 SNPs and Central Highland goat. On the other hand, inconsistent with the number population analysed, the admixture analysis classified the indigenous goats in Cameroon into three genetic backgrounds. Overall, the indigenous goats in Cameroon show weak genetic structure suggesting that interbreeding among the goat populations occurs.

Key Words: genetic admixture, goat, haplogroup, haplotypes, population expansion

MT312 Genetic characterization of the Hungarian water buffalo population using microsatellite DNA markers. C. Józsa*, B. Bán, and I. Péntek, *National Food Chain Safety Office, Animal Breeding Directorate, Laboratory of Genetics, Budapest, Hungary.*

Microsatellite markers are highly polymorphic and are widely used in genome mapping and population genetic studies in livestock species. Though only a limited number of microsatellite markers have been reported thus far in water buffalo, *Bubalus bubalis*. This species is an economically important livestock species in Hungary. Despite of this fact, genealogical control is still one of the problems of the Hungarian selection and breeding programs. The DNA test is important to develop a system that allows the animal genealogy certification as well as its undeniable individual and parentage identification. The present study was performed by using two panel of 23 microsatellite markers (BM1818, BM1824, BM2113, ETH3, ETH10, ETH225, INRA23, SPS115, TGLA53, TGLA122, TGLA126, TGLA227, CSRM60, CSSM60, ILSTS006, MGTG4B, RM067, SPS113, HEL13, ILSTS26, ILSTS28, ILSTS58, ILSTS61) in three Hungarian water buffalo populations (n = 555) in order to estimate the genetic variability and calculate the parentage exclusion probability. We used only 13 microsatellites for genetic structure analysis of water buffaloes. A probability of exclusion of 0,998 was tested for the set of 13 microsatellites. DNA markers were tested in Hungarian Water Buffalo. Due to their extremely large number and the more definite polymorphism of some loci, it can be used on higher level and more efficiently in numerous fields of animal

breeding, e.g. examination of genetic structure of populations, test of homozygosity, estimation of the degree of inbreeding of populations, maintenance of autochthonous populations (gene-reserve), parentage control, estimation of genetic distance between populations and breeds, planning of crossing programs (heterosis breeding). The high conservation of cattle microsatellite loci in water buffalo promises the usefulness of the cattle microsatellites markers on buffalo. The polymorphic markers characterised in this study will contribute to population genetic studies of water buffalo.

Key Words: *Bubalus bubalis*, microsatellite, polymorphism, parentage identification

MT313 Signature selection analysis reveals candidate adaptive genes in Iraqi cattle breeds. A. Alshawi*[1,2], S. Albayati[3], A. Essa[3], and O. Hanotte[1], [1]*School of Life Sciences, The University of Nottingham, Nottingham, UK;* [2]*College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq;* [3]*Animal Genetics Resources Department, Ministry of Iraqi Agriculture, Baghdad, Iraq.*

Indigenous Near East cattle are characterised by high genetic diversity and they are adapted to different agro-ecological zones including desert areas as well as humid regions. The region was one of the earliest and most significant areas of the domestication of cattle. They currently four main breed of Iraqi cattle recognised. Among these, the Jenoubi breed is found in the southern more humid part Iraq while the Rustaqi cattle is found in the middle region of the country. Here, we report at genome-wide level the diversity and genetic signature of selection of these two breeds. Thirty-five unrelated Jenoubi cattle, sampled in Maysan and Basra region, and 60 Rustaqi cattle, sampled in Baghdad and Babylon regions, were genotyped using the Illumina Bovine HD BeadChip (777,962 SNP markers). PCA and admixture analysis indicated that the two breeds are crossbreed zebu × taurine with more zebu background in Jenoubi cattle. Signature selection analysis – Extended Haplotype Homozygosity (EHH) based *iHS* and *Rsb* tests – identified 24 and 45 candidate regions under positive selection in Jenoubi and Rustaqi breeds, respectively. With only one region identified in both population. These include 68 genes. Functional annotation, identified 19 genes related to the innate and/or acquired immune response in cattle, including the PRKG1 gene, related to tick resistance and the ABCC2 gene, which has been linked to gastrointestinal nematodes resistance. Other genes may be related to the control of metabolic stress, e.g. UCN3, as well as NCAM2, which has been linked to the marbling score of the meat. Our results underline the importance of local adaptation of indigenous Near East Iraqi cattle breeds.

Key Words: Iraqi cattle, population genomics, single-nucleotide polymorphism (SNP), adaptive immunity, genomic selection

MT314 Congenital mandibular prognathia in Droughtmaster cattle. S. A. Woolley*[1], E. R. Tsimnadis[1], M. S. Khatkar[1], C. E. Willet[2], B. A. O'Rourke[3], and I. Tammen[1], [1]*Sydney School of Veterinary Science, The University of Sydney, Camden, NSW, Australia;* [2]*Sydney Informatics Hub, Core Research Facilities, The University of Sydney, Sydney, NSW, Australia;* [3]*The Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle, NSW, Australia.*

Congenital mandibular prognathia is an emerging inherited disease in Australian Droughtmaster cattle that causes a non-lethal craniofacial deformity where the mandible and maxilla fail to align. This presents animal welfare, productivity and economic issues as the affected animals are unable to meet their nutritional requirements, thus affecting the production capabilities and economic value of these animals. Preliminary pedigree analysis is suggestive of a recessive mode of inheritance. This study aimed to characterise a causative mutation by sequencing the coding and non-coding regions of the previously identified positional candidate gene *FOXI2*. SNP genotyping of 9 affected and 4 obligate heterozygote animals was performed using the bovine 80K SNP chip. Homozygosity mapping identified a 3.1Mb region of homozygosity on chromosome 26 that was shared by all 9 affected animals and the positional candidate gene *FOXI2* was identified within this region. Sanger sequencing of *FOXI2* in previous studies was only successful for one of the two exons and alignment to the bovine genome assembly UMD3.1 identified no disease-causing allelic variants. Amplification of exon 1 failed to generate a PCR product of expected size using various primer pair combinations in samples from clinically affected and clinically normal cattle. DNA samples from two affected Droughtmaster cattle were sent for next-generation sequencing (NGS) with 150bp paired-end reads at 30X coverage. Initial quality control of the NGS data is complete and has shown a per base sequence quality determined by a Phred score of Q >30 being on average 87.2% and 85.9% for each sample. Both sample sequences had an average GC content of 43.25% with no over-represented sequences or adaptor contamination identified. The data will now be aligned to the bovine reference genome and allelic variations will be identified and validated with a focus on the positional candidate gene *FOXI2* and the flanking 3.1 Mb region of homozygosity.

Key Words: cattle, DNA sequencing, genetic disorder

MT315 Towards accurate transcriptional analysis of important bovine natural killer receptors. R. Borne*, P. Ribeca, and J. A. Hammond, *The Pirbright Institute, Pirbright, Surrey, UK.*

Natural killer (NK) cells are a fundamental component of the mammalian innate immune system and crucial in the response against pathogens and tumours. The leukocyte receptor and natural killer complexes are highly diverse genomic regions that contain the majority of polymorphic natural killer receptor genes that control NK activation and function. However, examining gene expression over these regions using RNA-seq is challenging as they are highly repetitive; each region contains multiple genes sharing high sequence identity. We plan to use RNA-Seq technology to investigate transcriptional regulation of these receptor genes during various immune stimuli in livestock. Identifying unique regions of these genes through whole genome mappability and calculating coverage only of these unique regions provides confidence in mapping for the first time. To improve confidence to genes that do not possess suitable regions of uniqueness, we plan to group highly similar genes together and map to a representative of the group. Although this approach sacrifices some resolution, it will inform targeted full-length transcript sequencing by PacBio to enable differential expression analysis of over these important immune gene complexes for the first time. These methods are useful for other gene complexes/species where the underlying genome is of high confidence.

Key Words: cattle and related species, immunogenomics, RNA-seq, innate immunity, gene expression

MT316 Rambouillet sheep genome and FAANG RNA resources. Y. Liu[1], R. A. Harris[1], X. Qin[1], S. Richards[1], J. Rogers[1], Y. Han[1], Q. Meng[1], T. P. Smith[2], B. P. Dalrymple[3], S. N. White[4], B. Murdoch[5], J. Kijas[6], N. E. Cockett[7], D. M. Muzny[1], K. C. Worley*[1], [1]*Baylor College of Medicine, Houston, TX, USA;* [2]*USDA Agricultural Research Service, U.S. Meat Animal Research Center, Clay Center, NE, USA;* [3]*University of Western Australia, Institute of Agriculture, Perth, Western Australia, Australia;* [4]*Washington State University, Veternary Microbiology and Pathology, Pullman, WA, USA;* [5]*University of Idaho, Animal and Veternary Science, Moscow, ID, USA;* [6]*CSIRO, St. Lucia, Australia;* [7]*Utah State University, Logan, UT, USA.*

High quality reference genomes are fundamental resources for biology. We report here a new sheep reference genome for the Rambouillet breed generated using the latest methods for producing and

de novo assembling long reads and scaffolding to chromosomes. High quality annotation will be enabled by assays of the FAANG quality samples that have been collected from the reference animal. A total of 200 Gb of sequence was generated from a single ewe, Benz2616, using the Pacific Biosciences (PacBio) technology. The data have a 12.6 kb N50 and 8.9 kb mean subread length. We used both Falcon and Celera Assembler to error correct and assemble the error corrected reads and selected the more complete product of the Celera Assembler for further processing. The preliminary assembly has a contig N50 of 2.2 Mb, a total length of 2.85 Gb, 365 contigs contain half of the genome sequence, and the longest contig is 16.3 Mb. The majority (89%) of 338,551 EST sequences align to the genome, with most (90%) having nearly complete alignments, aligning over more than 95% of their length. This version of the genome is more complete and has more contiguous alignment to these expressed sequences than the Texel breed Oar4.0 reference. Base quality of this assembly is high, with error rates less than 1% following assembly polishing using Arrow. Hi-C proximity ligation data from the same individual is being used for scaffolding the preliminary contigs. An initial scaffolding attempt incorporated 97.4% of the assembly into 32 large scaffolds and 2,900 smaller scaffolds (< 100kb). This initial scaffold version captures more complete, single copy BUSCO genes than Oar-v4.0 or the input initial contigs. Further scaffold refinement is ongoing. Additional PBJelly gap filling and Pilon base error correction are planned before release. Over 100 tissues from the reference animal have been collected for additional FAANG assays. This large effort involved over 35 people in the sample collection, advance planning and coordination. In addition to the PacBio genome sequence and Hi-C data for the genome assembly, PacBio IsoSeq, miRNAseq, ATAC-Seq and other assays are planned.

Key Words: reference genome, sheep, RNAseq

MT317 Influence of a 1-Mb region of BTA 5 on beef cow stayability in *Bos indicus* × *Bos taurus* crossbred cows. B. N. Engle*, A. D. Herring, J. E. Sawyer, D. G. Riley, J. O. Sanders, and C. A. Gill, *Texas A&M University, College Station, TX, USA.*

Beef cow reproductive longevity is an economically important, complex quantitative trait strongly influenced by the environment. *Bos indicus-Bos taurus* crossbred cows have been shown to have high potential for long reproductive lifespan. They are generally also later maturing and experience greater reproductive difficulty early in life when compared to pure *Bos taurus* cattle. The aim of this analysis was to identify the underlying genetic architecture influencing beef cow reproductive longevity in *Bos indicus* influenced cattle. A herd of *Bos indicus-Bos taurus* crossbred cows (n = 303) of Nellore and Angus influence was assessed for this study. By GWAS we had previously identified a critical region from 40–50 Mb on BTA 5 associated with stayability, a cow's ability to successively give birth to five calves by age six. These findings corresponded to reports from other crossbred populations of similar composition and were related to similar traits, such as puberty and reproductive efficiency. Within the critical region on BTA 5, a 1 Mb haplotype block with SNP in moderate to high linkage disequilibrium explained 4.5% of the variance associated with cow stayability. Haplotype analysis of this 1 Mb region revealed a significant relationship between stayability and haplotype breed of origin (P < 0.05). Cows carrying a haplotype from each breed (Nellore and Angus) out-performed (probit of 0.39 ± 0.22) cows with Angus genotypes (0.27 ± 0.21), and cows with Nellore genotypes had the most negative phenotypic response (-0.40 ± 0.20). This region contains the genes *HELB*, *GRIP1*, *and TMBIM4*, which are functional candidates for this effect. These results suggest that there is a Nellore effect on a cow's ability to produce calves before six years of age at this region. Given the prior body of work, it is likely that this can be generalized as a *Bos indicus* effect on stayability and that the effect is conserved across *inter se* populations of *Bos indicus-Bos taurus* cattle. It appears that the effect is expressed early in a cow's reproductive lifetime. After bypassing this critical period, we would expect cows to remain productive in the herd until at least six years of age.

Key Words: stayability, beef cattle, *Bos indicus*

MT318 Differences in meiotic chromosome pairing characteristics in spermatocytes of hybrid beefalo. A. Rodriguez*, K. Davenport, H. Jaeger, M. Follett, B. Badigian, R. Sawyer, and B. Murdoch, *University of Idaho, Moscow, ID, USA.*

Hybrid animals provide an opportunity to combine desirable phenotypic traits from different species. In an effort to optimize hybrid mating, mating between cattle (*Bos taurus*) and bison (*Bison bison)* to produce beefalo (*Bison bison* × *Bos taurus*) has been used. However, mating of two different species can lead to reduced viable and fertile offspring from hybrid crosses. Chromosome pairing and crossovers (CO) during spermatogenesis creates an exchange of genetic material. The synaptonemal complex (SC) forms with synapsis and anchors homologous chromosomes together. There is a positive correlation between number of CO and the length of a chromosome, which can be determined measuring SC length. Immunofluorescent staining was used to identify chromosome synapsis (SYCP3) and CO (MLH1) in pachytene stage spermatocytes. Despite both species having 30 chromosomes, previous data (Murdoch laboratory, unpublished) show beefalo exhibit on average ~10% fewer CO per spermatocyte when compared to cattle. To evaluate why fewer overall CO occur, the number of CO per SC were examined in each spermatocyte. For a subset of corresponding spermatocytes, SC measurements from beefalo were examined and compared to cattle. Overall, cattle exhibited a higher occurrence of 3, 4, and as many as 5 CO per SC, whereas the maximum number of CO in beefalo was 4. Importantly, when compared to cattle, 11% of beefalo spermatocytes exhibited the structural defect of a circular/fused chromosome, and 9% of beefalo spermatocytes were lacking CO, both of which may lead to improper chromosome segregation. The consequence of improper chromosome segregation in spermatocytes is ultimately cell death; as defective spermatocytes will not develop into mature spermatozoa. Hybrid animals have the potential to provide valuable phenotypic traits from different species. However, due to chromosomal differences, this may result in breeding difficulties of the next-generation. Studying hybrid cattle spermatocytes will provide valuable insight and strategies for breeding these and other hybrids through understanding chromosome pairing, synapsis, and crossovers.

Key Words: beefalo, cattle, chromosome pairing, meiosis, recombination

MT319 Mapping of calf death in Japanese Black cattle. T. Hirano*[1], S. Nishimura[2], H. Hara[1], Y. Sugimoto[2], and K. Hanzawa[1], [1]*Tokyo University of Agriculture, Funako, Atsugi, Kanagawa, Japan;* [2]*Shirakawa Institute of Animal Genetics, Odakura, Nishigo, Nishi-shirakawa, Fukushima, Japan.*

Weak calf syndrome (WCS) is a major cause of calf death in Japanese Black cattle. Among IARS disorders, the *isoleucyl-tRNA synthetase* c.235G>C mutation has been identified as one of the causes of WCS. However, calf deaths differing from those attributed to IARS disorder has been occurring. To identify other genes potentially responsible for these calf deaths, we are performing a mapping and exome analysis for critical regions. In 35th ISAG conference (2016), we reported to have identified a critical region and candidate variants for weak calf syndrome on BTA4. Here we show identification of new critical regions for calf death in Japanese Black cattle. We constructed three populations of three bulls (Bull-1, Bull-2 and Bull-3) that did not carry the *IARS* mutation, and calves died before 20 days old (18, 28 and 31 calves) and healthy cattle (18, 15 and 10 cattle) sired by these bulls. To identify a critical region for the calf death with performing homozygosity mapping

and linkage analysis, the populations were genotyped using the BovineSNP50 BeadChip. Homozygosity mapping did not detect any associated genomic regions with calf death. Linkage analysis performed using each population as a paternal half-sib family of Bull-1, Bull-2 and Bull-3 revealed that, in the Bull-1 population, calf death was mapped to the 8.94 Mb - 14.53 Mb and 29.82 Mb - 33.77 Mb regions of BTA29 (Region-1 and Region-2, respectively). The findings suggested that the incidence of calf death in calves sired by Bull-1 was a hereditary disease exhibiting a dominant, not recessive, inheritance pattern. Furthermore, we compared reconstructed haplotypes in Bull-1, Bull-2 and Bull-3. Haplotypes of Bull-2 did not correspond with the risk-haplotype of Bull-1. However, an 11.91 Mb - 33.77 Mb region on a haplotype of Bull-3 indicated risk-type. The region indicating the risk-type haplotype overlapped with a part of Region-1 and all of Region-2, and a common ancestor was found between Bull-1 and Bull-3. Since calf death was not mapped on these regions with Bull-3 family, the causative region might be narrowed to an 8.94 Mb - 11.91 Mb region. We are performing an exome analysis for the causative region.

Key Words: calf death, SNPs, mapping, Japanese Black cattle

MT320 Melatonin target scondary hair follicle by RORα receptor gene in cashmere goat. Y. Zhao[1,3], Z. Liu*[1,2], J. Wu[1,3], Y. Wang[1,3], R. Wang[1,4], Y. Zhang[1,3], R. Su[1,3], J. Li[1,2], and H. Xiao[1,4], [1]*Inner Mongolia Agricultral University, Hohhot, Inner Mongolia, China;* [2]*The Ministry of Agriculture Key Laboratory of animal genetics and breeding of sheep, Hohhot, Inner Mongolia, China;* [3]*Inner Mongolia Key Laboratory of animal genetics, breeding and reproduction, Hohhot, Inner Mongolia, China;* [4]*Inner Mongolia Engineering Center of goat genetics and breeding, Hohhot, Inner Mongolia, China.*

Melatonin as a nerve secretion material based on photoperiod, seasonal and reproductive rhythm, which promotes the hair growth of mink, blue fox and goat, it is widely used in animal testing and wool production. In this study, we used it in Inner Mongolia cashmere goats. With RT-PCR technique and *in situ* hybridization technique, distribution and mRNA expression of melatonin receptor gene (MTNR1a, MTNR1b, RORα) in skin follicles of Inner Mongolia cashmere goat were detected to explore binding receptors of melatonin. Skin samples of different months of year (February, April, August, October and December) were determined. Results showed that only nuclear receptor RORα expression was detected in skin samples, membrane receptor MTNR1a and MTNR1b expression were not detected. With *in situ* hybridization, strong expression signals of RORα mRNA were found in the trunk of hair follicles, inner root sheath, outer root sheath and medulla, these parts are target organs of RORα receptor gene. RORα mRNA showed highest expression in February and lowest expression in December samples by qRT–PCR, but no significant difference among February, April, August and October Our experiment revealed that melatonin's role in promoting the growth of hair was achieved by nuclear receptors RORα. It laid a good foundation for further study of melatonin receptors and Cashmere relationship and it helped clarify the role of melatonin pathway and regulation mechanism.

Key Words: cashmere goat, melatonin, RORα, hair follicle

MT321 Expression quantitative trait loci mapping in dairy cattle. A. Chamberlain*[1], C. Vander Jagt[1], M. Goddard[1,2], C. Reich[1], C. Prowse-Wilkins[1], B. Mason[1], H. Daetwyler[1,3], and B. Hayes[1,4], [1]*Agriculture Victoria, Centre for AgriBiosciences, Bundoora, VIC, Australia;* [2]*Faculty of Veterinary & Agricultural Science, The University of Melbourne, Parkville, VIC, Australia;* [3]*School of Applied Systems Biology, La Trobe University, Bundoora, VIC, Australia;* [4]*Centre for Animal Science, The University of Queensland, St Lucia, QLD, Australia.*

There is increasing evidence that genetic variation affecting complex traits lies in regions of the genome regulating gene expression. Expression quantitative trait loci (eQTL) mapping is one way of finding *cis*-regulatory genetic variation, that could also affect complex traits. We have used RNA sequence data from milk and white blood cells (WBC) from 29 Jerseys and 112 Holsteins to identify eQTL that affect gene expression in lactating cattle. On average 119 (milk cells) and 51 (WBC) million reads per library were generated with 89% reads passing quality control and a mapping rate of 92%. Samples with >50 (milk cells) or >25 (WBC) million reads and a mapping rate >80% were used to generate a gene counts matrix. Whole genome sequence was imputed from BovineSNP50 genotypes for each animal, utilising the 1000 bull genomes dataset as reference population. 10,904,750 (milk cells) and 10,469,612 (WBC) variants were tested for association with expression of 12,772 (milk cells) and 11,577 (WBC) genes, where only variants on the chromosome that contained that gene were tested. The genome relationship matrix among cows was also fitted to control for population structure, as were fixed effects of breed, parity, days in milk, sampling day and RNA sequencing batch. Read counts were transformed as log(x+1), where × is the read count of a particular gene for a cow. Only genes that were expressed in more than 25% of the cows were analysed, to avoid spurious associations due to very low read counts. eQTL analysis revealed 361 (milk cells) and 554 (WBC) eGenes ($P < 1x10^{-6}$, false discovery rate of 0.016 and 0.013 respectively). There was a trend for the eQTL to be close to the gene transcription start site, with 23% of significant eQTL within 50kb. We also found some cases where the same variant was a significant eQTL for 2 genes. The eQTL discovered here will be investigated for their effects on a range of complex traits.

Key Words: gene expression, expression QTL, dairy, RNAseq

MT322 Concordance analysis for fine-mapping QTL in tropical beef cattle. L. R. Porto-Neto*[1], S. M. McWilliam[1], S. Harburg[2], S. A. Lehnert[1], and A. Reverter[1], [1]*CSIRO Agriculture & Food, Brisbane, QLD, Australia;* [2]*North Australian Pastoral Company, Brisbane, QLD, Australia.*

The availability of SNP genotyping arrays and whole-genome sequencing gives the opportunity to fine-map QTL impacting production-related traits in livestock. Since the release of the first generation of SNP arrays, several QTL have been detected and confirmed in other populations, but only a few have been narrowed down to their functional mutation. The integration of SNP genotypes used for QTL detection, and status determination (homozygous or heterozygous) with whole-genome sequence in a concordance analyses has been shown to assist this process. Here we exploit a population of tropical composite cattle (n = 964), with phenotype measurements for birthweight, scrotal circumference and sheath score. The SNP genotypes (n = 63,861) of these cattle were phased (Beagle 4.1) together with around 9,000 cattle of the same company (including 31 sires that were whole-genome sequenced). GEBV were estimated fitting a genomic relationship matrix in a mixed model equation approach. The GEBV and the individual SNP genotype data were merged to back-solve the SNP effect required to generate GEBV of un-phenotyped individuals. SNP effects were used to identify QTL regions confirming a known QTL on BTA5:47Mb affecting the three observed phenotypes. Furthermore, we applied the sire's phased SNP genotypes on the estimated SNP effect to obtain individual haplotype GEBV around the target QTL (sheath score), determining its status on each sire. There were fifteen qq, eleven Qq, and one QQ, the remaining four animals had questionable status, mainly due to potential recombination (or phasing error) across the haplotype. For the concordance analysis we used SNP called at genome sequence level at a critical interval of 114Kb. Among the 709 SNP at this interval, 217 agreed with the QTL status. Even

though this represents a huge reduction of potential functional mutations, there were still too many to be further tested. In a concordance analysis using 31 sequenced animals we could not ascertain the functional variant affecting sheath score in BTA5. Although promising, the approach requires larger number of sequenced individuals (either sequenced or SNP genotyped imputed to sequence) to pin down functional variants.

Key Words: cattle, genome-wide association, genome sequencing, complex trait, genetic improvement

MT323 A missense mutation of *ADAMTS3* causes pulmonary hypoplasia and anasarca (PHA) syndrome in Cika cattle. N. Wiedemar[1], T. Svara[2], V. Cociancich[3], K. Sest[4], M. Gombac[2], T. Paller[2], J. Staric[4], V. Jagannathan[1], and C. Drögemüller*[1], [1]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland;* [2]*Institute of Pathology, Wild Animals, Fish and Bees, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia;* [3]*National Veterinary Institute, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia;* [4]*Clinic for Reproduction and Large Animals, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia.*

Pulmonary hypoplasia and anasarca (PHA) syndrome is a rare lethal autosomal recessive disorder in cattle that is characterised by hydrops fetalis including extreme subcutaneous oedema (anasarca) and undeveloped or poorly formed lungs (pulmonary hypoplasia). Until now, sporadic cases of PHA were reported in different cattle breeds. Gene tests for unpublished causative mutations allow detection of PHA carriers in Dexter, Maine-Anjou, and Shorthorn cattle. We noticed two cases of PHA syndrome from consanguineous mating's in Slovenian Cika cattle emphasising how inbreeding in cattle populations with small effective population sizes can lead to emergence of recessive defects. We delimited the critical genome regions for PHA by homozygosity mapping using two cases, which were genotyped on the bovine high-density 777k SNP chip. A total of 12 shared genomic regions >1 Mb were located on different cattle chromosomes, including the largest homozygous region by far of 37.8 Mb on chromosome 6, predicted to harbour the causative mutation. We sequenced the genome of an affected Cika calf at 9x coverage using an illumina HiSEqn 2500 instrument. Filtering for private variants of the sequenced affected calf against control genomes of cattle from other breeds and dbSNP identified a total of 5 homozygous non-synonymous variants in the critical intervals with respect to the UMD3.1 reference genome sequence. Genotyping of a larger cohort of Cika cattle left one single variant perfectly associated with the disease: a missense variant in *ADAMTS3* encoding a metalloprotease playing an essential role in embryonic lymphangiogenesis and placental angiogenesis. The missense variant is predicted to change an evolutionary conserved (invariant in vertebrates) histidine into a tyrosine residue (p.His408Tyr). As *ADAMTS3* represents an excellent candidate gene for genetic diseases characterised by lymphedema the detected missense mutation most likely represents the causative mutation for PHA.

Key Words: cattle, genetic disorder, inbreeding, candidate gene, genome sequencing

MT324 The sheep gene expression atlas project. E. L. Clark*, S. J. Bush, M. E. McCulloch, L. Farquhar, C. B. Whitelaw, M. Watson, K. M. Summers, A. L. Archibald, and D. A. Hume, *The Roslin Institute, University of Edinburgh, Edinburgh, Midlothian, UK.*

Sheep are an important source of meat, milk and fibre globally. To support functional annotation of the sheep genome we have produced a high-resolution transcriptional atlas of the sheep using Scottish Blackface × Texel crossbred individuals. RNA-Seq libraries were generated by Edinburgh Genomics (http://genomics.ed.ac.uk) from tissues and cells representing all the major organ systems from adult sheep and from multiple juvenile, neonatal and prenatal developmental time points. The dataset includes 352 medium depth and 74 high depth 125bp stranded Illumina RNA-Seq libraries. The raw reads have been mapped using two pipelines, an alignment free method, Kallisto, and a conventional alignment based HiSat2-Stringtie pipeline. Of the protein coding genes currently annotated by Ensembl in the Oar_v3.1 reference genome, 96% are captured in the sheep atlas dataset. We have assigned gene names to hundreds of previously unannotated genes and are using network cluster analysis in Miru (http://kajeka.com) to assign putative function to many of these genes. Numerous clusters have tissue or cell specific signatures of gene expression. From the macrophage cluster, for example, we have identified many unannotated putative immune genes. We are also comparing the RNA-Seq data with SNPs identified in whole genome sequence data (10X coverage) for evidence of allelic expression imbalance in key genes, such as those involved in innate immunity. Details of all samples collected are included in the BioSamples database (http://ebi.ac.uk/biosamples) under project GSB-718. The sequence data have been deposited in the European Nucleotide Archive (http://ebi.ac.uk/ena), under study accession number PRJEB19199, along with detailed experimental metadata. We will make the data available via the BioGPS gene annotation portal (http://biogps.org) in which the pattern of expression for each gene can be visualised across a range of tissues. The Sheep Gene Expression Atlas has the potential to inform future improvements in productivity, efficiency and health in sheep and other small ruminants and is a valuable resource for the international Functional Annotation of Animal Genomes (FAANG) initiative.

Key Words: sheep and related species, Functional Annotation of Animal Genomes (FAANG), RNA-seq, gene expression, cross-breeding

MT325 A joint analysis of bovine tuberculosis resistance in dairy cattle. S. Wilkinson*[1], S. Bishop[1], A. Allen[2], S. McBride[2], R. Skuce[2,3], D. Bradley[4], D. Berry[5], M. Coffey[6], G. Banos[6,1], M. Mrode[6], J. Woolliams[1], and E. Glass[1], [1]*The Roslin Institute and R(D)SVS, The University of Edinburgh, Easter Bush, UK;* [2]*Agri-Food and Biosciences Institute Stormont, Stoney Road, Belfast, UK;* [3]*Queen's University Belfast, Medical Biology Centre, Belfast, UK;* [4]*Smurfit Institute of Genetics, University of Dublin, Trinity College, Dublin, Ireland;* [5]*Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Co. Cork, Ireland;* [6]*Scotland's Rural College, The Roslin Institute Building, Easter Bush, UK.*

Resistance to bovine tuberculosis (bTB) in cattle is complex and governed in part by host genetics. Studies have found bTB resistance loci and different genomic variants in cattle with detected bTB pathology versus undetected bTB pathology. By pooling independent bTB datasets, there is an opportunity to confirm previous findings and find novel variants. The aim of this study was to conduct a joint analysis of the genetic architecture of bTB resistance in dairy cattle. Pooled genotypes consisted of 2 058 and 1 310 animals genotyped with the BovineHD and BovineSNP50 chips, respectively, with the latter data imputed to higher density. The pooled bTB phenotypes were case-control cow data and sire estimated breeding values. Three bTB resistance phenotypes were defined: (1) skin-test positives with visible lesions (VLs), (2) skin-test positives with non-visible lesions (NVLs) and (3) skin-test positives (SICCTs). The number of animals were 2 126, 2 355 and 2 955 for the VL, NVL and SICCT phenotype, respectively, genotyped at 536 649 SNPs. To identify bTB resistance loci, a genome-wide association study (GWAS) and regional heritability (RH) mapping was conducted. Chromosome heritability (BTA_h^2) was estimated to partition genetic variation across the genome. GWAS and RH mapping detected previously identified bTB resistance loci and revealed novel genomic regions. The bTB resistance loci were distinct for the VL

and NVL phenotypes. BTA_h[2] analysis confirmed that certain chromosomes harbour variants associated with bTB resistance, especially BTA23 which has the bovine Major Histocompatibility Complex. The three bTB resistance phenotypes had shared along with distinct chromosomal variation. Regardless of phenotype, not all chromosomes harboured variants associated with bTB resistance and no chromosome explained a large proportion of phenotypic variation. The joint analysis confirmed previous findings: (i) genetic control of bTB resistance likely involves variants of small effect across many but not all chromosomes and (ii) the pathological outcome of bTB may differ to some extent with host genetics. The joint analysis also identified novel loci, demonstrating the power of pooling data to uncover additional genomic regions associated bTB resistance in dairy cattle.

Key Words: animal health, genome-wide association

MT326 Runs of homozygosity are unevenly located across the genome in highly inbred cattle. D. Goszczynski[1], A. Molina[2], G. Giovambattista*[1], H. Morales Durand[1], and S. Demyda-Peyras[1], [1]*Instituto de Genética Veterinaria 'Ing. Fernando N. Dulout' CCT La Plata, CONICET, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina;* [2]*Departamento de Genética, Universidad de Córdoba, Córdoba, Spain.*

Inbreeding depression has been recognised as a major cause of deleterious effects in individuals due to the loss of genetic variability. Nowadays, massive genotyping technologies and the analysis of runs of homozygosity (ROH) provide us with valuable tools to further understand this condition. Using *in silico* methods, it has been recently demonstrated that ROH size is correlated with the number of generations since the common ancestor, although experimental studies focused in this relation are scarce. Hereby, we characterised the ROH patterns of an extremely consanguineous cattle population with very reliable pedigree data (5.2 ECG). For this, 33 Retinta bulls (average Fped = 16.57%; ranging from 10.25% to 30.62%) were genotyped using the BOS 1 SNP Array. Recent inbreeding was estimated using data from the 3 last generations. ROH patterns were classified upon their size (0.5–1Mb; 1–2Mb; 2–4Mb; 4–8Mb; 8–16Mb; >16MB). FROH values (percentage of the genome covered by ROHs) were also determined per size category and per chromosome. ROHs showed an average size of 2.17 Mb (0.50 to 66.87 Mb), with 3.19 runs longer than 16Mb per individual. FROHs for the longest runs (8–16 and >16Mb) showed the highest values, which is consistent with the recent inbreeding events produced in this population. Interestingly, high FROH values were also observed in the [1–2Mb] cluster, which may be explained by traits fixed at the origin of the breed (50 generations ago). These results were confirmed by Fped3-FROH correlation, which increased towards long ROHs (-0.28 in [0.5–1Mb] to 0.43 in >16Mb). Therefore, we demonstrated for the first time that the association between long ROHs and generations since the common ancestor is conserved in highly inbred cattle. A further analysis showed that FROH values were highly variable among chromosomes (ranging from 0.1198 in BTA21 to 0.2178 in BTA14). More interesting, remarkable differences were observed in the >16Mb runs, where average FROH ranged from 0 (no runs on BTA26 and BTA29) to 0.076 on BTA22. In conclusion, we demonstrated that homozygosity is unevenly distributed across the genome in an inbred cattle population, potentially affecting different metabolic pathways in individuals with similar Fped

Key Words: ROH, cattle and related species, inbreeding, bioinformatics tools

MT327 Liver transcriptome profiling of beef steers divergent in feed intake or growth rate phenotype. R. Mukiibi[1], M. Vinsky[2], C. Fitzsimmons[1,2], S. M. Waters[3], P. Stothard[1], and C. Li*[1,2], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada;* [2]*Agriculture and Agri-Food Canada, Lacombe Research and Development Centre, Lacombe, Alberta, Canada;* [3]*Animal and Bioscience Research Department, Teagasc, Grange, Dunsany, County Meath, Ireland.*

Feed consumption and growth rate are economically important traits in beef production. In this study we analysed whole transcriptome profiles of liver samples of steers from three Canadian beef breeds, Angus (AN), Charolais (CH) and Kinsella Composite (KC). Liver samples were collected at slaughter and gene expression profiles of steers with high dry matter intake (DMI) (n = 6) and low DMI (n = 6) phenotypic values were obtained by RNAseq using Illumina HiSeq sequencing technology. Samples were then sorted based on their average daily gain (ADG) phenotypic values, and gene expression profiles of high (n = 6) and low (n = 6) ADG groups were also analysed. On average, RNAseq generated 34 million reads per sample with a Phred score of 36, and 89.7% of the reads per sample were aligned and mapped to the UMD3.1 bovine reference genome. Differential gene expression analyses between groups were performed using a linear mixed model as implemented in the R package edgeR. Differentially expressed (DE) genes were identified at a false discovery rate of < 0.05 and fold change >2. For DMI, 65 (27 down and 38 up-regulated), 49 (28 down and 21 up-regulated) and 54 (21 down and 33 up-regulated) DE genes were identified in AN, CH and KC, respectively. For ADG, 147 (87 down and 60 up-regulated), 60 (41 down and 19 up-regulated) and 40 (17 down and 23 up-regulated) DE genes were identified in AN, CH and KC steers, respectively. One DE gene (*PRAP1*) was common and down-regulated in low DMI steers of all the breeds. For ADG, two DE genes (*BOLA* and *TMEM45A*) were common among all three breeds. Both genes were down-regulated in AN and CH but up-regulated in KC high ADG steers. Ingenuity Pathway analysis results showed that DE genes identified across the three breeds for DMI were mainly involved in carbohydrate metabolism, vitamin and mineral metabolism, and cellular movement. DE genes between divergent ADG steers across all breeds were mainly involved in lipid metabolism, small molecule biochemistry, vitamin and mineral metabolism, and molecular transport. Our results provide insights into the genetic and molecular controls of feed intake and growth in beef cattle.

Key Words: transcriptome analyses, liver, dry matter intake, average daily gain, beef cattle

MT328 Detection of *de novo* mutations causing osteogenesis imperfecta and bulldog calf syndrome and assessment of mosaicism in the sires. C. Wurmser*[1], H. Pausch[1,2], S. Ammermüller[1], A. Heldmann[1], and R. Fries[1], [1]*Chair of Animal Breeding, TUM, Freising, Germany;* [2]*Animal Genomics, Institute of Agricultural Sciences, ETH, Zurich, Switzerland.*

We examined whole genome sequencing data in an attempt to identify the underlying mutations of two genetic diseases in cattle: osteogenesis imperfecta type 2 in German Fleckvieh (FV) and bulldog calf syndrome (lethal chondrodysplasia) in Holstein Friesian (HF). Out of 442 and 275 offsprings of a FV and HF bull, respectively, 32% and 21% were affected with osteogenesis imperfecta and the bull dog calf syndrome, respectively. Both sexes were afflicted. Whole genome re-sequencing of the two sires and two calves with bulldog calf syndrome was performed at a coverage of 10–20x. A frameshift mutation (p.A1049_P1050DelInsS) in *COL1A1* for osteogenesis imperfecta and a missense mutation (p.G996S) in *COL2A1* for the bulldog calf syndrome were detected. Both mutations alter the Gly-x-y motif of COL1A1 and COL2A1, respectively, which is essential for a proper assembly of the collagen triple-helix. The affected calves were heterozygous, whereas the genotypes of the sires could not be clearly assessed. Sanger- and pyrosequencing finally confirmed mosaicism in semen and blood derived DNA samples of both breeding bulls. Seven calves with

osteogenesis imperfecta and ten calves with bulldog calf syndrome were genotyped to validate the causality of the variants. All cases were heterozygous for the respective polymorphism. Furthermore, the mutations were neither found in healthy paternal half-sibs nor in 1577 animals from the 1000 bull genomes project. Deleterious mutations with dominant effects are less of a threat to breeding populations, since the defect alleles normally would not pass to another generation and allele frequency remains low. Nevertheless, timely identification of mosaic bulls is crucial. Excluding such bulls from breeding reduces economic losses and animal suffering.

Key Words: cattle and related species, animal breeding, DNA sequencing, genetic disorder, animal health

MT329 New output formats for Axiom genotyping arrays. J. Foster*, A. Davassi, A. Pirani, S. Kaushikkar, B. Wong, M. Patil, and L. Jevons, *Thermo Fisher Scientific, Santa Clara, CA, USA.*

The high-throughput agricultural genotyping landscape encompasses a broad range of applications and technical platforms. One of the major challenges of adopting a new platform or performing meta-analyses is data format congruity. Biallelic genotypes are recorded in one of three ways; 'AA', 'AB' and 'BB' call codes, '0', '1', and '2' numeric call codes and base calls. For call codes and numeric call codes the A and B alleles must be designated. Historically, two formats have dominated the designation of variant alleles; 'Forward' and 'TOP'. For a bi-allelic SNPs this can create a situation where the 'A' allele designated by one format differs from the other. To support cross-platform high throughput genotyping analysis, Affymetrix have developed the Axiom Long format Export tool (AxLE); a companion application to Axiom Analysis Suite (AxAS). The tool formats Axiom genotype data from native 'Forward' format to the top (TOP) and bottom (BOT) designations based on the polymorphism itself, or the contextual surrounding sequence and designates the A/B allele. A clear requirement for the homogenization of allele designation is in the downstream application of genotyping data to genetic evaluation systems where mixing of the formats could be disastrous to the prediction of economically important traits. To support this specific use case in dairy cattle, Affymetrix have developed the CDCB (Council on Dairy Cattle Breeding) export tool; a companion application to AxAS. Once a genotyping export has been formatted using AxLE, the CDCB export tool formats it and a sample sheet to enable direct upload to the Council on Dairy Cattle Breeding website. The tool is capable of consuming data from any Affymetrix catalogue bovine array and also custom bovine designs.

Key Words: cattle and related species, bioinformatics, genotyping, genomic prediction

MT330 Genetic diversity and population structure amongst commercial and native cattle breeds using a whole-genome SNP panel. I. Jasielczuk*, A. Gurgul, T. Szmatola, A. Radko, T. Zabek, and M. Bugno-Poniewierska, *National Research Institute of Animal Production, Balice, Poland.*

Knowledge of the genetic diversity and population structure is useful for managing and conserving farm animal genetic resources and provides information about the origin and history of cattle. The aim of this study was to investigate and compare interbreed diversity and population structure between ten both commercial (Angus Red- AR, n = 41; Charolaise- CH, n = 100; Hereford- HH, n = 82; Holstein- HO, n = 300; Limousin- LM, n = 197; Montbeliarde- MO, n = 93; Simmental- SM, n = 106) and native (Polish Red- PR, n = 195; Polish Black-White- ZB, n = 28; Polish Red-White- ZR, n = 72) cattle breeds maintained in Poland. Single nucleotide polymorphism (SNP) data were obtained using Illumina Bovine SNP50K BeadChip. After filtering, 41,269 (75.6% of all SNPs) high quality and common between studied breeds SNP markers were utilised for the final analysis. The calculated distribution of minor allele frequencies suggested that the SNP panel was sufficiently polymorphic for all studied breeds. The percentage of polymorphic SNP markers within breed ranged from 92.04% (AR) to 98.47% (RP). The average expected heterozygosity (H_E) ranged from 0.30 (MO) to 0.33 (RP, ZR) and observed heterozygosity (H_O) was between 0.31 (MO) and 0.34 (ZR) among all studied breeds. Inbreeding coefficient was low across the breeds ranging from -0.001 (LM) to -0.039 (ZB). Pairwise F_{ST} estimates between all pairs of cattle showed the greatest divergence between HH and HO (0.154) and the lowest level of genetic differentiation between native RP and ZR (0.046) cattle. A phylogenetic tree computed based on the breed pairwise F_{ST} showed that all studied breeds seemed to be clustered in relation to their primary geographical place of origin. It is in agreement with the principal component analysis (PCA) which showed that AR, HH, HO, RP breeds had separate (non-overlapping with the other breeds) clusters. LM, MO and SM formed a range of overlapping with each other clusters in close proximity to CH breed. Both ZB and ZR breeds formed conjoint cluster what may be an evidence of their high genetic similarity. Study funded from BIOSTRATEG project, contract number: BIOSTRATEG2/297267/14/NCBR/2016.

Key Words: cattle, genotyping, microarray, population genomics, single-nucleotide polymorphism (SNP)

MT331 Polymorphism of *FGF2* gene and its effect on reproduction traits in Czech Holstein cattle. A. Svitakova*, M. Brzakova, L. Vostry, and Z. Vesela, *Institute of Animal Science, Prague, Czech Republic.*

The fibroblast growth factor 2 (*FGF2*) gene is expressed by the uterine endometrium throughout the oestrus cycle and early pregnancy. *FGF2* gene has been implicated in ovarian function, embryonic development and mortality. Bovine *FGF2* gene with the total length of more than 52 kb is located in chromosome 17. The association of single nucleotide polymorphism SNP11646 (*A* to *G* alleles substitution) in the *FGF2* gene with fertility was examined in 149 individual Holstein sires born in period 2000 – 2006 in the Czech Republic. Genomic DNA was extracted from whole blood by proteinase K method. The PCR-RFLP method was used to identify this polymorphism by using restriction endonucleases *Csp6I*. The frequencies of alleles *A* and *G* were of 0.35 and 0.65, respectively, and the genotype frequencies were 0.13, 0.43 and 0.43 for genotypes *AA*, *AG* and *GG*. Only breeding values of evaluated traits were known, therefore we have calculated deregressed breeding values (DRP) to compensate for the fact that performance records were not available. DRP is calculated as estimated breeding values divided its accuracy. Using DRP instead of breeding values (BVs), it could separate more properly the influence of genetic background in animal effect. The evaluated traits were conception rate of daughters (maternal genetic effect) and ability to impregnate females (direct genetic effect). The estimated BVs were taken from the national genetic evaluation. Statistical analyses were performed using a linear mixed model (SAS software, ver. 9.4). Model equation was simple: the fixed effect was genotype and the random effect was the animal. Sires with the *AA* genotyped were linked to daughters of higher fertility (maternal genetic effect) compared to the sires with *AG* or *GG* genotypes ($P < 0.05$). The fertility of sires (direct genetic effect) was the best in the sires with *AA* genotype as well (not significant). The *FGF2* gene appears to be a candidate gene for determining reproduction traits. This work was supported by the project no. NAZV QJ1510217.

Key Words: candidate gene, cattle and related species, reproduction, genetic marker

MT332 mRNA-microRNA interaction network revealed that WNT signal pathway is a key to start the hair follicle in Cashmere goat. Z. Liu[1,2], M. Zhao[1,3], R. Nai[1,4], R. Wang[1,4], Y. Zhang[1,3], R. Su[1,3], Z. Wang[1,4], Y. Zhao[1,3], J. Li*[1,2], and Y. Xie[1,3],

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Cashmere quality and production have an important impact on textile industry. Inner Mongolia Cashmere, which produced by secondary hair follicle, is 14μm in diameter. Its growth has obvious cyclical in one year.We explored the effect of skin microRNA and mRNA interaction on hair follicle development. Skin samples of two different hair follicle growth phase, anagen and telogen, were collected and sequenced to obtain transcriptome and microRNA sequencing data. 12799 transcripts displayed significant expression difference between the samples. Among them, 5579 transcripts (2505 genes) expressed higher in telogen skin of two different periods, while 7220 transcripts (3706 genes) have higher expression in the anagen. There were 45 microRNAs with significantly differential expression between the two periods, 36 of them were expressed higher in anagen samples, while another 9 have higher expression in telogen samples.12927 target genes were found by two computational target prediction algorithms (TargetScan 50 and miRanda 3.3a). Messenger RNA-microRNAs interaction was analysed for detecting negative regulatory. 5114 transcripts showed both negative and positive expression pattern between DEGs and microRNA. And then we constructed the mRNA-microRNA GO annotation and KEGG pathway interaction network. The target genes and microRNAs, which are up-regulated and down-regulated in the earlier development period, are clustered into two groups. The regulation of skin microRNAs and target gene is important for the beginning of the growth. 350 up-regulated genes and 13 microRNAs were clustered as one group, 233 down-regulated genes and 33 microRNAs were clusteredinto the other group. This may indicate that the cycle start of hair follicles is controlled by micro-polygenes, the balance between genes is critical to cycle start. The Wnt signalling pathway associated with hair follicle development was found to be one of the most significant pathway. Construct Wnt signal interaction network,show that *SMAD2*, *SIAH1,FZD6* and CHP1 which are located in centre of network. The expression levels of them validated by qRT-PCR showed high concordance with the sequencing data, demonstrating our analysis is reliable.

Key Words: cashmere goat, hair follicle, WNT signal, mRNA-microRNA network

MT333 Identification of long noncoding RNAs by whole transcriptome analysis in the jejunum of pre-weaned calves. R. Weikard*[1], F. Hadlich[1], H. M. Hammon[1], D. Frieten[2], C. Gerbert[3], C. Koch[3], G. Dusel[2], and C. Kühn[1,4], [1]*Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany;* [2]*University of Applied Sciences, Bingen, Germany;* [3]*Educational and Research Centre for Animal Husbandry, Hofgut Neumühle, Münchweiler, Germany;* [4]*University Rostock, Faculty of Agricultural and Environmental Sciences, Rostock, Germany.*

Long noncoding RNAs (lncRNAs) emerged as important regulatory component of mechanisms involved in gene expression, chromatin modification and epigenetic processes, but they are rarely annotated in the bovine genome. Our study monitored the jejunum transcriptome of male German Holstein calves fed two different diets (ad libitum versus restricted milk replacer for 8 weeks; 5 and 6 animals per group) using whole transcriptome sequencing (RNA-seq). Total RNA was isolated from epithelial sections of jejunum samples of the calves slaughtered two weeks after terminating the divergent feeding regimes. Indexed, stranded sequencing libraries were prepared and paired-end sequenced (2x 80 bp, Illumina HiSEqn 2500). After quality control of raw sequencing data, the reads were aligned to the bovine genome. The transcripts were assembled with an annotation-guided approach enabling discovery of yet unannotated genes and transcripts. About 88% of reads (from an average of 56.8 million paired-end reads obtained per sample) mapped to the bovine genome. In addition to known protein coding genes varying between 18,395 and 20,904 per sample, 16,378 novel transcripts were identified in the jejunal transcriptomes of the calves. To identify potential lncRNAs from unknown assembled transcripts (14,328 class code 'u' transcripts) four bioinformatic lncRNA prediction tools (CNCI, PLAR, PLEK and FEELnc) were applied. Based on the intersection results, a total of 1,055 lncRNA transcripts were detected and classified according to their genomic location. Furthermore, a total of 48 novel mRNAs were retrieved from the unknown transcript dataset. Out of the 30 unknown transcripts that were differentially expressed in the jejunal mucosa between calf groups fed two different diets, three are contained in the intersection of the lncRNA prediction tools indicating functional relevance of these potential lncRNAs for the modulation of metabolic processes in intestine tissue associated with long-lasting adaptation to food restriction or different feeding regimes. For further functional annotation of lncRNAs expressed in the jejunum mucosa, co-expression analysis with mRNA transcripts was performed.

Key Words: RNA-seq, non-coding RNA, intestine transcriptome, calf, nutrition

MT334 Effect of plane of nutrition on the transcriptomic profile of subcutaneous adipose tissue in Holstein-Friesian bull calves. A. M. English*[1,2], S. Fair[2], P. Cormican[1], C. Byrne[1,3], S. M. Waters[1], and D. A. Kenny[1,3], [1]*Animal and Bioscience Research Department, Teagasc, Grange,, Dunsany, Co. Meath, Ireland;* [2]*Laboratory of Animal Reproduction, Department of Biological Sciences, School of Natural Sciences, Faculty of Science and Engineering, University of Limerick, Limerick, Ireland;* [3]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland.*

Adipose tissue is a metabolically dynamic organ serving many endocrinological and homeostatic functions. In particular it is thought to mediate, through targeted neuroendocrine signalling, the impact of nutritional status on many reproductive processes, including the timing of onset of puberty. However, there is a dearth of published information on the exact biochemical mechanisms involved. The aim of this study was to investigate the effect of plane of nutrition during early calf-hood on the global transcriptional profile of subcutaneous adipose tissue. Holstein-Friesian bull calves with a mean (± s.d.) age and bodyweight of 19 (± 8.2) days and 47.5 (± 5.3) kg, respectively, were assigned to either a high (n = 10) or low (n = 10) plane of nutrition, with target growth rates of 1.2 and 0.5 kg per day, respectively. Calves were killed at 16 weeks of age and subcutaneous adipose tissue was harvested from the flank of the carcass and snap-frozen in liquid nitrogen. RNA was extracted, cDNA libraries, generated and RNAseq analysis performed. There were 674 genes differentially expressed between the high and low plane of nutrition treatments, consisting of 164 up-regulated and 581 down-regulated genes ($P < 0.05$; False Discovery Rate < 0.05; fold change >2.0). Overall, a low of plane of nutrition resulted in the down-regulation of genes involved in energy production and branched chain amino acid degradation. Specifically, offering calves a low compared with a high plane of nutrition resulted in down-regulation of genes involved in adipogenesis *LPL, PLIN1* and *FABP4* as well as lower transcript abundance for genes such as leptin (*LEP*; -4.541 log fold change) and adiponectin (*ADIPOQ*, -1.62 log fold change). Both of these two latter genes are known to affect reproductive function, including gonadotropin synthesis, with *ADIPOQ* having direct effects on *LH* and *FSH*. These results provide insight into the effect of plane of nutrition during calfhood

on the transcriptome of adipose tissue during a critical window of sexual development.

Key Words: cattle, RNA-seq, animal nutrition, reproduction, gene expression

MT335 A copy number variant (CNV) scan in the autochthonous Italian Valdostana Red Pied cattle and comparison with specialized dairy populations. M. G. Strillacci[1], E. Gorla[1], M. C. Cozzi[1], M. Vevey[2], F. Bertolini[3,4], L. Fontanesi[3], and A. Bagnato*[1], [1]*Department of Veterinary Medicine, University of Milan, Milano, Italy;* [2]*Associazione Nazionale Allevatori Bovini Razza Valdostana, Gressan, Italy;* [3]*Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Bologna, Italy;* [4]*Department of Animal Science, Iowa State University, Ames, IA, USA.*

CNVs are an important source of genomic structural variation, recognised to affect phenotypic variation in many mammalian species. Here we report on a high-resolution CNV scan from Illumina's 777k BovineHD Beadchip data for Valdostana Red Pied (VRP) bulls, an autochthonous Italian dual-purpose cattle population reared in the Alps, that did not undergo strong selection for production traits, as compared to Holstein and Brown Swiss. CNVs were called across 108 bulls using the PennCNV software after stringent quality control and filtering using default parameters. Specific breed selection signatures were identified using the CNVs here mapped in the Valdostana Red Pied, and those available from published studies in the Italian Brown Swiss (BS - 164 sires) and in the Mexican Holstein (MH - 124 males and females). In order to describe the distribution of CNV within and among breeds we used the V_{ST} statistic as $(V_T - V_S)/V_T$, an analogue of F_{ST} for CNV, where V_T is the variance in copy numbers among individuals and V_S is the average variance within each breed, weighted for population size. Go analysis of genes included in CNV regions (after their definition) was performed using DAVID database. In the VRP we identified a total of 6,784 CNVs that were summarised to 1,723 CNV regions on 29 autosomes covering a total of ~59 Mb of the UMD3.1 autosome. Among the mapped CNV regions 812 resulted loss, 832 gain and 79 complex. A total of 171 CNV regions were the same in all the three breeds. Between PRV and BS there were 486 CNV regions overlapping while between VRP and MH only 311 indicating a more similar genetic background among populations with common origins, the Alps. These CNV regions harbor genes related to functional traits as the ones on BTA11 (mastitis resistance), immune functions on BTA23 (BoLA ClassII complex) and on BTA28 (development of mammary gland). Data were generated as part of the FP7 project QUANTOMICS contract n. 2226642.

Key Words: cattle, CNV, genetic diversity

MT336 Congenital cataract formation in Holstein Friesian cattle is associated with a nonsense mutation in bovine CPAMD8 gene. A. K. Hollmann*[1], I. Dammann[2], W. M. Wemheuer[3], W. E. Wemheuer[1], A. Chilla[1], A. Tipold[4], W. J. Schulz-Schaeffer[3], J. Beck[5], E. Schütz[1,5], and B. Brenig[1], [1]*Institute of Veterinary Medicine, University of Goettingen, Goettingen, Germany;* [2]*Prion and Dementia Research Unit, Department of Neuropathology, University Medical Center Goettingen, University of Goettingen, Goettingen, Germany;* [3]*Institute of Neuropathology, University of the Saarland, Germany;* [4]*Dept. Small Animal Medicine and Surgery, University of Veterinary Medicine Hannover, Hannover, Germany;* [5]*Chronix Biomedical, Goettingen, Germany.*

Congenital cataracts are lens opacities reducing sight of affected individuals and being present from birth. In humans and mice more than 290 genes and 19 non-gene loci have been described that harbor causative mutations for hereditary cataracts. However, knowledge about the aetiology of cataract development in cattle is still relatively scarce. We investigated 31 Holstein Friesian (HF) cattle with bilateral complete congenital cataracts. Pedigree analysis revealed a relationship of all cataract cases indicating an autosomal recessive inheritance of the disorder. A case-control study was performed using genotyping data of 26 cases and 88 controls to check for associations with the cataract phenotype. The genome-wide analysis revealed an association at position 12.4Mb (Bonferroni-adjusted *P*-value 1.85×10^{-32}) and 6.2Mb (Bonferroni–adjusted *P*-value 5.44×10^{-30}) and a 4.7Mb region of extended homozygosity (5,639,104 to 10,406,009) on bovine chromosome 7 (BTA7) (UMD_3.1). Whole genome re-sequencing of one case and four closely related cattle revealed a nonsense mutation in *CPAMD8* (g.5995966C>T, p.Q74X) gene (C3 and PZP like, α-2-macroglobulin domain containing 8), located at the proximal end of the detected region of extended homozygosity. Subsequent genotyping of SNP g.5995966C>T in *CPAMD8* was performed on a cohort of 1,248 animals. All cataract cases were tested homozygous affected. All heterozygous tested cattle ($n = 161$), including all tested parents of cataract cases, were related to a common ancestor detected by pedigree analysis. Immunohistochemical analysis revealed the presence of CPAMD8 protein in the ciliary body epithelium of healthy cattle, but not in cataract cases. These findings indicate a secretion of CPAMD8 from the epithelium into aqueous humour under normal circumstances. Our data provide convincing evidence that the absence of CPAMD8 protein leads to congenital cataract formation in HF cattle and are in accordance with previous findings of *CPAMD8* being associated with anterior segment dysgenesis in humans.

Key Words: cattle, functional genomics, HTS, candidate gene, animal health

MT337 Allele-specific gene expression in liver of Nelore cattle extremes for feed efficiency. M. Rocha*[1], M. de Souza[1], A. Zerlotini-Neto[2], P. Tizioto[3], P. de Oliveira[4], A. de Lima[1], J. Afonso[1], L. Coutinho[3], L. Regitano[4], and S. Niciura[4], [1]*Departament of Genetics and Evolution, Federal University of São Carlos/UFSCar, São Carlos, São Paulo, Brazil;* [2]*Embrapa Informática Agropecuária, Campinas, São Paulo, Brazil;* [3]*Department of Animal Science, University of São Paulo/ESALQ, Piracicaba, São Paulo, Bazil;* [4]*Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil.*

Feed efficiency is an economically important trait, and several candidate genes involved in biological processes associated with residual feed intake (RFI) have been identified. It is also known that allele-specific expression (ASE) has an important role in regulating multifactorial traits such as RFI; however, studies identifying these mechanisms are still scarce. We analysed the profile, presence and distribution of ASE in liver tissue transcriptomes from 30 Nelore cattle steers genetically divergent for RFI. The survey of the genome-wide distribution of the ASE was assessed by applying the ALEA software, using the bovine reference genome (UMD3.1) and sample data from 777k SNPs genotyped by the Illumina BovineHD BeadChip. ALEA creates a diploid reference genome for each individual and detects RNA-Seq reads that are uniquely aligned in one of the two haploid parental genomes constructed. The frequency of reads mapped to each allele was computed and a binomial statistical test was applied to identify ASE. Functional annotation was performed using DAVID 6.8. We detected seven SNPs presenting complete ASE (i.e. consistently identified in at least 90% of the samples tested) and 33 SNPs presenting incomplete ASE (i.e.: identified in less than 90% of the samples tested). These SNPs were located within or close to *GB, KNG1, CRP, LDHB, ORM1, FMO3* and *CHP2* genes. The *FGB* and *KNG1* genes stood out because they are involved in the cascade of the complement and coagulation over-represented pathway, which was also identified in our previous differentially expression analysis performed using these same animals. The obtained results suggest a possible regulation mechanism of this pathway. However, more studies are needed to elucidate

it better. The knowledge of ASE profile of these genes may help to explain the differences in gene expression identified in Nelore steers genetically divergent for RFI. This project was supported by FAPESP (12/23638-8, 15/17802-8).

Key Words: allele-specific expression, cattle and related species, bioinformatic tools, genome regulation, genetic improvement

MT338 Accuracy of genomic prediction of economically important traits in Brangus cattle using original and imputed low-density SNP genotypes. F. B. Lopes[1,2], X. Wu*[2], H. Li[1,2], J. Xu[2,3], T. Perkins[4], J. Genho[5], R. Ferretti[2], M. Wells[2], R. J. Tait[2], S. Bauck[2], and G. J. M. Rosa[1], [1]*University of Wisconsin, Madison, WI, USA;* [2]*GeneSeek (An Neogen company), Lincoln, NE, USA;* [3]*University of Nebraska, Lincoln, NE, USA;* [4]*International Brangus Breeders Association, San Antonio, TX, USA;* [5]*Livestock Genetic Services LLC, Woodville, VA, USA.*

Reliable genomic prediction of breeding values for quantitative traits requires a sufficient number of animals with genotypes and phenotypes in the training set. At the initial stage of a genomic selection program, however, the training population size is often very limited. As of October 31, 2016, there were 3,797 Brangus animals genotyped with varying sizes of SNP chips. The largest group consisted of 1,535 animals genotyped with the GGP-LD-V4 SNP chip (40,660 SNPs) and with expected progeny differences (EPD) information for ten traits. To expand the training data, it was possible to add 2,262 animals by imputing these SNP genotypes to the same SNP content as the GGP-LD-V4 chip. The present study showed that, by pooling animals with both original and imputed 40K SNP genotypes, genomic prediction accuracies (GPA) were substantially increased for all ten traits. The relative gains in GPA on EPDs varied from 12.51% to 26.27%, and those on de-regressed EBV (estimated breeding values) were from 18.17% to 61.83%. Nevertheless, GPA on de-regressed EBV (0.5811 – 0.7953) were not greater than those on EPD (0.7038 – 0.8929). Possible reasons included a substantial loss of data during the de-regression procedure and the impacts of removing parental averages from individual EBV and the presence of unadjusted bases. The present study also compared the performance of five genomic prediction models and two cross-validation methods. The five genomic models performed very similarly when predicting EPD for the ten traits. Of the two cross-validation methods, LOOCV (leave-one-out cross-validation) gave GPA close to those using the whole dataset whereas the *K*-fold cross-validation ($K = 3$ in the present study) tended to underestimate genomic prediction accuracies. GPA on the ten quantitative traits were validated in 1,106 newly genotyped Brangus animals using SNP effects estimated in the previous 3,797 animals, and such GPA were slightly decreased. The present study was the first to leverage currently available genotype and phenotype resources in order to harness effective genomic prediction in Brangus beef cattle.

Key Words: beef cattle, cross-validation, genomic prediction, quantitative traits, SNP array

MT339 Gene mapping and genomic prediction of sire conception rate in US dairy cattle. F. Peñagaricano*[1], Y. Han[1], P. Nicolini[1,2], G. Morota[3], and R. Abdollahi-Arpanahi[1,4], [1]*University of Florida, Gainesville, FL, USA;* [2]*Universidad de la República, Tacurembó, Uruguay;* [3]*University of Nebraska-Lincoln, Lincoln NE, USA;* [4]*University of Tehran, Tehran, Iran.*

Reproductive performance greatly impacts the profitability of the dairy farm. Most studies have focused on cow fertility, while bull fertility has been largely ignored. However, the service sire affects not only the fertilization process but also the viability of the pre-implantation embryo, and represents indeed an important source of variation for conception rate. The goal of this study was to perform a comprehensive genomic analysis of dairy bull fertility including gene mapping and genomic prediction. Sire conception rate (SCR) was used as a measure of bull fertility. Data consisted of 50k SCR records from 13k US Holstein and Jersey bulls. Whole-genome single nucleotide polymorphism (SNP) data were available for 9k animals. The association mapping included the application of alternative statistical methods and the subsequent use of gene set tools to unravel the genomic architecture of SCR. Furthermore, the predictive ability of alternative prediction models including both genomic and biological data was evaluated in cross-validation. Specifically, different sets of SNPs were evaluated for prediction, including SNPs in genic regions, SNPs linked to functional categories such as Gene Ontology or Medical Subject Headings terms, and SNPs that were marginally associated with SCR. The association analyses identified many genomic regions associated with bull fertility. Most of these regions harbor genes, such as *CCT6A*, *CKB*, *IGF1R*, *KAT8* and *TDRD9* with functions closely related to sperm development, sperm motility and fertilization. Moreover, genomic models achieved decent SCR predictions in the testing set, suggesting that genomic prediction of bull fertility is feasible in dairy cattle. Models with genic or gene set SNPs did not outperform their counterparts using random sets of SNPs. Interestingly, prediction models fitting significant SNPs showed better predictive ability than the whole-genome approach. Overall, our study contributes to the identification of genetic variants and biological pathways responsible for the genetic variation in bull fertility. In addition, we provide evidence that dairy bull fertility can be improved via marker-assisted selection.

Key Words: association analysis, bull fertility, gene sets, prediction of complex traits

MT340 The Ovine Functional Annotation Project. B. Murdoch*[1], S. White[2,3], M. Mousel[2], A. Massa[3], K. Worley[4], A. Archibald[5], E. Clark[5], B. Dalrymple[6], J. Kijas[7], S. Clarke[8], R. Brauning[8], T. Smith[9], T. Hadfield[10], and N. Cockett[10], [1]*University of Idaho, Moscow, ID, USA;* [2]*USDA, ARS, Animal Disease Research Unit, Pullman, WA, USA;* [3]*Washington State University, Pullman, WA, USA;* [4]*Baylor College of Medicine, Houston, TX, USA;* [5]*The Roslin Institute and R(D)SVS, University of Edinburgh, Edinburgh, Scotland;* [6]*University of Western Australia, St Lucia, Queensland, Australia;* [7]*CSIRO Agriculture, St Lucia, Queensland, Australia;* [8]*AgResearch, Invemay Agricultural Centre, Mosgiel, New Zealand;* [9]*USDA, ARS U.S. Meat Animal Research Center, Clay Center, NE, USA;* [10]*Utah State University, Logan, UT, USA.*

The overarching goal of the Ovine FAANG Project is to deliver enhanced functional annotation of the sheep genome in order to enable research in this globally important food and fibre species. This research will facilitate the understanding of gene regulation in sheep by generating and distributing curated transcriptome gene regulation data. We will characterise the complexity of the ovine transcriptome and the regulatory signals using assays for coding and non-coding transcript isoforms and alternative splicing, promoters and cis-acting regulatory elements, histone modifications, DNA methylation and open chromatin across a wide range of sheep tissues. Members of International Sheep Genomics Consortium (ISGC) and other researchers at Utah State University collected ~100 tissues from a Rambouillet female, Benz 2616, using protocols outlined by the FAANG Consortium. This animal was selected for collection because her genomic DNA had been used for the *de novo* assembly of the sheep whole genome sequence. Tissue samples were either snap frozen in liquid nitrogen for RNA and DNA based assays or slowly frozen for use in chromatin accessibility assays. Using a method that targets poly-A+ mRNAs and microRNAs, a total of 94 tissues will be sequenced to determine the transcriptome. Cap analysis of gene expression (CAGE), will be performed on 100 tissues to complement the gene expression data and will be used to identify active promoters and enhancers and confirm transcription start sites. Histone modification (H3K4me3,

H3K4me1, H3K27me3 and H3K27ac) will be used with ChIP-seq procedures for 59 tissues to determine transcription activation, repression and enhancer sites. Chromatin accessibility will be determined by ATAC-seq for 100 tissues. The methylation status of all 100 tissues from Benz 2616 will be established utilising epiGBS, a reduced representation bisulfite sequencing method. In total, this project will provide tissue-specific detailed understanding of the genes and gene products that contribute to the evolution, development and function of sheep.

Key Words: sheep and related species, Functional Annotation of Animal Genomes (FAANG), genome annotation

MT341 Effects of SRLV infection on the expression of acute phase protein and cathelicidin genes in goat blood leukocytes and milk somatic cells. D. Reczynska*[1], J. Jarczak[1], M. Czopowicz[2], D. Sloniewska[1], K. Horbanczuk[1], W. Jarmuz[1], J. Kaba[2], L. Zwierzchowski[1], and E. Bagnicka[1], [1]*Institute of Genetics and Animal Breeding PAS in Jastrzebiec, Magdalenka, Poland;* [2]*Warsaw University of Life Sciences-SGGW, Warsaw, Poland.*

Maintaining homeostasis is essential for normal cell function. Defence of life organisms against pathogens is vital for homeostasis. Among virtually 1000 antimicrobial peptides belonging to different families so far identified ~30 members of cathelicidin family have been found in mammals. Cathelicidins constitute the first line of defence since they can act at a physiological salt concentration which is characteristic for healthy tissues. Moreover, alow salt concentration, typical for infected tissues, inhibits their activity. Following cathelicidins have been identified in goats: bactenecin 7.5 (BAC7.5), bactenecin 5 (BAC5), myeloid antimicrobial peptide 28 (MAP28), myeloid antimicrobial peptide 34 (MAP34A and B), goat bactenecin 3.4 (ChBac3.4). Acute phase proteins (APPs) also participate in immune defence of organisms. The level of APPs changes during infection, trauma and injury. In goats five positive APPs (haptoglobin (Hp), serum amyloid (SAA), ceruloplasmin (Cp), fibrinogen (Fb), α_1- acid glycoprotein (AGP)) and one negative APP (lactalbumin (LALBA)) have been identified. Caprine arthritis-encephalitis (CAE) caused by small ruminant lentivirus (SRLV) is a worldwide economic problem. Main CAE symptoms are arthritis, pneumonia and in durative mastitis (significant elevation of the somatic cell count and deterioration of some technological parameters), and weight loss, leading to emaciation. The study was conducted on 24 dairy goats of which 12 were SRLV-infected and the rest were uninfected. Blood and milk samples were collected five times during lactation: on the 1st, 30th, 60th and 140thand 200th day of lactation. The levels of selected gene transcripts in blood leucocytes and milk somatic cells were measured using qPCR method. Levels of expression of *SAA* and *Hp* genes in blood leukocytes and *BAC5*, *Hp*, *AGP* genes in milk somatic cells were higher in the SRLV-infected group. The decreased expression was observed only for *LALBA* gene in blood leukocytes. The study was financed by a grant from the National Scientific Center No. 2013/09/B/NZ6/03514.

Key Words: goat, CAEV, acute phase proteins, cathelicidins, blood leukocytes

MT342 Sex chromosome-linked cancer/testis antigens (CTAs) and male fertility in cattle. W. Liu*, *The Pennsylvania State University, University Park, PA, USA.*

CTAs are proteins expressed only in the germline in testis and a variety of tumours. Among the 228 CTAs reported in mammals, 52% of which map to the X chromosome (termed CTX), a few CTAs map to the Y chromosome, and the remaining CTAs are distributed on the autosomes. CTAs have been extensively studied in cancer biology, but their role in male reproduction remains unclear. The objectives of this study are i) to identify the X- and Y-linked CTA gene in the bovine genome by a comparative mapping approach; and ii) to investigate the copy number variation (CNV) of a Y-linked CTA gene, named preferentially expressed antigen in melanoma, Y-linked (PRAMEY), and its association with bull fertility in Holsteins. We searched bovine orthologs of the human CTX genes by BLASTp and retrieved 86 proteins corresponding to 18 CTX families. This result differs radically from the human CTX annotation in which at least 35 CTX families, including a total of 128 CTX genes, have been identified. The three most amplified CTX families are GAGE, MAGEA, and CT47 with 18, 13, and 12 copies respectively in the human genome. However, no bovine orthologs were identified for GAGE, and only 9 and 4 copies of MAGEA and CTAG were found in bovine, suggesting that the number of CTXs is probably underestimated due to incomplete sequence in bovine. Only two CTAs, TSPY and PRAMEY that are multicopy gene families, map to the bovine Y. TSPY is conserved in human and several other mammalian lineages, while PRAMEY is bovid-specific, derived from an autosome-to-Y transposition during evolution. Copy number of PRAMEY were estimated for 257 Holstein bulls by a qPCR method with an average of 12 copies on the Y, ranging from 2 to 20. Association analysis revealed that the PRAMEY CNVs was correlated negatively with scrotal circumference (SC), relative scrotal circumference (RLSC), percentage of normal sperm (PNS), and non-return rate (NRR). Our preliminary data suggest that the sex-linked CTAs may play a functional role in male fertility in cattle.

Key Words: sex chromosome, cancer/testis antigen, copy number variation, male fertility, cattle

MT343 Effects of stage of lactation on the expression of acute phase protein and cathelicidin genes in goat blood leukocytes and milk somatic cells. D. Reczynska*[1], J. Jarczak[1], M. Czopowicz[2], D. Sloniewska[1], M. Mickiewicz[2], K. Horbanczuk[1], W. Jarmuz[1], J. Kaba[2], L. Zwierzchowski[1], and E. Bagnicka[1], [1]*Institute of Genetics and Animal Breeding Polish Academy of Sciences in Jastrzebiec, Magdalenka, Poland;* [2]*Warsaw University of Life Sciences-SGGW, Warsaw, Poland.*

Animals are constantly exposed to pathogens and physical effort and they must have a complex system of defence. Antimicrobial peptides (AMP) such as cathelicidins take part in the first line of defence. So far, 30 peptides belonging to this family have been found in mammals, but in goats only bactenecin7.5 (BAC7.5), bactenecin5 (BAC5), myeloid antimicrobial peptide 28 (MAP28), myeloid antimicrobial peptide 34 (MAP34 A and B), and goat bactenecin3.4 (ChBac3.4) were identified. Moreover, acute phase proteins (APP), which are a heterogeneous group of proteins, are involved in the immune response. In goats five of them –haptoglobin (Hp), serum amyloid A (SAA), ceruloplasmin (Cp), fibinogen (Fb), α_1- acid glycoprotein (AGP) are named positive APP, because their level increase during inflammation or infection, and only lactalbumin (LALBA) is negative. Both, AMPs and APPs are important mediators or inhibitors of inflammation. The study was conducted on 24 dairy goats. Blood and milk samples were collected five times during lactation: on the 1st, 30th, 60th and 140th and 200th day. The levels of transcripts of selected genes in blood leucocytes and milk somatic cells were measured using qPCR method. The changes in AMP and APP expression levels during lactation were observed for *SAA*, *Hp*, *LALBA*, *AGP*, and *BAC5* genes in milk somatic cells and blood leukocytes. In both materials the lowest level was observed postpartum, however the highest expression was detected in leukocytes in the peak of lactation while in full lactation in milk somatic cells. Moreover, differences in *MAP34* expression were observed in milk somatic cells and differences in *Crp*, *BAC7.5*, and *MAP28* were identified in blood leukocytes. These results indicate possible disturbances of homeostasis in organisms due to metabolic effort during milk production. The study was financed by a grant from the National Scientific Center No. 2013/09/B/NZ6/03514.

Key Words: goat, acute phase proteins, cathelicidins, blood leukocytes, milk somatic cells

MT344 The level of expression of immune system and milk protein genes in bovine mammary epithelial tissue infected with coagulase-positive and coagulase-negative staphylococci. E. Kawecka*[1,2], M. Zalewska[1], D. Reczynska[1], E. Kosciuczuk[1], D. Sloniewska[1], S. Marczak[1], W. Jarmuz[1], and E. Bagnicka[1], [1]*Institute of Genetics and Animal Breeding PAS in Jastrzebiec, Magdalenka Poland;* [2]*Warsaw University of Life Sciences -SGGW, Warsaw, Poland.*

Mastitis caused by coagulase-positive (CoPS) or coagulase-negative (CoNS) bacteria is one of the major diseases of dairy cattle, which causes chemical and physical changes in milk quality such as increased somatic cells count and decreased casein content. The study was performed to determine the differences in the level of expression of genes associated with the immunological response such as interleukin-8 (IL-8) and interleukin-18 (IL-18) in mammary epithelial tissue infected with different species of *Staphylococcus*. The expressions of α-S1-casein and kappa-casein genes that strongly affect milk technological features were also studied. The mammary gland tissue (parenchyma) samples were obtained from 20 quarters of black and white Holstein-Friesian infection-free (N = 8) cows or infected with coagulase-negative or coagulase-positive staphylococci (N = 12). Microbiological examination of quarter milk samples was performed two days before culling of the animals due to a chronic mastitis or reproduction problems. The qPCR was conducted using LightCycler480 (Roche) devices. Higher expression level of kappa-casein gene and lower level of IL-18 gene were observed in the samples derived from infection-free quarters ($P \leq 0.05$). While the expressions of both α-S1-casein and IL-8 genes did not differ between infected and infection-free tissues. Kappa-casein is a major milk protein and the lower the expression of its gene the worse the technological parameters of the milk. IL-8 is proinflammatory cytokine produced mainly by macrophages, but also by lymphocytes and monocytes. Although the number of macrophages decreased, the number of lymphocytes increased during mastitis. The elevated expression of IL-18 is probably connected with the high level of lymphocytes in a response of the mammary gland to the bacterial infection. The study was financed by a grant from the National Scientific Center 2015/17/B/N29/01561

Key Words: cattle, gene expression, bacteria, inflammatory response

MT345 Characterization of copy number variation in European cattle. M. Upadhyay*[1,2], H. Megens[1], V. Silva[1,2], V. Marleen[1], M. Groenen[1], and R. Crooijmans[1], [1]*Wageningen University and Research, Wageningen, the Netherlands;* [2]*Swedish Institute of Agricultural Sciences, Uppsala, Sweden.*

Copy number variation (CNV) is characterised by large-scale losses and gains of sequences and contributes significantly to genetic and phenotypic variation. Assessing CNVs across different European cattle breeds might reveal genetic changes responsible for phenotypic differences, which have accumulated throughout the domestication history of cattle as result of natural and artificial selection. To explore the pattern of CNVs across various European cattle breeds, we genotyped 149 individuals, which represents five European regions, using Illumina Bovine HD Genotyping array. A total of 9,944 autosomal CNVs were identified in 149 samples using a Hidden Markov Model (HMM) as employed in PennCNV. On average, animals originating from breeds of the Iberian, Balkan and Italian, and British region displayed higher abundance of CNVs compared to animals of Dutch or Alpine breeds. A total of 923 CNV regions (CNVRs) were identified by aggregating overlapping CNVs with overlap identified in at least two animals. The hierarchical clustering of CNVRs indicated low differentiation and sharing of high frequent CNVRs between different European cattle populations. The CNVRs identified in the present study overlapped with more than 900 genes involved in many traits. In addition, we also detected and validated a CNV overlapping the *Kit* gene in English Longhorn cattle which has been identified previously in Belgian blue, white park and Galloway cattle and is associated with colour-sidedness. We provide a comprehensive overview of the distribution of CNVs in the genomes of various European cattle breeds. Our results also hint at the important role of purifying selection and genomic drift in the distribution of CNVs in genome of various European cattle breeds.

Key Words: CNV, European cattle, *Kit*, drift

MT346 Somatic structural and numerical aberrations in bovine leukemia virus induced tumors. K. Durkin*[1], M. Artesi[1], V. Hahaut[1], N. Rosewick[1,2], P. Griebel[3], N. Arsic[3], A. Burny[2], M. Georges[1], and A. Van den Broeke[1,2], [1]*Unit of Animal Genomics, GIGA-R, University of Liège, Liège, Belgium;* [2]*Laboratory of Experimental Hematology, Institut Jules Bordet, Universite Libre de Bruxelles, Brussels, Belgium;* [3]*VIDO, University of Saskatchewan, Saskatoon, Canada.*

Bovine leukemia virus (BLV) is a deltaretrovirus that integrates into B-cells producing a lifelong infection in cattle. Like its close relative Human T-cell leukemia virus-1 (HTLV-1), BLV induces an aggressive leukemia/lymphoma in about ~5% of infected individuals. While not a natural host it is possible to infect sheep with BLV and in contrast to cattle, all infected sheep develop tumours at an accelerated rate (~18 months). Historically research into both viruses has primarily focused on their transcripts/proteins. However secondary events are likely to be important as only a subset of infected individuals, following many decades of infection, develop a tumour. At the current time little is known about the landscape of somatic changes in BLV induced tumours. To examine gross numerical and structural variants (SVs) we assayed 12 bovine tumours on the BovineSNP50 Illumina BeadChip as well as 22 ovine tumours on the OvineSNP50 Illumina BeadChip. We also carried out whole genome sequencing (~30X) on 4 ovine tumours with matched normal tissue. Initial examination of the tumours revealed frequent aneuploidy, with orthologous regions of the genome involved in both species. Focal SVs identified included an amplification (>4 copies) of the terminus of BTA16 in three tumours (contains *PTPRC* & miR-181), while the tumour suppressor *CDKN2A* on OAR2 was deleted in multiple ovine tumours. For the 4 sequenced tumours multiple time points over the course of infection were available allowing us to determine when these SVs arose via nested PCR. Interestingly we observed that the SVs involving well know cancer driver genes generally appear many months before tumour development. These preliminary results indicate that tumours induced by HTLV-1 and BLV display somatic structural changes that impinge on overlapping sets of genes and point to the emergence of SVs affecting cancer driver genes in the preleukemic clone, well before the clone undergoes rapid expansion.

Key Words: cancer, structural variants, somatic

MT347 Genetic profiles, history and signatures of selection of the Russian native cattle breeds. N. S. Yudin[1], A. Yurchenko[1], R. Aitnazarov[1], P. Plysnina[1], and D. Larkin*[1,2], [1]*The Federal Research Center Institute of Cytology and Genetics, The Siberian Branch of The Russian Academy of Sciences, Novosibirsk, Russia;* [2]*Department of Comparative Biomedical Sciences, Royal Veterinary College, University of London, London, UK.*

The genetic structure, history and signatures of selection were revealed in the genomes of 18 Russian native cattle breeds: Bestuzhevskaya, Black-Pied, Buryatian, Kazakh Whitehead, Kalmykian, Kostromskaya, Kholmogorsakaya, Tagilskaya, Ukrainian Whitehead, Yakutian, Yaroslavskaya, Red-Stepped, Alatauskaya, Red Gorbatovskaya, Istobenskaya, Yurinskaya, Red-Pied, Ukrainian Grey. The number of samples varied from two to 39 with the average of 20 per breed. Genotyping was performed either on the

150K GGP or the 50K Illumina bovine arrays. The phylogenetic analysis using SNPs shared between the arrays combined with the data obtained for 134 world breeds (Decker *et al*. 2014) suggests close relations between majority of the Russian native breeds and breeds of the European origin. However, the Yakutian, Kalmykian and Buryatian cattle have formed a cluster close to Asian breeds. The three major clusters of breeds: (1) Yakutian, Kalmykian and Buryatian, (2) Ukrainian Gray and (3) the remaining breeds. The combination of admixture and MDS analyses allowed for detection of the optimal number of populations (K) and related breeds within our set. Admixture analysis allowed to distinguish breeds with a high level of admixture from the non-admixed ones. We used Fst approach to look for signatures of selection within the Yakutian, Kalmykian and Buryatian cattle cluster and the haplotype-based (hapFLK) approach for the largest cluster of breeds (eliminating admixed breeds from the analysis) using ~139,000 SNPs. Yakutian cattle contains genome regions under selection enriched for genes related to GO-terms *immune response* including *response to interferon gamma*. The hapFLK analysis shows that multiple genome regions were subjected to selection between Russian breeds. Many of these contain known genes related to colouring (KIT, KITLG), behaviour (IMPAD1) carcass (PDE1B) and muscle mass (FAM184B). This work is a first step towards revealing the population structure, history, and signatures of selection within the native Russian cattle made to facilitate application of genomic technologies to improve breeding procedures and to increase the effectiveness of genomic selection for livestock in Russia.

Key Words: cattle, native breeds, signatures of selection, Russia, genotyping

MT348 Cis-perturbation of cancer drivers by the HTLV-1/BLV Proviruses is a major early determinant of leukemogenesis in humans, cattle, and sheep. M. Artesi*[1], N. Rosewick[1,2], K. Durkin[1], A. Marçais[3], V. Hahaut[1], P. Griebel[4], N. Arsic[4], A. Burny[2], C. Charlier[1], O. Hermine[3], M. Georges[1], and A. Van den Broeke[1,2], [1]*Unit of Animal Genomics, GIGA-R, University of Liège, Liège, Belgium;* [2]*Laboratory of Experimental Hematology, Institut Jules Bordet, ULB, Brussels, Belgium;* [3]*Service d'hematologie, Hopital Universitaire Necker, Paris, France;* [4]*VIDO/Intervac, USASK, Saskatoon, Canada.*

An estimated 10–20 million humans and 50 million dairy cattle are infected with the closely-related human T-cell leukemia virus type-1 (HTLV-1) and bovine leukemia virus (BLV) respectively. These retroviruses infect T (HTLV-1) and B (BLV) lymphocytes, provoking an asymptomatic polyclonal expansion that will evolve into an aggressive lethal monoclonal leukemia in ~5% of individuals following decades of latency. It is generally assumed that early oncogenic changes are largely dependent on virus-encoded products, while progression to acute leukemia/lymphoma involves the accumulation of somatic mutations, with both apparently independent of proviral integration site. We utilised the BLV experimental model in sheep to track the tumour evolution in a compressed time frame. Genome-wide mapping of BLV and HTLV-1 proviral integration sites of both full-blown tumours and polyclonal asymptomatic samples was performed via NGS-based DNA sequencing. We mined stranded RNA-seq data obtained from 47 ruminant B-cell tumours and 44 Adult T-cell leukemia (ATLs) to explore transcriptional interactions between HTLV-1/BLV and the host genome. In our sheep experimental model, we demonstrate the occurrence of genic and intergenic hotspots of proviral integration and a strong signature of preferential proviral orientation within hotspots, both in tumours and during the polyclonal asymptomatic stage of the disease. In addition, we show that both BLV and HTLV-1 proviruses are integrated in the vicinity of cancer drivers, which they affect either by provirus-dependent transcription termination or as a result of viral antisense RNA-dependent cis-perturbation via virus-host chimeric transcripts. Our data strongly support the notion that cis-perturbation of cancer drivers by the provirus contributes to the initial selection of the multiple clones characterising the asymptomatic stage and is a major determinant of early clonal expansion in both BLV and HTLV-1 induced leukemia.

Key Words: cancer, retrovirus

MT350 Effect of supplementation with n-3 PUFA and modulation of post-insemination plane of nutrition on bovine uterine endometrial gene expression. C. Surlis*[1], S. Waters[1], J. Evans[1], P. Cormican[1], D. Doyle[1], and D. Kenny[1,2], [1]*Teagasc, Animal and Bioscience department, Dunsany, Co. Meath, Ireland;* [2]*UCD, School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland.*

Supplementing diets with n-3 PUFAs has been postulated to have beneficial effects on reproduction in ruminants. A significant cause of reproductive wastage in cattle is early embryonic loss, occurring in 80% of cases between days 14–16 of pregnancy. The objective of the study was to examine (i) the effect of supplementation by n-3 PUFA and post-insemination plane of nutrition on uterine endometrial gene expression, and (ii) alterations in key genes and pathways that may affect pregnancy outcome. A total of 60 oestrous synchronised crossbred heifers were fed either PUFA supplemented (n = 32) or a control (n = 28) diet before insemination. Following insemination animals were allocated one of two post-insemination diets, either remaining on the high plane of nutrition, or fed a low plane of nutrition diet. Diets were maintained until slaughter and embryo recovery on Day 16 post-insemination or pregnancy diagnosis by ultrasonic scanning on Day 30. Uterine endometrial tissue was collected, RNA isolated and gene expression analysis conducted by RNAseq. There was no effect of either pre-insemination supplementation by n-3 PUFA or post-insemination plane of nutrition on pregnancy. Comparison of transcript levels across groups however highlighted a significant effect of diet on uterine endometrial transcript levels, with a notable effect of PUFA supplementation on levels of differentially expressed genes (DEG). Of particular interest, the comparison of PUFA supplemented and control fed pregnant heifers on the low plane of post-insemination nutrition resulted in 561 DEG, including the altered expression of genes previously demonstrated to be involved in early pregnancy recognition such as *UPK3BL* and *IF1T3*, and in reproductively important pathways including mTOR signalling and oxytocin signalling, important to establishing pregnancy. Results indicate that despite the lack of a directly observed effect on pregnancy outcome, supplementation with n-3 PUFA is shown to positively alter several key fertility genes and pathways involved in early pregnancy, providing crucial knowledge towards improving reproductive success in cattle.

Key Words: PUFA, reproduction, cattle

MT351 Expression of key genes of the lipogenesis pathway in adipose tissue of beef cattle phenotypically divergent for RFI. C. Mckenna*[1,2], S. Waters[1], K. Keogh[1], R. Porter[2], and D. Kenny[1], [1]*Teagasc Animal and Grassland Innovation and Research Centre, Dunsany, Co. Meath, Ireland;* [2]*School of Biochemistry, Trinity College Dublin, Dublin, Ireland.*

Feed accounts for up to 75% of costs in beef production systems and improvements in feed efficiency can benefit the profitability of beef systems, by reducing the cost of production. Residual feed intake (RFI) is a measure of FE that is independent of level of production. Adipose tissue is the primary energy storage reservoir and modulates a variety of processes related to feed intake. This study was aimed at determining how mRNA profiles of adipose tissue were altered by divergence in RFI. DMI and ADG were measured in bulls (n = 28) with an initial BW of 381kg ± (s.d. = 51.5). RFI was calculated and animals were ranked by RFI into high (HRFI; inefficient), medium and low (LRFI; efficient) groups. This

resulted in 9 HRFI bulls and 9 LRFI bulls. ADG and DMI for bulls was 1.8kg (s.d. = 0.13) and 9.5kg (s.d. = 0.2), respectively. High RFI bulls consumed 10% more ($P < 0.05$) than low RFI bulls. Adipose tissue was collected at slaughter and qPCR was performed. There was an effect of RFI on gene expression of *ACLY, ACAT1* and *HMGCS* ($P < 0.05$). HRFI bulls had a higher expression of *ACLY* ($P = 0.017$) while LRFI bulls tended to have a higher expression of *ACAT1* ($P = 0.07$) and had significantly higher expression of *HMGCS* ($P = 0.01$). *ACLY* is related to fatty acid biosynthesis and the present study suggests that HRFI bulls are directing their substrate partitioning towards fat deposition. *ACAT1*, vital in cellular cholesterol homeostasis was higher in LRFI bulls which could indicate that LRFI animals are more efficient at maintaining cholesterol homeostasis. *HMGC S*, involved in the ketogenesis pathway, was higher in LRFI animals suggesting these animals direct their metabolism towards increased ketogenesis in order to facilitate their lower feed intake. The present study suggests altered lipid metabolism is contributing to variation in RFI in cattle.

Key Words: RFI, cattle, adipose, ACAT1, ACLY

MT352 Differential expression of microRNA in the peripartum of Holstein Friesian cattle. C. Cambuli[1], R. Puglisi[1], F. Fusi[2], L. Bertocchi[2], A. Galli[3], G. Bongioni*[1], and M. Montedoro[1], [1]*Istituto Sperimentale Italiano Lazzaro Spallanzani, Rivolta d'Adda, CR, Italy;* [2]*Italian National Reference Centre for Animal Welfare, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna Bruno Ubertini, Brescia, Italy;* [3]*Centro di ricerca per le produzioni foraggere e lattiero-casearie CREA, Lodi, Italy.*

Intensive genetic selection in dairy cows has affected the onset of a variety of metabolic disorders. Moreover, it is known that the peripartum period is of particular relevance with respect to metabolic and oxidative stress and general increased susceptibility to disorders. MicroRNAs (miRNA), small non-coding RNAs with a role in regulating gene expression, are of interest in understanding the different capacity of response of the immune system. The aim of this study was to investigate miRNAs differential expression in order to characterise the molecular basis of immune imbalances in dairy cattle during the peripartum. Forty metabolic and immunological parameters classically used as indicators of increased risk of peripartum-related diseases were recorded in 100 Holstein Friesian cows during 4 time-points of the peripartum (-21 ± 2; -3 ± 1; +3 ± 1; +21 ± 2, days). Animals were housed in a farm monitored according to the model of the National Reference Centre for Animal Welfare. Based on the simultaneous variation found in the levels of non-esterified fatty acids, albumin, cholesterol and haptoglobin, six cows were separated into a control group (values included in the corresponding reference range) and a group with altered values. The miRNAs were extracted from buffy coat of 24 blood samples relatively to the 4 time-points and sequenced by Illumina HiSEqn 2000. Statistical analysis, performed with EdgeR, identified 30 differentially expressed miRNAs in the group with altered values respect to control group (False Discovery Rate, FDR, at $P < 0.05$). Among them, the bta-mir-2415 (logFC = -2.98, FDR = 0.004), bta-mir-126 (logFC = 3.93, FDR = 0.004), bta-mir-6522 (logFC = 2.14, FDR = 0.003) and bta-mir-10b (logFC = 3.25, FDR = 0.02) were differentially expressed 3 weeks before the birth. The bta-mir-6522 (logFC = 3.032, FDR = 0.01) and bta-mir-6531 (logFC = -3.535, FDR = 0.03) were differentially expressed 3 days after birth. The bta-mir-10b (log FC = -2.744, FDR = 0.007) and bta-mir-143 (logFC = -1.687, FDR = 0.06) were differentially expressed 3 weeks after the birth. The miRNAs identified in the present work are potential candidates to improve the understanding on the ability to respond more efficiently to critical phases.

Key Words: MicroRNA, cattle, RNA-seq

MT353 Identification of genes associated with copper-deficient fatty acid increase in Nelore cattle. J. Afonso[1], P. Tizioto[2], P. Oliveira[3], W. Diniz*[1], A. Lima[1], M. Souza[1], M. Rocha[1], J. Silva[1], C. Buss[1], C. Gromboni[4], G. Mourão[2], A. Nogueira[3], L. Coutinho[2], and L. Regitano[3], [1]*Department of Genetics and Evolution, Federal University of São Carlos, São Carlos, São Paulo, Brazil;* [2]*Department of Animal Science, University of São Paulo, Piracicaba, São Paulo, Brazil;* [3]*Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil;* [4]*Bahia Federal Institute of Education, Science and Technology, Valença, Bahia, Brazil.*

Copper is a modulator of fatty acid metabolism (FAM) known to impact meat quality traits like fat percentage, flavor and tenderness. An increase in FAM activity is associated with low copper concentrations, but the genes and metabolic processes involved are not well characterised. In this study we analysed transciptome data measured with RNA-seq in *Longissimus dorsi* muscle from 12 animals split in groups of low and high muscular copper content (LC and HC). We identified 132 differentially expressed genes (DEGs) between the groups, among which we found five genes (*ACACA*, *FASN*, *SCD*, *ELOV5* and *ELOV6*) up-regulated in the LC group that are in FAM pathway. These genes are involved in the synthesis of arachidonic acid, known to promote the repair and growth of skeletal muscle tissue and for being the mainly polyunsaturated fatty acid in muscle. *ACACA* and *FASN* genes have been previously associated with copper-deficient fatty acid increase, whereas no such relationship has been reported for *SCD*, *ELOV5* and *ELOV6*. This may suggest that the upstream-acting ACACA and FASN induce the downstream-acting *SCD*, *ELOV5* and *ELOV6* via increase in substrate concentration. Functional enrichment analysis on the other DEGs further revealed two more LC up-regulated genes involved in fatty acid biosynthesis (LEP and ACSM1). LEP expression was already associated to copper concentration and both are related to obesity. Other over-represented pathways found in the DEGs are Protein digestion and absorption and ECM-receptor interaction. This study provides evidence for induction of arachidonic acid and other fatty acids synthesis at low copper concentrations and thus contributes to our understanding of the processes involved in copper-deficient fatty acid increase. This project was supported by FAPESP 2012/23638–8.

Key Words: cattle and related species, genome regulation, gene expression, meat production, RNA-Seq

MT354 Identification of pleiotropic loci for daily weight gain and intramuscular fat in cattle using bivariate genome-wide association analysis. C. Buss[1], W. Diniz[1], B. Andrade[3], M. Rocha[1], A. Lima[1], L. Geistlinger[3], J. Afonso[1], R. Tullio[3], P. Tizioto[2], J. Petrini[2], L. Coutinho[2], J. Wolf[4], G. Mourão[2], and L. Regitano*[3], [1]*Departament of Genetic and Evolution, Federal University of São Carlos, São Carlos, São Paulo, Brazil;* [2]*Department of Animal Science, University of São Paulo/ESALQ, Piracicaba, São Paulo, Brazil;* [3]*Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil;* [4]*Departament of Biology & Biochesmistry, University of Bath, Milner Centre for Evolution Bath, Bath, Somerset, UK.*

Pleiotropic loci influencing two or more seemingly unrelated phenotypic traits have been repeatedly detected by genome-wide association studies (GWAS) in cattle. However, single-trait GWAS are not *per se* suitable for systematically identifying such loci. In this study, we used a bivariate GWA approach for detecting pleiotropic effects between average daily gain (ADG) and intramuscular fat (IMF). Based on Illumina High-Density SNP-chip data for an ADG and IMF-phenotyped population of 387 Nelore steers, we found two loci significantly associated with ADG and IMF. These loci reside in genes encoding the serine/threonine kinase MAST4 and the transcription factor TAL1. In agreement with our results, perturbation of MAST4 has been previously found associated with

increased fat accumulation during adipogenesis. The detected SNP in MAST4 explains up to 3.61% of the variance observed for IMF and 0.40% for ADG. On the other hand, TAL1 is known to be involved in omental fatty tissue deposition. Here, the detected SNP explains 3.73% and 0.02% of the observed variance for IMF and ADG, respectively. Both loci identified in this study indicate pleiotropic effects were acting in the process of adipogenesis, which provides further evidence for the genetic interrelation between intramuscular fat deposition and weight gain. This project was supported by FAPESP 2012/23638–8.

Key Words: fat/lipid, feed efficiency, pleiotropy, genome-wide association, transcription factor

MT355 Complete mitogenome analysis supports multiple origin domestication hypothesis for European cattle. V. Cubric-Curik*[1], D. Novosel[1], V. Brajkovic[1], S. Krebs[2], J. Sölkner[3], D. Salamon[1], S. Ristov[4], S. Triviziaki[5], I. Bizelis[6], M. Ferenčaković[1], S. Rothammer[7], E. Kunz[7], M. Simčič[8], P. Dovč[8], G. Bunevski[9], H. Bytyqi[10], B. Marković[11], M. Brka[12], K. Kume[13], S. Stojanović[14], V. Nikolov[15], N. Zinovieva[16], M. Cacic[17], I. Curik[1], and I. Medugorac[7]
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The domestication of wild Aurochs (*Bos primigenius*) is among the greatest achievements of mankind that had immense impact on the development of human society. Our understanding of the transformation from Aurochs to modern breeds is still not resolved as neither of the two hypotheses, single *v.* multiple origin domestication, have not been decisively rejected. To improve our understanding of cattle domestication we have sequenced the complete mitogenome of 196 individuals using NGS. Our sample encompassed 50 'new' breeds and covered a large number of Central and South East European cattle breeds thus fulfilling the missing gap on the route from the Fertile Crescent to the Western Europe. We have performed the comprehensive complete mitogenome phylogenetic analyses, comprising 498 bovine samples (300 from the GenBank) from more than 100 cattle breeds. With the exception of very rare haplogroups (C, E, R, I and T7), mitogenomes sequenced here were classified within all known haplogroups (T1'2'3, T1, …, T5 and T6), including even haplotypes assigned to the haplogroup P and Q. Up to now, haplogroup P was found only in the ancient West European Aurochs samples and the one sample from the modern Korean cattle. The existence of the haplogroup P ('Aurochs') was confirmed on the original sample and its four female relatives, sampled additionally. This is a direct evidence of the continuity and presence of Aurochs genes in modern breeds that further increases the possibility of multiple origin of cattle domestication, while we were not able to exclude post-domestication admixture. The haplogroup Q, here found in three individuals with two unique haplotypes, has diverged from the 'macro-haplogroup T' more than 35 thousand years ago and also indicates the presence of Aurochs genes in modern breeds. We have observed a large, newly arisen, divergence within 'macro-haplogroup T', with the new, rather deeply, rooted branches (non-classified haplogrups) prompting for the urgent reclassification of the cattle mitogenomic systematics.

Key Words: cattle and related species, evolutionary genomics, high-throughput sequencing, animal domestication, breed/population identification

MT356 Genome-wide association analysis for β-hydroxybutyrate concentration in milk using mid-infrared spectroscopy and whole-genome sequence genotypes in North American Holstein dairy cattle. S. Nayeri*[1], F. Schenkel[1], V. Kroezen[1], A. Fleming[1], M. Sargolzaei[1,2], C. Baes[1], J. Squires[1], and F. Miglior[1,3], [1]*Centre for Genetic Improvement of Livestock, Department of Animal Bioscience, University of Guelph, Guelph, Ontario, Canada;* [2]*The Semex Alliance, Guelph, Ontario, Canada;* [3]*Canadian Dairy Network, Guelph, Ontario, Canada.*

As a result of genetic improvement, milk production in dairy cattle has greatly increased during the last 30 years. This increase in milk production is accompanied by enormous metabolic changes and challenges in high-yielding dairy cows early in lactation. A failure of the cow adapting to these metabolic changes and maintaining their internal homeostasis and homeorhetic regulation can lead to metabolic and fertility disorders, and can detrimentally impact the health and welfare of the cow. Several studies have shown that there is a negative genetic correlation between milk yield and ketosis (0.26–0.65), mastitis (0.15–0.68), and lameness (0.24–0.48) in dairy cattle. Abnormal concentrations of ketone bodies (for example β-hydroxybutyrate, BHB) in blood and milk of the cow is a good indicator of clinical and subclinical ketosis. There has been, however, little information on the genomic regions associated with this indicator trait. Routine phenotyping tools, such as mid-infrared (MIR) spectroscopy for milk BHB concentrations, may provide accurate evaluations for ketosis. BHB measurement is now available for all recorded animals in Canada. About 40% of herds, record clinical health data for at least one health trait, and genetic evaluations have been calculated for BHB and clinical ketosis by the Canadian Dairy Network since December 2016. The purpose of this study is to use such data to perform a genome-wide association analysis in Holstein dairy cattle using imputed whole-genome sequence genotypes to identify regions and candidate genes that may affect MIR predicted BHB concentration in milk associated with ketosis. The results of this study can provide a better understanding of biology, genes and metabolic pathways that may affect ketosis and correlated traits, as well as developing a cost-effective approach to improve the welfare of animals in dairy herds by decreasing the prevalence of metabolic disease.

Key Words: genome-wide association analysis, ketosis, milk β-hydroxybutyrate, whole-genome sequence

MT357 Association of candidate SNPs with feed efficiency in Israeli-Holstein lactating cows. M. Cohen-Zinder*[1], J. Miron[2], Y. Ben-Meir[2], E. Lipkin[3], and A. Shabtay[1], [1]*Dept. of Ruminant Science, Agricultural Research Organization, Newe-Ya'ar Research Center, Ramat Yishay, Israel;* [2]*Dept. of Ruminant Science, Institute of Animal Science, Agricultural Research Organization, Beit Dagan, Israel;* [3]*Dept. of Genetics, Silberman Life Sciences Institute, The Hebrew University of Jerusalem, Jerusalem, Israel.*

Feed efficiency is a major component determining the profit of livestock production. One of the most acceptable measures of

feed efficiency in livestock is residual feed intake (RFI). Marker assisted selection (MAS) will accelerate the genetic progress. Recent advances in DNA technologies allowed cost-effective mapping of genetic variants associated with RFI. In a previous study, we detected 48 SNPs associated with RFI in Holstein beef calves. All SNPs were present in genes harboring known QTL regions of feed efficiency and RFI related traits. In the current study, the association of eight of these SNPs was tested on feed efficiency, feeding behaviour and milk performances of Holstein dairy cows. A total of 41, 42 and 31 Israeli-Holstein lactating cows were held on the same ration in three separate periods (spring 2015, autumn 2015 and spring 2016), and were phenotyped in real time for milk yield (MY), milk fat content, milk lactose content, milk protein content, fat corrected milk (FCM), dry matter intake (DMI), energy corrected milk (ECM), feeding rate, feeder occupancy, lying and rumination duration, bodyweight feed conversion ratio (FCR) and RFI. All 114 cows were genotyped for eight candidate SNPs of seven genes, involved in energy, amino acid, carbohydrate and lipid metabolism: (FABP4, PPAR-gamma, UCP1, MYH3, MYH13, HIF1AN, ATP5A1). Taking all periods together, association test with fixed effects of the heifer status (1 *v.* more lactation), period, period average temperature, average humidity, age at start of the period and average days in milk, yielded suggestive ($0.10 \geq P \geq 0.05$) to highly significant ($P \leq 0.01$) results for various marker-trait combinations, both for production and feed efficiency traits. Large excess of small significant P values indicate the presence of true effects. FCR, RFI and milk protein content were each significantly associated with four of the markers. Markers FABP4_5 and ATP5A1 were significantly associated with five and six of the traits. Patterns of significant test changed substantially between periods. If confirmed, these results may be applied in a selection index.

MT358 Production of goat milk lacking an allergenic protein (αs1-casein) is possible due to a micro-chromosomal deletion comprising the entire *CSN1S1* gene. V. Bâlteanu*, *University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Cluj, Romania.*

Alpha αs1-casein is considered one of the main allergenic proteins among the milk casein fraction and in ruminants it is encoded by the *CSN1S1* gene that is 16.7 kb-long. In this paper is reported the first known case of a micro-chromosomal deletion comprising the entire goat *CSN1S1* gene and its effect on the main milk components. Null (00) *CSN1S1* Carpathian she-goats were identified by screening of milk samples through an isoelectric focusing (IEF)-based approach. The lack of transcripts in null *CSN1S1* goats only, as compared with AA goats, was confirmed by cDNA analysis. Using a PCR-based screening approach several regions located between 3′ ends of *SULT1E1* and *CSN2* genes (approx. 109.57 kb), including parts of *CSN1S1* gene and promoter, were analysed in several AA and null samples. By sequencing of certain amplicons the micro-chromosomal deletion was precisely located and, besides the lack of the entire *CSN1S1* gene, it included significant parts from both intergenic regions in null goats. For rapid genotyping of null goats, a duplex PCR test was designed and was successfully tested in homozygous and heterozygous *CSN1S1* samples. To investigate the association of this *CSN1S1* deletion with milk composition, two groups of goats were tested (*CSN1S1* AA and 00, respectively). Total protein, total casein, fat, non-fat solids and lactose contents were determined six times during lactation using a MilkoScan device (Foss Electric, Denmark). A mixed repeatability model was used for the analyses of milk components. As anticipated, results show a significant effect of the *CSN1S1* deletion on all studied variables: total protein % (AA: 4.07 ± 0.13; null: 3.60 ± 0.20); total casein % (AA: 3.00 ± 0.10; null: 2.61 ± 0.15); fat % (AA: 4.49 ± 0.18; null: 4.01 ± 0.27), non-fat solids % (AA: 8.81 ± 0.12; null: 8.49 ± 0.18); lactose % (AA: 3.91 ± 0.07; null: 4.056 ± 0.10). Improving goat milk casein content and cheese yield was applied in several goat breeds by promoting in selection strong expression *CSN1S1* alleles (in particular A allele). These null *CSN1S1* goats could be exploited for production of milk lacking αs1-casein, suitable for human subjects that are allergic to this milk protein.

Key Words: goats, milk production, allergy, deletion, genetic marker

MT359 When *taurus* met *indicus.* Exploring admixture events in ancient cattle. M. Verdugo*, *Trinity College Dublin, Dublin, Ireland.*

The Bronze Age is an important period in prehistory that comprised many socio-economic changes including the rise and fall of city states in Mesopotamia. In this period the establishment of trade routes allowed for the exploitation of raw materials and animals. From archaeology, there is evidence of zebu cattle moving into in the Near East from the Indus Valley from the end of the Late Bronze Age. Ancient DNA provides a direct view into the past of these early domestic animals. We present a dataset of ~50 high and low coverage ancient Near Eastern cattle genomes through time and space. From comparison using both 700K Bovine SNP chip data and whole genomes we observe at least one main admixture event in the Bronze Age. This contrasts with mitochondrial data in which such admixture is invisible. We also show the power of low coverage data from ancient domestic cattle to confidently detect admixture between taurine and indicine animals, increasing the utility of sequencing of archaeological samples from hot regions with poor preservation.

Key Words: cattle and related species, evolutionary genomics, ancient DNA, admixture

MT360 Origin and evolutionary history of the European bison unraveled through ancient DNA. T. Grange*, D. Massilani, S. Guimaraes, and E.-M. Giegl, *Institut Jacques Monod, CNRS, University Paris Diderot, Paris, France.*

The European bison or wisent (*Bison bonasus*), one of the last wild European large mammals, narrowly escaped extinction at the onset of the 20th century. It shares a common ancestry with both American bison (*Bison bison*) and modern cattle (*Bos primigenius* f. *taurus*), although its evolutionary history since the divergence of these three lineages during the Early Pleistocene has never been well characterised. Here we describe complete and partial mitogenomes from 57 ancient Eurasiatic bison specimens dating from 50 kiloyears ago (kya) to the early 20th century, just preceding the last major bottleneck, and covering the area from Western Europe to the Caucasus and to Siberia. Our results reveal several waves of population expansion, contraction and extinction. Three bison populations successively occupied Western Europe during this time frame and their presence can be correlated with major climatic and environmental fluctuations. First, an ancestral, now extinct, *Bison bonasus* clade was dominant during most of the temperate Marine Isotope Stage (MIS) 3 (ca. 57 – 29 kya). Second, a steppe bison (*Bison priscus*) population originating from North-East Eurasia recolonized Western Europe during the following cold period of the last glaciation (MIS2 ca. 29 – 14 kya). We hypothesise that the population overlap we observed in southern France during this transition period (39–34 kya) is reflected in the contemporaneous rock paintings of the Chauvet cave in the same area. Third, the steppe bison was replaced after the last glacial maximum at the beginning of the Holocene (MIS1) by a separate *Bison bonasus* population that previously occupied a refuge encompassing the southern Caucasus. This last population survived up to the Middle Ages in France, while a related population survived into the 20th century in Eastern Europe, where it can still be found today. The present-day pattern of reduced genetic diversity of the wisent preceded the last major bottleneck of World War I. Climatic oscillations, environmental

changes and, recently, anthropogenic pressure have both shaped the evolutionary history of this emblematic species in different ways.

Key Words: bison, ancient DNA, Europe

MT361 Whole-genome structural analysis of Caribbean hair sheep reveals quantitative link to West African ancestry. G. Spangler*[1], B. Rosen[1], O. Hanotte[2], T. Sonstegard[3], and C. Van Tassell[1], [1]*USDA/ARS/AGIL, Beltsville, MD, USA;* [2]*U of Nottingham/School of Life Sciences, Nottingham, UK;* [3]*Acceligen of Recombinetics, St Paul, MN, USA.*

Hair sheep of Caribbean origin have become an important part of the USA sheep industry. Lack of wool eliminates several health concerns and drastically reduces the cost of production. More importantly, Caribbean hair sheep demonstrate robust performance even in the presence of drug resistant gastrointestinal nematodes, a rising concern to the industry. Despite the growing importance of hair sheep in the Americas their genetic origins have remained speculative. Prior to this report no genetic studies were able to identify a unique geographical origin of hair sheep in the New World. Our study clarifies the African and European ancestry of Caribbean hair sheep. Whole genome structural analysis was conducted on four established breeds of hair sheep from the Caribbean region. Using breeds representing Africa and Europe we establish an objective measure indicating Caribbean hair sheep are derived from Iberian and West African origins. Caribbean hair sheep result from West African introgression into established ecotypes of Iberian descent. Genotypes from 47,750 autosomal single nucleotide polymorphism markers scored in 290 animals were used to characterise the population structure of the St Croix, Barbados Blackbelly, Morada Nova, and Santa Ines. Principal components, admixture, and phylogenetic analyses results correlate with historical patterns of colonization and trade. These results present an important basis for further investigation into desirable traits attributed to hair sheep of the region such as heat tolerance and nematode resistance.

Key Words: sheep, genome, genotyping, admixture, breed identification

MT362 A worldwide investigation of the effects of climate selection on goat genomes. F. Bertolini*[1], E. Rochat[2], S. Joost[2], B. Servin[3], P. Crepaldi[4], A. Stella[5], and M. F. Rothschild[1], [1]*Department of Animal Science, Iowa State University, Ames, IA, USA;* [2]*LASIG, EPFL, Lausanne, Switzerland;* [3]*INRA, Castanet-Tolosan, France;* [4]*DIMEVET, University of Milan, Milan, Italy.;* [5]*PTP, Lodi, Italy.*

Climate factors can cause genomic selection pressures that can affect several traits in livestock. To investigate these effects on the goat genome, the ADAPTMAP consortium compiled a dataset of high-throughput genotyped animals collected from more than 30 countries. For the purpose of this project, each animal was classified according to its GPS location, discarding animals with no GPS coordinates, and an individual Köppen climate group was assigned. Then the following filters were applied: 1) For each group only breeds with at least 10 animals were considered and 2) If two breeds were located in different climate groups only the animals that belonged to the groups of known breed origin were considered. The filtered groups were as follows: 160 animals/7 breeds for group A (= Tropical), 1,020 animals/30 breeds for group B (= Arid), 744 animals/33 breeds for group C (= Temperate) and 136/4 breeds for group D (= Cold). The single SNP Fst analyses were performed comparing one group against the others merged together. The top 20 SNPs of each analysis were compared with the results provided by the landscape genomics approach to the same dataset filtered with independent criteria and performed using the Sambada software. At least 20 SNPs detected with the Fst were concordant for Fst and landscape genomics results, and were analysed to find genes nearby (± 100Kb). Among these 20 SNPs, 7 SNPs were found to differentiate the A groups from the others. Particularly, two SNPs on chromosome 5 were close to members of the HOX gene family that controls body plan of an embryo along the craniocaudal axis, has been linked to reproductive behaviour and is subject to selective pressure in several species. A total of six SNPs were detected for the B and C groups, separately. The allele frequency analyses of these SNPs revealed that the two groups have opposite major alleles that confirmed the different selection that may occur in temperate and arid environments. The regions nearby these SNPs contain genes that are linked to many functions, such as feed intake, growth phenotypes and pubertal development in cattle.

Key Words: goat, climate, adaptation, ADAPTmap

MT363 Signals of adaptive introgression between European taurine and indicine cattle revealed by local ancestry inference. M. Barbato*[1], M. Del Corvo[1], T. Sonstegard[2], and P. Ajmone-Marsan[1], [1]*Istituto di Zootecnica, Universitá Cattolica del Sacro Cuore, Piacenza, Italy;* [2]*Recombinetics Inc., St. Paul, Minnesota, USA.*

European taurine cattle (*Bos taurus*) was domesticated in the Fertile Crescent around 8,000 year BC and later colonised Europe, Asia and Africa following the agriculture wave. A second domestication involving the ancestors of the modern humped zebu cattle (*Bos indicus*) occurred 2,000 years later in the Indus valley, in Central Asia. Admixture between taurine and indicine species occurred extensively in the past, the indicine species sometimes contributing to taurine's genetic pool with a better adaptation to tropical climate and diseases, and an improved ability to thrive with very poor fodder. Interestingly, a small percentage of indicine ancestry can be detected in several modern taurine breeds. With the aim to identify the putative adaptive nature of such indicine × taurine introgression, we analysed Illumina BovineHD SNP genotypes of 16 Chianina cattle sampled in central Italy, along with 187 individuals from six reference breeds of taurine (3) and indicine (3) origin. Local ancestry investigations involving nine reference population combinations were performed and smoothed using the CIWI (Consistently Introgressed Windows of Interest) analytical framework, able to identify concordant and reference-independent genomic regions of a given ancestry. Among the CIWIs of indicine ancestry identified in Chianina, the strongest signal was recorded in chromosome 18. Haplotype homozygosity-based selection sweep analysis evidenced signatures of selection occurring within the same genomic region. Here, we infer the putative adaptive nature of this ancestral indicine genome portion, and suggest its association to indicine cattle's superior ability to efficiently use poor quality fodder.

Key Words: cattle, local ancestry, adaptive introgression, SNP array, selection signature

MT364 The ruminant biology and evolution revealed by a flock of ruminant *de novo* genomes. W. Wang[1], Q. Q. Qiu[1], G. Zhang[3,4], R. Heller[3], H. R. Siegismund[3], and Y. Jiang*[2], [1]*Northwestern Polytechnical University, Xi'an, Shaanxi, China;* [2]*Northwest A&F University, Yangling, Shaanxi, China;* [3]*University of Copenhagen, Copenhagen, Denmark;* [4]*Beijing Genomics Institute at Shenzhen, Shenzheng, Guangdong, China.*

The ruminants form one of the most ecologically important hehbivorous animal groups on Earth, with two major families, the bovids (Bovidae) and the deer (Cervidae). Furthermore, the ruminants include five of the most important livestock species: the cow, water buffalo, yak, sheep and goat. Despite the remarkable diversity and evolutionary success of the ruminants relatively little is known about the evolutionary genomics of the group, let alone how did they evolve. In an international consortium including Danish and Chinese research groups, we are *de novo* assembling 45 ruminant genomes, covering all of the six exist families and most of the 82 exist genera of ruminant suborder. With this big dataset we plan to

bring our knowledge about the evolutionary genomics in this important animal group to a whole new level. We will look at several specific ecological and physiological adaptations, such as the evolutionary innovation of the rumen and the horn, the ruminantia population dynamics correlated with the climate change in past millions of years. Combined with comparative genomics and ENCODE genomic features, the ruminant specific conventional regions would be also revealed and used for the cattle/sheep/goat functional genome annotation.

Key Words: ruminant biology, de novo genome, comparative genomics, rumen, horn

MT365 Genotyping by sequencing for genomic selection in dairy goats (*Capra hircus*). S. Clarke*[1], K. Dodds[1], R. Brauning[1], T. Van Stijn[1], R. Anderson[1], M. Wheeler[2], B. Foote[3], A. Cameron[4], and J. McEwan[1], [1]*AgResearch, Mosgiel, Dunedin, New Zealand;* [2]*AgResearch, Ruakura, Hamilton, New Zealand;* [3]*Foote Dairy, Hikurangi, Northland, New Zealand;* [4]*Meredith Dairy, Meredith, Victoria, Australia.*

High-throughput genotyping by sequencing (GBS) methodology produces SNP genotypes that are supported by varying depth of sequence reads, dependent on the number of samples and proportion of the genome assayed within a lane of sequencing. Although additional samples per lane is more cost effective, a balance is needed to achieve the required sequence read depth to support the SNP genotype for downstream applications such as genetic diversity and differentiation, parentage assignment, inbreeding estimation, genomic selection and genome wide association studies. Advances in statistical methods tailored to GBS data have enabled the use of GBS-derived genomic relationship matrices to implement genomic selection via GBLUP for the dairy goat industry. GBS is a cost effect alternative to chip arrays that are further compromised by large and inflexible setup costs. Although GBS has suffered from a variety of technical challenges (increased complexity of data processing, a high proportion of missing genotypes, and low accuracy of genotype calls), these have been overcome enabling this technology to be implemented in the dairy goat herd. Here we present an example in both a New Zealand and Australian dairy goat herd utilising a methodology that comprises ~60,000 SNPs for less than US$20/sample. The use of GBS has considerable implications for future goat genomic research especially when utilising this technology for high-density genotyping (>200k SNPs), due to the lack of a suitable HD chip.

Key Words: genomic selection, GBLUP, dairy goats, genotyping by sequencing, GBS

MT366 Cattle on the Western Atlantic edge of Europe: A time series of ancient cattle genomes through Ireland and Britain. V. Mullin*, *Trinity College Dublin, Dublin, Ireland.*

The domestication of cattle marks a significant period of time in human prehistory. The initial neolithic movement of domestic cattle across western Europe concluded with the movement of animals to the Western Atlantic edge; the islands of Ireland and Britain. One approach to understanding this past is the study of variation in modern cattle genomes to model past demography, admixture and selection. However, an alternative, more challenging and promising approach is the the direct study of archaeological genomes. Ancient genomes provide a snapshot of the genetic diversity present in the past, allowing for the exploration of the timing of population events such as an admixture, migration and turnover. The combination of technological advancements in next-generation sequencing and improved sampling techniques of archaeological samples enables the sequencing of many more ancient individual animals than previously possible, and has allowed us to compare the genomes of multiple animals across space and time. We have sequenced a time series of ~20 ancient Irish and British cattle genomes with genome coverage ranging from 0.2X to 17X sampled from the Neolithic (3500BC) to the Middle Ages (1200AD). The application of population genomics techniques to these data provide new insights into the demography of the prehistoric livestock of these islands.

Key Words: cattle and related species, palaeogenomics, ancient DNA, animal domestication

MT367 Genome-wide association study for monocyte count at day 7 post-challenge with bovine viral diarrhea virus in F_2 and F_3 Nellore-Angus halfblood steers. K. M. Sarlo Davila*[1], A. D. Herring[1], J. E. Sawyer[1], J. F. Ridpath[2,3], and C. A. Gill[1], [1]*Texas A&M University, College Station, TX, USA;* [2]*National Animal Disease Center, Ames, IA, USA;* [3]*Ridpath Consulting, Ames, IA, USA.*

Bovine viral diarrhoea viruses (BVDV) are prevalent worldwide, and outbreaks in USA beef herds are estimated to cost between $50 and $100 per animal. BVDV infections are associated with varying degrees of immunosuppression. Monocyte counts have been shown to drop at day 7 or 8 of infection. The objective of this study was to identify genetic variants associated with monocyte counts following BVDV challenge in crossbred cattle. A population of 372 F_2 and F_3 Nellore (*Bos indicus*)-Angus (*Bos taurus*) halfblood steers were balanced across sires regarding modified-live, killed and non-vaccinated experimental treatments and subsequently intra-nasally challenged with a BVDV type 1b noncytopathic field strain. . Monocyte count at day 7 post-challenge was evaluated and adjusted for vaccine type, calf type and weaning temperament score. Genotypes were imputed within family to high density and after quality control filtering there were 555,670 SNP available for a genome-wide association study for bovine monocyte count applying the univariate procedures of GEMMA that fitted the genomic relationship matrix to account for genetic covariance among animals. To correct for multiple tests, the Benjamini and Hochberg false discovery rate was constrained to 0.05 and there were 37 SNP associated with bovine monocyte count on bovine chromosomes (BTA) 2, 3, 14, 17, 19 and 29. There were 11 significant SNP within a 100kb region on BTA 17:72,729,721 - 73,483,993, which explained 7.5% of the variation in monocyte count at day 7. This region contains 2 genes, SLC5A1 and SLC5A4, which code for glucose co-transporter family proteins. In human studies the expression of glucose transporter genes in monocytes and other leukocytes has been shown to be vital to providing the necessary cellular fuel to mount an immune response. The genomic region identified may be important to immune response to viral challenge or vaccination.The identification of genetic variants associated with reduced impact of BVDV infection in *Bos indicus* influenced cattle would be of great economic importance globally to cattle producers in tropical and sub-tropical regions.

Key Words: *Bos indicus*, BVDV, monocytes

MT368 Evidence from the bovine of major differences between individuals in the rate of *de novo* single nucleotide mutation and transposon mobilization in the germ-line. C. Harland[1,2], K. Durkin[1], M. Artesi[1], L. Karim[1,3], N. Cambisano[1,3], M. Deckers[1,3], N. Tamma[1,3], E. Mullaart[4], W. Coppieters[1,3], M. Georges[1], and C. Charlier*[1], [1]*Unit of Animal Genomics, GIGA-R, University of Liège, Liège, Belgium;* [2]*Livestock Improvement Corporation, Research & Development, Hamilton, New Zealand;* [3]*GIGA-Genomics Platform, University of Liège, Liège, Belgium;* [4]*CRV, Research & Development, Arnhem, Netherlands.*

To study the process of *de novo* mutations in the bovine germ line, we have sequenced the whole genome of >750 individuals constituting 130 sire-dam-offspring trios with at least five grand-offspring each. A first study using four pedigrees revealed

the common occurrence of somatic and germ-line mosaicism for *de novo* mutations pointing towards mutation-prone early cleavage cell divisions (http://biorxiv.org/content/early/2016/10/09/079863). We herein characterise *de novo* mutations in the remaining 126 pedigrees. Two observations point towards major inter-individual differences in the rate of *de novo* mutations. We first identify one sire characterised by a mutation rate that is ~3-fold larger than the population average. We show that this remarkable increase is due to a ~7-fold excess of mutations occurring at the very early stages of development (on the basis of observed mosaicism). The corresponding mutations are characterised by a ~8-fold excess in C to T transitions outside the CpG context. The corresponding animal was shown to be the only individual of the pedigree to be homozygous for a rare disruptive mutation in components of the DNA repair or replication machinery: a $P > L$ substitution in the REV1 DNA Directed Polymerase. The causality of this mutation is presently being examined. We further developed a pipeline to detect *de novo* transposition and pseudogene mobilization events. We identified a family of LTR elements that are still active in the bovine genome. We detected five corresponding *de novo* transposition events, of which three occurred in the same individual including two in the same gamete. Latest results of both studies will be presented.

Key Words: de novo mutations, mosaïcism, transposable elements, pseudogenes, whole genome sequences

MT369 Identification of polymorphisms modifying gene expression regulation in cattle. G. Guillocheau* and D. Rocha, *GABI, INRA, AgroParisTech, Université Paris Saclay, Jouy-en-Josas, France.*

Thanks to the advent of novel sequencing technologies, an increasing number of polymorphisms have been identified in genic regions. These polymorphisms can play an important role in gene expression regulation. Allele-specific expression (ASE) analysis is a robust approach to detect *cis*-regulatory variations of gene expression. Because of its economic importance, cattle were one of the first mammals to have its genome sequenced. During this sequencing more than 2.2 million putative Single Nucleotide Polymorphisms (SNPs) have been detected. Since many bovine genomes have been sequenced and there is currently more than 99 Million SNPs. Polymorphisms showing allele-specific expression could be linked to economic important traits and therefore could help to improve genetic selection. The aim of our project is to develop a pipeline to predict polymorphisms that modify the regulation of gene expression. Association studies between these predicted polymorphisms and important phenotypes will later be performed. We used RNA-seq data from muscle samples of 19 Limousin bull calves (77 Million reads in average per samples) and from eight different tissue samples (heart, kidney, liver, lung, muscle, ovary, spleen and uterus) of six Holstein cows (65 Million reads in average by samples). We had also the whole-genome DNA sequences for these 25 animals (an average coverage of 15 Limousin samples and 5 Holstein samples). The RNA-seq data was aligned with STAR, an ultrafast RNA-seq aligner and we predicted polymorphisms (SNPs and small insertions/deletions) with GATK for DNA and RNA sequence data. The ASE detection was performed using ASEReadCounter and binomial test. We detected more than 150,000 SNPs showing ASE in all samples with this method. Currently, our pipeline can detect SNPs in genes with an allelic imbalance for species with a reference genome sequence available. We select interesting polymorphisms to perform a experimental validation using pyrosequencing.

Key Words: bioinformatics, polymorphisms, genome regulation, sequencing, transcriptomics

MT370 GWAS for response to vaccination in Angus calves. L. Kramer*[1], M. Mayes[1], J. Williams[1], E. Fritz-Waters[1], E. Downey[2], R. Tait[3], A. Woolums[4], C. Chase[5], J. Ridpath[6], and J. Reecy[1], [1]*Iowa State University, Ames, IA, USA;* [2]*Elanco Animal Health, Larchwood, IA, USA;* [3]*Neogen GeneSeek Operations, Lincoln, NE, USA;* [4]*Mississippi State University, Mississippi State, MS, USA;* [5]*South Dakota State University, Brookings, SD, USA;* [6]*Ridpath Consulting, Gilbert, IA, USA.*

Bovine respiratory disease complex (BRDC) is an economically important disease and an animal welfare issue. While vaccines have been shown to be efficacious, morbidity and mortality still persists. To examine the genetics of response to vaccination, Iowa State University Angus calves (>2000 head) were vaccinated for bovine viral diarrhoea virus 1 and 2, bovine respiratory syncytial virus, and bovine herpes virus 1. Serum neutralization scores for each virus were collected across multiple time points to allow for identification of genomic regions associated with response to vaccination. The response to vaccination traits were initial serum neutralization score (week 0), initial response to vaccination (week 3 – week 1), response to booster vaccination (week 6 – Week 3), overall response to vaccination (week 6 – week 0), and final antibody titer score (week 6). In addition, maternal decay and pre-vaccination titer scores were collected on a subset of individuals for bovine viral diarrhoea virus 1 and 2 only. A genome wide association study was performed using imputed 770k (BovineHD beadchip) markers and a BayesB statistical model with pi of 0.999 (575 most associated markers). 1-Mb windows with a posterior probability of inclusion (PPI) 0.9 or greater were identified from the BayesB analysis, with each window accounting for a minimal portion of the total genetic variation. Genes within the 1-Mb windows were examined for potential candidacy for causality. These associated windows may give insight into the genetic control of response to vaccination, and indicate avenues of advancement in improving vaccines and vaccination protocols against BRDC.

Key Words: cattle, genome-wide association, antibody response, bovine respiratory disease complex

MT371 Sheep parchment as a genetic resource. M. Teasdale*, *Trinity College Dublin, Dublin, Ireland.*

Before the mass production of paper, parchment was the major medium for codices and until the widespread adoption of typewriters, they were a clerk's preferred medium for many formal legal documents and records. Unlike modern parchment, which is typically made from goat or calf, ~1,000 English legal documents that we have analysed via peptide mass fingerprints (MALDI-TOF mass spectrometry), were made of sheepskin. Parchment has been shown to harbour sufficient concentrations of DNA to allow for high throughput sequencing and therefore offers tremendous scope for documenting the recent genetic history of British sheep breeds. Documents contained within the British archival collections span the transition from a wool to a meat based economy and the beginnings of intense artificial selection. We have sequenced a range of parchment samples from the county of Yorkshire (UK) that date from the 14th to the 19th century to low coverage. These animals were then compared to modern breeds from the Sheep HapMap and the Sheep Genomes Database and scanned for evidence of recent positive selection between the time points.

Key Words: genomics, ancient DNA, selection

MT372 SheepGenomesDB: Towards 1000 Genomes. S. McWilliam*[1], R. Brauning[2], S. Clarke[2], A. McCulloch[2], N. Cockett[3], G. Saunders[4], M. N. Sanchez[1], H. Daetwyler[5,6], and J. Kijas[1], [1]*CSIRO, St Lucia, QLD, Australia;* [2]*AgResearch Ltd, Invermay Mosgiel, New Zealand;* [3]*Utah State University, Logan, UT, USA;* [4]*EMBL-EBI, Cambridge, UK;* [5]*Department of Economic*

Development, Jobs, Transport and Resources, Bundoora, VIC, Australia; [6]La Trobe University, Bundoora, VIC, Australia.

The SheepGenomesDB is a repository of genome wide variants produced from publically available sheep genomes. The project applied a harmonised pipeline for raw read filtering, mapping and variant detection. Run 1 captured nearly 500 sheep genomes, and generated nearly 100 million unfiltered variants which were pruned to define a high confidence set. Run 2 increases the genome count to almost 1000 across a wider range of sheep breeds. Both variant collections (raw and high quality) are available via the European Variation Archive (EVA), with the high quality variants annotated against OARv3.1 using the Ensembl Variant Effect Predictor (VEP). We report on the data diversity, variation across breeds and geographical location and the applications to imputation, analysis of domestication, disease mutation identification and linkages with the emerging FAANG project.

Key Words: sheep, genome, variants

MT373 The water buffalo gene expression atlas. R. Young*[1], L. Lefevre[1], S. Bush[1], J. Williams[2], S. Gokhale[3], S. Kumar[4], A. Archibald[1], and D. Hume[1], [1]*The Roslin Institute and Royal (Dick) School of Veterinary Studies (RDSVS), Easter Bush, Midlothian, UK;* [2]*School of Animal and Veterinary Sciences, The University of Adelaide, Adelaide, South Australia, Australia;* [3]*BAIF Development Research Foundation, Central Research Station, Pune, Maharashtra, India;* [4]*Centre for Cellular and Molecular Biology, Hyderabad, Telangana, India.*

The domestic water buffalo (*Bubalus bubalis*) contributes significantly to the global agricultural economy through milk, meat, hides and draught power, and a larger part of the human population depends on domestic water buffalo than on any other livestock species in the world. Despite its agricultural importance, the buffalo genome is not fully annotated. We have generated a fine-scale gene expression atlas of 220 tissue and cell types collected from adult riverine water buffalo (Mediterranean, Pandharpuri and Bhadawari breeds). Accompanying whole genome sequence data were also generated for each breed. Gene expression was quantified from RNA-Seq data and visualised using the network analysis tool Miru, allowing the co-expression of genes to be explored across tissues. The atlas data are also being used to analyse alternative splicing, candidate expressed SNPs and allelic expression imbalance and compared to other ruminant species. The sample metadata have been loaded into BioSamples and the sequence data will be deposited in ENA. This data will be a valuable resource for the international Functional Annotation of Animal Genomes (FAANG) initiative. This study is the largest gene expression atlas generated in water buffalo to date. Potential variation identified between domestic breeds will form the basis for the development of predictive marker-assisted selection and breed improvement in the buffalo industry.

Key Words: water buffalo, transcriptome, genome annotation, FAANG, breed improvement

MT374 Ancient whole mitochondrial genomes and insights into the prehistory of goats. K. Daly*, *Smurfit Institute of Genetics, Trinity College, Dublin, Ireland.*

The domestication of goats (*Capra hircus*) from bezoar (*Capra aegagrus*) is thought to have occurred in the Near East ~10,000 years ago. As one of the earliest domesticated animals, elucidating the patterns, pace and major events of the process is of great interest. However, such analyses using genomic data from modern goats are hampered by 10,000 years of human-mediated movement of goat. Ancient DNA allows populations before this be directly sampled. We present an initial analysis of whole mitochondria data from goat sampled from a range of time depths. We observed a high degree of mitochondrial diversity at earlier periods followed by a significant restriction which has shaped modern goat mitochondrial diversity. We also report a 14 thousand year old caprid mitochondrial lineage most similar to the Caucasian Tur (*Capra caucasica*), having diverged from it over 100,000 years ago.

Key Words: goats and related species, ancient DNA, animal domestication

Applied Genetics of Companion Animals

WT1 Genetic trend of the junctional epidermolysis bullosa (JEB) in the German Shorthaired Pointer in Italy. S. Frattini*[1], S. P. Marelli[1], A. Picchi[1], F. Danelli[1], J. Riva[2], E. Moretti[2], A. Talenti[1], G. Gandini[1], G. Pagnacco[1], M. Polli[1], and P. Crepaldi[1], [1]*Department of Veterinary Medicine, University of Milan, Milan, Lombardy, Italy;* [2]*Vetogene - Spin Off University of Milan, Milan, Lombardy, Italy.*

Junctional epidermolysis bullosa (JEB), one of the major forms of epidermolysis bullosa (EB), is characterised by skin and mucous membranes fragility. In humans, EBs are estimated affecting one in 17,000 live births, while in several animal species the frequencies of these diseases are still unknown. In German Shorthaired Pointers (GSPs), JEB is an autosomal recessive disease caused by an insertion (4,818+207 ins 6.5 kb) of repetitive satellite DNA within intron 35 of the *LAMA3* gene. The insertion causes abnormal mRNA transcript with an insertion of 227 nucleotides in position 4,818 at the junction among exon 35 and 36 of the *LAMA3* gene. This 227 bp sequence carries a nonsense (TAA) codon 33 bp downstream of the insertion site. Considering that conventional treatments are ineffective for homozygous dogs and that heterozygous animals are symptomless, the genetic identification of carrier animals is important. For this study, we monitored the genetic trend of JEB in the Italian population of the GSPs in a period of ten years. We analysed 945 animals (498 males and 447 females) out of more than 28,000 dogs registered in the Italian Kennel Club, determining the presence of the 6.5 kb insertion by PCR amplification of the genomic DNA. Overall, less than 1% of the animals analysed resulted homozygous for the mutation. This frequency is expected to be underestimated because afflicted animals are suppressed after birth. The mutant allele showed a frequency of almost 9% in the population, with 150 carrier subjects (~16%). During the 10 years period, the frequency of the mutant allele decreased from ~10% in 2007 to ~5% in 2016. This encouraging tendency was observed only in the 3.3% of the entire recorded Italian population. The simplicity and the low cost of the analysis for the detection of this pathology suggests that a deeper identification of carrier dogs will allow better breeding strategies and management, leading to a rapid JEB eradication. This simple genetic analysis can efficiently increase the welfare of the GSP in Italian population.

Key Words: dog and related species, genetic identification, PCR, allele specific expression, animal welfare

WT2 Spread of concepts of animal genetics for buyers of purebred dogs in Brazil: A contribution to the improvement of cynophilia. F. Stortti, M. Soares, J. M. S. Nunes, J. Neves, and F.

M. de Andrade*, *Centro Universitário Ritter dos Reis, Uniritter, Porto Alegre, RS, Brazil.*

Unofficial estimates show that in the city of São Paulo (Brazil) alone, more than 500 thousand dogs from illegal breeders are sold per year due to lack of legislation and inspection. Moreover, most of the official breeders do not know anything about genetics; veterinarians have insufficient training in this area and the buyers of purebred dogs are completely uninformed. Therefore, this set of deficiencies stimulates a poor quality market, and leads to an increase in the prevalence of diseases with genetic influence among all breeds. In order to contribute to the change of this reality, a website has been created as part of the activities of an extension project. This site (www.geneticacanina.com) contains basic concepts of animal genetics in order to assist the buyer in finding a breeder who works focusing on genetic improvement geared towards animal health and welfare. The material contains general information on the process of breeding dogs, plus a form where the user can select their breed of interest, out of thirteen available so far. For each breed, the most common diseases with genetic influences, both monogenic and multifactorial, have been chosen. By selecting one of the diseases, the visitor can find information about its aetiology in colloquial language, as well as advice for evaluating the work of the breeder of the puppy to be purchased. Information such as the availability of DNA tests and other tests required for the correct choice of breeding stock are also available to aid in process of choosing a breeder. Google Analytics data shows that since its publication in December 2016, the site has been visited by 670 users, with 20.2% of visitors returning to the website. Among the visitors, 24.4% originated from other Brazilian states, which demonstrates a great capacity of dissemination of knowledge in the country. With this type of spread of scientific data throughout the society, it is expected to contribute to the improvement of the quality of cynophilia in Brazil.

Key Words: dogs and related species, animal breeding, genetic disorder, spreading of science

WT3 Genetic variation at the K locus and resultant phenotypes. R. Grahn*, J. Grahn, and M. Torres Penedo, *University of California, Veterinary Genetics Laboratory, Davis, CA, USA.*

Canine coat colour phenotypes often result from numerous epistatic interactions. The Kurokami (K) locus is involved in black/brindle pigmentation and results from mutations in β-defensin. A dominant, 3 bp deletion mutation prevents pigment switching by the agouti locus resulting in a black (B) phenotype while the brindle (br) colour pattern, with black and yellow stripes, results from a tandem duplication of the β-defensin gene. The allelic series for the K locus is reported as $K^B > k^{br} > k^y$ with k^y representing wild type. A ddPCR copy number assay to interrogate the nature of the duplication of the β-defensin brindle mutation has identified additional complexity at the K locus. The common causal brindle allele is a tandem duplication of the β-defensin gene consisting of one recessive wild-type allele and one dominant deletion allele. Whether this results from non-homologous recombination or tandem duplication with gene conversion remains unclear. Additional rare alleles at this locus include a duplication and/or triplication of the dominant black mutation allele. Among the rarer alleles, nearly 88% are restricted to a limited number of bulldog, retriever, and Great Dane breeds with the remaining 12% occurring in five unrelated breeds. The ddPCR assay provides clear detection of the brindle mutation and thus improves genotyping accuracy in genetic diagnostic testing for the K locus in dogs.

Key Words: brindle, dominant black, K locus, β-defensin

WT4 Application of multiplex microsatellite panel in Felidae family. A. Podbielska*[1], A. Radko[1], W. Nizanski[2], J. Kochan[3], A. Nowak[3], and M. Bugno-Poniewierska[1], [1]*National Research Institute of Animal Production, Department of Animal Genomics and Molecular Biology, Balice, Cracow, Poland;* [2]*Wroclaw University of Environmental and Life Sciences, Faculty of Veterinary Medicine, Department of Reproduction and Clinic of Farm Animals, Wroclaw, Poland;* [3]*University of Agriculture in Krakow, Institute of Veterinary Science, Faculty of Animal Sciences, Cracow, Poland.*

The aim of the study was a preliminary assessment of polymorphism domestic cats and free-living cats by using multiplex microsatellite panel recommended by ISAG for cat identification and parentage control. Ten microsatellite markers: FCA310, FCA220, FCA069, FCA441, FCA075, FCA229, FCA678, FCA149, FCA105 and AMEL were used in Laboratory of Molecular Genetics. Forty-four individuals were tested in the family *Felidae* of species: different breeds a domestic cat (*Felis catus)*, five tiger *(Panthera tigri),* two lynxes *(Lynx lynx)* and one of animal, which represent the species wildcat (*Felis silvestris),* manul *(Otocolobus manul),* clouded leopard *(Neofelis nebulosa),* and snow leopard *(Panthera uncia)*. Genomic DNA was extracted from hairs and buccal swabs. DNA isolates were amplified by one multiplex PCR. Amplification of genes was amplified using the QIAGEN Multiplex PCR Kit, the amplified products were separated on 3100xl Genetic Analyzer and genotyped using GeneMapper Software (Applied Biosystems). PCR products for all markers were obtained for a domestic cat. Studied wild cats, except for a wildcat and snow leopard were different than domestic cats. PCR products were not observed for FCA441 marker in clouded leopard and lynx. The same results obtained for FCA149 marker for manul, clouded leopard and tigers. Identified alleles of the lynx were out of range in FCA220 marker. The same situation was with manul alleles in FCA441 marker. Additionally, in manul we observed different variant in AMEL marker, which produced a 216-bp X allele, while others species had 214-bp X allele. We conclude, based on the presented studies that we should apply other known markers to the assessment of biodiversity free-living cats. Our preliminary studies allow stating that manul is the most different feline but we need further study on much larger population. Financed by: NCBiR PBS3/B8/16/2015.

Key Words: feline, identification, microsatellite

WT5 Pedigree and genomic-based relationships in a dog population. A. Talenti*[1], D. L. Dreger[2], F. Danelli[1], S. Frattini[1], B. Coizet[1], S. P. Marelli[1], G. Pagnacco[1], G. Gandini[1], M. Polli[1], R. Caniglia[3], M. Galaverni[3], E. A. Ostrander[2], and P. Crepaldi[1], [1]*Department of Veterinary Medicine, University of Milan, Milan, Italy;* [2]*National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA;* [3]*Laboratorio di Genetica, Istituto Superiore per la Protezione e la Ricerca Ambientale, Ozzano dell'Emilia, Italy.*

In many dog breeds, small population sizes, associated with intense selection schemes, have led to considerable losses of genetic diversity. This complicates the production of accurate genomic estimates of parentage. The Lupo Italiano, with ~300 living dogs, is an Italian breed created in 1966 by crossing the Apennine grey wolf (*Canis lupus italicus*) with German Shepherd dogs (GSD). The aim of this work was to compare calculated relationships from genomic and pedigree data, using the Lupo Italiano as an example of a small population. The entire pedigree of the Lupo Italiano is known, consisting of up to 12 generations and dating to the founder animals. The pedigrees of 28 Lupo Italiano dogs (provided by AAALI) were used to build an additive relationships matrix (A) (CFC software). These 28 dogs were genotyped on the Illumina CanineHD 170K SNP chip (University of Milan and National Institutes of Health in Bethesda, MD), and the resultant genotypes were used for the estimation of the within-breed Genomic Relationships Matrix (GRMa) (GCTA64 software). The mean parentage values for GRMa (-0.02 ± 0.05) and A (0.80 ± 0.05) were not equivalent, however, they did

display a significant positive correlation (R = 0.75; $P < 0.001$). Four additional populations, genotyped on the same panel, consisted of 20 Apennine grey wolves (ISPRA), 30 GSDs, 14 grey wolves, and 31 village dogs (publicly available in Dryad, Shannon *et al*. 2015). The GRM produced with the combined set (GRMb) led to a higher correlation with A (R = 0.80; $P < 0.001$) and to higher estimates of parentage between Lupo Italiano individuals (0.53 ± 0.05). The calculation was expanded a final time to include an additional 250+ GSDs (GRMc). This matrix showed a decrease in the correlation with A (R = 0.76; $P < 0.001$) balanced by a strong increase in the parentage values (0.82 ± 0.08), making it the closest to the A matrix. These results show that estimation of genomic relationships from populations with greater allelic diversity can improve the correlation and accuracy with pedigree-derived estimates. Consideration of these implementations can allow for better management of mating schemes and conservation of genetic variation in dog breeds with small population sizes. The authors thank AAALI for the kind collaboration.

Key Words: dog, parentage, SNP

WT6 Can-ID: The genetic Identification system for Canine samples based on SNPs. O. Ramírez*[1], A. Cuscó[1], A. Sánchez[2], O. Francino[2], and L. Altet[1], [1]*Vetgenomics, Barcelona, Spain;* [2]*Molecular Genetics Veterinary Service (SVGM), Barcelona, Spain.*

Genetic identification establishes a secure and permanent DNA profile that is very useful in cases of lost or stolen dogs or to prove parentage. In this study we present a Canine Identification method (Can-ID) based on SNPs genotyping using a TaqMan OpenArray platform. Can-ID is the genetic identification system for canine samples, developed for the purpose of reducing the problem of unhygienic and unpleasant canine excrement in public places, one of the biggest and most widespread antisocial issue that is very hard to eliminate or even curb. Can-ID panel contains three types of markers. First, contains 100 highly polymorphic SNPs to obtain a unique DNA profile for each dog. These highly polymorphic SNPs were selected from two different datasets: (i) the whole genome sequence (5–22 of final coverage) of 22 dogs of 13 breeds and (ii) the massive sequence after enrichment and capture of 0.4 Mb from 335 dogs (7 breeds) and 100 wolves (2 populations). Second, 15 mitochondrial highly polymorphic SNPs that allow Can-ID guarantees that the biological sample comes from a single animal, and has not been contaminated with exogenous DNA (e.g. faeces contaminated with the urine of another dog), and so avoid false positives. And third, 13 SNPs associated to phenotype traits (as body size, sex, head shape, colour and type of fur) allow obtaining for a 'composite picture' of the dog to be formed, in those cases where the genetic profile found in the excrement is not included in the database. Can-ID have been validated in more than a thousand of dogs and it is the identification method used in the first village from Catalonia that established the obligatory dog DNA testing for all dog owners within the community.

Key Words: dogs and related species, genetic identification, genotyping, genetic marker, parentage

Applied Sheep and Goat Genetics

WT7 Analysing the genetic diversification among the sheep breeds of Balochistan by utilizing the mitochondrial cytochrome *b* gene. A. Hameed*[1], M. Mohsin[2], A. N. Khosa[1], N. Bangulzai[1], and I. B. Marghazani[1], [1]*Lasbela University of Agriculture, Water and Marine sciences, UThal, Balochistan, Pakistan;* [2]*Livestock and Dairy Development Department, Quetta, Balochistan, Pakistan.*

Current study was performed on 4 sheep breeds of Balochistan including Balochi, Bibrik, Harnai and Rakhshani breeds. The primary objective of the study was to analyse the genetic diversification among the breeds raised under different circumstances by utilising the mitochondrial Cytochrome *b* gene that has extensively been used to study for genetical analysis. Balochistan covers the major land area (44%) of Pakistan with least population. Small ruminant farming is a common practice among the local population and more than 50% of the total sheep population of Pakistan reared in the range lands of Balochistan. For this purpose a total of 40 animals (10 from each breed) were selected randomly. 5–10 ml intravenous blood was collected aseptically from all the selected animals in 15 ml falcon tube containing EDTA as anticoagulant. Genomic DNA was extracted from the samples through an inorganic method. Primers were designed for Cytochrome *b* gene by using the Primer3 sofware. The DNA samples were amplified through PCR and then amplified products were sequenced for analysing the genetic variations among the breeds. The sequencing results revealed 3 genetic variations including c.243T>C (p.T81T) observed in a sample of Harnai breed, c.309G>A (p.A103A) observed in a sample of Balochi breed and c.366T>C (p.A122A) observed in all samples of Rakhshani breed.The phylygenetic tree of all the sheep breeds under study was constructed by using MABL online with the reported sequences of *Ovis aries* KP 22891.1, *Capra hircus* AB044308.1. In conclusion, mitochondrial Cytochrome *b* gene is a better tool for analysing the genetic diversification among breeds.

Key Words: sheep, PCR, Cytochrome *b* gene, genomic DNA, Primer3

WT8 Genomic architecture of Punjab Urial. T. Hussain*, F. Marikar, M. Babar, M. Musthafa, and K. Periasamy, *Virtual University of Pakistan, Lahore, Pakistan.*

Punjab urial (*Ovis vignei punjabiensis*) is endemic to wild northern Punjab, Pakistan and also an endangered species according to red list categories of International Union of Conservation of Nature and Natural Resources. The taxonomic status of this sub-species remains uncertain and the present study is an attempt to assess its mitochondrial DNA diversity and evolutionary relationship with closely related taxa. Analysis of mitochondrial DNA D-Loop of *O. vignei punjabiensis* revealed wide variations in the number and pattern of tandem repeats as compared to other Moufloniform wild sheep. With the exception of tandem repeat regions, a total of 117 sequences covering an overlapping region of 748 bp from nine wild and domestic sheep populations were analysed. The nucleotide and haplotype diversity was low to moderate within different sheep populations and Punjab Urial sheep exhibiting a mean of 0.0043 and0.829 respectively. The average pairwise differences within population varied from 0.67 (*O. musimon*) to 23.69 (Kachi) while the average differences between Urial sheep and other Moufloniform wild sheep populations was high indicating the distinctness of *O. vignei*. Bayesian and maximum likelihood phylogeny clearly established the divide between the lineages of *O. vignei* and *O. orientalis* populations. Also, the average pairwise differences between *O. vignei bochariensis* and *O. vignei punjabiensis* were significantly higher ($P < 0.01$) despite both the populations being classified as sub-species of Urial type sheep. To compare *O. vignei punjabiensis* with Argaliform and Pachyceriform wild sheep, pairwise FST between populations were utilised to perform principal components analysis (PCA). PCA revealed clustering of Asiatic (*O. orientalis*), European (*O. musimon*) and Captive Mouflon type sheep together, while *O. nivi-cola* and *O. canadensis* clustered separately. *O. ammon* and *O. vignei bochariensis* were observed to be clustering together while *O. vignei punjabiensis* was located distinctly. The tests for selective neutrality indicated purifying selection in Punjab Urial

sheep while Asiatic Mouflon appears to have experienced a strong population bottleneck in the recent past.

Key Words: Punjab Urial, conservation genomics, DNA sequencing, animal domestication, genomic selection

WT9 Construction and functional analysis of expression vector for Tibetan sheep *Fzd4* gene. B. B. Qu, L. Huang, Y. Wang, X. D. Zi, Y. Q. Lin, and Y. C. Zheng*, *College of Life Science and Technology, Southwest University for Nationalities, Chengdu, Sichuan, China.*

Transmembrane Frizzled (Fzd) proteins are the principal receptors for the Wnt family of signalling molecules. Ten family members of Fzds have been identified in mammals, of which Fzd4 belongs to a separate subfamily 4. The functions of Fzd4 were studied mostly in mice. The objective of this study was to explore possible functions of Fzd4 of Tibetan sheep (*Ovis aries*) at cellular level. RNA was extracted from skin tissues of Tibetan sheep (n = 5), and the cDNA sequence of *Fzd4* gene was cloned by RT–PCR. The coding sequence of Tibetan sheep *Fzd4* was 1620 bp in length, coding 539 amino acids. Sequence alignments showed that Tibetan sheep *Fzd4* is highly conserved and shows 99.63% coding sequence similarity to that of predicted sheep *Fzd4* (XM_012143519.2), resulting one amino acid change (Met65Val). A eukaryotic expression vector (pCDsRed2-KF) containing skin-specific KAP6.1 promoter was constructed for Tibetan sheep *Fzd4*, and transfected into human hair inner root sheath cells *in vitro*. The transfection rate reached 20% through optimizing related procedures, sufficient for quantitative detection of gene expressions. Quantitative real time PCR assay using *GAPDH* as reference gene proved that the expression vector was overexpressed in human hair inner root sheath cells by ~4.8 folds after transfection, and two major downstream genes in canonical Wnt signalling pathway, *Dvl* and β-catenin genes, were up-regulated by ~5.8 and 1.5 folds, respectively. Western blot analysis also confirmed that both Dvl and β-catenin were up-regulated significantly at protein levels ($P < 0.05$). Our data suggest that *Fzd4* gene may play an important role in regulating hair follicle development through canonical Wnt signalling pathway.

Key Words: sheep, functional assay, animal breeding, qPCR

WT10 *ASIP* and *MC1R*: Dominant black and recessive black alleles segregate in native Swedish sheep populations. C. Rochus*[1,2], K. Westberg-Sunesson[1], E. Jonas[1], S. Mikko[1], and A. Johansson[1], [1]*Department of Animal Breeding and Genetics, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [2]*UFR Génétique, Élevage et Reproduction, Sciences de la Vie et Santé, AgroParisTech, Université Paris Saclay and GenPhySE, Animal Genetics Division, INRA, Paris and Castanet-Tolosan, France.*

Many coat colour genes have been found under selection in livestock because breeds are defined by their coat colour and pattern. By studying genes associated with coat colour we can further understand the role of these genes in pigmentation but also gain insight into selection history. North European short-tailed sheep including native Swedish breeds are defined in part by their coat colour variation making them good models to expand our current knowledge of mutations associated with coat colour in sheep. We studied mutations in *ASIP* and *MC1R*, two genes with known roles in pigmentation, and their association with black coat colour. We did this by sequencing the coding regions of *ASIP* in 154 animals and *MC1R* in 132 animals from seven native Swedish sheep breeds in individuals that had black, white or grey fleece. Previously known mutations in *ASIP* (recessive black allele: g.100–105del (D_5) and/or g.5172T>A) were associated with black coat colour in Klövsjö and Roslag sheep breeds and mutations in *ASIP* and *MC1R* (dominant black allele: c.218T>A or c.361G>A) were associated with black coat colour in Swedish Finewool. In Gotland, Gute, Värmland, and Helsinge sheep breeds, coat colour inheritance was more complex: only one third of individuals with black fleece had genotypes that could explain their black coat colour. These breeds have grey individuals in their populations and the grey allele is believed to be a duplication of *ASIP* with mutations, which could be a possible explanation for the lack of a clear inheritance pattern in these breeds. Finally, we found a novel missense mutation in *MC1R* (c.452G>A) in Gotland, Gute and Värmland sheep and evidence of a duplication of *MC1R* in Gotland sheep: further studies are needed to understand their role in pigmentation.

Key Words: sheep, coat colour

WT11 Tracing the effect of *FecB* gene on transcriptome profile in ovine oocyte and cumulus cells using single-cell RNA-Seq. X.-F. Guo*[1,2], X.-Y. Wang[1], R. Di[1], Q.-Y. Liu[1], W.-P. Hu[1], X.-Y. He[1], X.-H. Cao[1], and M.-X. Chu[1], [1]*Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China;* [2]*College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Fecundity Booroola gene (*FecB*) is a major gene responsible for high ovulation rate and litter size in sheep. Chinese native breed Small Tail Han sheep (STH) is one of prolific sheep carry the mutant of FecB. The molecular mechanisms of *FecB* gene on ovulation were still unclear. Follicular development and ovulation rely on continuing cross talk between the oocyte and the cumulus cell. Single-cell mRNA-sequencing (scRNA-Seq) method now enables providing an unbiased view of the gene expression heterogeneity of oocyte and cumulus cell within an ovary. To comprehend the effect of *FecB* gene on ovulation rate, oocyte and cumulus cell in STH of wild type (++) and homozygous carriers of *FecB* for high fecundity (BB) (each genotype including 3 biological replicates) were collected and separated to investigate the transcriptome profile using scRNA-Seq. Based on the oestrous synchronization with CIDR for 12 days, all of the oocytes and cumulus cells were picked by mouth pipette at 45 h after CIDR removing. Single-cell RNA was reverse transcripted and amplified by SMART-sEqn 2 method, 12 libraries were sequenced using the Illumina HisEqn 4000 platform. An average total of 12G clean bases were obtained and ~80 percent of the total reads in each library were mapped to the reference genome. Transcriptome profiles of oocyte and cumulus cells in the same genotype were different. Compared oocytes with cumulus cells, 4131 and 4882 were found in ++ and BB genotype, separately (FDR < 0.05), Bone Morphogenetic Protein 15 (*BMP15*) and Granulysin (*GNLY*) genes etc. specially expressed in oocyte and cumulus cells. Contrast ++ with BB, the 683 and 786 differentially expressed genes (DEG) were identified in oocytes and cumulus (FDR < 0.05), respectively. Functional annotation of the DEG by KEGG pathway showed that oxidative phosphorylation and oocyte meiosis in oocyte were significantly enriched. In cumulus cells, ovarian steroidogenesis pathway was significantly enriched. Transcriptome profiles of oocyte and cumulus cells in single-cell level will help us to understand the *FecB* gene effect on the difference in ovulation rate and other reproductive phenotypes.

Key Words: *FecB*, oocyte, cumulus cell, single-cell RNA-sequencing

WT13 Preliminary differential transcriptomic analysis of abomasal mucosa from resistant and susceptible sheep to gastrointestinal nematodes (GINs) after an experimental infection with *T. circumcincta*. P. K. Chitneedi*[1], J. J. Arranz[1], A. Suarez-Vega[1], M. Martínez-Valladares[2,3], and B. Gutiérrez-Gil[1], [1]*Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, León, Spain;* [2]*Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de León, León,*

Spain; [3]Instituto de Ganadería de Montaña, CSIC-ULE, León, Spain.

This study presents the preliminary results of a differential transcriptomic analysis between abomasal mucosa samples obtained from two groups of sheep classified as resistant or susceptible to gastrointestinal nematodes (GINs) after an experimental infection with *T. circumcincta.* Initially based on the faecal egg counts (FEC) of 119 Spanish Churra ewes sampled in a flock, 10 animals with the highest FEC values and 14 with the lowest FEC values were included in the study. After a first experimental infection (EI1) 18 of these animals with *T. circumcincta*, and based on the accumulative FEC estimated from day 14 to 31 post-infection, eight ewes were classified as 'Susceptible' and seven ewes as 'Resistant'. After an antihelminthic treatment, a second experimental infection (EI2) of these animals was performed. On day 7 after EI2 the animals were sacrificed and abomasal mucosa tissue samples were collected. The total mRNA extracted from these samples was were later sequenced using an Illumina Hi-SEqn 2000 sequencer by generating 'paired-end' reads of 75 bp, with a depth of 30M reads. The bioinformatics work flow analysis included the assessment of raw sequence data quality using FastQC, the alignment against the sheep reference genome (Oar_v.3.1), the quantification and normalization of gene expression performed with Cufflinks and finally the differential expression analysis performed with two different R-based packages, DESEqn 2 and edgeR. DESEqn 2 identified one differentially expressed (DE) gene, *SYT8* ($P\text{-}value_{adj}$ = 0.020) whereas the edgeR analysis found 18 DE genes among which *SYT8* was the second highly expressed gene (FDR_{adj} = 0.0023). *SYT8* is involved in trafficking and exocytosis of secretory vesicles in non-neuronal tissues. Many of the genes highlighted by the edge R are related to muscular excitation and contraction whereas one belongs to the Major Histocompatibility Complex. Because one of the proposed mechanisms of parasite resistance in sheep is the increase in peristalsis, some of the genes highlighted by the edgeR analysis may be further investigated in future studies.

Key Words: sheep and related species, RNA-seq, transcriptome, complex trait, bioinformatics tools

WT14 Underdominant KCC3b R31I association with blood sodium concentration in domestic sheep suggests role in dimerization. S. N. White*[1,2], R. D. Oliveira[2], M. R. Mousel[1,4], M. V. Gonzalez[2,5], M. A. Highland[1], J. B. Taylor[6], and D. P. Knowles[1,2], [1]*USDA-ARS Animal Disease Research, Pullman, WA, USA;* [2]*Dept. Veterinary Microbiology & Pathology, Washington State University, Pullman, WA, USA;* [3]*Center for Reproductive Biology, Washington State University, Pullman, WA, USA;* [4]*School for Global Animal Health, Washington State University, Pullman, WA, USA;* [5]*Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA;* [6]*USDA-ARS Range Sheep Production Efficiency Research, Dubois, ID, USA.*

The potassium chloride cotransporter 3 protein (KCC3) is an electroneutral ion cotransporter strongly linked to hypertension. The KCC3 protein is encoded by solute carrier family 12 member 6 (SLC12A6) gene, and two main isoforms differ by alternate promoters and first coding exons. The longer isoform, KCC3a, is expressed throughout the body and is involved in cell volume homeostasis. The shorter isoform, KCC3b, is highly expressed in the kidney proximal convoluted tubule and is involved in recycling ions that would otherwise be excreted in urine. It is also indirectly involved in sugar retention through coordinated action with the sodium-potassium pump and glucose transporters including the sodium-glucose transporter. We genotyped KCC3 amino acid substitutions in 300 Suffolk, Rambouillet, Polypay, and Columbia sheep with whole blood sodium and potassium concentrations determined by atomic absorbance spectrometry. Association was determined by mixed models including blood sodium or potassium concentration as the dependent variable, breed and genotype for the variant of interest as independent fixed effects, and sire nested within breed treated as random. The KCC3b R31I substitution was associated with blood sodium in an underdominant manner. Specifically, blood sodium was significantly higher in both homozygotes than in heterozygotes ($P <= 0.05$). While we did not observe association with blood potassium, other potassium chloride cotransporters provide partially redundant cotransport function in some, but not all, contexts. The underdominant pattern suggests allelic incompatibility, and since KCC3 functions as a dimer these data suggest the charged R31I substitution may interfere with KCC3b dimer function.

Key Words: sheep and related species, biochemical genetics, candidate gene, biomedical model

WT15 Non-linked SNPs in promoter and exon 2 of *MTNR1A* gene are associated to reproductive seasonality in Rasa Aragonesa sheep breed. J. H. Calvo*[1,3], M. Serrano[2], A. Martinez-Royo[1], B. Lahoz[1], P. Sarto[1], A. Ibañez-Deler[1], J. Folch[1], and J. L. Alabart[1], [1]*Centro de Investigación y Tecnología agroalimentaria de Aragón (CITA)-IA2, Zaragoza, Spain;* [2]*Instituto Nacional de Investigaciones agrarias (INIA), Madrid, Spain;* [3]*ARAID, Zaragoza, Spain.*

Sheep breeds from Mediterranean area show reproductive seasonal patterns of oestrous behaviour and ovulatory activity, mainly regulated by changes in the photoperiod. To investigate the responsibility of the *MTNR1A* gene in the reproductive seasonality behaviour variability of Rasa Aragonesa ewes, characterisation and an association study of polymorphisms in its promoter and whole coding region with reproductive seasonality traits was carried out. The flock was composed of 268 ewes classified in three age groups: lambs (0.9 years, n = 51), young (1.9 years, n = 79) and mature (5.2–7.2 years, n = 138). The flock was controlled from January to August. No hormonal treatments were applied to ewes during the study. Individual liveweight and body condition score were assessed every three weeks. Two reproductive seasonality traits were considered: the total days of anoestrus (TDA), based on weekly individual plasma progesterone levels, and defined as the sum of days in anoestrus, considering anoestrus those periods with three or more consecutive progesterone concentrations lower than 0.5 ng/mL; and the oestrus cycling months (OCM), defined for each ewe as the rate of months cycling based on daily oestrous records. A promoter region of 733 bp and total exons 1 and 2 were sequenced in all animals, detecting a total of 35 SNPs. SNPs in the promoter were not in linkage disequilibrium with those located in exon 2. Significant associations with TDA were found for 9 SNPs located in the promoter region and 4 SNPs pertaining to exon 2. Differences between alternative genotypes were up to 32 days and 52 days for promoter and exon 2 polymorphisms. However, the SNPs located in the promoter region only affected young animals: lambs and young ewes; while the SNPs placed in exon 2 showed association with reproductive seasonal traits irrespective of the age of the animals. In the same way, the SNPs located in exon 2 were also associated with a difference of 15% of OCM between alternative genotypes. Haplotype association analysis confirmed these results. The highest significant associations were found for the SNPs rs403212791 (OARP00000008886.1: p.Arg336Cys) and rs400830807 (3′UTR region) in exon 2.

Key Words: sheep and related species, candidate gene, genetic improvement

WT16 Association analysis between *ABCG2* gene and milk production in dairy sheep breeds kept in the Czech Republic: Preliminary results. J. Rychtarova*, A. Svitakova, and Z. Sztankoova, *Institute of Animal Science, Prague, Czech Republic.*

The ATP-binding cassette sub-family G member 2 (*ABCG2*) belongs to the ABC (ATP- binding casette) transporter family which

includes proteins responsible for the transport of various molecules across cell membranes. The *ABCG2* gene has been mapped on chromosome 6 in sheep together with a microsatellite OarAE101, which was highlighted in an earlier QTL studies and associated with fat and protein percentage, as well as somatic cell score (SCS). A single 35-base insertion/deletion, (c.683–80_46del) were chosen for association study between polymorphism and milk production traits. We used a PCR test for genotyping of the 35 basis insertion/deletion in intron 5 of the *ABCG2*. The length of the PCR product was 267 bp if the deletion was not present (genotype *II*), and 232bp if present (genotype *DD*). The frequencies of alleles *D* and *I* were 0.38 and 0.62, respectively, and the genotype frequencies were 0.19, 0.38 and 0.43 for genotypes *DD, ID* and *II*. The dataset of performance testing obtained 264 individuals of two breeds - East Friesian and Lacaune sheep. The observed period was between 2009–2017 and contained 2691 records (average 11.5 records per sheep). The mixed model with repeatability was used to estimate the impact of polymorphism *ABCG2* gene on milk production traits (milk yield, fat and protein percentage, SCS). The fixed effects were genotype, herd-year-season of lactation, age, age^2, breed, month of performance test and parity number, depend on evaluated trait. The random effect was the animal (repeated-measurements per goat). A significant effect of *ABCG2* gene on milk yield, fat percentage and protein percentage was found. The animals carrying the *II* genotype have the highest milk production ($P < 0.001$) but with the lowest fat and protein content (not significant) and they have tendency for lower somatic cell score ($P < 0.07$). Otherwise animals with *DD* genotype produced milk with the highest fat percentage ($P < 0.001$). Genotype *ID* had positive significant influence on protein percentage ($P < 0.001$). The *ABCG2* gene appears to be a strong candidate gene for determining milk production traits. This work was supported by the project no. NAZV QJ1510137.

Key Words: sheep and related species, genetic marker, milk production, somatic cell score

WT17 Are *TMEM154* and *CCR5* variants promising markers for selection against maedi-visna susceptibility in German sheep flocks? V. Molaee, M. Eltanany, and G. Lühken*, *Department of Animal Breeding and Genetics, Justus-Liebig University, Giessen, Germany.*

Maedi-visna (MV) disease is considerably spread among German sheep flocks. Variants in the genes *TMEM154* (E35K) and *CCR5* (promoter indel "aatg") have been reported to be associated with the serological status of and/or the provirus concentration in U.S. sheep. The overall aim of the current study was to evaluate associations of those markers with MV antibody titer in MV-positive German sheep flocks. A total of 543 sheep (three years or older) from 17 flocks in different German regions was sampled. According to the regional origin and the dominating breed composition, the flocks were grouped in four sample sets. Serology (ELISA) for MV antibodies resulted in 323 MV-positive and 209 MV-negative samples. *TMEM154* and *CCR5* variants were genotyped by PCR-based methods. For statistical analyses, chi-square/Fisher's exact test and one-way analysis of variance were used over all samples as well as separately for each sample set. Over all samples, *TMEM154* genotype frequencies showed highly significant differences ($P < 0.0001$) between MV-positive (42% EE, 43% EK, 15% KK) and MV-negative (19% EE, 33% EK, 48% KK) sheep. Except for sample set 3, *TMEM154* genotype frequencies were also significantly different within each sample set. For *CCR5*, the difference in genotype distribution between MV-positive and MV-negative sheep was significant ($p = 0.038$) over all sheep (MV-positive: 76% ins/ins, 21% ins/del, 3% del/del; MV-negative: 66% ins/ins, 30% ins/del, 4% del/del), but not within sample sets 1, 3 and 4. Mean MV antibody titer values were significantly different between *TMEM154* genotype groups (EE: 189.99 ± 7.29, EK: 163.33 ± 7.07, KK: 84.30 ± 8.66; $P < 0.001$). This was also significant within each sample set except for sample set 3. The same analysis model did not show any significant results for *CCR5*. According to these initial results, *TMEM154* (E35K) seems to be a more promising marker for selection against MV susceptibility in the German sheep population than the promoter variant in *CCR5*. However, this will have to be verified by deeper statistical analysis and inclusion of other phenotypes, e.g., provirus load.

Key Words: sheep and related species, animal breeding, genotyping, animal health

WT18 Allelic and genotypic frequencies of *PRNP* gene polymorphisms in some Italian goat populations. C. Sebastiani[1], M. Torricelli[1], M. Ciullo[1], G. Vaccari[2], E. Lasagna[3], F. Sarti[3], S. Ceccobelli[3], N. D'Avino[1], M. Paniccià[1], and M. Biagetti*[1], [1]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy;* [2]*Istituto Superiore di Sanità - Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare, Roma, Italy;* [3]*Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università degli Studi di Perugia, Perugia, Italy.*

Scrapie is a transmissible spongiform encephalopathy of sheep and goats, characterised by the deposition, in the central nervous system, of an abnormal isoform of a host-encoded cellular prion protein (PrP). In sheep polymorphisms of *PRNP* gene at codons 136, 154, 171 have been associated to scrapie resistance/susceptibility. In Europe, sheep genetic selection plans were set up in order to control the disease. In goats, more than 50 putative polymorphisms, including both conservative and missense mutations, were described. Among these, Q222K has been associated with resistance to the disease. Genetic selection plans based on *PRNP* polymorphisms could become a tool to control and eradicate scrapie in caprine herds, too. The aim of this work was to evaluate the frequencies of polymorphisms of *PRNP* gene, with particular attention to codon Q222K, in 5 Italian populations (*Grigia Ciociara, Bianca Monticellana, Capestrina, Fulva del Lazio and Facciuta della Valnerina*) and in crossbred goats (125 animals sampled). DNA from whole blood was extracted and the whole CDS region was amplified by PCR. Amplification products were sequenced using ABI PRISM 3130 and 3500 Genetic Analyzers and then analysed with Bioedit software. Allelic and genotypic frequencies were calculated from a spreadsheet. Preliminary data on allelic and genotypic frequencies are reported. At codon 222, allelic frequencies were 92.8% for Q and 7.2% for K alleles. Furtheremore, among the other mutations, we observed at codon 154 97.6% for R and 2.4% for H alleles and at codon 211 allelic frequencies were 96.8% for R and 3.2% for Q alleles.. Regarding genotypic frequencies, at codon 222 87.2% of animals were homozygote for Q, 11.2% heterozygote Q/K and 1.6% were homozygote for K. At codon 154 95.2% of animals were homozygote for R, 4.8% heterozygote R/H and none was homozygote for H. Finally, at codon 211 93.6% of animals were homozygote for R, 6.4% heterozygote R/Q and none was homozygote for Q. These data could be useful for planning future genetic selection programmes to control and eradicate scrapie in goats. This study is funded by Italian Ministry of Health RCIZSUM 04/2015.

Key Words: goat and related species, single-nucleotide polymorphism (SNP), animal health

WT19 Accelerating genetic improvement in sheep by increased pedigree accuracy. H. Koshinsky, A. Pirani, M. Patil*, V. Missirian, V. Joshi, and J. Curry, *Thermo Fisher Scientific, Santa Clara, CA, USA.*

Genetic management is a key driver for increased revenue in agri-genomics production systems. The amount of genetic information required for a decision depends on the application. At the low end is genotypes on a few hundred markers for a targeted SNP parentage and the associated reduction in pedigree errors and more

efficient herd improvement. Correct parentage assignment increases the success of any breeding program by facilitating linkage of production performance to the correct families to improve estimates of breeding values. At the high end is full genome sequence for high value individuals. Eureka Ovine Parentage Panel is a comprehensive parentage panel for sheep and provides superior power to accurately verify parentage. It provides an affordable next-generation sequencing (NGS)-based panel for both parentage testing and traceability in diverse sheep breeds. The availability of over 3,000 barcodes enables processing of over 3,000 samples in a single sequencing run for fast turnaround time. Thus this genotyping panel may be used as a tool in an ovine breeding and production system that has the potential to increase overall revenue.

Key Words: parentage, SNP-genotyping, NGS, ovine

WT20 Molecular study of the melanocortin 1 receptor gene in association with coat color variation of Iranian native sheep. M. A. Eshghabadi[1], A. A. Masoudi*[1], H. Emrani[2], and S. Amirina[2], [1]*Tarbiat Modares University, Tehran, Tehran, Iran;* [2]*Animal Sciene Research Institute, Tehran, Tehran, Iran.*

A tremendous variety is observed in coat coloration of the Iranian indigenous sheep populations, whereas a few molecular based studies have been carried out to identify the responsible genes in this area. Coat colour in vertebrates is determined by function and distribution of melanin, categorized as phaeomelanin and eumelanin, which are primarily regulated by interactions of the two main loci, agouti and extension. In this study we focused on analysis of the upstream region and a part of the coding sequence of the MC1R gene in three Iranian sheep breeds, Zandi, Baluchi and Zel, through molecular methods and in silico predictions. Random selection of the individuals from various populations with different coat colours was followed by genomic DNA and total RNA extraction and purification, PCR amplification, direct sequencing and genotyping through the standard methods. Complementary DNA synthesis and Semiquantitative RT-PCR were carried out while the SNPs were verified by sequence analysis. PCR-SSCP results of the 5′ UTR flanking region showed a clear banding pattern, in which the Eab was the most frequent pattern (67%) observed in the individuals with light coloured phenotypes, while the two homozygous patterns, Eaa and Ebb were rare in the populations. Sequencing the fragments revealed that the pattern is consistent with the -206G>A polymorphic site in the upstream region and four putative transcription binding sites close to this site were defined and characterised. The 12A>G and 51G>A transitions were also detected and verified as silent mutations in the 5′ end of the coding region, with synonymous effect on amino acid sequence. Semiquantitative RT-PCR of various phenotypes resulted in acquisition of the highest expression in the dark grey phenotype of the Zel sheep

Key Words: indigenous sheep, coat color variation, MC1R, transcription factor binding site, gene expression

WT21 Detecting selection footprints from production system-driven genomic divergence of South African sheep breeds. E. Dzomba*[1], M. Snyman[2], M. Chimonyo[1], and F. Muchadeyi[3], [1]*University of KwaZulu-Natal, Pietermaritzburg, KwaZulu-Natal, South Africa;* [2]*Grootfontein Agriculture Development Institute, Middelburg, Eastern Cape, South Africa;* [3]*Agriculture Research Council–Biotechnology Platform, Onderstepoort, Gauteng, South Africa.*

Niche production of speciality products such as wool, pelts and mutton and intense selective breeding have contributed immensely to sheep (*Ovis aries*) breed development in South Africa. Consisting of derivatives of native as well as exotic breeds introduced during eras of colonialism and pastoral agriculture, adaptability-focused and production-driven selective pressure have produced the phenotypic diversity and genomic footprints evident in the sheep population today. A genomewide scan using the OvineSNP50 Beadchip of 400 animals belonging to the Dorper (n = 23), Blackhead Persian (n = 14), indigenous Nguni (n = 30), Namaqua Afrikaner (n = 12) and Meatmaster (n = 48), Afrino (n = 51), four sub-populations of the Karakul-derived Swakara (n = 96) and three Merino-type breeds (n = 116) was performed. Ten Karakul sheep samples obtained from Halle Germany were also included in the analysis. The number of SNPs used in the analysis ranged from 34,006 to 46,477 in the Nguni and the Dohne Merino, respectively. The Nguni had the most monomorphic SNPs (13,316) followed by the South African (SA) Mutton Merino (9,824) and Namaqua Afrikaner (8,692) while the Meatmaster had the least number of SNPs of MAF = 0. High F_{IS} values above 10% were observed in the SA Mutton Merino (F_{IS} = 0.127); Dorper (F_{IS} = 0.167); Namaqua Afrikaner (F_{IS} = 0.154) and all Swakara sub-populations (F_{IS} > 0.14). Per population pairwise F_{ST} indicated highest divergence (F_{ST} > 0.30) between the Nguni and all other breeds except for the Dorper, Meatmaster and Namaqua Afrikaner. Per-marker F_{ST} analysis revealed outlier loci, presumably under selection, in various pairwise breed comparisons. Annotation of breed differentiating SNPs as well as gene and pathway analyses suggested selection pressures consistent with the diverse sheep production systems of South Africa. The study illustrates how the genomic architecture of sheep breeds in South Africa is responding to the combined impact of environmental differences and artificial selection for primary production traits and adaptability.

Key Words: selection footprints, genomewide SNPs, sheep breeds, production systems

WT22 Effect *ACACA*, *FASN* on fat acid and somatic cells in sheep— Preliminary results. Z. Sztankoova*[1], M. Borkova[2], A. Svitakova[1], J. Kyselova[1], and T. Kott[1], [1]*Institute of Animal Science, Prague, Czech Republic;* [2]*Institute of Dairy Research, Prague, Czech Republic.*

The aim of research study was to determinate effect of the genetic polymorphism at the ACACA and FASN locus on the milk traits including composition of fat acids and find out whether, there is any effect of these genes on somatic cells score. Acetyl-coenzyme A carboxylase α (*ACACA*) is a major regulatory enzyme of fatty acid biosynthesis and fat acid synthase (*FASN*) is the central enzyme of the *de novo* fatty acid biosynthesis pathway. The frequencies of alleles C and T at the FASN locus were of 0.88 and 0.12, respectively, and the genotype frequencies were 0.78, 0.20 and 0.02 for genotypes CC, CT and TT. The total genotype combination at ACACA locus were 18, from the most frequent combinations were: AGGGCCCC (0.28), followed AGGTCCCT (0.26) and AGGTGGCC (0.13). The dataset contained 440 sheep with 6411 records in time period between 2009–2017. The mixed model with repeatability was used to estimate the impact of polymorphism *FASN* and *ACACA* gene on milk production traits (milk yield, fat and protein percentage, somatic cell score) including composition of fat acid. The fixed effects were genotype, herd-year-season of lactation, age, age^2, breed, month of performance test and parity number, depend on evaluated trait. The random effect was the animal (repeated-measurements per sheep). Locus FASN (SNP at position 257C/T) was associated with protein and fat percentange and somatic cells score ($P \leq 0.05$). Locus ACACA was also associated with protein and fat percentange,milk yield and somatic cells score ($P \leq 0.05$). Statistical analysis confirmed association between genetic polymorphism of the ACACA and FASN locus and observed fat acid (PUFA, suma C18:1; C, suma C18:2; T (linoelaidová, C18:2n6t, cis-11, 14-eikosadienová (C20:2), α-linolenová (C18:3n3). Statistical analysis also confirmed association between somatic cell score and FASN and ACACA locus, resp. ($P \leq 0.05$). This work was supported by the na-

tional Agency of Agriculture of the Czech Republic (NAZV) project no. QJ1310107 and QJ 1510137.

Key Words: genetic marker, genetic polymorphism, fat acid, somatic cell score

WT23 Genetic investigation of sheep and goat families demonstrating the entropion eye condition. T. Hadfield* and N. Cockett, *Utah State University, Logan, UT, USA.*

A condition called entropion, reported in several mammalian species, results in the lower eyelid being inverted, causing the bottom eyelashes to rub on the cornea which can lead to blindness if not treated. This condition in sheep is commonly treated by unrolling the eyelid and surgically stapling it in correct alignment for a few weeks. Previous reports on entropion have indicated that it is genetically controlled. In this study, samples from seven paternal half-sibling families segregating for entropion were collected in 2014 to 2016. Four of the seven sires were born at the Utah State University sheep facility. Of these four rams, three were from a flock with high incidence of entropion and two were born with entropion. The final USU ram did not have entropion and was from a flock with no recorded entropion births in the last seven years. The other three rams were purchased and their eye condition at birth is unknown. Seventy-one (42%) of the 169 lambs produced by these seven rams were born with entropion. In an attempt to identify genetic regions involved with the entropion eye condition, genomic DNA was extracted from all lambs, sires and dams in the seven families and the DNA samples genotyped with the Illumina HD SNP chip. Analysis of the SNP genotypes and entropion was done using SNP & Variation Suite v8. (Golden Helix Inc.). Preliminary results indicated only marginal associations between the entropion condition and SNP markers across the genome. Additional lambs born in 2017, including lambs resulting from entropion by entropion matings, will be added to the analysis. Samples from a flock of Boer goats with a high incidence of entropion will be genotyped with the Caprine HD SNP chip and analysed for associations.

Key Words: ovine, caprine, entropion eye

WT24 DNA polymorphism of melanocortin 4 receptor gene in Bligon goat. L. Latifah[1], D. A. Priyadi[1], K. Kustantinah[2], and T. Hartatik*[1], [1]*Departement of Breeding and Reproduction, Faculty of Animal Science, University of Gadjah Mada, Yogyakarta, Indonesia;* [2]*Department of Animal Nutrition and Feed Science, Faculty of Animal Science, University of Gadjah Mada, Yogyakarta, Indonesia.*

Melanocortin 4 receptor (MC4R) are associated with growth traits and feed intake in mammals. MC4R gene plays a key role in the hypotalamic control of food intake and anergy balance. The aim of this research was to identify DNA Polimorphism of Melanocortin 4 Receptor gene in Bligon goat. Primer was designed based on alignment 12 GenBank. Primer PF:5′- TCGGGCGTCTTGTTCATCAT'3 and PR:5′- CAAGACTGGGCACTGCTTCA -'3 were used to amplify 642 bp of PCR product. We investigated 2 DNA polymorphism of MC4R in exon region (g.998 A/G and g.1079 C/T). In case of 998 A/G was changed of amino acid methionine (Met) to isoleucine (Ile). DNA sequence of MC4R gene in Bligon goats were used for restriction map of specific enzyme using Bioedit version 7.2.0. Based on rectriction mappping, *Kpn1* (G_GTAC'C) was detected to recognise SNP in region 1079 C/T. Rectriction enzyme *Kpn1* may be used for genotyping of targeted gene using PCR-RFLP method and it will be assosiated with growth traits and feed intake in Bligon goat in the future research.

Key Words: melanocortin 4 receptor (MC4R), Bligon goat, PCR, sequencing, restriction enzyme

WT25 Using genomic estimated breeding values to detect (cryptic) microevolution in body weight of a wild mammal. B. Ashraf*[1], J. Slate[1], J. Pemberton[2], C. Berenos[2], J. Pilkington[2], and S. Johnson[2], [1]*Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK;* [2]*IEB, University of Edinburgh, Edinburgh, UK.*

Understanding the maintenance of genetic variation in natural populations is one of the main goals of evolutionary quantitative genetics research. Soay sheep on the St Kilda archipelago, Scotland, have been the subject of a long-term individual-based study since 1985. Previous research has shown that despite selection favouring larger, heavier sheep, the average body mass of adult sheep has declined. Here we use genomic estimated breeding values (GEBVs) for bodyweight to resolve this paradox. Trait genetic architecture and GEBVs were estimated with the Ovine SNP50 BeadChip (Illumina) and 1080 sheep, by a Bayesian method that simultaneously estimates the contribution of all SNPs to phenotypic variation. We show that GEBVs have increased over the lifetime of the study, and that by creating gene-dropped simulated datasets, we show these trends are stronger than can be explained by genetic drift. Our results are robust to earlier concerns about the use of pedigree-derived EBVs to infer evolutionary trends. In summary, we confirm a previous suggestion of cryptic microevolution of bodyweight of Soay sheep.

WT26 Detection of selection signals between Merino and Churra sheep breeds. B. Gutierrez-Gil*[1], P. K. Chitneedi[1], A. Suarez-Vega[1], P. Wiener[2], C. Esteban-Blanco[1], and J. J. Arranz[1], [1]*Department of Animal Production, Faculty of Veterinary Sciences, University of León, León, Spain;* [2]*Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush Campus, Midlothian, UK.*

Selection affecting desired phenotypes has left detectable signatures of selection within the genomes of modern sheep. The aim of this study was the identification of selection signals related to wool production. To achieve this, we contrasted the OvineSNP50 BeadChip genotypes of 332 samples from three Australian Merino sheep populations, characterised by the production of extremely fine wool, and 378 samples of Spanish Churra sheep, a coarse wool sheep breed phylogenetically close to Merino. With the aim of identifying genomic regions harboring selection signatures in the two breeds, we performed four different analyses. In addition to genetic differentiation (F_{ST}) and reduced heterozygosity (*ObsHtz*) analyses, we also performed two complementary analyses based on haplotype structure using the *hapFLK* and *reHH* (*XPEHH* test) programs. We defined selection signature candidate regions (CR) by grouping the positions showing extreme values from the four approaches into discrete regions, based on the extent of linkage disequilibrium in the two breeds. Subsequently, convergence candidate regions (CCRs) were identified as those CRs that overlapped between the different approaches, i.e. to qualify as a CCR, overlap was required between a region identified by at least one of the methods based on allele/genotype frequencies (F_{ST}/*ObsHtz*) and at least one of the methods based on haplotype analysis (*hapFLK/XPEHH)*. The F_{ST} analysis identified a total of 49 CRs, whereas the *ObsHtz* analysis identified 96 and 72 regions in Merino and Churra respectively. The *hapFLK* and the XPEHH analyses identified seven and 98 significant (P-value < 0.001) selection sweeps regions, respectively. Overlap between these regions defined a total of 18 CCRs, on chromosomes 2, 3, 6, 8, 10, 11, 15 and 25. Five of the CCRs were related to positive selection in Merino while the rest were related to positive selection in Churra. Further study of genetic variation within these regions may help to identify candidate mutations underlying the selection signals reported here, some of which are expected to be related to the specialization of the Australian Merino for wool quality traits.

Key Words: sheep and related species, single-nucleotide polymorphism (SNP), selection scan, wool production

WT27 Introgression of wool-shedding genes into the Romane breed sheep. L. Drouilhet*[1], B. Pena[1], C. Huau[1], D. Marcon[2], Y. Bourdillon[2], and D. Allain[1], [1]*GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France;* [2]*INRA UE0322, La Sapinière, Bourges, France.*

Wool production in Europe became unprofitable compared to the meat or milk productions. One major reason is that the wool income is lower than the cost of shearing. The Romane breed, a French composite breed between the Berrichon du Cher breed (meat breed) and the Romanov breed (prolific breed), showed little ability to shed. We decided to introgress in the Romane breed the phenotype of wool-shedding of the Martinik Hair breed. Indeed, the Martinik Hair sheep have the ability to annually naturally wool-shed. The experimental trial of introgression was realised on 4 successive backcrosses from the Martinik Hair (MH) to the Romane breed (RM): F1 (MH*RM), then BC1 (F1*RM), BC2 (BC1*RM) and BC3 (BC2*RM). Two traits were considered: the ability to shed at least a part of the fleece or not, as a binary trait and the extent of shedding as the ratio of wool shed area to total body area. During those backcrosses, animals were measured and selected on their ability to shed at 7 months of age. The BC3 population represents the first generation (G1) of the introgressed population for the wool-shedding phenotype. This introgressed breeding stock (n = 150 ewes) was then selected using estimated breeding values based on shedding extension. A high heritability estimate (0.50 ± 0.09) and a large genetic gain (2.2 genetic standard deviations) on wool-shedding were observed after 6 generations of selection, without impairing the production fitness on the Romane sheep. We did not observe a bimodal distribution of wool-shedding extension phenotype, suggesting that not only one mutation is segregating in our population, but more probably a few major genes with large effects due to the large genetic gain observed. At the G6, 96 animals (9 family sires, 6 to 11 progeny per sire, 10 dams) with extreme phenotypes (total wool shedding or not) including some full-sibs and their dam were selected and genotyped on 50K SNP chip. This dataset is currently analysed using linkage analysis (LA), linkage disequilibrium (LD) and joint LD-LA mapping using QTLMAP software. The first results showed at least 3 different loci influencing the ability to shed on chromosome OAR3, OAR12 and OAR15. The analysis are in progress to precise those intervals of localization.

Key Words: sheep, genetic introgression, genome wide-association, wool shedding

WT28 Investigating genetic associations with meiotic recombination in rams. K. M. Davenport*, A. M. Rodriguez, R. J. Sawyer, T. M. Badigian, H. K. Jaeger, M. A. Follett, and B. M. Murdoch, *University of Idaho, Moscow, ID, USA.*

Meiotic recombination is an important process during gametogenesis that contributes to genetic variation. Understanding the process of recombination will lead to enhanced genetic predictions that will promote the sustainability of the sheep industry. It is clear from previous studies that recombination is not random, and at least one recombination event or crossover (CO) per chromosome arm is necessary for proper chromosome segregation. In addition, CO experience location preferences termed 'hotspots,' as well as interference in that one CO cannot occur in too close proximity with another. Previous studies in sheep have identified loci associated primarily in females with recombination rate derived from linkage data. Our objective was to investigate genetic associations with CO counts in rams acquired cytogenetically. We quantified the number of CO in Suffolk, Icelandic, and Targhee rams using a cytogenetic approach because it allows us to accurately identify all recombination events during meiosis without a large number of offspring. In total, we examined over 165,000 CO events from ~2,600 spermatocytes. We identified significant differences in CO number between individual rams within Suffolk, Icelandic, and Targhee breeds (P < 0.05), as well as differences between breeds (P < 0.01). Using the mean CO counts obtained from the spermatocytes from individual rams as a quantitative phenotype, we performed a genetic association study. The OvineSNP50 BeadChip was used to genotype rams and an association study was performed with PLINK v1.09. The results of the association study identified genomic regions of interest on chromosomes 1, 4, 6, 9, 14, 22, 23, and 24 after a Bonferroni correction (P < 1E-06). This study identifies potentially important genomic regions of interest associated with the number of CO in these rams. These data contribute important information towards the understanding of individual and breed recombination differences. Furthermore, this research will advance breed specific selection strategies that support the sustainability of the sheep industry.

Key Words: sheep, recombination, quantitative trait locus (QTL), genome-wide association, genetic improvement

WT29 Genomic regions associated with entropion in Columbia, Polypay, and Rambouillet breeds of sheep. M. R. Mousel*[1,2] and S. N. White[1,3], [1]*Animal Disease Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Pullman, WA, USA;* [2]*Paul G. Allen School of Global Animal Health, Washington State University, Pullman, WA, USA;* [3]*Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.*

Entropion is an inward rolling of the eyelid allowing the eyelashes and cornea to have direct contact, potentially causing abrasions which may lead to infections and blindness if not treated. Typically in domestic sheep entropion only occurs with the lower eyelid and is a congenital defect. Entropion has a wide frequency (0–80%) worldwide in domestic sheep, was found to be heritable (0.08–0.21) and it is speculated to be recessive in inheritance. To eliminate this condition from their flocks, producers must cull afflicted sheep and their parents. Identification of genomic regions or genes associated with entropion could lead to the development of genetic marker(s) to reduce entropion through selective breeding. Therefore, a genome-wide association scan was conducted with 473 Columbia, Polypay, and Rambouillet sheep genotyped using the Illumina OvineSNP600 BeadChip. Entropion status was recorded within 48 h of birth and corrected if present. The overall prevalence was 6.1% in these 473 sheep. Data was analysed using a mixed model with EMMAX that accounted for relatedness, breed, and SNP minor allele. Heritability was estimated to be 0.28. Five genome-wide significant ($P < 1\times10^{-8}$) SNP were identified on chromosomes 2, 3, and 15 as well as nine SNP that were genome-wide suggestive ($P < 1\times10^{-7}$) on chromosomes 2, 3, 7, 14, 15, 20 and 22. Previously, locations on ovine chromosome 2, 3, and 15 were found to be associated with entropion by Mousel *et al*., 2015 and/or Hadfield *et al*., 2016. We are working to narrow the range of these associated regions and identify the underlying casual mutations for the benefit of sheep producers.

Key Words: entropion, genome-wide association, domestic sheep, breeds

WT30 Community-based sheep breeding programs in Ethiopia resulted in substantial genetic gains. A. Haile*[1], T. Mirkena[3], G. Duguma[2], S. Gizaw[2], M. Wurzinger[4], J. Solkner[4], O. Mwai[2], T. Dessie[2], A. Abebe[6], M. Mamiru[8], T. Tadesse[7], R. N. B. Lobo[5], and B. Rischkowsky[1], [1]*International Center for Agricultural Research in the Dry Areas, Addis Ababa, Ethiopia;* [2]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, and Nairobi, Kenya;* [3]*FAO, Addis Ababa, Ethiopia;* [4]*BOKU University, Vienna, Austria;* [5]*EMBRAPA-goat and sheep, Sobral, Brazil;* [6]*Debre Berhan Agricultural Research Center, Debre*

Berhan, Ethiopia; [7]Bako Agricultural Research Center, Bako, Ethiopia; [8]Bonga Agricultural Research Center, Bonga, Ethiopia.

In small ruminants, centralized breeding schemes, entirely managed and controlled by governments – with minimal, if any, participation by farmers – were developed and implemented in many developing countries. Such programs have generally failed to sustainably provide the desired genetic improvements to smallholder livestock keepers. Community-based breeding Programs (CBBPs) have been suggested as an alternative and are being implemented in a few pilot countries. A team of international and national scientists designed and implemented sheep CBBPs in three sites/breeds (Bonga, Horro and Menz) in Ethiopia. The team developed an innovative methodological framework on how to design, implement and sustain CBBPs. Selection traits identified through participatory approaches were six month weights in all the three sites, and in Horro and Bonga, where resources, particularly feed and water, permit larger litter sizes, twinning rate was included. Eight years (2009–2016) performance data from the programs were analysed using WOMBAT (Meyer, 2007). The results indicate that the birthweight of lambs has not improved over the years in Menz and Bonga. In Horro, there is even a slight decrease. Given that we have not selected for birthweight in the community flocks we did not expect genetic change. However, there could have been an effect through correlated responses which was not the case in all the three breeds. Six months weight, the major selection trait in our CBBPs, increased over the years in all breeds. In Horro the average increase was 0.31 ± 0.060 kg per year, followed by average increase of 0.26 ± 0.058 kg per year in Bonga and 0.14 ± 0.006 kg for Menz. This is quite substantial in an on-farm situation. In Horro and Bonga sheep, where twinning rate was one of the selection traits, the litter size of lambs born increased over the years in both breeds: the increase was 12% (from 1.28 to 1.46) in Horro and 8% (from 1.48 to 1.61) in Bonga. This increase combined with the increased bodyweight has made a substantial impact on the incomes of the farmers. Our results show that CBBPs are technically feasible and result in measurable genetic gains in performance traits.

Key Words: sheep and related species, animal breeding, genetic improvement

WT31 Identification of two major genes affecting prolificacy in the French Noire du Velay sheep. L. Chantepie*, L. Bodin, F. Woloszyn, J. Sarry, and S. Fabre, *GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France.*

Classical selection methods of prolificacy in sheep assume a full polygenic inheritance, each gene having an infinitesimal effect. However, in the last decades, many mutations having a major effect on prolificacy were discovered in four major fecundity genes namely *BMP15 (FecX)*, *BMPR1B (FecB)*, *GDF9 (FecG)* and *B4GALNT2 (FecL)*. When present, these mutations should be taken into account to obtain relevant prolificacy breeding values for the selection process. Based on litter size records (LS), we suspected the segregation of such major gene in the French Noire du Velay (NdV) sheep population. The first approach was to genotype selected highly prolific ewes for the already known mutations at the four major loci. We evidenced the segregation of the $FecL^L$ mutation in the *B4GALNT2* gene originally discovered in the Lacaune breed. By a specific genotyping of 1224 NdV ewes, we observed 10% of $FecL^L$ carriers with a mutated allele effect of +0.4 lambs/lambing. Nevertheless, it still existed extremely prolific NdV ewes non-carrier of the $FecL^L$ allele. Then in a second approach, we genotyped 150 $FecL^+$ NdV ewes using the ovine 50k SNP array, followed by an association analysis under a case (n = 100, mean LS 2.2) *v.* control (n = 50, mean LS 1.1) design. A unique significant signal was located on the X chromosome (53.8Mb), near the *BMP15* candidate locus. The whole genome sequencing of 3 ewes heterozygous, homozygous carrier and non-carrier of the supposed prolific allele, identified a novel A>T SNP 290bp upstream of the *BMP15* gene. Using a specific RFLP test, we genotyped 756 $FecL^+$ NdV ewes and evidenced 125 ewes as carriers of the new polymorphism (16%). We observed that this variant increased significantly the prolificacy by +0.2 lambs/lambing. By real-time PCR analysis, we showed that the A>T nucleotide change was associated to a decrease of the oocyte-specific expression of the *BMP15* gene. According to the nomenclature of *Fec* genes, this new mutation was named $FecX^N$. In conclusion, by a combination of candidate gene and whole genome scan approaches, we identified $FecL^L$ and $FecX^N$ mutations essential for improving the genetic evaluation and selection of prolificacy in Noire du Velay sheep.

Key Words: sheep, genome-wide association, monogenic trait, reproduction, genetic improvement

WT32 Genome-wide scan reveals *NF1* locus is associated with fat tail phenotype rather than high-altitude adaptation in Asian sheep. K. Dong[1,2], M. Yang[1], N. Gorkhali[1], Y. Ma[1], and L. Jiang*[1], [1]*Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China;* [2]*USDA, Agricultural Research Service, Avian Disease and Oncology Laboratory, East Lansing, MI, USA.*

The main morphological changes such as tail length and distinct patterns of tail fat deposition are considered to be some of the major changes that followed domestication. However, nowadays, a decrease in the size of sheep fat-tail is desirable for both producers and consumers. To identify genes underlying the ovine fat tail phenotype, we performed a genome-wide scan for the highly differentiated loci between seven thin-tailed sheep breeds from Asia and six fat-tailed breeds from Middle East using Illumina Ovine 50KSNP Beadchip. By combing an independent dataset of fat-tailed and thin-tailed Chinese indigenous sheep breeds, we found a total of 90 common SNPs corresponding to 251 genes. Among those were several genes known to be involved in lipid metabolic process, including *RAB7A*, *ENPP6*, *PLA2GA*, *INSIG2*, *PEX2*, *BMP2*, *VDR* and etc. GO analysis showed one of the most significant GO categories was 'lipid glycosylation' (P = 9.33E-4). Intriguingly, we found that the locus of *NF1* (containing *NF1* and three small genes, *EVI2A*, *EVI2B* and *OMG*), previously linked to the high-altitude adaptation, displayed the highest differentiation across the ovine genome. Our study demonstrated that sheep living at different altitudes showed no evidence of divergence at this locus and therefore, *NF1* locus is likely the predominant factor for the fat tail phenotype, rather than high-altitude. This finding suggested caution in drawing a conclusion in genomic studies without excluding the potential confounding factors. Additionally, by combining re-sequencing data for the *NF1* locus and RNA-sequencing (RNA-seq) data of adipose tissues from divergent tail types, we found that the potentially functional genes within the *NF1* locus were *EVI2A* and *OMG*, rather than *NF1*. Furthermore, two missense mutations at the well-conserved positions in *EVI2A* and *OMG* genes were identified in fat-tailed sheep. As the function of *EVI2A* and *OMG* are currently little known, the two missense mutations represent a unique opportunity to study the function of these two genes in an organismal context, especially for the potential novel roles in fat deposition.

Key Words: fat-tailed, genomic selection signature, sheep, NF1

WT33 Diversity of sheep breeds in Russia based on SNP analysis. T. Deniskova*[1], A. Dotsev[1], M. Selionova[2], K. Wimmers[3], H. Reyer[3], V. Kharzinova[1], E. Gladyr[1], G. Brem[1,4], and N. Zinovieva[1], [1]*L.K. Ernst Institute of Animal Husbandry, Podolsk, Moscow region, Russia;* [2]*All-Russian Research Institute of Sheep and Goat, Stavropol, Stavropol region, Russia;* [3]*Institute of Genome Biology, Leibniz Institute for Farm Animal Biology, Dum-*

merstorf, Germany; [4]Institute of Animal Breeding and Genetics, VMU, Vienna, Austria.

Due to a huge territory, Russia is characterised by great variety of climate and relief conditions. The combination of these features led to creation of wide range of sheep breeds with high adaptive capacity to local environmental conditions and variety of productivity qualities. High-throughput SNP arrays allowed to investigate genetic traits of animals at the genome level and to understand more fully the character of relationships between breeds. Nevertheless, such a detailed study has not been performed up to now on Russian sheep breeds. In this regard, the aim of our work was to evaluate the genetic diversity and to study genetic relationships between the most widespread sheep breeds in Russia using whole-genome analysis. We have genotyped 411 individuals from the most popular sheep breeds in Russia, including 11 coarse wool (CW, n = 205), 5 semi-fine wool (SFW, n = 78) and 9 fine wool breeds (FW, n = 128) by using Illumina OvineSNP50 BeadChip. Statistical indicators were calculated in PLINK 1.07, GENETIX 4.05, HP-Rare 1.1. The lowest level of genetic diversity was found for CW group (Ho = 0.374; Ar = 1.89), whereas the FW and SFW groups showed similar levels of observed heterozygosity (Ho = 0.383…0.385) and allelic richness (Ar = 1.92). Among all the breeds the maximum measures of diversity were detected for Baikal fine wool (Ho = 0.394; Ar = 1.93), whereas minimum values were estimated in Romanovs (Ho = 0.350; Ar = 1.86). The breeds were characterised by insignificant heterozygote excess from 1.1% in the SFW group to 1.6% in the CW and the FW groups. Phylogenetic tree showed that breed`s distribution corresponded to their wool type. Thus, the CW breeds formed distant cluster from FW and SFW and had more branchy structure. The most CW originated from unique local sheep whereas the SFW were created with using English Long Wool rams, and Australian Merino improved the FW. The whole genome SNP data on sheep breeds is the first step to design effective selection and conservation programs for local breeds. Besides introduction of new methods, this will lead to prosperity of Russian sheep breeding. The research was funded by Russian Scientific Foundation (14–36–00039).

Key Words: sheep, biodiversity, single nucleotide polymorphism

Comparative and Functional Genomics

WT34 Maternal nutrient restriction in early gestation upregulates myogenic genes in cattle fetal muscle tissue. A. K. Ward*[1], M. S. Crouse[1], R. A. Cushman[2], K. J. McLean[3], C. R. Dahlen[1], P. P. Borowicz[1], L. P. Reynolds[1], and J. S. Caton[1], [1]*North Dakota State University, Fargo, ND, USA;* [2]*USDA-ARS-MARC U.S. Meat Animal Research Center, Clay Center, NE, USA;* [3]*University of Kentucky, Lexington, KY, USA.*

Prenatal myogenesis is a critical factor in determining the muscle growth potential of cattle. We hypothesised that maternal nutrient restriction during early gestation would alter the transcriptome of fetal primordial muscle tissue in cattle. A total of 14 Angus-cross heifers were oestrus synchronized and assigned at breeding to one of two dietary treatments (CON- 100% of nutrient requirements; RES- 60% of CON). At d 50 of gestation heifers were ovariohysterectomized, and fetal muscle tissue from the hind limb was dissected and flash frozen. RNA was extracted and RNA-seq analysis was conducted on the Illumina HiSEqn 2500 platform using 50-bp paired-end reads at a depth of 2 × 10.4M reads/sample. Transcriptome analysis was performed in collaboration with USDA-ARS-MARC using the Tuxedo Suite, and KEGG pathways were analysed with DAVID 6.8. A total of 317 genes (P < 0.01) were used for pathway analysis, of which 92 were false discovery rate protected (q < 0.10). Within the fetal muscle KEGG cluster, 22 genes were identified as differentially expressed with all but three of being up-regulated in RES. These include the myogenic genes *MYOG* and *MYOD1* (1.49 and 1.39 fold greater than CON, respectively), both of which play important roles in skeletal muscle cell differentiation and fibre development. Four members of the Wnt signalling pathway, namely *WNT5A*, *FZD1*, *APC2*, and *FZD10*, were up-regulated in RES fetuses (1.32–2.11 fold greater than CON). The Wnt pathway is critical in promoting the differentiation of myocytes from progenitor stem cells. Additional genes up-regulated in RES include members of the troponin (*TNNC1, TNNC2, TNNI1, TNNI2, TNNT1, TNNT2*, *TPM2*), myosin (*MYL1, MLY2, MLY4, MLY7, MYL6B, MLY9, MYH8, MYLPF*), and actin (*ACTA1, ACTA2, ACTG2*) families. These data support our hypothesis that moderate maternal nutrient restriction within the first 50 days of gestation alters the fetal muscle transcriptome, specifically up-regulating myogenic genes in RES fetuses. Therefore we conclude that early gestation is an important period of myogenic developmental programming in cattle. USDA is an equal opportunity provider and employer.

Key Words: cattle and related species, RNA-seq, development, nutrigenomics, muscle

WT35 Altered gene expression of the appetite regulation pathway in low-birth-weight piglets. M. Vázquez-Gómez*[1], C. García-Contreras[2], R. Benítez[2], Y. Nuñez[2], A. Fernández[2], A. Gónzalez-Bulnes[2], B. Isabel[1], and C. Óvilo[2], [1]*UCM, Madrid, Spain;* [2]*INIA, Madrid, Spain.*

Intrauterine growth restriction (IUGR) causes Low Birth-Weight (LBW) offspring due to inadequate maternal nutrition and/or placental efficiency. In Pig Production, LBW piglets are a problem because of a higher perinatal morbidity and mortality than Normal Birth-Weight (NBW) piglets. The IUGR process is related to asymmetrical development to preserve the growth of vital organs, like the brain which regulates essential functions to survive, at the expense of others, but this does not guarantee a normal development. The aim of this study was to evaluate the effects of birthweight and sex on the expression of genes involved in energy balance, with anorexigenic (*LEPR, INSR, POMC, CART, MC4R*) and orexigenic (*NPY, AGRP*) roles in newborn Iberian piglets. After birth, 40 LBW piglets (20 males and 20 females; 626 ± 152 g bodyweight) and 26 NBW piglets (13 males and 13 females; 1367 ± 313 g bodyweight, P < 0.0001) of Iberian × Duroc genotype, were obtained from 56 multiparous (3th and 4th pregnancy) Iberian sows. Newborns were weighed, classified according to their weight in NBW or LBW and sacrificed. Hypothalamic samples were used for total RNA extraction using RiboPure kit and gene expression was measured by RT-qPCR. Differential expression conditional on weight and sex was tested by a linear mixed model and fold changes (FC) were calculated. Regarding the IUGR effect, LBW piglets had lower expression levels of *AGRP*, but higher of *NPY* (P < 0.05, for both) although both genes are involved in food intake stimulation. These effects are significant in females, with *AGRP* showing 0.36X down-regulation (P < 0.005) and *NPY* showing 2.87X up-regulation in LBW (P < 0.05). On the other hand, LBW males had lower *POMC* gene expression (FC = 0.31X) than NBW males (P < 0.05), which suggest a down-regulation of anorexigenic signal in LBW males. High levels of orexigenic genes, which increase the appetite, are normal in newborns, and an increase of these peptides in IUGR could be expected due to prenatal programming. Our results indicate a different hypothalamic transcriptional response to the IUGR

process between sexes. Females showed an interesting and unexpected response with opposite effects on orexigenic genes.

Key Words: Iberian pig, functional genomics, qPCR, gene expression, energy balance

WT36 Machine learning based model selection strategy for powerful and efficient genomic prediction. L. Yin[1,2], X. Zhou[3,4], Y. Ma[1,2], M. Zhu[1,2], X. Li[1,2], X. Liu*[1,2], and S. Zhao[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding, and Reproduction, Ministry of Education; Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture; Huazhong Agricultural University, Wuhan, Hubei, China;* [2]*The Cooperative Innovation Center for Sustainable Pig Production, Wuhan, Hubei, China;* [3]*Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA;* [4]*Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA.*

The advances of high-throughput sequencing technologies have dramatically reduced the cost of genotyping and have made genomic prediction (selection) widely applied in animal and plant breeding programs, as well as in human genetics. GBLUP (Genomic Best Linear Unbiased Prediction), owing to its computational efficiency, becomes one of the most popular genomic prediction methods. However, GBLUP assumes that all available markers have the same effect-size distribution, which is used further to build the random effect term (K) that reflects genetic similarities between pairs of individuals. The assumption limits the prediction accuracy, especially in cases where objective traits are controlled by several major genes. In contrast, Bayesian methods assume that variants underlying a trait may have large, moderate, small, or zero effect, where the effect sizes are treated as unknown parameters that are obtained by a MCMC (Markov Chain Monte Carlo) procedure. Although Bayesian methods outperform GBLUP in terms of prediction accuracy in most cases, MCMC procedure takes a long time and cannot be paralleled in theoretical, which limits the application of Bayesian methods in practice. Inspired by the computational efficient of GBLUP and high prediction accuracy of Bayesian methods, we propose a new method named MRBLUP (Multi-loci Regressed Best Linear Unbiased Prediction), which incorporates pseudo QTNs (Quantitative Trait Nucleotides) as fixed effects and a trait-specific K matrix as random effect in a mixed linear model. Both pseudo QTNs and trait-specific K matrix are optimized using a parallel-accelerated machine learning strategy. We compared MRBLUP with GBLUP, Bayes B, BayesC, BayesLASSO, BSLMM, and BayesR using 3 public datasets that including human (WTCCC1, 7 diseases), cattle (German Holstein, 3 traits), and 3 simulated traits (16th QTL-MAS workshop). MRBLUP has higher or similar prediction accuracy in 12 traits and 1 trait, respectively. In addition, taking the advantages from parallel acceleration, MRBLUP is computationally efficient. Using 10 CPUs, a dataset that contains 5 thousand individuals and half a million markers can be analysed in 1 h by MRBLUP while Bayesian methods take ~100 h.

Key Words: MRBLUP, genomic selection, genomic prediction, machine learning

WT37 Characterization of perirenal fat transcriptome from suckling lambs. A. Suárez-Vega*[1], J. Arranz[1], J. Mateo[2], and B. Gutiérrez-Gil[1], [1]*Department of Animal Production, Faculty of Veterinary Sciences, University of León, León, Castilla y León, Spain;* [2]*Department of Food Science and Technology, Faculty of Veterinary Sciences, University of León, León, Castilla y León, Spain.*

Suckling lamb meat is a valuable secondary product from Mediterranean sheep dairy farms due to its high edible quality. Suckling lambs are raised exclusively on maternal or artificial milk from birth to slaughter (19–22 days) and their meat is highly appreciated by its palatability tenderness and juiciness. Total lipids and fatty acid composition contribute significantly to the latter mentioned quality aspects of suckling lambs meat. The aim of this study is characterising the perirenal fat transcriptome of suckling artificially reared lambs. To that goal, RNA was extracted from perirenal fat collected from seven male Assaf lambs at slaughter (age: 17–23 days; weight: 9.03–17.64 kg). Through massive sequencing with an Illumina Hi-SEqn 2000 sequencer a total of 307.11 million reads pairs were generated. The quality of our reads was assessed with FastQC and they were mapped against the Sheep Genome (Oar_v3.1) using the STAR aligner. The percentage of unique aligned reads ranged between the 60.84% and the 74.08%. Using specific tools from the software Cufflinks for normalization and quantification of gene transcripts we detected a total of 17,791 expressed genes (>0.01 FPKM in one sample). Of these, the 633 highly expressed genes (>180 FPKM) were considered as core genes of the perirenal fat transcriptome and were used for the KEGG functional enrichment analysis. Among the 32 enriched pathways, the highest enriched pathways for these genes were related to energy metabolism such as 'Oxidative phosphorylation', 'Citrate cycle (TCA cycle)', 'Glyoxylate and dicarboxylate metabolism', 'Glycolysis / Gluconeogenesis', etc. We also found other pathways related to fat metabolism such as 'PPAR signalling pathway', 'Glycerolipid metabolism', 'Biosynthesis of unsaturated fatty acids'. The genes involved in these pathways may be considered as candidate genes underlying some of the specific features related to palatability and fatty acid content that characterizes suckling lamb meat.

Key Words: sheep and related species, RNA-seq, gene expression, fat/lipid, meat production

WT38 Whole blood RNA-Seq profiling of early lactation dairy cows of diverse fertility clusters. M. Salavati*[1] and G+E Genotype Plus Environment Consortium[2], [1]*Royal Veterinary College, London, UK;* [2]*Names and addresses are listed on the GplusE website: http://www.gpluse.eu/index.php/project/partners/.*

Poor fertility, often associated with negative energy balance and/or uterine inflammation in early lactation, is a major concern to the dairy industry. We compared the transcriptomic profiles of white blood cells (WBC) from multiparous Holstein Friesian cows with differing fertility related phenotypes. Blood samples (47 cows, 3 herds) were taken at 14 days in milk (DIM) and WBC processed for total RNA-Seq using Illumina NextSEqn 500 platform. Libraries were sequenced at 75 nt length single end reads to reach average 30 million reads/sample. Fastq raw files were quality controlled and mapped on *Bos taurus* UMD3.1.1 (Ensemble87 gene tracks). Phenotype data were captured as follows: calving ease, body condition score (BCS) at calving, BCS and circulating IGF1 at 14 and 35 DIM, change in BCS and IGF1, total milk yield 0–50 DIM (TMY) and fertility (days to first service, DFS) and conception categorised as in calf by <100, 100–200 or >200 DIM (ICB). Component analysis was performed in R. Residuals were calculated based on REML prediction of phenotypes with diet nested within herd as a random effect. K-mean clustering over centre scaled phenotypes produced significantly discriminant fertility clusters. Differentially expressed genes (DEG) between clusters were identified using Cufflinks. The three fertility clusters contained 16, 22 and 9 cows, with 211 DEG overall. There were 97 DEG between the clusters with best and worst fertility (#3: shortest ICB, lowest DFS, highest BCS gain, least TMY and highest IGF1 at 14 DIM *v.* #2: longest ICB, lowest BCS and medium TMY). Gene set ontology enrichment analysis showed involvement in four main pathways and functions: Platelet degranulation and blood coagulation (*R-BTA-114608, CD9, ECM1, PPBP, VWF, PROCR*); C-Lectin and sugar binding *(R-BTA-114608, GO:0030246);* Innate immunity *(R-BTA-936440, FOXO signalling, CLECT, R-BTA-1433557)* and Regulation of defence response to virus (*R-BTA-1169408, ISG15, HERC5, IL15, MB21D1)*. These data suggest that fertility outcome is related to the functionality of circulating WBC, which can influence key inflammatory processes

(e.g. uterine involution, ovulation) when recruited to the reproductive tract.

Key Words: RNA-Seq, fertility, cattle

WT39 Comparative genomic identification and functional characterisation of β-defensin genes in the *Ovis aries* genome. T. J. Hall*[1,2], C. McQuillan[1], E. Finlay[1], C. O'Farrelly[3], S. Fair[4], and K. G. Meade[1], [1]*Animal & Bioscience Research Department, Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co Meath, Ireland;* [2]*Vet sciences centre, School of agriculture, UCD, Dublin, Ireland;* [3]*Comparative Immunology Group, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland;* [4]*Laboratory of Animal Reproduction, School of Natural Sciences, Faculty of Science and Engineering, University of Limerick, Limerick, Ireland.*

β-defensins are small, cationic, antimicrobial peptides discovered in species across the plant and animal kingdoms with roles in immunity and reproduction. However, β-defensin genes in domestic sheep have been poorly annotated, with genes identified only by automatic gene prediction algorithms. The objective of this study was to use a comparative genomics approach to identify and functionally characterise the β-defensin gene repertoire in sheep. Results: All 57 currently predicted bovine β-defensin genes were used to find orthologous sequences in the most recent version of the sheep genome. Forty-three genes were found to have close genomic matches between sheep and cattle. The orthologous genes were located in four clusters across the genome, with 4 genes on chromosome 2, 19 genes on chromosome 13, 5 genes on chromosome 20 and 15 genes on chromosome 26. Conserved gene order was apparent for the β-defensin genes in the two smaller clusters. Complete conservation of gene order was observed for chromosome 13 β-defensin orthologs. More structural differences were apparent between chromosome 26 genes and the orthologous region in the bovine reference genome, which is known to be copy-number variable. The Defensin-β 1 gene matched to eleven Bovine Neutrophil β-Defensin genes on chromosome 27 with almost uniform similarity, as well as to tracheal, enteric and lingual anti-microbial peptides suggesting that annotation of the bovine reference sequence is still incomplete. Thirty-four of the genes representing each of the four clusters were profiled in the male reproductive tract using qPCR. Distinct site-specific and differential expression profiles were detected across the reproductive tract of mature rams with preferential β-defensin gene expression in the epididymis, recapitulating observations for orthologous genes in other species. This is the first comprehensive analysis of β-defensin genes encoded by the ovine reference sequence, and the first report of an expanded repertoire of β-defensin genes in this species. The preferential expression of these genes in the epididymis suggests a role in fertility and possibly immunoprotection of sperm within the female reproductive tract.

Key Words: bioinformatics, sheep, fertility, qPCR

WT40 Modulating the genetic expression of canine mesenchymal stem cells (MSCs) on two types of scaffolds: Contact lenses and equine amniotic membrane. A. R. Remacha*[1], A. L. Ortillés[2], A. de Torre[2], M. E. Lebrero[2], P. Zaragoza[1], and C. Rodellar[1], [1]*LAGENBIO, Departamento de Anatomía, Embriología y Genética Animal, Facultad de Veterinaria, Universidad de Zaragoza, Zaragoza, Spain;* [2]*Departamento de Patología Animal, Área de Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad de Zaragoza, Zaragoza, Spain.*

Eye injuries suppose a very common disease in dogs. Although the eye has its own repair mechanisms, they become disrupted at times; so developing innovative therapies that could contribute to the treatment of this disease would be of great interest. Regenerative medicine is a new field of medicine that is getting promising results in the treatment of a wide range of disorders in different species, including canine species. Mesenchymal Stem Cells (MSCs) represent an excellent tool to improve the healing of corneal ulcers. MSCs are capable of self-renewal, differentiation along multiple cell lineages and immunomodulation ability. This way, they contribute to the treatment of canine ocular pathologies, thus achieving tissue regeneration, which maintains the properties of the original tissue. The main difficulty when using MSCs to treat canine ocular diseases, such as corneal ulcers, is their application and maintenance in the injured area. To solve this handicap, organic or inorganic scaffolds can be used. However, organic scaffolds such as amniotic membrane need to be sewn to the injured area. These surgical procedures require general anaesthesia in usually aged patients in which anaesthesia is an additional risk. Instead of exposing the animal to a surgical procedure, contact lenses imply a noninvasive alternative, which can be applied without surgery. In this study, MSCs behaviour was analysed in two types of scaffolds: contact lenses and equine amniotic membrane. We studied the effect of both scaffolds on the gene expression profile of *CD90, CD105* and *CD73* by RT-qPCR assay, which confirming the mesenchymal origin of the cells. Moreover, the expression of the cell surface adherence markers *ICAM1* and *VCAM1* was also analysed. Adhesion capacity and proliferation on both scaffolds was assessed by fluorescent staining of the MSCs cultured on contact lenses and on equine amniotic membrane, respectively. MSCs showed adhesion capacity and proliferation on both types of scaffolds and also a positive expression of specific MSCs markers, thus confirming their mesenchymal origin.

Key Words: mesnchymal stem cells, canine, ocular diseases, contact lenses, equine amniotic membrane

WT41 A simple, cheap and universal nucleic acid integrity assay based on the ubiquitin C gene (UBC). M. Van Poucke* and L. Peelman, *Ghent University, Merelbeke, Belgium.*

Nucleic acid integrity assessment is an important aspect of quality control for many applications in molecular biology. Several methods exist (electrophoresis-, microfluidics- or PCR-based), but they are not universally applicable. Some of them need huge amounts of input, expensive equipment, only work specifically on (c)DNA, (m)RNA, certain species or certain tissues, or produce fragments covering a small length range. We investigated if the ubiquitin C gene (UBC) could be used to develop a simple, cheap and universal integrity assay. UBC gene analysis (in human, mouse, pig, cow, horse, sheep, dog and cat) shows that UBC is a highly conserved and widely expressed gene (reference gene in RT-qPCR), that encodes a polyubiquitin precursor (containing at least 5 ubiquitin monomers of 228 bp) in a single exon. On average, ubiquitin monomers show a nucleic acid sequence identity of around 96% at intraspecies level and around 93% at interspecies level. Based on a multiple alignment of all monomer ubiquitin sequences of all investigated species, we could design a single degenerated primer pair generating PCR amplicons of 137, 365, 593 and 821 bp on low amounts of high quality DNA of all investigated species and on cDNA reverse transcribed from high quality RNA from different tissues (e.g. heart, intestine, liver, kidney, cerebellum). Increasing levels of nucleic acid degradation resulted in a decrease of amplification products starting with the longer amplicons. We conclude that UBC was suited to develop a simple, cheap and universal assay to estimate the level of integrity of nucleic acids.

Key Words: multispecies, conservation, PCR, integrity

WT42 Effect of fetal genotype and weight on muscle transcriptome in Iberian pigs. C. García-Contreras[1], O. Madsen[2], M. Vázquez-Gómez[3], S. Astiz[1], R. Benítez[1], Y. Núñez[1], A. Rey[3], B. Isabel[3], M. Groenen[2], A. González-Bulnes[1], and C. Óvilo*[1],

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The Iberian pig breed is characterised by higher adipogenic potential and lower lean growth than commercial breeds. Also, Iberian pigs are more susceptible to changes in maternal nutrition, with growth and tissue composition being highly dependent on genetic background and diet, even at early developmental stages. The aim of this study was to evaluate the transcriptomic profiles of the *longissimus dorsi* muscle of pure Iberian and crossbred fetuses, with different bodyweight values, in order to identify genes and pathways involved in their phenotypic differences. To achieve this goal, we used Iberian sows, inseminated with heterospermic semen (from Iberian and Large White boars). At day 77 of gestation, 51 fetuses were obtained and 32 (16 from each genotype, and selecting individuals with extreme bodyweight values in each genotype), were selected for the study of *l. dorsi* transcriptome by RNAseq. The results showed that 368 genes were differentially expressed (DE) between genotypes, of which 303 showed higher expression in Iberian (FC = 2.9 to 835; $q < 0.05$) and 65 in crossbred fetuses (FC = 2 to 857; $q < 0.05$). DE genes were related to lipid metabolism or glycolysis (such as *PFKFB3* and *APOD*, overexpressed in crossbreed fetuses); response to excess diet, catabolic processes of cholesterol, adipogenesis or lipid transport (such as *APOE* or *PPARG*, overexpressed in Iberian fetuses). DE results were employed to infer transcriptional regulators, and several were predicted to be activated in muscle from pure iberian piglets and potentially involved in the transcriptome differences, such as *CEBPA*, *CEBPB*, *HNF1A* or *HNF4A*. The observed DE of genes related to lipid metabolism are consistent with the significantly higher total blood cholesterol ($P < 0.01$) and blood LDL level ($P < 0.05$) observed in pure Iberian fetuses. The transcriptome comparison between individuals with extreme bodyweight values yielded 26 DE genes in Iberian fetuses; while in crossbreds 73 DE genes were found. The identified genes indicate differential regulation of relevant functions and pathways mainly involved in lipid metabolism and muscle development, which may explain the phenotypic differences among the compared groups.

Key Words: RNA-seq, growth and development, iberian pig, functional genomics, transcriptome

WT43 RT-qPCR to analyze the expression of oligosaccharides metabolism genes (*ST3GALT1*, *ST6GALT1*, *LALBA* and *B4GALT1*) during goat lactation. A. Crisà*, C. Marchitelli, and B. Moioli, *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA), Animal Production Research Centre, Monterotondo (RM), Italy.*

Milk is a natural source of prebiotics in the diet of mammals during infancy and the sialylated oligosaccharides play crucial roles in many biological processes. Goats produce milk containing free oligosaccharides (OS) at much higher levels than cattle or sheep, specifically 3′sialyllactose (3′-SL) and 6′sialyllactose (6'-SL). The production of oligosaccharides is genetically determined by the expression of glycosyltransferases and the availability of sugar nucleotide substrates, but the precise mechanisms regulating differential expression of milk oligosaccharides are not known. The aim of this work was to study the expression of 4 candidate genes involved in synthesis of lactose and sialylated oligosaccharides (*B4GALT1, LALBA*, *ST3GALT5*, *ST6GALT1*). To this purpose we used milk somatic cells samples corresponding at 4 lactation stages (colostrum, day1, day 60, day 120) of 6 Maltese and 6 Garganica goats. Total RNA was extracted by using TriReagent (Sigma) and DNAse treated. RNA quality and quantity were assessed before to proceed with reverse transcription and qPCR. Expression analyses, normalized with two reference genes selected by geNorm (*ATP5B, SDH*), were performed by using the qBase^{PLUS} software. Least Square means of breed and lactation stages were calculated using Proc GLM in SAS. The results showed differential expression patterns both between genes as well as lactation stages. *LALBA* and *ST3GALT5* were the most expressed genes and showed a statistically significant increase from colostrum to day 1 and 60. A statistically significant difference between breeds was observed for *B4GALT1* and *ST3GALT5* expression in colostrum with higher level found in Garganica; moreover the expression of these genes is correlated in both breeds (b = 0,76; $P = 0{,}0041$). The increased *LALBA* expression that we found starting from colostrum stage is consistent both with an increase of milk protein output and with lactose synthesis. The higher expression of *ST3GALT5* respect to *ST6GALT1* corresponds to what reported in literature of a higher concentration of 3′-SL compared to 6'-SL in goat colostrum and milk.

Key Words: oligosaccharide gene, goat colostrum, goat milk, ST3GALT5, ST6GALT1

WT44 Building the sequence map of the Caprini pan-genome and their ancestor genome. Y. Jiang*, R. Li, S. Gao, Y. Zhao, and W. Fu, *College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China.*

The tribe Caprini, including genus *Ovis*, *Capra*, *Pseudois* and *Ammotragus*, is of particular interest for studying the adaptation to the harshest terrestrial habitats, such as snowy mountainous regions and subtropical deserts. We have generated *de novo* genome assemblies of four wild Caprini species including *Pseudois nayaur*, *Ammotragus lervia*, *Capra ibex* and *Ovis ammon*. Together with the other six available Caprini assemblies (ARS1 and CHIR_2.0 of domestic goat, Oar4.0 of domestic sheep, CapAeg1.0 and Caeg1 of wild goat, Ooril of wild sheep), a Caprini pan-genome was constructed, comprised of ~2.5 Gb core sequences and ~600 Mb dispensable sequences. Among the dispensable sequences, we identified ~56 Mb and 45 Mb sequences respectively specific to genus Capra and genus Ovis based on our pan-genome and over a thousand re-sequencing data from wild/domestic goat and sheep samples. The common ancestor genome of Caprini species (~six million years ago) was also constructed. The frequency of dispensable sequences intersecting with generic regions demonstrates a clear population specific pattern fitting well with their geographical boundaries. Notably, we found dozens of highly divergent alleles characterised by varied length and gene contents in goats and sheep respectively some of which are related with crucial biological functions including host immune response (MHC region) and high altitude adaption (β-globin and MYADM). In summary, our results have revealed abundant presence/absence variations within the tribe Caprini, and summarised the general pattern of their distribution in diverse Caprinae species. The Caprini pan-genome demonstrates a great advantage over a single reference assembly in exploring genomic variations and expands our understanding of environment adaptation associated with gene gain and gene loss.

Key Words: pan-genome, sheep and goat, tribe Caprini, adaptation, divergent alleles

WT45 Late fetal blood transcriptomic approach to get insight into biology related to birth survival. L. Liaubet*[1], V. Voillet[1], Y. Lippi[2], N. Iannuccelli[1], C. Lascor[1], Y. Billon[3], M. San Cristobal[1], and L. Canario[1], [1]*GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France;* [2]*Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, Toulouse, France;* [3]*INRA UE1372 GenESI, Surgères, France.*

In recent decades, improvement of prolificacy and body composition has been accompanied by a substantial increase in the mortality of piglets before weaning. The most critical period is the perinatal period, mostly during the first 24–48 h following birth. The maturity of piglets, defined as the state of full development for survival at birth, is an important determinant of early mortality. The

objective of our project is to take advantage of current knowledge about two pig breeds, Large White (LW) pigs selected for prolificacy and body composition and Meishan (MS) pigs being more robust. Maturity of several tissues and metabolite profiles of various fluids are analysed on the fetuses (LW, MS and reciprocal F1) at day 90 or 110 of gestation (birth at day 114). Here we presented the transcriptomic analysis done on total blood samples (N = 63). Microarray analysis identified 265 differentially expressed genes for the interaction between gestational age and genotype and revealed differential pathways for mitochondrial ATP synthesis, negative regulation of cell proliferation, and response to hypoxia overexpressed at day 110 of gestation. A clustering analysis (hclust) misclassified 6 LW fetuses at 110 days of gestation with fetuses at 90 days. This was followed by a differential analysis between the two obtained classes to identify genes for a lesser maturity at birth in LW underlying several biological processes related to gene expression regulation. All p-values were adjusted with a Bonferroni correction of 1%. Overall, genes expression profiles underlined the delay of maturity for LW just before birth as already observed across the transcriptomic analysis of the muscle tissue (Voillet et al., 2014). Acknowledgment: ANR-09-GENM-005 PORCINET.

Key Words: pigs, microarray, blood, fetal maturity

WT46 Association of desmosomal gene polymorphisms and arrhythmogenic right ventricular cardiomyopathy in *Pan troglodytes* (chimpanzees): A genetic investigation. J. Gorzynski*[1,2], E. Flach[3], E. Ashley[1], R. Shave[4], and A. Boswood[2], [1]*Stanford University, Stanford, CA, USA;* [2]*Royal Veterinary College, London, UK;* [3]*Zoological Society London, London, UK;* [4]*Cardiff Metropolitan University, Cardiff, UK.*

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is characterised by a fibrofatty replacement of the myocardium resulting in right heart failure and ventricular arrhythmias. In humans, ARVC is a genetic disorder that can be caused by mutations in desmosomal genes. ARVC-like cardiomyopathy has been observed in a related population of captive *Pan troglodytes* (chimpanzee) and led to sudden cardiac death. Using targeted sequencing, we identified potential causative mutations in the desmosomal genes of two chimpanzees with ARVC phenotypes after sudden cardiac death. To test the hypothesis that the identified mutations were causative, we went on to determine the prevalence of these mutations in family members of the two ARVC positive chimpanzees [Office1], as well as a population of wild Congan chimpanzees. Echocardiographic information was also obtained from the 95 wild chimpanzees. While no correlation between the presence of the desmosomal polymorphisms and echocardiographic indexes was found, we anticipate that whole genome sequencing from these two populations will reveal the role genetics plays in ARVC.

Key Words: *Pan troglodytes*, cardiomyopathy, whole-genome sequencing

WT47 RNA transcript biomarker development for bovine tuberculosis using the NanoString nCounter Analysis System. S. L. Faherty-O'Donnell*[1], K. E. McLoughlin[1], C. N. Correia[1], N. C. Nalpas[2], D. A. Magee[1], J. A. Browne[1], K. Rue-Albrecht[3], H. M. Vordermeier[4], B. Villarreal-Ramos[4], E. Gormley[5], S. V. Gordon[5,6], and D. E. MacHugh[1,6], [1]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland;* [2]*Quantitative Proteomics and Proteome Centre Tübingen, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany;* [3]*NDM Research Building, University of Oxford, Oxford, UK;* [4]*Animal and Plant Health Agency (APHA), Weybridge, Addlestone, Surrey, United Kingdom;* [5]*UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland;* [6]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland.*

Current diagnostics for bovine tuberculosis (BTB), caused by infection with *Mycobacterium bovis*, have had limited success in control of BTB in Ireland and the UK. To facilitate improved epidemiological surveillance and management of tuberculosis in cattle, it is important that new diagnostics with enhanced specificity and sensitivity are developed. For example, recent work has focused on panels of expressed RNA biomarkers that exhibit differential expression in cattle confirmed to be infected with *M. bovis* compared to control animals. NanoString nCounter technology facilitates midplex analysis of up to 800 gene expression targets and represents a useful platform for validation of putative transcriptional biomarkers discovered using high-plex techniques such as gene expression microarrays and RNA-sequencing (RNA-seq). In the present study, we used the nCounter platform to validate a panel of mRNA targets observed to be differentially expressed across a 14-week *M. bovis* (strain AF2122/97) infection time-course experiment using 10 age-matched male Holstein Friesian cattle. Previous work by our group used RNA-seq to generate high-resolution transcriptomics data for each animal/time point. Analysis of these data identified 19 mRNA transcripts consistently differentially expressed in infected animals (+1 wk, +2 wk, +6 wk, +10 wk and +12 wk post-infection) compared to the same animals at the pre-infection time point (-1 wk). The nCounter platform was then used to examine and validate expression of these 19 targets using animal samples from the same infection time-course experiment. This study validated 19 mRNA transcript biomarkers that may have potential application as a diagnostic tool to augment existing tests for *M. bovis* infection in cattle. These results also provide novel immunobiological information relevant to human tuberculosis, which is caused by infection with *Mycobacterium tuberculosis*, a mycobacterial strain that shares 99.95% genome sequence identity with *M. bovis*.

Key Words: cattle and related species, functional genomics, biomarker, infectious disease, animal health

WT48 Selective genotyping for genome-enabled prediction in mice. R. P. Savegnago*[1], S. B. Ramos[2], A. P. Sbardela[1], G. B. Nascimento[1], L. A. Freitas[1], P. A. Bernardes[1], and D. P. Munari[1], [1]*Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil;* [2]*Universidade de Franca, Franca, São Paulo, Brazil.*

The objective of the present study was to evaluate the predictive ability of the Bayesian Lasso and GBLUP genomic models for body mass index (BMI, h^2 = 0,13) and cholesterol (CHOL, h^2 = 0,38) using selection candidates from different genotyping strategies in a mice population. The quality control of the genomic data removed 277 SNPs with call rates lower than 95% and 1,853 SNPs with MAF less than 3%, remaining 10,415 SNPs markers. The phenotypic data contained 1,817 records for BMI and 1,751 for CHOL and they were used as response variables in the genomic prediction models. The training sets were chosen according to the genotype strategy: (1) 50% of the animals randomly chosen; (2) 50% of the animals with the highest phenotypic values; (3) 50% of the animals with the lowest phenotypic values; (4) 25% of the animals with the highest and 25% with the lowest phenotypic values; (5) The 50% less related and (6) the 50% more related in the training set. The validation set was composed of 30% of the dataset. The correlation between the observed and predicted values was used as a measure of predictive performance of the models in the training and validation sets. The correlation in the training sets were higher than in the validation, regardless the genotyping strategy, the model used and the evaluated trait. It was found that the correlations in the validation set for BMI were similar for both models in each scenario. The lowest correlations in the validation set for BMI and CHOL were in scenarios (2) and (3), respectively. This was probably due to the non-representativeness of the data in these training sets. The highest

correlation in the validation set was in scenario (1) for BMI using the Bayesian Lasso and GBLUP and for CHOL using the Bayesian Lasso. Acknowledgments RPS, PAB and LAF thanks FAPESP for a postdoctoral fellowship, doctoral and master scholarships, (process numbers 2013/20091- 0, 2015/25096–6, and 2016/10583–1, respectively). APS thanks CAPES for a doctoral scholarship and DPM thanks CNPq for a productivity fellowship research.

Key Words: genome-enabled breeding, single-nucleotide polymorphism, genomic selection

WT49 Cross-Species Genomics Explorer for disease mutation identification through cross-species analysis. S. Wong*, S. Häkkinen, M. Kuisma, H. Edgren, and K. Ojala, *Medisapiens Ltd, Helsinki, Finland.*

Recent developments in NGS technologies have made them widely used in genetic analyses in animal research, ranging from captive to natural populations. However, for some non-model organisms, genomes are not well annotated, and the genes are not thoroughly characterised. As such, causative mutations cannot be pinpointed with high certainty. In these cases, the use of human phenotypic data with targeted genetic alterations is an intriguing possibility to narrow down on a specific mutation as likely causative. However, effective cross-species use of genotype-phenotype data is hampered by the difficulty in effectively accessing this data and using it together with the sequencing data itself. To solve this problem, we have developed Cross-Species Genomics Explorer, a browser based system that enables efficient and flexible analysis of genomics data in a cross-species context. It incorporates a variant storage and analytic system for big variant data together with efficient exploration of genetic variants. This exploration is done in the context of the variants' orthologous positions in the genomes of other species, through multi-species genome tracks and on the fly cross-species genome build conversion. With an extensive database of the known phenotypic consequences of genetic alterations in human, this allows researchers to quickly test hypotheses against data on target species such as mouse, zebrafish and dog. From target organisms' genotype-phenotype observations and variant consequences, researchers can cross-reference to human reference to explore for similarity in phenotype association and genetics polymorphism, and generate hypotheses about target organisms' gene functions, traits and diseases.

Key Words: databases/repositories, genome-wide association, comparative genomics

WT50 Introduction to a new online database of animal rDNA loci. J. Sochorová*[1], S. Garcia[2], F. Gálvez[3], R. Symonová[4], and A. Kovarík[1], [1]*Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic;* [2]*Institut Botànic de Barcelona (IBB-CSIC-ICUB), Barcelona, Catalonia, Spain;* [3]*Bioscripts - Centro de Investigación y Desarrollo de Recursos Científicos, Sevilla, Andalusia, Spain;* [4]*Research Institute for Limnology, Mondsee, University of Innsbruck, Mondsee, Austria.*

Ribosomal DNA (rDNA) loci encoding 5S and 45S (18S-5.8S-26S) rRNA are important components of eukaryotic chromosomes varying both in numbers and locations. In animals, the 18S-5.8S-28S genes are always linked in a single 45S rDNA unit. The 5S genes occur as separate tandems or linked to the 45S units. Here, we set up the Animal rDNA database containing cytogenetic information about these loci in more than 1200 animal species (almost 250 families). The collected data are based on fluorescence situ hybridization (FISH) studies (in 500 papers) of metaphase chromosomes carried out in major groups of vertebrates (fish, reptiles, amphibians and mammals), invertebrates (insects and molluscs), minor represented groups (birds, annelids, flatworm) and other groups with just few representatives. Fishes have the greatest variability in the number of rDNA loci (1 to 27 5S sites and 1 to 27 45S sites per haploid genome). The terminal position of loci was the most prevalent (>60% karyotypes) for 45S rDNA loci with the exception of arthropods, where the pericentromeric location was more frequent, particularly in Orthoptera. The pericentromeric location of 45S rDNA loci was often restricted to karyotypes bearing prevalent telocentric and acrocentric chromosomes while the terminal positions occurred in mixed karyotypes suggesting that: (i) chromosome morphology may influence rDNA position and (ii) there may be functional constraints favouring a reduced distance of 45S loci from chromosome ends. The position of 5S rDNA loci was more variable. The database is accessible via a web-based interphase at http://www.animal-rdnadatabase.com/.

Key Words: rDNA, FISH, animal

WT51 Transcriptomic analysis of the immune response to vaccination and vaccine components by high-throughput RNA sequencing (RNA-Seq) in sheep. E. Varela-Martínez[1], N. Abendaño[1], J. Asín[2], M. Sistiaga-Poveda[1], R. Reina[3], D. de Andrés[3], L. Luján[2], and B. M. Jugo*[1], [1]*Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Spain;* [2]*Veterinary Faculty, University of Zaragoza, Zaragoza, Spain;* [3]*Institute of Agrobiotechnology (CSIC-UPNa), Pamplona, Spain.*

The sheep is an important domestic animal and it is a useful model organism for cardiology research, respiratory and reproductive medicine. Sheep can be affected by a variety of infectious diseases and vaccinations are an integral part of a flock health management program. Aluminum is the most widely used adjuvant in both human and veterinary vaccines under the form of aluminum salts. Whereas they function as an excellent adjuvant, their toxic effects are only partially known. In fact, a form of autoimmune/autoinflammatory syndrome induced by adjuvants has been described in commercial sheep, linked to the repetitive inoculation of aluminum-containing adjuvants through vaccination. In order to characterise the immune response to vaccination in general, and to adjuvants in particular, blood samples were taken from animals involved in a repetitive vaccination experiment at the beginning and at the end of the treatment. RNA was extracted from PBMC and total RNA and miRNAs were analysed by RNA-Seq technology. A total of 845,89 milllions paired-end reads were obtained from the total RNA sequencing and 206 millions reads from the miRNA sequencing of the 12 samples analysed. An alignment of the reads to the *Ovis aries* Oar_v3.1 genome yielded a mean of 62.57% of the reads per total RNA-Seq sample that aligned to unique locations in the ovine genome. In the case of miRNAs, 43.35% and 37.35% were aligned to unique locations and multiple locations respectively. Functional characterisation of differentially expressed genes and regulatory elements is currently underway. To the best of our knowledge, this study represents the first application of RNA-Seq technology for transcriptomic studies in response to vaccination in sheep. The identification of gene signatures that are activated by vaccines and adjuvants will provide insight into the mechanisms that underlie the immune response and could help in improving them. Moreover, the sheep could be used also as a model to disentangle the autoimmune/autoinflammatory syndrome induced by adjuvants or other similar diseases affecting both human and animals.

Key Words: sheep, functional genomics, non-coding RNA, RNA-seq, animal health

WT52 Globin mRNA depletion is an unnecessary step in RNA-seq transcriptomics studies of peripheral blood from cattle. C. N. Correia*[1], K. E. McLoughlin[1], N. C. Nalpas[2], D. A. Magee[1], J. A. Browne[1], K. Rue-Albrecht[3], H. M. Vordermeier[4], B. Villarreal-Ramos[4], S. V. Gordon[5,6], and D. E. MacHugh[1,6], [1]*Animal Genomics Laboratory, UCD School of Agriculture and*

Food Science, University College Dublin, Belfield, Dublin, Ireland; [2]*Quantitative Proteomics and Proteome Centre Tübingen, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany;* [3]*NDM Research Building, University of Oxford, Oxford, UK;* [4]*Animal and Plant Health Agency (APHA), Weybridge, Addlestone, Surrey, United Kingdom;* [5]*UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland;* [6]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland.*

Transcriptomics of peripheral blood using RNA-seq is useful for disease pathology studies and biomarker development. However, humans and some other mammals have a high abundance of globin transcripts that introduces compositional bias in gene expression studies and can compromise detection of lowly expressed RNA transcripts. For example, reticulocyte-derived haemoglobin, α subunit 1 and 2 (*HBA1* and *HBA2*), and haemoglobin, β (*HBB*), can account for ~60% of total mRNA transcripts in human whole blood samples. To investigate this phenomenon, whole-blood RNA-seq libraries from published globin depletion studies in 12 piglets and six human subjects were obtained. In addition, whole blood samples from ten age-matched male Holstein-Friesian calves were used for strand-specific RNA-seq. Sequence reads from all three species groups were quality-checked, adaptor and quality filtered, and aligned to the appropriate reference genome: *S. scrofa* 10.2, *H. sapiens* GRCh38.p9, or *B. taurus* UMD3.1.1. Following summarisation and retrieval of gene annotations, raw gene counts for *HBA* (subunit genes *HBA1* and *HBA2* were combined) and *HBB* were obtained for each species. Note: the current pig genome annotation has a single *HBA* locus (*LOC100737768*) because orthologs have not yet been assigned. Results revealed mean values of 524,343 raw counts for *HBA* and 12,132 for *HBB* in non-globin depleted (NGD) human samples. After globin depletion (GD), mean raw counts for human globin genes were reduced to 45,104 for *HBA* and 3,694 for *HBB*. Porcine NGD samples generated 1,982,264 and 1,704,868 mean *HBA* (*LOC100737768*) and *HBB* raw counts, respectively. Mean raw counts for GD-treated porcine samples were 277,491 *HBA* and 530,338 *HBB*. In comparison, nontreated cattle samples exhibited only 1.35 and 1,950 mean raw counts for *HBA* and *HBB*, respectively. Globin depletion is therefore an unnecessary step in RNA-seq library preparation for blood-based transcriptomics in cattle, which greatly simplifies design of experiments to study gene expression in bovine peripheral blood.

Key Words: cattle and related species, comparative genomics, RNA-seq, gene expression, animal health

WT53 Effects of diet supplementation with oleic acid or carbohydrates on *Biceps femoris* transcriptome in growing Iberian pigs. R. Benítez*[1], B. Isabel[2], A. Fernández[1], Y. Núñez[1], E. De Mercado[3], E. Gómez Izquierdo[3], J. García-Casco[1], C. López-Bote[2], and C. Óvilo[1], [1]*INIA, Madrid, Madrid, Spain;* [2]*UCM, Madrid, Madrid, Spain;* [3]*Centro Pruebas Procino Itacyl, Hontalbilla, Segovia, Spain.*

Tissue composition largely determines the quality of meat and meat products and is influenced by factors as diet, genetic type, age or sex. Diet influences animal body and tissue composition due to direct deposition and to the nutrients effects on metabolism. In this study we evaluated the effects of a diet supplemented with 6% high oleic sunflower oil or carbohydrates as energy source on muscle composition and quality traits of growing Iberian pigs. A comparative study of the *Biceps femoris* transcriptome with RNA-seq between animals fed with both types of diet was carried out. A total of twenty nine Iberian males started the dietary treatment at 19.9 kg of average live weight (LW) and were kept under identical management conditions and fed with two different isocaloric and isoproteic diets (3.3 Kcal of digestible energy and 15.6% of crude protein) provided *ad libitum*: HO diet enriched with 6% high-oleic sunflower oil and CH diet with carbohydrates as energy source. All animals were slaughtered after seven days of treatment, with 24.1 kg of average LW. Fatty acid composition of animal tissues reflected the diet composition and indicates higher lipogenesis in CH group, as expected. We detected 25 differentially expressed (DE) genes, 17 were overexpressed in HO (FC = 2.3 to 23.5; $q < 0.1$) (i.e. *ALB* and *APOC3*) and 8 in CH diet (FC = 2 to 11.2; $q < 0.1$) (i.e. *microRNA143, INSIG* and *TBXAS1*). We performed a functional analysis (metabolic pathways and GO enrichment) of the DE genes, which showed the enrichment of functions related to lipid metabolism (synthesis and accumulation of fatty acids), cellular differentiation and proliferation, inflammatory response and metabolic processes related to oxidative stability. The bioinformatic analysis also allowed to predict potential regulators (*ATF4, PPARGC1A* and *TNF*) for the expression differences observed. The results indicate the direct deposition of nutrients and a small effect of the diet on gene expression, affecting relevant biological pathways, in agreement with previous results.

Key Words: Iberian pig, RNA-Seq, tissue composition, diet, transcriptome

WT54 Transcription factor binding sites enrichement in ruminant and cetartiodactyl specific conserved non-coding elements. L. Buggiotti*, M. Farrè, and D. Larkin, *Royal Veterinary College, London, UK.*

Gene regulatory sequences (e.g. transcription factor binding sites (TFBSs)) contribute to differences in gene expression patterns within and between species and from *de novo* in clade specific-evolution. To investigate association between lineage-specific conserved non-coding elements (CNEs) and potential TFBSs, we have done identification of CNEs from several mammalian lineages and looked for association between these elements and TFBSs found in the cattle genome. A multiple-species alignment of 28 mammalian genomes, CNEs, and TFBSs detections were done as follows: i) lastZ pairwise alignments of each genome against the cattle genome assembly (bosTau6); ii) mulitZ was used to combine pairwise alignments in multiple alignment files; iii) conserved elements (CEs) were estimated using phastCons; iv) TFBStools with JASPAR CORE vertebrate motifs was utilised to perform the whole-genome scan for TFBS; v) CNEs were defined as those CEs that did not overlap with the coding parts of all known genes. Genomic association test was done to test for enrichment of TFBSs in the ruminant-, cetartiodactyl- and mammalian-specific CNE sets compared to the rest of the cattle genome. Overall, mammalian CNEs (>20bp) cover 1.3% of the cattle genome, while cetartiodactyl and ruminant CNEs cover ~1% and ~1.5%, respectively. *In silico* analysis of TFBS predicted over 25.9 million binding sites in the three groups of CNEs or ~3% of all TFBSs. The enrichment analysis revealed that >200 TFBS motifs were significantly overrepresented (qvalue = 0.001) in the ruminant, cetartiodactyl and mammalian CNE sets. The TFBS motif EWSR1-FLI1 had the highest overrepresentation in the ruminant CNEs compared to the mammalian CNE set, while multiple interferon-regulatory factor (IRF) and heat shock factor (HSF) TFBSs were most overrepresented both in the cetartiodactyl and ruminant CNEs compared to the mammalian set.

Key Words: multispecies, comparative genomics, bioinformatics tools/data mining, regulatory element

WT55 Integrative genomics of human and bovine tuberculosis. K. E. Killick*[1,2], M. P. Mullen[3], T. Hall[1], N. C. Nalpas[4], I. W. Richardson[5], D. A. Magee[1], C. N. Correia[1], J. A. Browne[1], D. P. Berry[6], D. Bradley[7], V. Naranbhai[8], A. Hill[8], E. Gormley[9], S. V. Gordon[2,9], D. E. MacHugh[1,2], [1]*University College Dublin, UCD College of Health and Agricultural Sciences, University College Dublin, Dublin, Ireland;* [2]*University College Dublin, UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland;* [3]*Athlone Institute of*

Technology, Department of Life and Physical Sciences, Athlone Institute of Technology, Athlone, Ireland; [4]University of Tübingen, Quantitative Proteomics and Proteome Centre Tübingen, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany; [5]IdentiGEN Ltd, IdentiGEN Ltd., Blackrock Business Park, Blackrock, Dublin, Ireland; [6]Teagasc, Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Cork, Ireland; [7]Trinity College, Smurfit Institute of Genetics, University of Dublin, Trinity College, Dublin, Ireland; [8]University of Oxford, Wellcome Trust Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, Oxford, UK; [9]University College Dublin, UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland.

Human tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent. Bovine TB (BTB), caused by the closely related *Mycobacterium bovis* (99.95% sequence identity), is a major endemic disease affecting global cattle production, particularly in many developing countries. In the current study we used a network-based approach to integrate host gene expression data with high-density single-nucleotide polymorphism (SNP) genome-wide association (GWA) data to enhance detection of genomic variants for susceptibility/resistance to both *M. tuberculosis* and *M. bovis* infection. The host gene expression data used consisted of human and bovine RNA-seq data from macrophages infected with *M. tuberculosis* and *M. bovis*, respectively. A base gene interaction network of the mammalian host response to mycobacterial infection was generated using 213 genes identified from GeneCards (www.genecards.org). Differential gene expression data (FDR *P* value < 0.001) were superimposed on to this base network and the JActiveModules Cytoscape (www.cytoscape.org) plugin was used to extract functional modules with DE gene sets from macrophage infection experiments. SNP array population data was obtained from large human and bovine TB susceptibility/resistance studies, including the Wellcome Trust Case Control Consortium (WTCCC – www.wtccc.org.uk) resource and a published GWAS study in dairy cattle. SNPs from the top functional modules (5 kb up- and downstream of each gene) were identified for both human and bovine gene expression data. These analyses identified new genomic variants in humans and cattle associated with susceptibility and resistance to tuberculosis disease in both species. Comparison and integration of human and bovine gene expression data with GWAS data for TB and BTB can be used to identify shared and specific mechanisms underlying the mammalian host response to mycobacterial infection. In summary, the integrative genomics approach described here can be used to generate new knowledge by leveraging distinct but complementary omics datasets from a wide range of biological contexts.

Key Words: integrative genomics, bovine tuberculosis, *Mycobacterium tuberculosis*

WT56 Hypothalamus transcriptome during the early rise in LH secretion related to puberty age in bull calves. J. Liron[1], M. Fernández*[2], A. Prando[3], A. Baldo[3], and G. Giovambattista[2], [1]*Center of Veterinary Research (CIVETAN, CONICET), Faculty of Veterinary Sciences, UNCPBA, Tandil, Buenos Aires, Argentina.;* [2]*Institute of Veterinary Genetics (IGEVET, CONICET), Faculty of Veterinary Sciences, National University of La Plata, La Plata, Buenos Aires, Argentina;* [3]*Cátedra de Zootecnia Especial (II Parte)), Faculty of Veterinary Sciences, National University of La Plata, La Plata, Buenos Aires, Argentina.*

Cattle puberty influences reproduction rates and profitability. In pre-pubertal bull calves there is an early transient rise in gonadotropin secretion between 10 and 20 weeks of age. The early elevation in mean circulating concentrations of LH and FSH most likely causes the proliferation of Sertoli and Leydig cells, respectively. This post-natal gonadotropin rise is considered one of the main factors in determining the age at which bulls reach puberty. In order to enhance our knowledge about genes and regulatory pathways involved in this phenomenon, we characterised the hypothalamus transcriptome from six Angus calves along this early expression pattern of LH (4, 6 and 14 wk of age) using the RNA-Seq technology. Of 37 million RNA-Seq reads per sample generated using the Illumina HiSEqn 2000 sequencer, at least 95% were mapped to the customized reference genome BosTau6. The gene annotation revealed that 13,976 genes were expressed in the hypothalamus. Tophat2, EdgeR, DESEqn 2, Bioconductor and R packages were utilised to performed differential expression (DE) analysis between groups. We detected 915 DE genes (*P* adjusted values < 0.05). The top gene ontology term enrichment of the highest expressed genes in the hypothalamus included cellular synapse, ion channel complex, neuron projection and plasma membrane part (Cellular component category); cell-cell signalling, transmembrane transport, behaviour and organism process (Biological process); metal ion transmembrane transporter activity and neuropeptide hormone activity (Molecular function). Enrichment analysis identified 40 KEGG significantly enriched pathways. Based on the observation of the lowest *P*-value, calcium, oxytocin, circadian entrainment, cholinergic, glutamatergic, dopaminergic, serotonergic and GABAergic synapse, GnRH, oestrogen, Rap1, MAPK, ErbB, Ras and cancer signalling and several drugs addiction were among the most significant enriched pathways. The list of highest DE genes includes OTP, AVP, OXT, CRH and TH, known for their physiological roles associated with lactation and mammalian social behaviours.

Key Words: cattle, functional genomics, RNA-seq, puberty, genetic improvement

WT57 Generating customized integrated functional annotation datasets with BovineMine. C. Elsik*, D. Unni, A. Tayal, and D. Hagen, *University of Missouri, Columbia, MO, USA.*

BovineMine is the data mining resource of the Bovine Genome Database (BGD, http://BovineGenome.org). The objective of this presentation is to show how BovineMine can accelerate genomics research by enabling scientists without scripting skills to create and export customized annotation datasets merged with their own research data for use in downstream analyses. BovineMine allows researchers to leverage the curated gene pathways of model organisms (e.g. human, mouse and rat) based on orthology, and is especially useful for GO and pathway analyses in conjunction with GWAS and QTL studies. BovineMine also includes the reference genomes of sheep and goat so researchers can leverage information across ruminants. BovineMine uses the InterMine platform to integrate data from a variety of sources, including reference genome assemblies, genes (NCBI, Ensembl, Official Gene Set), proteins (UniProt), protein families and domains (InterPro), orthologs and paralogs (EnsemblCompara, Homologene, OrthoDB, TreeFam), pathways (BioCyc, KEGG, Reactome), interactions (BioGRID, IntAct), Gene Ontology (GO), QTL (AnimalQTLdb), variation (dbSNP, dbVar) and publications (PubMed). Pre-computed data from BGD, including variant effects and RNAseq-based gene expression, allow users to query tissue specific gene expression levels together with genomic variation data. BovineMine provides simple and sophisticated data mining tools. Built-in query templates provide starting points for data exploration, while the QueryBuilder tool supports construction of complex queries. The List Analysis and Genomic Regions search tools execute queries based on uploaded lists of identifiers and genome coordinates, respectively. BovineMine supports meta-analyses by tracking identifiers across gene sets and genome assemblies, which will be particularly valuable with the release of the upgraded bovine reference genome assembly. Future plans include the incorporation of FAANG datasets

to enable fine-grained data mining of functional elements in combination with gene annotations and additional biological data.

Key Words: cattle and related species, functional genomics, comparative genomics, bioinformatics tools, data mining

WT58 Identification of regulatory elements in livestock species. H. Zhou*[1], P. Ross[1], C. Kern[1], P. Saelao[1], Y. Wang[1], M. Halstead[1], K. Chanthavixay[1], I. Korf[1], M. Delany[1], H. Cheng[2], J. Medrano[1], A. Van Eenennaam[1], C. Tuggle[3], and C. Ernst[4], [1]*University of California, Davis, Davis, CA, USA;* [2]*USDA-ARS, Avian Disease and Oncology Laboratory, East Lansing, MI, USA;* [3]*Iowa State University, Ames, IA, USA;* [4]*Michigan State University, East Lansing, MI, USA.*

Regulatory elements play an essential role in understanding how an organism's genotype determines its phenotype. The technologies and assays developed in the human and mouse ENCODE projects provide a solid foundation to functionally annotate farm animal genomes. The recent international FAANG (Functional Annotation of ANimal Genomes) initiative has stimulated such efforts on livestock species, which will ultimately be leveraged to improve production efficiency, animal welfare, and food safety. The overall objective of this study is to functionally annotate regulatory elements in three major livestock species: chicken, cattle, and pig. The first key step is to identify regulatory elements in the genomes by integrating RNA-seq, DNase-seq and ChIP-seq data. As one of FAANG pilot projects coordinated by UC Davis, we will present the current progress in generating and analysing data from these three important species. We have collected samples from adipose, cerebellum, cortex, hypothalamus, liver, lung, muscle, and spleen in two male biological replicates from each species, allowing us to identify both universal and tissue-specific functional elements. High depth of RNA-seq has allowed the identification of 9,393 long non-coding RNAs in chicken, 7,235 in cattle, and 14,428 in pig. From DNase-seq sequencing in chickens, we have identified 132,362 open chromatin regions across the genome, many of which are tissue-specific or only present in some tissues. Per tissue, the values range from a high of 57,703 regions in spleen to the lowest of 14,700 in cerebellum. Genes present in these open chromatin regions show generally higher expression in our RNA-seq data. ChIP-seq for the H3K4me3 histone modification from all eight tissues in chicken identified a total of 31,174 peaks, while 35,081 were identified from liver, lung, and spleen in pig. H3K27me3 from the same three tissues in pig identified a total of 104,640 peaks. Preliminary results from H3K27me3 assays in chickens identified a total of 16,247 peaks. In the future, an integrative analysis of five ChIP-seq assays and DNase-seq will produce genome-wide chromatin state predictions and allow the identification of promoters, enhancers, and silencers in all three species.

Key Words: bioinformatics, Functional Annotation of Animal Genomes (FAANG), ChIP-seq, epigenomics

WT59 The Vertebrate Gene Nomenclature Committee (VGNC). P. Denny*, B. Yates, S. Tweedie, B. Braschi, K. Gray, R. Seal, and E. Bruford, *European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Hinxton, Cambridgeshire, UK.*

Standardised gene nomenclature is essential for effective communication and provides a critical resource for all biomedical researchers. However, an ever-increasing number of vertebrate genomes are being sequenced and the data released into the public domain without systematic annotation or gene naming. There are only six vertebrate model organisms with an established gene nomenclature committee (mouse, rat, chicken, Anolis, *Xenopus* and zebrafish), all of which base their gene names on those approved by the HUGO Gene Nomenclature Committee (HGNC) for human genes. The Vertebrate Gene Nomenclature Committee (VGNC) is a new initiative that extends the remit of the HGNC to approve consistent gene names and symbols across other vertebrates. Our naming strategy for each species starts by identifying a high confidence set of gene annotations with 1:1 human orthologs, using our HCOP tool (http://www.genenames.org/cgi-bin/hcop) that combines orthology predictions from multiple sources. The human gene nomenclature is transferred in a semi-automated way to these 1:1 orthologs. Genes with multiple predicted orthologs, members of complex gene families, pseudogenes and RNA genes require additional manual curation, such as review of phylogeny, synteny, gene structures and encoded proteins. Our pilot species for VGNC naming has been chimpanzee and we have named over 14,000 protein-coding chimp genes with a 1:1 human orthologue. During this process, we have taken the opportunity to simplify and improve the consistency of our human gene names, taking care to minimise transfer of species-specific information, such as susceptibility to pathogens. This naming process will soon be expanded to other species, including dog, horse and cow. We plan to prioritize species based on the quality of genome assembly and annotations, perceived importance as a model for humans and demand from the research community. An online vertebrate gene nomenclature portal has been created that stores, displays and makes this new nomenclature data accessible both to individual researchers and available for dissemination to other key resources including Ensembl and NCBI Gene. Further information and requests for individual gene names and symbols can be made via: http://vertebrate.genenames.org.

Key Words: nomenclature, comparative, bioinformatics, annotation, website

WT60 Circulating microRNAs as potential novel biomarkers to diagnose *Mycobacterium avium* ssp. *paratuberculosis* infection in cattle. K. Zhao[1], S. Hendrick[2], and L. Guan*[1], [1]*Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, AB, Canada;* [2]*Coaldale Veterinary Clinic, Lethbridge, Canada.*

Johne's disease (JD) is an infectious disease caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in ruminants. The circulating microRNAs are promising biomarkers for prediction and diagnosis of variety of diseases in humans. Therefore, the purpose of this study is to explore the potential of using the circulating microRNAs as diagnosis markers for early MAP infection detection. The microRNAomes of sera collected from three-year-old cows with clinical symptoms (CC, $n = 20$) and subclinical carriers (SC, $n = 24$) were generated using RNA-seq. In total, 116 expressed miRNAs were detected across all samples, and 11 of them were differentially expressed (DE) between CC and SC (fold change >2 and FDR < 0.05). Among them, seven miRNAs (miR-1468, 296–3p, 2284x, 2284y, 181a, 181b, and let-7f) were up-regulated in SC and four miRNAs (miR-192, 22–5p, 24–3p, and 361) were up-regulated in CC. Functional analysis showed that function of highly expressed miRNAs in SC was enriched to 'Metabolic pathway' by inhibiting the expression of genes related to glycolysis, fatty acid metabolism, and ATP synthesis. The function of those DE miRNAs in CC was enriched in 'Phagosome', including the genes regulate phagosome formation and function. Moreover, principal component analysis showed that sera miRNAome profiles of 24 SC cattle segregated into two groups (SC1 and SC2, $n = 12$, respectively), with profile of SC2 overlapped with those of the CC. This suggests that SC2 cattle may potentially progress to CC, while the SC1 may retain as SC. Moreover, 51 DE miRNAs were identified between SC1 and SC2 with 16 up-regulated in SC1 and 35 up-regulated in SC2. Functional analysis of up-regulated miRNAs in SC1 and SC2 showed similar pattern with those DE miRNAs between SC and CC, including 'Metabolic pathway' and 'Platelet activation'. Our results indicate that the decreased glucose/energy metabolism and innate immune response contributed partially to the molecular mechanism of MAP infection in SC and CC, respectively. The panels of circulating miRNAs (miR-2284x, 2284y, 181a, 181b, 24–3p, and 361) can be

potentially used as novel biomarkers for early diagnosis of MAP infection at subclinical stage.

Key Words: biomarker, miRNAome, serum, Johne's disease

WT61 The reconstruction and evolutionary history of eutherian chromosomes. J. Kim[1], M. Farre[2], L. Auvil[3], B. Capitanu[3], J. Ma[4], H. A. Lewin[5], and D. M. Larkin*[1], [1]*Department of Biomedical Science and Engineering, Konkuk University, Seoul, Korea;* [2]*Royal Veterinary College, University of London, London, UK;* [3]*Illinois Informatics Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA;* [4]*Computational Biology Department, School of Computer Science, Carnegie Mellon University, Pittsburgh, PA, USA;* [5]*Department of Evolution and Ecology, University of California, Davis, CA, USA.*

Whole genome assemblies of 19 placental mammals and two outgroup species were used to reconstruct the order and orientation of synteny blocks in chromosomes of the eutherian ancestor and six other descendent ancestors leading to human. For ancestral chromosome reconstructions, we developed a new algorithm (DESCHRAMBLER) that probabilistically determines the adjacencies of syntenic blocks using chromosome-scale and fragmented genome assemblies. The reconstructed chromosomes of the eutherian, boreoeutherian and euarchontoglires ancestor each included >80 percent of the entire length of the human genome, while reconstructed chromosomes of the most recent common ancestor of simians, catarrhini, great apes, and humans and chimpanzees included >90% of human genome sequence. These high coverage reconstructions permitted reliable identification of chromosomal rearrangements over ~105 million years (My) of eutherian evolution. Orangutan was found to have eight chromosomes that were completely conserved in homologous sequence order and orientation with the eutherian ancestor, the largest number for any species. Ruminant artiodactyls had the highest frequency of intrachromosomal rearrangements, while interchromosomal rearrangements dominated in murid rodents. A total of 162 chromosomal breakpoints in evolution of the eutherian ancestral genome to the human genome were identified; however, the rate of rearrangements was significantly lower (0.80/My) during the first ~60 million years of eutherian evolution, then increased to greater than 2.0/My along the five primate lineages studied. Our results significantly expand knowledge of eutherian genome evolution and will facilitate greater understanding of the role of chromosome rearrangements in adaptation, speciation, and the aetiology of inherited and spontaneously occurring diseases.

Key Words: ancestral chromosome reconstruction, primates, human, chromosome evolution, algorithm

WT62 iTRAQ-based proteomic analysis reveals key proteins affecting muscle growth and lipid deposition in pig. Z. Wang*[1], P. Shang[1,2], Q. Li[3], L. Wang[1], H. Zhang[1], and C. Wu[1], [1]*China Agricultural University, Beijing, China;* [2]*Tibet Agriculture and Animal Husbandry University, Linzhi, China;* [3]*Anhui Academy of Agricultural Sciences, Hefei, China.*

Pig growth rate and meat quality that are the main economic traits may be involved in multiple genes and biological pathways. The Tibetan pig (TP) and Diannan Small Ear pig (DSP) are indigenous Chinese breeds; they have significantly lower growth rates, higher lipid deposition ability, and better meat quality than those of introduced pig breeds such as Yorkshire (YY) and Landrace (LL). Nowadays, the proteomic analysis is a powerful method to identify key functional genes for the complex quantitative traits. Parallel reaction monitoring (PRM) is a recent development in targeted mass spectrometry and involves the use of a quadrupole-equipped Orbitrap. In present study, *the longissimus dorsi* muscle tissues were collected from the TP, DSP, YY and LL pig breeds at the age of six month and were performed the iTRAQ-based quantitative proteome analysis. The protein expression obtained using iTRAQ analysis was confirmed by quantifying the expression levels of twelve selected proteins by a PRM-MS analysis. Totally, 4,815 peptides corresponding to 969 proteins were detected. Comparison of expression patterns between TP-DSP and YY-LL revealed 288 differentially expressed proteins (DEPs), of which 169 were up-regulated and 119 were down-regulated. Functional annotation suggested that 28 DEPs were related to muscle growth and 15 to lipid deposition. Protein interaction network predictions indicated that differences in muscle growth and muscle fibre morphology between TP-DSP and YY-LL breeds were regulated by ALDOC, ENO3, PGK1, PGK2, TNNT1, TNNT3, TPM1, TPM2, TPM3, MYL3, MYH4, and TNNC2, while those in lipid deposition ability were regulated by LPL, APOA1, APOC3, ACADM, FABP3, ACADVL, ACAA2, ACAT1, HADH, and PECI. Twelve DEPs (up-regulated: UQCRC1, ACAT1, ACADM, PECI, MYL3, NNT, ACAA2, TTN, and HADH; down-regulated: PRDX4, MYL1, and LDB3) were selected for the PRM to confirm the reliability of the iTRAQ analysis. The fold changes and *P* values for these proteins were significantly different between the TP-DSP and YY-LL groups at $P < 0.10$, which was in agreement with the findings of the iTRAQ analysis. Our expression profiles provide new insights into the key proteins involved in muscle growth and lipid deposition in the pig.

Key Words: iTRAQ, PRM, muscle growth, lipid deposition, pig

WT63 Gene regulation in sheep alveolar macrophages: Genome-wide identification of active enhancers. A. Massa*[1], M. Mousel[2,1], B. Murdoch[3], and S. White[2,1], [1]*Washington State University, Pullman, WA, USA;* [2]*United States Department of Agriculture-ADRU, Pullman, WA, USA;* [3]*University of Idaho, Moscow, ID, USA.*

Lung macrophages provide a first line of defence for the cell-mediated innate immune system against many inhaled pathogens. Annotation of regulatory elements within these cells aids advanced understanding of gene regulation and genetic priming of the immune system. Acetylation of lysine 27 on histone protein three (H3K27ac) is one of the most dynamic histone modifications, denoting active enhancer regions of the genome. Changes in H3K27ac correspond to changes in gene expression that control cellular differentiation. In this study, alveolar macrophages were harvested from the lungs of 2 healthy, one-year-old, Suffolk-cross sheep. Chromatin immunoprecipitation with high throughput sequencing (ChIP-seq) was performed for histone modification H3K27ac. Approximately 50,000 peaks were identified in the immunoprecipitation dataset over control input DNA. Peaks with a false discovery rate of less than 5% included 12,984 peaks with an average enrichment of 18-fold. This is comparable with published data from humans and other mammalian species for active enhancer marks. Overall, 51% of active enhancers were either within genes or were less than 500 base pairs upstream of genes. While 13% were located greater than 50,000 base pairs from any gene. Peak length was similar to expected values of ~200–2000 base pairs for mammalian enhancers. However, sequence motif discovery in sheep suggested many are unique when compared with known enhancer motifs in humans, indicative of sequence divergence between species. These data provide a basis for regulatory landscape comparison among sheep cell types and for cross-species comparative regulome analysis in macrophages and immune cells.

Key Words: ChIP-seq, H3K27ac, sheep, enhancers

Gene Function

WT64 Genome-wide survey by ChIP-seq explores the regulatory mechanism of c-Myc in chicken skeletal muscle differentiation. W. Luo* and X. Zhang, *Department of Animal Genetics, Breeding and Reproduction, College of Animal Science, South China Agricultural University, Guangzhou, Guangdong Province, China.*

c-Myc is an important transcription factor in animal development. However, its roles in skeletal muscle development has not been well understood. By using chick as model system, we systematically investigated the functions and action mechanisms of c-Myc during skeletal muscle differentiation. c-Myc is down-regulated its protein expression during chick primary myoblast differentiation. Notably, c-Myc localization was shown to change from nuclear to cytoplasmic during myoblast differentiation to myotube. Analysis of c-Myc function in myoblast proliferation and differentiation was assessed using both gain and loss of function approaches. Results shown that c-Myc is able to promote myoblast proliferation and repress myoblast differentiation. To better understand the mechanisms of c-Myc-regulated target gene expression, we studied genome-wide binding sites of c-Myc in chick myoblast and myotube by using ChIP-seq. After analysed the sequencing results, we found that c-Myc can regulate the transcription of numerous muscle development related genes through binding to their promoters. Additionally, the transcription of miRNAs related to skeletal muscle differentiation and cell proliferation was also found to be regulated by c-Myc. Functional and rescue assays indicated that the roles of c-Myc in myoblast proliferation and differentiation are partly depend on the transcriptional regulation of these target miRNAs. Besides, gga-miR-1814, gga-miR-211, gga-miR-26a-5p and gga-let-7a-5p, which are regulated by c-Myc in myoblast, can inhibit c-Myc expression by directly binding to its mRNA 3′UTR. Therefore, an auto-regulate feedback loop includes c-Myc and its target miRNAs may play roles during skeletal muscle differentiation. Finally, we also found that c-Myc can bind to the promoters of 1104 lncRNAs. In these lncRNAs, lnc-2949 and lnc-1369, which are transcriptional regulated by c-Myc, can regulated myoblast proliferation and differentiation by binding to muscle development related miRNAs. Together, this study argue that c-Myc regulates skeletal muscle differentiation by directly interaction with its target genes, miRNAs and lncRNAs.

Key Words: c-Myc, skeletal muscle, differentiation, miRNA, lncRNA

WT65 Insights into the dynamic proteomic changes of bovine mammary gland between peak and late lactation stages with tandem mass tags assay. X. Zheng*[1], C. Ning[1], Y. Yu[1], L. Jiang[1], Y. Dong[2], P. Zhao[1], H. Wang[3], and J. Liu[1], [1]*China Agricultural University, Beijing, China;* [2]*Haian County Agricultural Commission, Nantong, Jiangsu, China;* [3]*Yang Zhou University, Yangzhou, Jiangsu, China.*

Mammary gland is an important organ for milk synthesis and secretion. It undergoes dramatic physiological adaptations during the transition from peak to late lactation stage. Protein expression is the final executant of life functions, and the knowledge of the protein changes during different lactation stages helps us better understanding the biology of lactation and mammary function in cows. To investigate proteome changes occurring in bovine mammary gland from peak to late lactation stage transition, samples taken at two lactation points from four individual cows were analysed by tandem mass tags (TMT) mass spectrometry (MS)/MS. A total of 3,753 proteins were quantified, out of which 92 proteins were expressed differentially and were mapped to important biological pathways involved in lactation. Compared with the peak lactation stage, 63 proteins were significantly up-regulated (>1.3-fold) while 29 were significantly down-regulated (< 0.77-fold) in the late lactation stage. The top three down-regulated proteins were PTX3, GPA33 and SLC28A3, while SFRP4, OLFML1and LRRC8D were the most up-regulated proteins. Gene Ontology (GO) enrichment analysis revealed that amino acid transport, asymmetric protein localization and homeostatic process were strongly overrepresented in peak lactation stage and immune and development as well as their related processes were overrepresented in late lactation stage. These findings will constitute to the reference dataset of dairy cows for improving both milk quantity and quality.

Key Words: mammary gland, cow, proteome, different lactation points, TMT

WT66 The Functional Annotation of Animal Genomes (FAANG) Project: Metadata, data sharing, and the FAANG data portal. L. Clarke*[1], P. Harrison[1], L. Eory[2], R. Kuo[2], G. Cochrane[1], A. Archibald[2], D. Zerbino[1], and P. Flicek[1], [1]*European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK;* [2]*The Roslin institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edniburgh, UK.*

The Functional Annotation of Animal Genomes (FAANG) project is a coordinated international effort to produce high-quality transcriptional, regulatory and epigenomic data from a set of livestock species. FAANG standardises core assays, experimental protocols and analysis methods to maximise effectiveness and inter-comparability of assay data. This provides the community with a rich resource enabling the investigation of genome to phenome links. A primary focus for the FAANG data coordination centre is the collection of high-quality metadata to describe the project's animals, specimens, cell cultures and experimental assays. This is made possible by clear metadata rules (http://www.ebi.ac.uk/vg/faang/rule_sets/FAANG) and tools to validate submissions meet those standards. FAANG also encourages early registration of samples and pre-publication data release in the appropriate archives in line with FAANG's data release policy (http://www.faang.org/data-share-principle). The FAANG data and metadata can be discovered using the FAANG data portal (http://data.faang.org). This portal displays the project's samples, experimental data and analysis results. It also enables visualisation of those results in the UCSC or Ensembl genome browsers through the use of TrackHub technology. The portal itself provides dynamic search and filtering tools, allowing users to explore both data generated for FAANG and existing livestock genomic data sourced from EMBL-EBI's archives. The data coordination centre builds these tools drawing on advances from resources at EMBL-EBI's including major data archives and Ensembl.

Key Words: FAANG, metadata, data sharing, portal, trackhub

WT67 Identification and characterization of miRNAs for milk protein in Chinese Holstein cows. W. Cai*, C. Li, C. Zhou, H. Yin, S. Liu, and S. Zhang, *College of Animal Science and Technology, China Agricultural University, Beijing, China.*

MicroRNAs (miRNAs) serve as key post-transcriptional regulators of gene expression, involved in various biological process in livestock. our previous study has revealed some functional genes responsible for milk protein biosynthesis in mammary using ssRNA-seq technology, yet miRNA-mediated gene regulatory network for the synthesis of milk protein in mammary is poorly understood. Mammary samples were biopsied from 12 multiparous and healthy mastitis-free Chinese Holstein cows with 3 extremely high and 3 low phenotypic values for milk protein percentage in peak and non-lactation period were selected for our study. We constructed small RNA libraries and subjected them to deep sequencing.A total of 512 known and 212 novel miRNAs were identified by bioinformatics analysis, of which the greatest expression of miRNAs in peak stage was bta-mir-148a compared to bta-mir-143 expressed in non-lactation stage. The differentially expressed analysis detected 9 at peak lactation and 29 in the non-lactating period with a high-

ly significant correlation with milk protein concentration, and 179 significant miRNAs for lactation secretion. In addition, 4 differential miRNAs were detected in all the three compare groups. Gene annotation revealed many target genes of differential miRNA were associated with the process of milk protein synthesis and transport. Integration of differentially expressed miRNAs, previous RNA-seq study, relative QTLs, target gene function and known or significant GO and pathways suggested bta-mir-494 and bta-mir-2419 were the most promising candidate miRNAs for milk protein as well as bta-mir-375 may act as important role in milk secretion. This integrated study on the transcriptional and post-transcriptional regulatory profiles between extremely differential phonotype of milk protein concentration provides new insights into the mechanism of milk protein synthesis, which should contribute to revealing the regulatory mechanisms of milk secretion.

Key Words: cattle, epigenomics, microRNA, candidate gene, milk production

WT68 Degree of coat color reddening in F_2 Nellore-Angus cattle demonstrates multilocus influences. K. Scienski*, T. Womack, and C. A. Gill, *Texas A&M University, Texas A&M AgriLife Research, College Station, TX, USA.*

Variation in base coat colour in cattle has been attributed to enzymatic activity governed by the melanocortin-1 receptor (*MC1R*) locus, with alleles coding for black (E^D), wildtype (E^+), or red (*e*). Based on the dominance series, E^DE^+ heterozygotes are expected to be black, but we have previously shown in our Nellore-Angus F_2 population that these heterozygotes display degrees of pigmentation ranging from light red to black. We identified a major recessive locus on bovine chromosome 6 that interacts with *MC1R,* and haplotypes carrying the reddening allele were of Nellore origin. The objective of this study was to identify a functional candidate for the reddening locus by characterising expression of genes within the reddening critical interval (*KIT*, *PDGFRA*, *CORIN*, *KDR*, *CHIC2*) and other genes with known roles in melanogenesis (*MC1R*, *ASIP*, *KITLG*, *MITF*, *TYRP1*). We used realtime qRT–PCR on total RNA isolated from skin biopsies for biological triplicates of each genotype by breed of origin at the reddening locus (NN, NA, AA) for heterozygotes (E^DE^+) at *MC1R* (n = 9). Combinations of homozygotes by breed of origin at the two loci (NN E^DE^D, AA E^DE^D, NN E^+E^+, AA E^+E^+) were compared as controls (n = 12). Differential expression of *MC1R, ASIP* and *TYRP1* between reddened and black animals was consistent with known differences in expression of these genes in pheomelanogenesis and eumelanogenesis. Expression patterns for *CHIC2* suggest it is the reddening locus. CHIC2 has a cysteine-rich palmitoylated motif similar to the motif in melanoregulin that stably targets it to the melanosome membrane. Melanoregulin is a negative regulator of melanosome transfer from melanocytes to keratinocytes, and we propose that CHIC2 also has a role in exocytosis of melanosomes.

Key Words: reddening, gene expression

WT69 Long-non coding RNAs repertoires in liver and two T lymphocyte cell types in four livestock species [FAANG pilot project ‘’FR-AgENCODE”]. K. Muret*[1], S. Djebali[2], T. Derrien[3], C. Cabau[2], C. Klopp[4], D. Esquerré[2,5], K. Munyard[6], G. Tosser-Klopp[2], H. Acloque[2], E. Giuffra[7], S. Foissac[2], and S. Lagarrigue[1], [1]*UMR PEGASE INRA, Agrocampus Ouest UMR PEGASE, Rennes, France;* [2]*UMR GenPhySE, INRA, INPT, ENVT, Université de Toulouse, Castanet-Tolosan, France;* [3]*IGDR, CNRS-University Rennes 1, Rennes, France;* [4]*SIGENAE, INRA, Castanet-Tolosan, France;* [5]*Plateforme GENOTOUL, INRA, Castanet-Tolosan, France;* [6]*School of Biomedical Sciences, Curtin Health Innovation Research Institute, Western Australian Biomedical Research Institute, Curtin University, Perth, Western Australia, Australia;* [7]*UMR GABI, INRA, AgroParisTech, Université Paris Saclay, Jouy-en-Josas, France.*

Understanding genome-to-phenome relationships requires deep and cross-disciplinary genetic analyses among which functional annotation provides crucial insights. The development of High Throughput Sequencing and RNA-seq now help us to find a large number of heterogeneous and low-expressed transcripts known to be long non-coding RNAs (lncRNAs). One of the aims of the FAANG pilot project ‘FR-AgENCODE’ is to identify and characterise the long non-coding RNAs of multiple tissues and cell lines in 4 farm animals (chicken, bovine, pig and goat) of both sexes. Here, we focus our analysis on the liver tissue and two blood T-cell types (CD3+CD4+, CD3+CD8+) where samples were collected through 4 biological replicates (2 males and 2 females). It allows us to compare lncRNA repertoires between tissues, sex and species in relation with fundamental biological functions like energy storage and immunity. High depth strand-specific RNA-seq produced ~200M paired-end reads for each of the 16 RNA-seq datasets. After transcriptome reconstruction, we used the recently published FEELnc program (Wucher *et al*., 2017, Nucleic Acid Research) to identify lncRNAs longer than 200 bp and without protein-coding capabilities. FEELnc also classifies lncRNAs based on their genomic localizations with respect to the ENSEMBL protein-coding annotation: intergenic lncRNAs are categorized depending on the distance and orientation with respect to the closest mRNAs and the intragenic lncRNAs are extracted based on their overlap with mRNAs exons and introns. We will report these lncRNA repertoires in terms of intergenic/intragenic lncRNA class, structure and expression and comparing these features between livestock species, tissues and sexes. By profiling the transcriptional landscape of lncRNAs in these 4 species, this data will further contribute to the global action for annotating functional elements of livestock genomes.

Key Words: long non-coding RNAs, multispecies, Functional Annotation of Animal Genomes (FAANG), comparative genomics, RNA-seq

WT70 Combining transcriptome and epigenetic analysis of H3K36me3 and H3K4me3 marks to explore mechanisms of liver-specific gene expression in pigs. J. Huang, M. Schroyen, N. Gabler, J. Dekkers, and C. Tuggle*, *Department of Animal Science, Iowa State University, Ames, IA, USA.*

Post-translational covalent modifications on the histones in nucleosomes play important roles in regulating gene expression at the chromatin level. To study the interplay between RNA expression and histone modification in pig tissues, we selected putative tissue-specific genes using RNAseq analysis of liver, muscle, spleen and ileum collected from 6 Yorkshire pigs. We found 148, 108, 17 and 109 genes were specifically or highly differentially expressed in liver, muscle, spleen and ileum, respectively. Using Q-RT–PCR, we validated these predictions for 11 liver, 10 muscle, 9 spleen, and 6 ileum genes. Trimethylation of lysine 36 of histone H3 (H3K36me3) and trimethylation of lysine 4 of histone H3 (H3K4me3) are associated with specific transcriptional states, and we predicted that H3K4me3 marks would be enriched in liver chromatin at liver-specific gene promoters, but not at muscle-specific gene promoters. Likewise, we predicted H3K36me3 would be enriched in the 3′UTR only for liver-specific genes. Liver chromatin preparations from two replicates were immunoprecipitated (ChIP) using antibodies to these two marks. We developed Q-PCR tests (at the promoter or the 3′UTR) for 17 expression-validated genes (9 liver-specific and 8 muscle-specific) to test whether H3K36me3 (gene body/3′UTR) and H3K4me3 (active promoter) marks were associated with RNA-seq predictions. Results showed that the H3K36me3 levels measured at the 3′UTR were high for all liver-specific genes but low for muscle-specific genes. Conversely, H3K4me3 levels measured at the promoter were high for only liver-specific genes. Q-PCR assays for gene deserts were negative for both marks, while a broadly-ex-

pressed gene (RPL30) was positive for both marks. These data indicated that H3K36me3 and H3K4me3 are active marks that correlate well in pigs with their known connections to functional gene components in human and thus are likely to play a vital role in epigenetic control of porcine gene expression. Therefore, these marks should be highly valuable for functional annotation of the porcine genome.
Funding acknowledgment: NIFA-AFRI-2011–68004–30336.

Key Words: pig, Functional Annotation of Animal Genomes (FAANG), gene expression, epigenetics, liver

Genetics and Genomics of Aquaculture Species

WT71 The distribution of repetitive DNAs between regular and supernumerary chromosomes in the cavefish genome. S. F. Ahmad*[1], M. Jehangir[1], A. Cardoso[1], G. T. Valente[2], and C. Martins[1], [1]*Department of Morphology, Institute of Biosciences, UNESP, São Paulo State University, Botucatu, SP, Brazil;* [2]*Bioprocess and Biotechnology Department, Agronomical Science Faculty, UNESP, São Paulo State University, Botucatu, SP, Brazil.*

Approximately 15% of eukaryotes contain supernumerary (extra) B chromosomes. Despite thousands of reports describing their distribution in various taxa, a comprehensive theory for the origin, maintenance, and evolution of B chromosomes has not emerged. A remarkable feature of these extra genomics elements is the accumulation of repetitive DNA sequences. We have sequenced the genomes of two individuals (with and without B chromosomes) of the cave fish, *Astyanax mexicanus*, to investigate B-related distinct repetitive contents and their relative abundance. Repeats, were identified and annotated using *RepeatExplorer* and *RepeatMasker,* a collection of software tools for characterisation of repetitive elements. The exploration of *A. mexicanus* genome concluded several transposable elements (TEs), dominated by Gypsy, TC1 Mariner, Helitron, LINEs, Rex and 45S rRNA. Fluorescent insitu hybridization (FISH) mapping revealed: The B microchromosome of *A. mexicanus* presented gypsy and Rex elements; These TEs showed a dispersal pattern on regular chromosomes (A complement); 45S rRNA evidenced eight sites and manifested the clustering type pattern on A chromosomes. We expect to explain the possible role of these repeats in the maintenance, composition and evolution of B chromosomes. Further development of our project will also allow the identification of genes present on B chromosomes and their possible biological role.

Key Words: B chromosome, genome, evolution, next-generation sequencing

WT72 Mate selection accounting for genetic variability of progeny in coho salmon. G. Yoshida*[1,2], J. Yáñez[2], S. Queiroz[1], J. Lhorente[3], and R. Caralheiro[1], [1]*School of Agricultural and Veterinarian Sciences, São Paulo State University (Unesp), Jaboticabal, São Paulo, Brazil;* [2]*Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile;* [3]*Aquainnovo S.A, Puerto Montt, Chile.*

The objective of this study was to compare the mate selection results in coho salmon, considering functions of the genetic variability of future progeny, genetic merit, coancestry and inbreeding. Pedigree information and standardized economic index of 79,144 coho salmon from the even population, comprising eight generations, were used. The mate selection was defined with the application of an evolutionary algorithm for the optimization of objective functions (OF): OF = w_1*x'EBV + w_2*x'Ax + w_3*F + w_4*Ntop + w_5*vEBVt + w_6*vEBVb, where x'EBV is the expected merit of the future progeny; x'Ax is the weighted mean coancestry of selected parents; is the expected average inbreeding coefficient of the future progeny; Ntop is the number of animals in the future progeny with expected genetic merit greater than 3 deviations; vEBVt and vEBVb are, respectively, the means of the deviations elevated to the cube of the expected genetic values of the future progeny of females classified as 50% higher and 50% lower; w_1 to w_6 are the corresponding weighting factors and × is the vector to be optimized of genetic contributions for each candidate. The weights w_1 to w_3 (OF1; w_1 = 1, w_2 = -100 and w_3 = -1) were fixed in this study and different values for the w_4 to w_6 that contemplate the genetic variability of the progeny were used and the OF1 was used as a parameter of comparison for the other OFs. The results showed that the algorithm applied allowed changing the distribution of expected genetic values of the future progeny with the genetic merit and the coancestry in similar levels to the OF1. The OF5, when the weights w_4 to w_6 were optimized simultaneously (w_4 = w_5 = w_6 = 0.01), the greater dispersion of EBVs were observed, with a higher proportion of animals with EBVs upper than 3.50 and lower than 2.50. The OF6 (w_4 = 10, w_5 = 0, w_6 = 10) was effective in increasing the expected proportion of animals with EBV above three deviations. Thus, it would be possible to define the mate selection in order to optimize the average genetic merit, coancestry and inbreeding, besides producing progeny with more or less uniformity of production, depending on the objective.

Key Words: evolutionary algorithm, inbreeding, uniformity of production

WT73 Predictive sexing of African catfish based on body and morphometric measurements using statistical discriminant methods. O. T. Abanikannda[1], L. A. Awosanya[1], A. O. Giwa[1], O. N. Ottun[2], K. Z. Awusinu[1], and F. H. Abanikannda*[2], [1]*Lagos State University, Ojo, Lagos, Nigeria;* [2]*University of Lagos, Akoka, Lagos, Nigeria.*

Catfish, one of the most commonly cultivated species of fish in aquaculture because of its high biological value and hardiness, being omnivores engage in cannibalism and aggressive behaviour more frequently than other species in mixed-sex tanks or ponds. It is almost practically impossible to visually distinguish between sexes before sexual maturity, thus this study aims at using some body and morphometric measurements of Catfish to predict its sex before sexual maturity. Out of the 240 Catfish sampled, a total of 219 comprising 125 Male and 94 Female, sexed by anatomical inspection were studied. The data were first used as training data to build the discriminant function and later as test data for the predictive classification using the linear common covariance discriminant method. In all, nine measurements and one index were investigated which include body weight (BW), total length (TL), standard length (SL), pre dorsal length (PDL), dorsal fin length (DFL), anal fin length (AFL), pre anal length (PAL), heart girth (HG) and condition factor (CF). All statistical analyses were done using JMP statistical software for exploratory, descriptive, correlation, regression and discriminant analyses. Mean BW for Male, Female and Combined was 1035.84 ± 17.09 g, 988.05 ± 24.56 g and 1015.33 ± 14.42 g respectively. Multivariate correlation of all variables revealed that there was statistically significant ($P < 0.001$) relationship between majority of the variables with the exception of the pairs of HL/CF, PDL/CF and PAL/CF. Sex had the biggest influence on CF, while BW and PAL were least affected by Sex. There was statistical difference ($P < 0.05$) across the sexes for all variables except for BW and PAL. Overall across sexes, the accuracy of prediction was 93.6% (205/219), females were better predicted correctly (94.7% accuracy) and male had 92.8% percent accuracy (Table 1). The study revealed that body and morphometric measurements of Catfish are good discriminating factors that can be employed at earlier stages

to separate fish and rear as single sex for improved productivity and survival rates.

Table 1. Summary of classification

	True Group	
Classified as:	Female	Male
Female	89	9
Male	5	116
Total	94	125
Number correct	89	116

Key Words: fish, phylogeny, sex determination, aquaculture

WT74 Genome sequencing of Tasmanian Atlantic salmon used to characterize multiple sex determination loci. S. McWilliam*[1], M. N. Sanchez[1], B. Evans[2], H. King[3], P. Kube[3], K. Verbyla[4], M. Menzies[1], and J. Kijas[1], [1]*CSIRO, St Lucia, QLD, Australia;* [2]*SALTAS, Hobart, TAS, Australia;* [3]*CSIRO, Hobart, TAS, Australia;* [4]*Data61, Canberra, ACT, Australia.*

Farmed Atlantic salmon is globally important, including in Australia where selective breeding has been in progress since 2000. Accurate sex determination is important for efficient farming, and evidence exists for the presence of multiple sex determination loci in our Tasmanian population. We performed SNP chip based GWAS using 4716 animals to map sex determination to regions on six different chromosomes. We assessed each in comparison to the location of their homeologous partner regions. This identified evidence to support true association at three loci, with the remainder representing false positive signals. To characterise sex determination loci at the sequence level, we performed whole genome sequencing of 20 fish using Illumina short read technology. This resulted in 38 × average mapped coverage per fish when mapped against the reference genome assembly ICSASG_v2. Variants were called from the mapped sequences, identifying over 8 M SNP. Raw reads were also mapped against an available *sdY* BAC contig generated from a male fish. Analysis of read depth, SNP heterozygosity and paired end sequence information in male and females revealed the precise breakpoints bounding the male specific region. Further, *sdY* region SNP haplotypes were compared between males. This revealed aspects of the likely evolutionary history that has generated multiple sex lineages in Tasmanian Atlantic salmon.

Key Words: salmon, sex, variants, evolution, genome

WT75 Prediction of the genetic sex in *Seriola lalandi lalandi* using whole-genome sequencing. V. Martinez*[1], P. Dettleff[1], S. Escobar[1], and P. Zamorano[2], [1]*FAVET-INBIOGEN, Faculty of Veterinary Science, University of Chile, Santiago, Chile;* [2]*University of Antofagasta, Antofagasta, Chile.*

Sex assignment is a critical issue in aquaculture for the development of broodstock populations. We used whole genome sequence of ~45 individuals with phenotypic sex in order to assess to what extent is possible to discover regions of the genome explaining sex determination. We further assembled one male and one, using different library preparations with an expected genome coverage of 50x. Reads were mapped to either genome assembly in order to retrieve specific sex sequences and SNPs based on the expected sex determination mechanisms (WW (male) WZ (female)) as observed for the *Seriola* genus. Several regions appear to be specific for the female assembly. We further perform GWAS as well as FST analysis, based on the expected alleles distribution observed in each sex for more than 1.2 million high-quality sequence SNPs. A single region of ~2 kb harbored two haplotypes in close proximity, can fully predict the sex of the broodfish. We developed a HRM protocol that accurately predicted the phenotypic sex observed in a broodfish population. Furthermore, these findings closely match histological data and different populations. Overall, we have found two haplotypes that can precisely predict the genetic sex in this species, the specific region is highly consistent with information from other mammalian species. This data is being incorporated in the current development of the Seriola array and will be an important step towards the development of this resource for aquaculture. Funding: Programa Nacional para la diversificacion de la acuicultura Chilena CORFO 09PDAC-7020; Programas tecnologicos estrategicos desarrollo de la acuicultura CORFO 15PTEC-45861

Key Words: fish, aquaculture, sex determination, genome sequencing

WT76 Evaluation of microsatellite markers for the study of genetic contribution of broodstock in communal spawnings of Yellowtail kingfish (*Seriola lalandi*). P. Dettleff* and V. Martinez, *FAVET-INBIOGEN, Faculty of Veterinary Science, University of Chile, Santiago, Chile.*

The Yellowtail kingfish (*Seriola lalandi*) is a new fish species aim at diversifying fish farming. Althougb the complete productive cycle has been developed in the last years in the north of Chile, little is known regarding the reproductive biology of this species. This species have communal spawning, which makes it difficult to developed efficient breeding programs. The aim of this study was to evaluate the genetic contribution of the broodstock, used information of microsatellites developed in other species but tested within the lalandi species, using whole genome sequence and population diversity. On the whole, 20 microsatellite markers, identified from genomic information of the species as well as comparative genomics using markers found in related species of the genera. We obtain DNA from samples of the breeding nucleus of the company Acuinor ($n = 100$), as well as larvae coming from different spawning seasons of each breeding group. Twelve markers which showed to be polymorphic, showing specific amplification were selected. Important variability was observed in the genetic contribution of the different parents through the spawning events evaluated, with events in which few males and females dominate. This pattern was observed across the different broodstock tanks. Females tend to show pulses of egg production, while males show a more even contribution during the spawning season. These set of markers allowed to assign the offspring to their respective parents, as well as the predominance of the contribution of certain broodstock during different spawning events under productive conditions in *Seriola lalandi*. Funding: Programa de Diversificación de la acuicultura chilena (PDACH).

Key Words: fish, aquaculture, microsatellite

WT77 Allele-specific expression analysis related with jaw deformities in Yellowtail kingfish (*Seriola lalandi*) larvae. P. Dettleff*, A. Patel, and V. Martinez, *FAVET-INBIOGEN, Faculty of Veterinary Science, University of Chile, Santiago, Chile.*

Seriola lalandi is a globally distributed species with increasing relevance for Chilean aquaculture. Skeletal deformities represent an important issue on this species. The aim of this study was to identify SNPs that present Allele-specific expression (ASE) exclusively in normal or deform *S. lalandi* larvae using RNA-seq data. We used samples of four normal and four jaw deform larvae of 23 days posthatch for Illumina sequencing. Reads were mapped to a previous *de novo* transcriptome assembly of *S. lalandi* larvae and *de novo* SNPs identification was performed across all samples with CLC Genomics Workbench. To identify ASE, the deviation of the expected ratio 50:50 of the expression of each allele was evaluated using a Chi-squared test (FDR < 0.01), determining those SNPs with ASE in each sample. Subsequently, we identify those SNPs that present ASE exclusively on normal or deform group. A total of 5815 SNPs were identified across all samples. We identify 597 SNPs with ASE exclusively on normal group, corresponding to 190 transcripts.

We determined 41 SNPs with ASE exclusively on deforms larvae, corresponding to 41 transcripts. Interestingly, we found differences in *cis*-regulation between deform and normal larvae. These includes genes related to extracellular matrix as *PLOD1* and *COL10A1* (involved in endochondral ossification); protein synthesis initiation factors as *EIF3* and *EIF6*; with muscle structure and function as *MYBPC1K*, *TNNI2, DNM2* (previously associated with morphological abnormalities during development); the calcium ion-binding protein *PVALB* (previously observed in other fish that is affected in skeletal deformities). Finally, we observed *cis* regulation only in deform larvae of the *RARG* gene, a retinoid receptor with role in the normal embryonic development and previously associated with jaw and vertebral column deformities in other fish species. This study provides relevant information about *cis*-acting factors involved in jaw deformities in *S. lalandi*. Funding: Programa de Diversificación de la acuicultura chilena (PDACH).

Key Words: fish, aquaculture, RNA-seq, allele-specific expression

WT78 Exploiting linkage disequilibrium information in turbot selection programs. M. Saura*[1], A. Fernández[1], J. Fernández[1], M. Toro[2], P. Martínez[3], A. Millán[4], M. Hermida[3], A. Blanco[3], S. Cabaleiro[5], A. Doeschl-Wilson[6], and B. Villanueva[1], [1]*Departamento de Mejora Genética Animal, INIA, Madrid, Spain;* [2]*Departamento de Producción Agraria, ETS Ingenieros Agrónomos, Madrid, Spain;* [3]*Departamento de Xenética, Facultade de Veterinaria, Universidade de Santiago de Compostela, Lugo, Spain;* [4]*Geneaqua SL, Lugo, Spain;* [5]*CETGA, Cluster de Acuicultura de Galicia, Aguiño-Ribeira, Spain;* [6]*Division of Genetics and Genomics, The Roslin Institute and Royal (Dick) School of Veterinary Studies, Midlothian, UK.*

The turbot aquaculture sector holds a top position in the international market and breeding programs associated to the culture of this fish is a well established process. The recent availability of a high quality genome for this species opens new opportunities to study the genomic architecture of complex traits and to improve selection efficiency through genomic selection. The success of these approaches depends however on the magnitude and extent of the linkage disequilibrium (LD) across the genome. The aim of this study was to characterise genomic LD patterns in a Spanish turbot population challenged with the parasite *Philasterides dicentrarchi,* in order (1) to investigate the potential of within-family genomic selection, and (2) to identify QTL regions for disease resistance using genome-wide association analysis (GWAS). RAD-sequencing was used to identify and genotype 18,824 SNPs in 1,393 fish belonging to 36 families. Linkage disequilibrium was estimated as the squared correlation between SNP pairs (r^2). GWAS for resistance was performed by analysing each SNP independently. LD was moderately high between closely linked markers at the within-family level (r^2 ~0.40 between markers separated by 5 Kb), while it was considerably lower at the population level (r^2 ~0.10). With increasing distance between SNP pairs, r^2 decreased by half over the first 4 - 5 Mb and 1 Mb at within-family and population level, respectively. Results from GWAS revealed 60 associations with the disease trait. Our preliminary results suggest that within-family genomic evaluation for disease resistance could have great potential in turbot aquaculture. The consensus physical map currently under development will allow to identify candidate QTL regions and explore the genetic content within them.

Key Words: aquaculture, disease resilience, genome-wide association, genomic selection, linkage disequilibrium

WT79 Optimum-contribution selection increases genetic gain in Atlantic salmon breeding schemes. B. Hillestad*[1] and M. Henryon[2], [1]*SalmoBreed AS, Bergen, Norway;* [2]*Seges, Copenhagen, Denmark.*

We tested the hypothesis that genetic gain in an Atlantic-salmon breeding population will be increased by changing from truncation selection (TS) to optimum-contribution selection (OCS). This was tested by simulating salmon breeding populations under OCS and TS. Selection was performed for a single trait with $h^2 = 0.25$ and $\sigma^2_P = 0.722$. The schemes were run over 10 generations, with a rate of inbreeding (ΔF) equal to 1%. For each following generation 300 new families were produced with a litter size of 40 fish. The TS schemes were setup with a 2x1 factorial design, where each male where allowed to mate with two females. The maximum number of individuals per family that was selected to mate varied from five to 40. There were two different factorial designs set up for the OCS schemes: 3x3 and 300x300, where each selected animals was mated to three or 300 individuals, respectively. The second OCS scheme then allowed for full OCS. For OCS schemes as well, the number of individuals selected to mate per family varied from 5 to 40. Preliminary results showed that ΔG can be increased by at 8–15% when the ΔF is set to approx. 1%. Further results will also give us insight on how the number of males and females allowed to mate per family will affect both ΔG and ΔF, and how restriction on the number of mating sires and dams are allowed to do in OCS. This suggests that genetic gain in Atlantic salmon breeding population would be increased by implementing OCS.

Key Words: fish, animal breeding

WT80 Comparative genomics of disease resistance traits in salmonids. J. M. Yáñez*, *Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile.*

Infectious diseases generate large economic losses in salmon farming. For instance, *Piscirickettsia salmonis,* the causal agent of Salmon Rickettsial Syndrome, affects several salmon species and is considered one of the major pathogens of the salmon farming industry. A feasible and sustainable alternative to prevent disease outbreaks is represented by genetic improvement for disease resistance. The information from causative mutations involved in resistance against diseases may be used to accelerate the genetic progress for these traits. Comparative genomics can provide useful information from common functional variants associated with disease resistance traits in salmonids. Here, we perform genome-wide association studies (GWAS) for resistance against *Piscirickettsia salmonis* in three commercial salmonid species: Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*). The analyses were performed using phenotypic data from challenged fish and genotypes obtained from 50K and 57K Affymetrix single nucleotide polymorphisms (SNPs) arrays available for Atlantic salmon and rainbow trout, respectively, and from a Double Digest Restriction Associated DNA (ddRAD) Sequencing experiment for coho salmon. We found loci significantly associated with *P. salmonis* resistance in the three species. There were five SNPs in chromosomes *Ssa*01 and *Ssa*17, one SNP in chromosomes *Omy*25 and one SNP in scaffold *Oki*01025 significantly associated with *P. salmonis* resistance in Atlantic salmon, rainbow trout and coho salmon, respectively. However, the proportion of the phenotypic variance explained by each marker was small (< 5%) for each species. A comparison of the most important genomic regions associated with resistance against *P. salmonis*, representing 1% of the most significant associated SNPs for each species, was carried out in order to identify common genomic regions among species. Biological candidate including genes related with immune response and iron metabolism have been found to be physically linked with these genomic regions and may play an important role in the differential immune response against this pathogen in salmonids.

Key Words: *Piscirickettsia salmonis*, genome-wide association analysis, coho salmon, Atlantic salmon, rainbow trout

WT81 Transcriptomic profile of *Salmo salar* skin in response to the Chilean sea louse *Caligus rogercresseyi* using *de novo* transcriptome assembly. K. NEUMANN*[1], D. CICHERO[2], and V. MARTINEZ[1], [1]*FAVET-INBIOGEN-Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile;* [2]*Blue Genomic SPA, Puerto Varas, Chile.*

The Chilean sea louse *Caligus rogercresseyi* is the most important copepod ectoparasite responsible for significant economic losses in the salmon industry. In order to better understand the biological mechanisms involved in the response of the host to the parasite, the main objective of this study was to characterise the transcriptome profile of *Salmo salar* skin in response to *C. rogercr*esseyi through RNA-Sequencing analysis using a *de novo* transcriptome assembly. A challenge trial was conducted on a pedigree population of *S. salar* from the genetic program of AquaGen Chile SA (Puerto Varas, Chile), which were challenged with *C. rogercresseyi* in its infestive stage. The sea lice were allowed to develop until the chalimus III – IV stage. Chalimus counts were conducted on all fish, and individuals with extreme phenotypes were classified as having low (L) or high (H) lice count. The skin samples were RNA-sequenced on a MiSeq platform (Illumina, USA). A *de novo* assembly using Trinity was annotated using Trinotate. The RNA sequences were then mapped to the *de novo* assembled transcriptome using Salmon software, which also it allows quantifying the expression of transcripts. Differential expression analysis was performed with EdgeR with a false discovery rate p-value < 0.05. The enrichment analysis on Gene Ontology (GO) terms were performed by a Fisher's exact test using TopGo with a p-value < 0.01. Among the down-regulated transcripts in the L group compared to the H group, we found 19 GO terms enriched for biological process (BP), 9 terms for molecular functions (MF) and 4 terms for cellular component (CC). While the up-regulated transcripts in the L group, had 10 GO terms enriched for BP, 4 terms for MF and 2 for CC. Some important terms are related to protein metabolism, immune system processes and cell death which were down-regulated in the skin from L group. These results show the complexity of local host response to the sea louse, in term of processes involved and its relationship with the background of fish. This work contributes to better understanding the response of *S. salar* to sea louse *C. rogercresseyi*.

Key Words: fish, functional genomics, RNA-seq, gene expression, genomic selection

WT82 Rapid cold shock induces only slight shift in gene expression of rainbow trout *(Oncorhynchus mykiss*). T. Goldammer*[1], A. Borchel[1,2], M. Verleih[1], and A. Rebl[1], [1]*Leibniz Institute for Farm Animal Biology, Inst. f. Genome Biology, Dummerstorf, Germany;* [2]*University of Bergen, SLCR-Sea Lice Research Centre, Bergen, Norway.*

A rapid decline in temperature poses a major challenge for poikilothermic fish, as their entire metabolism depends on ambient temperature. We compared the gene expression of rainbow trout (Oncorhynchus mykiss) having undergone such a cold shock (0°C) to a control (5°C) using microarrays and quantitative real-time PCR. The number of genes found to be regulated at 0°C was surprisingly low. Instead of classical genes involved in temperature shock, the three genes encoding fibroblast growth factor 1 (fgf1), growth arrest and DNA-damage-inducible, α (gadd45a) and sclerostin domain-containing protein 1 (sostdc1) were up-regulated in the liver upon cold shock in two different rainbow trout strains, suggesting that these genes may be involved in the response to cold shock in rainbow trout.

Key Words: stress response, fgf1, gadd45a, microarray, sostdc1

WT83 Mining the European Sea Bass (*Dicentrarchus labrax*) genome for the characterization of tandem repeat variability. F. Bertolini*[1,2], S. Bovo[2,3], M. F. Rothschild[1], and L. Fontanesi[2], [1]*Department of Animal Science, Iowa State University, Ames, IA, USA;* [2]*Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Bologna, Italy;* [3]*Biocomputing Group, Department of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy.*

Dicentrarchus labrax L., the European Sea Bass (also known as European Seabass), is a teleost fish mainly distributed along the coasts of the North-Eastern Atlantic Ocean and of the Mediterranean and Black Seas. This species of the family Moronidae is one of the most relevant marine species for professional and sport fisheries and aquaculture production, raising attention and concerns for conservation and management of natural genetic resources on one hand and for adaptation to aquaculture conditions, domestication and selective breeding on the other hand. Therefore, the development of a large number of microsatellite markers could provide useful tools for these purposes. Microsatellites (with motif length < 10) are considered a subgroup of tandem repeats that traditionally includes minisatellites (with motif length of 10–60) and macrosatellites (with motif length >60). Tandem repeats been have frequently utilised in forensics, population genetics, and low-cost genetic scans in many different species. In this study, we mined the current draft version of the European Sea Bass genome encompassing 25 assembled chromosomes to identify tandem repeat regions and then we complemented this information using next-generation sequence data we produced from DNA pools obtained from 12 fish of this species. The European Sea Bass genome were scanned for tandem repeats with the Tandem Repeats Finder software. A total of 75,805 microsatellites, 12,790 minisatellites and 410 macrosatellites were identified across the genome with at least the 95% of identity. All these regions were profiled using the lobSTR software in 2 seabass DNA pools composed of 6 animals each and derived by 2 different hatcheries, and sequenced with Proton Torrent sequencer. A total of 1,471 regions for the pool 1 and 4,102 regions for the pool 2 had a minimum depth of 3. Among these regions, that on the whole intersected 381 genes, a subgroup of them, i.e. 1,313 for pool 1 and 3,724 for pool 2, showed variation from the profile of the reference genome. This study produced a whole tandem repeat map of the European Sea Bass genome useful for the characterisation of the genetic variability and future breeding programs.

Key Words: European Sea Bass, tandem repeats, NGS

WT85 GWAS reveals the architecture of two maturation traits in Tasmanian Atlantic salmon. J. Kijas*[1], A. Mohamed[1], S. McWilliam[1], B. Evans[2], H. King[3], P. Kube[3], and K. Verbyla[4], [1]*CSIRO, Brisbane, Queensland, Australia;* [2]*SALTAS, Hobart, Tasmania, Australia;* [3]*CSIRO, Hobart, Tasmania, Australia;* [4]*Data61, Canberra, ACT, Australia.*

A key developmental transformation in the life of all vertebrates is the transition to sexual maturity, whereby individuals are capable of reproducing for the first time. In the farming of Atlantic salmon, unwanted maturation that occurs before harvest size has a serious negative impact as it retards growth while severely diminishing flesh quality. Recent findings in European Atlantic salmon report the presence of a gene that exerts a large effect on age of maturation (*VGLL3*). We performed two genome wide association studies in Tasmanian animals, which are derived from North American stock, to map genetic loci that contribute to variation. First, a total of 2721 fish with trait data describing maturation in the marine environment were genotyped using a custom SNP50 array. Second, genotypes were collected from 1846 fish with trait data describing maturation in freshwater. For both experiments, a case-control design lineage regression analysis was performed to identify associated regions. Neither GWAS suggests *VGLL3* plays a major role in the two maturation traits as measured in the Tasmanian population. Further, the two traits have different architecture as few highly associated SNP were common to both experiments. We present findings

from a systematic assessment of the gene content of associated genomic regions. This revealed genes involved with energy metabolism, neuroendocrinology and steroid biosynthesis.

Key Words: salmon, GWAS

WT86 Reconstructing the complex structure of the sex determination locus in Atlantic herring using SMRT sequencing. N. Rafati*[1], C.-J. Rubin[1], C. Feng[1], M. Petterson[1], A. Bario Martinez[2], S. Lamichhaney[1], I. Bunikis[3], and L. Andersson[1,5], [1]*Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden;* [2]*Science for Life Laboratory, Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden;* [3]*Science for Life Laboratory, National Genomics Infrastructure, Uppsala University, Uppsala, Sweden;* [4]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [5]*Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA.*

The mechanism for sex determination considerably varies among species. Fish (similar to some of reptiles) have established two distinct sex determination systems: environmental sex determination (ESD) and genetic sex determination (GSD), and sometimes both systems work in concert. GSD can be under control of sex chromosomes or master genes on autosomal chromosomes, yet in most fish species genes with predominant roles in sex determination have not been reported. An exception is the *dmrt1* paralogous copy (*dmy)* on medaka's (*Oryzias latipes*) Y-chromosome governing sex determination. It is now clear that a GSD system has evolved independently in several lineages of teleosts. However in the majority of them, including Atlantic herring (*Clupea harengus*), the sex determination system is still unknown. Herring is among the most abundant species with vast economical and ecological importance in Northern Atlantic. Atlantic herring reproduces throughout the Baltic Sea and Atlantic Ocean in different salinities (2–35‰) and seasons. We generated a high-quality draft genome assembly by short read sequencing technology to unravel the genetic basis of ecological adaptation to both salinity and seasonal reproduction. We identified a large region (~100 Kb) for which males and females showed significant differentiation (male specific region). Our study on unmapped reads revealed male unique sequences belonging to a member of the *cation channel sperm-associated protein* (*CATSPER*) gene. But our efforts in linking these two segments by PCR failed. To gain further insight into the herring genome, we generated a new assembly by single-molecule real-time (SMRT) sequencing technology. In this new assembly, we revealed the organisation of the previously identified signals indicative of early stages of sex chromosome evolution. This is the first report on identifying a sex determination locus and proto-Y chromosome in Atlantic herring. This study enhances our understanding of the evolution of sex chromosome in this species and other teleosts.

Key Words: sex determination, evolution, sex chromosome, reproduction, SMRT sequencing

Livestock Genomics for Developing Countries

WT87 Genomic diversity and population structure analysis reveal few genetic differences among Ethiopian indigenous sheep populations. A. Ahbara*[1,2], J. Mwacharo[3], H. Bahbahani[4], S. Mastrangelo[5], F. Pilla[6], E. Ciani[7], and O. Hanotte[1], [1]*School of Life Sciences, University of Nottingham, Nottingham, Nottinghamshire, UK;* [2]*Department of Zoology, Faculty of Sciences, Misurata University, Misurata, Libya;* [3]*Small Ruminant Genetics and Genomics Group, International Center for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia;* [4]*Department of Biological Sciences, Faculty of Science, Kuwait University, Safat, Kuwait;* [5]*Dipartimento di Scienze Agrarie e Forestali, University of Palermo, Palermo, Italy;* [6]*Dipartimento Agricoltura, Ambiente e Alimenti, Università degli Studi del Molise, Campobasso, Italy;* [7]*Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica, Università degli Studi di Bari 'Aldo Moro,', Bari, Italy.*

African sheep, like other domestic sheep, are domesticated from the Asiatic mouflon (*Ovis orientalis*). They entered the continent through the North and the Horn of Africa regions following maritime and terrestrial trading routes. Ethiopia is one of the main entry points of various plant and animal domesticates into Africa. It is characterised by diverse agro-ecologies, ancient human ethnic diversity and the presence of indigenous sheep breeds/populations of unprecedented morphological diversity (e.g. tail types). Here, we investigate the genome diversity and population structure of 146 unrelated animals from 11 Ethiopian indigenous sheep populations. DNA was extracted from ear tissue punches and genotyped with the Illumina Ovine 50K SNP BeadChip assay. Sheep populations from The Caribbean, Europe, Middle East and China as well as from western, northern and southern Africa were included to clarify the genetic history of origin, introduction and dispersal of the species into Ethiopia. Principal component analysis (PCA), clearly separated all Ethiopian sheep from the other populations. Population structure and phylogenetic (neighbour-joining tree) analysis subdivided the Ethiopian indigenous sheep into three genetic clusters corresponding to their tail morphology (rump fat-tailed, short fat-tailed and long fat/thin-tailed population). It supports a common genetic ancestry for populations of each tail type in the country. Genetic distances among the Ethiopian populations were positively correlated with geographic distances (Mantel test, $P < 0.001$, $r = 0.465$) and the highest genetic diversity was recorded in the fat-tailed (short, rump and/or long fat-tailed) close to the Bab-el-Mandeb strait. However, despite their distinct morphology and separate geographic distribution, little genetic differentiation between Ethiopian populations are observed. This is most likely a consequence of their ancient and modern intermixing following their introduction into the country.

Key Words: sheep, genome-wide association, population structure, breed diversity, population

WT88 Genetic admixture in indigenous Ugandan goat breeds using genome-wide SNP data. R. B. Onzima*[1,3], M. R. Upadhyay[1], R. Mukiibi[2], E. Kanis[1], and R. Crooijmans[1], [1]*Wageningen University and Research Animal Breeding and Genomics, Wageningen, the Netherlands;* [2]*Department of Agriculture, Food and Nutritional Sciences (AFNS), Faculty of Agriculture, Life and Environmental Sciences University of Alberta, Alberta, Canada;* [3]*National Agricultural Research Organization (NARO), Entebbe, Uganda.*

Well-adapted indigenous goats are an important genetic resource for future sustainable production in marginal areas. The introduction of exotic Boer goats for meat production in Uganda has had an effect on the diversity of the goat genetic resources in the country. Little is known about the effect of exotic Boer goats on the genetic diversity and population structure of the goat breeds in Uganda. The objective of this study was therefore to assess genetic admixture and population structure in goat population in Uganda. Five indigenous Ugandan goats of Mubende ($n = 29$), Kigezi ($n = 29$), Small East African ($n = 29$), Sebei ($n = 29$) and Karamojong ($n = 15$) and exotic Boer ($n = 13$) were assessed using caprine SNP50 bead chip. The polymorphism (MAF >0.05) across the breeds was 93.4% with the highest polymorphism observed in Sebei (92.8%) and lowest in Kigezi (88.5%). The average heterozygosity across

the populations was (0.384 ± 0.143) with the lowest heterozygosity recorded in Kigezi (0.377 ± 0.189) and the highest in Karamojong (0.410 ± 0.192). Principle component analysis (PCA) and ADMIXTURE analysis inferred Boer as an outlier population from the Ugandan indigenous goat breeds, whereas ADMIXTURE clustered Ugandan indigenous goat populations into Small East African and two indistinct clusters of Kigezi & Mubende and Karamojong & Sebei. These clusters may reflect the similarity of the breeds by ancestry separated by distance.. Admixture analysis and four population tests further revealed introgression of the Boer into indigenous animals. . Information derived from the level of admixture will be useful in maintaining genetic diversity and designing appropriate breeding programs to exploit within breed selection and heterotic advantage in cross-breeding schemes.

Key Words: breed diversity, admixture, heterozygosity, population structure, goats

WT89 Genome-wide association study of growth traits in Nellore cattle. R. B. Costa*[1], A. P. N. Terakado[2], G. M. F. Camargo[1], R. Carvalheiro[2], and L. G. Albuquerque[2], [1]*Federal University of Bahia, Salvador, BA, Brazil;* [2]*Sao Paulo State University, Jaboticabal, SP, Brazil.*

The objective of this work was to study the association between the SNP markers with birthweight (BW), weight gain from birth to weaning (WGBW) and from weaning to yearling (WGWY), and hip height at yearling (HY) in Nellore cattle. Data from 5,064 animals belonging to the breeding programs DeltaGen and PAINT were used. The animals were genotyped with a panel of 777,962 SNPs (Illumina BovineHD Beadchip) and after the quality control of genomic data 412,993 SNPs remained in the analysis. The genome association analysis (GWAS) was performed using a single-step methodology and analyzes were performed using BLUPF90 family programs. A vector of weights of SNPs was considered in the analysis and the iteration process was done twice, so the SNPs and the animal effects were recalculated in order to increase the weight of SNPs with greater effects and lessen those with small effects on the traits. The results of GWAS were based on the proportion of the variance explained by the 50 adjacent SNPs windows. Based on two iterations, only windows of SNPs that explained more than 1.5% of the additive genetic variance were considered significant and were discussed. There were associations with BW in BTA 14, with WGBW in BTA 5 and 29, with WGWY in BTA 11 and HY in BTA 8, highlighting the *TMEM68* gene (transmembrane protein 8B) associated with BW and HY, *XKR4* (XK, Kell blood group complex subunit-related family, member 4) associated with BW, *NPR2* (natriuretic peptide receptor B) associated with the HY and *REG3G* (Regenerating islet-derived 3-gamma) associated with WGWY. These genes are strongly associated with feed intake, weight gain and the regulation of skeletal growth in previous studies.

Key Words: beef cattle, single-step, SNP, weight, weight gain

WT90 Linkage disequilibrium, linkage phase and effective population size estimates in four Philippine riverine buffalo populations. J. R. V. Herrera*[1,3], E. B. Flores[2], C. Gondro[3], and J. H. van der Werf[3], [1]*Philippine Carabao Center-University of the Philippines College, Laguna Philippines;* [2]*Philippine Carabao Center National Headquarters, Muñoz, Nueva Ecija, Philippines;* [3]*School of Environmental and Rural Science, University of New England, Armidale, NSW, Australia.*

The success of genome-wide association studies (GWAS) and genomic selection (GS) studies depends on the strength of linkage disequilibrium (LD) between genetic markers and quantitative trait loci (QTL). The extent of LD determines the minimum distance between markers for effective coverage for GWAS. Knowledge of effective population size (Ne) is also important since this affects the genomic prediction accuracy. Estimates of linkage phase across populations are also essential for determining the success of using pooled reference populations in multi-breed GS programs. The objective of this study was to estimate LD, linkage phase and Ne in four riverine buffalo populations found in the Philippines, namely Bulgarian Murrah (BUL), Brazilian Murrah (BRA), Italian Mediterranean (ITA) and American Murrah (AME). A total of 182 animals were genotyped using the Axiom 90k Buffalo genotyping array. Polymorphic SNPs found in autosomes for the BUL, BRA and ITA and AME populations were 56,818, 57,796, 46,154 and 45,599, respectively. Useful LD ($r^2 \geq 0.20$), determined using the PLINK software, extended up to ~70 kb, ~70 kb, ~400 kb and ~200 kb for BUL, BRA, ITA and AME, respectively. With average inter-marker distance of 44 kb, 43.3 kb, 54.1 kb and 54.8 kb for BUL, BRA, ITA and AME, respectively, there is sufficient LD to do GWAS and GS in the four populations. Using the NeEstimator software, recent Ne sizes were 125, 209, 79 and 49 for BUL, BRA, ITA and AME populations, respectively. Using the PLINK software, linkage phase was based on the correlation of r values for the same SNP pairs between populations. There was a moderate positive correlation of 0.42 at ~70 kb for the BUL: BRA pair while the BUL: ITA pair resulted to a weak positive correlation of 0.24 at the same distance. This suggested that the BRA population can be combined with the BUL population as one reference population while the ITA population would be a separate reference population.

Key Words: river buffalo, SNP chip, linkage disequilibrium, linkage phase, effective population size

WT91 Genomic diversity and autozygosity within the SA Drakensberger beef cattle breed. S. F. Lashmar[1,2], C. Visser*[1], and F. C. Muchadeyi[2], [1]*University of Pretoria, Pretoria, Gauteng, South Africa;* [2]*Biotechnology Platform (Agricultural Research Council), Pretoria, Gauteng, South Africa.*

The employment of genomics-assisted breed improvement in the developing world is dependent on genetic characterisation of indigenous livestock resources. End goal- (genomic selection) and intermediary (imputation) genomic applications require an understanding of both genome-wide diversity and uniformity. This study aimed to quantify population-specific genomic parameters that may influence the accuracy of downstream processes for the SA Drakensberger, a Sanga breed of unknown genetic composition. A sum of 338 cattle genotypes was generated with the GeneSeek Genomic Profiler (GGP) 150K SNP bead chip. Samples and autosomal, mapped SNP were quality filtered using PLINK. Inter-chromosomal variation in minor allele frequency (MAF) and linkage disequilibrium (LD) was investigated. The average MAF across autosomes was 0.26 ± 0.14. BTA21 and BTA14 harbored the lowest- (~6%; mean MAF = 0.28) and highest (~16%; mean MAF = 0.25) percentage of low-MAF SNP, respectively. Marker density averaged at 1 SNP per ~23.5kb; average LD for inter-SNP distances ≤ 23.5kb was $r^2 = 0.27$. LD of ≥ $r^2 = 0.2$, however, persisted only up to an inter-SNP distance of ~30kb; dropping to 0.18 and 0.14 when SNPs were separated by < 100kb and < 1Mb, respectively. Autozygosity was investigated through the identification of runs of homozygosity (ROH) in PLINK. Inbreeding coefficients were calculated based on pedigree- (F_{PED}), SNP- (F_{SNP}) and ROH (F_{ROH}) information using Pedigraph (F_{PED}) and PLINK (F_{SNP}, F_{ROH}). All coefficients indicated a low level of inbreeding; F_{PED}, F_{SNP}, $F_{ROH>1Mb}$, $F_{ROH>4Mb}$, $F_{ROH>8Mb}$ and $F_{ROH>16Mb}$ were estimated as 0.041, 0.005, 0.079, 0.063, 0.042 and 0.018, respectively. Shorter ROH contributed the most to ROH-based coefficients, indicating more distant inbreeding. Using *R*, moderate correlations ($r = 0.677$; *P*-value < $2.2x10^{-16}$) of F_{PED} with F_{SNP} and $F_{ROH>1Mb}$, respectively, were estimated. A degree of loss in genomic diversity will facilitate closer within-breed relatedness, which could enhance the accuracy of downstream applications. Inter-chromosomal variation in MAF and LD may influence how

different chromosomes are treated when implementing genomic strategies such as imputation for the Drakensberger breed.

Key Words: cattle, single-nucleotide polymorphism (SNP), breed diversity, inbreeding

WT92 Genome-wide SNP diversity of the South African domestic and wild pig populations. N. Hlongwane*[1,2], E. Dzomba[2], K. Mdladla[1,2], P. Soma[3], and F. Muchadeyi[1], [1]*Agricultural Research Council, Biotechnology Platform, Onderstepoort, South Africa;* [2]*Discipline of Genetics, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa;* [3]*Agricultural Research Council, Animal Production Institute, Irene, South Africa.*

South African (SA) pig populations are of mixed diversity because of large pig numbers of European, Asian and American origin and native wild breeds that are co-existing. Different founder pig populations could harbour unique genetic variants. The objective of this study was to investigate the genetic diversity, population structure and level of pairwise population differentiation of SA pigs using the PorcineSNP60 genotyping array. In total, 208 individuals from 13 diverse populations consisting of villages from Limpopo (Mopani, $n = 27$ and Capricorn, $n = 24$) and Eastern Cape provinces (Alfred Nzo, $n = 16$ and O.R. Tambo, $n = 22$), commercial (Large White, $n = 20$; Duroc, $n = 20$; SA Landrace, $n = 20$), indigenous (Kolbroek, $n = 20$; Windsnyer, $n = 20$), wild (Warthog, $n = 7$; Wild Boar, $n = 4$; Bushpig, $n = 3$) and Vietnamese ($n = 5$) pig breeds were genotyped. Expected diversity (He), Inbreeding (Fis) and Minor Allele Frequency (MAF) were used to estimate within population diversity. Pairwise F_{ST} and hierarchical analysis of molecular variance were used to determine the level of population differentiation and partitioned variation into within and between subpopulations. Moderate He levels, ranging from 0.337 to 0.433 were reported across populations. MAF varied from 0.021 for Warthogs to 0.264 for Capricorn. Fis was generally low in all the populations except for village pigs whose Fis was as high as 0.189 (Mopani). AMOVA analysis revealed that 92% of the genetic variation was within the populations. F_{ST} values ranged from 0.021 between the villages to 0.841 between the Warthog and Vietnamese populations. Population structure analysis showed the highest levels of admixture between village populations. Sharing of genetic materials was observed between villages, commercial and indigenous populations. Both PCA and ADMIXTURE clustering presented a clear separation of domestic pigs from the wild populations with the exception of the Wild Boar that clustered with the villages, Windsnyer, Large White and SA Landrace pigs. Inferences were made on levels of population hybridisation among breeds and the risk of extinction of given populations.

Key Words: genetic variation, PorcineSNP60, pig breeds, population differentiation

WT93 Genetic characterization of Argentine and Bolivian creole cattle using HD SNPs microarray. M. Fernandez[1], M. Ortega Masague[2], J. Orellana[3], F. Valdez[4], S. Peña[5], A. Rogberg Muñoz[1,6], L. Gutierrez[2], M. Baudoin[5], D. M. Posik[1], E. E. Villegas Castagnasso[1], I. P. Manrique Osinaga[3], F. D. Holgado[2], J. P. Lirón[7], D. E. Goszczynski[1], P. P. García[1], C. Bomblat[5], E. Salas[4], J. A. Pereira Rico[3], and G. Giovambattista*[1] [1]*Instituto de Genética Veterinaria (IGEVET), CCT La Plata CONICET - Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina;* [2]*Instituto de Investigación Animal del Chaco Semiárido (IIACS – INTA Leales), Leales, Tucumán, Argentina;* [3]*Facultad de Ciencias Veterinarias, Universidad Autónoma Gabriel René Moreno, Santa Cruz de la Sierra, Santa Cruz, Bolivia;* [4]*Centro de Investigación Agrícola Tropical (CIAT), Santa Cruz de la Sierra, Santa Cruz, Bolivia;* [5]*CEASIP, Fundación Simón I Patiño, Santa Cruz de la Sierra, Santa Cruz, Bolivia;* [6]*Departamento de Producción, Facultad de Agronomía, Universidad de Buenos Aires, CABA, Argentina;* [7]*Center of Veterinary Research (CIVETAN, CONICET) Faculty of Veterinary Sciences, UNCPBA, Tandil, Buenos Aires, Argentina.*

Latin American Creole cattle, direct descendants of bovines introduced by Europeans during the conquest of the Americas, are Taurine cattle adapted for more than 500 years of selection in tropical and subtropical environments. Although its population has suffered a drastic reduction during the last century, these native breeds still constitute a valuable animal genetic resource of this region. For this reason, the objective of the present study consisted in carrying out the genetic characterisation of five Argentine and Bolivian cattle populations, for which 73 samples were analysed using a 640K SNPs microarray. The results obtained showed an average expected heterozygosity value of 21.23, ranging from 20.40 to 24.60 in all populations. Molecular inbreeding, estimated through F index, evidenced that individual inbreeding values varied between -0.151 to 0.159. A subset of 4556, evenly-spaced SNPs among the 29 autosomal chromosomes, were filtered and used to analyse the relationship among breeds and estimated the breed admixture. The Principal component analysis evidenced that Creole cattle populations comprised a cluster that clearly diverge from the rest of the taurine and zebuine breeds included in the study. In addition, it showed low levels of cebu gene introgression and revealed the presence of two Creole components, one including the Argentine Creole breed and another comprising the Bolivian populations. In conclusion, the present results support the hypothesis that American creole cattle have unique characteristics and reinforce the need to conserve this valuable animal genetic resource.

Key Words: cattle and related species, population genomics, microarray, breed diversity, breed/population identification

WT94 Genomic population structure and relationship between the South African Nguni Sheep. K. S. Nxumalo*[1,2], J. P. Grobler[1], K. Ehlers[1], K. Ncube[3], F. C. Muchadeyi[3], and N. O. Mapholi[2], [1]*University of Free State, Department of Genetics, Bloemfontein, Free State, South Africa;* [2]*Agricultural Research Council-Animal Production Institute, Irene-Pretoria, Gauteng, South Africa;* [3]*Agricultural Research Council-Biotechnology Platform, Onderstepoort-Pretoria, South Africa.*

In this study, 146 (Zulu, Pedi, Swazi, Doper, Namaqua Afrikaner and Damara) sheep individuals were analysed to access genetic variation among Nguni sheep using ovine50KSNP Chip. Genetic diversity and population structure among Nguni sheep breed types is essential to understand and identify genetic improvement, understanding of environmental adaptation as well as utilisation and conservation of Nguni sheep breed. The South African Nguni sheep breed types (Pedi, Zulu and Swazi) forms part of the South African indigenous sheep breeds variation in the country. It has been reported that Nguni sheep breed is threatened with extinction, due to terminal crossbreeding effect. Blood samples ($n = 25$) were collected from six populations of unrelated individuals. Genomic DNA was extracted using the Qiagen DNeasy extraction kit. Animals DNA samples were genotyped using ovine 50KSNP chip. All genotype calls were obtained from the raw genotypic data using Genome Studio (Illumina). Genotypic data was subjected to quality control (QC) measures using PLINK software, sample call rate >90% and SNPs with a call rate (Geno >0.05), minor allele frequency (MAF < 0.05) or violated Hardy–Weinberg Equilibrium (HWE p-value < 0.001) were removed for further analysis. After QC of 54 241SNPs, 48429 SNPs remained for analysis. Expected heterozygosity (*He*) ranged between 0.28 (Namaqua Afrikaner) and 0.34 (Swazi) while observed heterozygosity (*Ho*) varied between 0.21 (Damara) to 0.38 (Swazi). Namaqua Afrikaner was clearly discriminated from other populations on the principal component analysis (PCA). The distance between Pedi and other Nguni populations was observed with the Damara being the closest to them instead. The distance pairwise

revealed the closest relationship between Damara and Swazi sheep, which was also observed on PCA. The clustering of populations clearly demonstrated the low levels of admixture among them.

Key Words: indigenous sheep, genetic variation, conservation, SNP genotyping

WT95 Genome-wide analysis for signature of selection in domestic chicken and red jungle fowl. R. A. Lawal*[1] and O. Hanotte[1,2], [1]*The University of Nottingham, Nottingham, Nottinghamshire, UK;* [2]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia.*

The red jungle fowl *Gallus gallus* is the main maternal ancestor of modern chicken having been domesticated from multiple subspecies of *Gallus gallus* more than 5400 years ago. Following domestication, environmental and human selection pressures have shaped the domestic chicken making them one of the most diverse livestock species with population of more than 50 billion across the world. Here, we report the analysis of indigenous chicken population from Ethiopia, Saudi Arabia and Sri Lanka, alongside the red jungle fowl, for signature of positive selection across the autosomes. Using the pool heterozygosity and composite likelihood ratio methods, we identified several candidate genomic regions that may correspond to local adaptation along with five candidate domestic regions shared and unique to all domestic populations. Only two candidate regions located on chromosomes 7 and 23 out of the 191 found in the red jungle fowl were shared with all domestic chicken populations. Gene ontology show that most of the seven genes located within the sweep region on chromosome 23 are associated with development with particular emphasis on central nervous system, memory, emotion and learning traits. Finally, this study identify several regions unique to each chicken ecotypes and several regions linked to production and growth traits in Saudi Arabia chicken

Key Words: genome sequencing, genomic selection, candidate gene, gene ontology, animal domestication

WT96 Genetic admixture and identity by descent in Senegalese dairy cattle. P. J. N. Ema[1,3], A. Missohou[1], K. Marshal[2], S. F. Tebug[2], J. Juga[4], and M. Tapio*[5], [1]*Interstate School of Veterinary Science and Medicine of Dakar, Dakar, Senegal;* [2]*International Livestock Research Institute, Nairobi, Kenya;* [3]*University of Ngaoundere, Ngaoundere, Cameroon;* [4]*University of Helsinki, Helsinki, Finland;* [5]*Natural Resources Institute Finland, Jokioinen, Finland.*

Cattle keeping is an important livelihood in Senegal. As a part of non-transhumant dairy production analysis to understand factors influencing farmer's net benefit, we analysed the composition of production stock of small and moderate scale farms in two Senegalese study sites. The data included genotypes of 624 Senegalese cows based on BovineSNP50 BeadChip. Published data of 455 genotypes from 26 breeds was used as a reference. This set included several improved taurine and indicine breeds and African cattle populations. The interviews and field visits suggested the presence of several breeds and crossbreeds. Trained Bayesian clustering analysis supported idea of heterogeneity and uneven influence of non-native taurine and indicine cattle was detected. The main types were *Indigenous zebu*, *Indigenous zebu by Guzerat*, *Indigenous zebu by improved taurine*, and *High improved taurine*. Identity by descent analysis revealed that cows that were more crossbred also shared a larger proportion of their genomes with each other. Thus, the crossbred cattle are related to each other. This may reflect the higher relatedness within the improved breeds. While the average IBD sharing was low in *Indigenous zebu* group, the variation across genome regions was more pronounced. Pedigree recording and managed crossbreeding schemes may combine benefits from local and exotic cattle, while limit the increase in the relatedness.

Key Words: cattle and related species, genome-enabled breeding, population structure, milk production, crossbreeding

WT97 Can genomics be used in the smallholder livestock sector? Case studies from South Africa. F. C. Muchadeyi*, *Agriculture Research Council – Biotechnology Platform, Pretoria, South Africa.*

High-density SNP genotyping technology has numerous possible applications in livestock improvement programs including breed characterisation, genetic diversity analysis, genome-wide association studies as well as genomic selection. In South Africa, dissection of livestock genome structures particularly in the marginalized smallholder sector is increasingly turning to SNP genotypes. Case studies are presented wherein population genomic tools have been applied in a range of livestock species to determine intra-species diversity and population genetic structures as well as infer on genetic adaptation using signatures of selection. Examples are given where genome-wide SNP data is utilised in estimating effective population sizes and genomic inbreeding levels in populations where pedigree information is unavailable. Determination of causal mutations for livestock genetic disorders through application of genome-wide association analysis is presented. The value of these genomic tools to smallholder livestock populations of diverse nondescript phenotypes, limited breeding records because of random and unmonitored mating systems is demonstrated. SNP array data has been useful in assessing as the genomic response of animals to exposure to extreme and fluctuating environmental conditions.

Key Words: SNP arrays, livestock production systems, diversity, breed improvement

WT98 Towards the unraveling of the genomic basis of milk production traits in African dairy zebu cattle. A. Tijjani*[1,4], J. Kim[3], R. Mrode[2], B. Salim[5], N. Oyekanmi[4], H. Kim[3], and O. Hanotte[1,2], [1]*School of life Sciences, University of Nottingham, Nottingham, United Kingdom;* [2]*International Livestock Research institute (ILRI), Nairobi, Kenya;* [3]*C&K genomics, Seoul National University Research Park, Seoul, South Korea;* [4]*National Biotechnology Development Agency, Lugbe, Abuja, Nigeria;* [5]*Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum, Khartoum, Sudan.*

Among the few African *Bos indicus* cattle considered as major milk producers is the Kenana cattle population, which are indigenous to northern Sudan. Together with the Butana cattle breed, they account for ~25 percent of the total cattle population in Sudan. In this study, we carried out full genome sequencing of nine Kenana cattle belonging to a single population in order to provide insight into the genomic basis of their milk production capability in comparison to European dairy cattle (Holstein and Jersey) and African zebu beef breeds (Boran and Ogaden). A total of 26 million autosomal biallelic single nucleotide polymorphisms (SNPs) were identified after comparison to the UMD3.1 bovine reference assembly. Three different signature of positive selection approaches were used (pooled heterozygosity (hp), integrated haplotype score (iHS) and global fixation index (FST)). We identified a total of 109, 61, and 71 significant candidate loci showing the signal of positive selection in Kenana, Holstein, and Jersey respectively, of which 85, 58, and 55 regions have overlap with known cattle quantitative trait loci (QTL) associated with milk traits. Five regions, located on BTA 1 and 7 are shared with Jersey, one region on BTA 20 is shared with Holstein and no candidate region is commonly detected in the three breeds. Several protein coding genes such as LEMD3, WIF1, GPCPD1, ZCCHC11, and CCND2 were found to overlap the Kenana milk traits linked candidate regions, these genes may have roles in milk production and have been documented to be under selection in

other cattle breeds and livestock. This study suggests that Kenana and Jersey cattle may have witnessed partially common selection pressure for better milk production traits distinct from the selection mechanisms which have shaped milk production in Holstein.

Key Words: Africa dairy zebu, milk traits, Kenana, positive selection, full genome sequencing

WT99 Development of genomic tools to select for economic traits in tropical adapted cattle breeds. F. F. Cardoso*[1,2], G. S. Campos[2], C. C. Gulias-Gomes[1], B. P. Sollero[1], and A. R. Caetano[3], [1]*Embrapa Pecuária Sul, Bagé, RS, Brazil;* [2]*Universidade Federal de Pelotas, Pelotas, RS, Brazil;* [3]*Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil.*

Tropical areas in developing countries have great potential to help supply increasing demands for livestock products, as cattle and other ruminants have the ability to convert fast-growing tropical grasses into high-quality protein for human consumption. Nevertheless, sustainability of beef production systems requires the use of livestock that are both highly productive and adapted to tropical environments. Work to apply genomic tools to enhance beef cattle productivity and tropical adaptation is well underway in Brazil. Brazilian Braford and Hereford cattle herds have been systematically measured for key phenotypes related to parasite resistance: tick counts (TC); heat tolerance: eye pigmentation (EP), hair coat at weaning (HCW) and at yearling (HCY); growth: birth (BW), weaning (WW) and long-yearling (LYW) weight, and post-weaning gain (PWG); and reproduction: scrotal circumference (SC). Phenotypes and historical pedigree data of 169,839 animals were combined with 3,954 genotypes for 41,011 SNP markers using different methods (single and multi-step) to derive prediction equations for genomic selection. Estimated heritability (h^2) and accuracy (r) of best performing single-step genomic predictions within this population were 0.19 ± 0.03 and 0.56 for TC, 0.46 ± 0.02 and 0.74 for EP, 0.44 ± 0.03 and 0.93 for HCW, 0.41 ± 0.02 and 0.67 for HCY, 0.28 ± 0.01 and 0.78 for BW, 0.22 ± 0.01 and 0.65 for WW, 0.26 ± 0.01 and 0.78 for LYW, 0.10 ± 0.01 and 0.57 for PWG, and 0.48 ± 0.03 and 0.83 for SC, respectively. These moderate to high r estimates were largely proportional to the respective trait h^2 and represented gains of up to 93% when compared to traditional pedigree-based predictions. Moreover, genome-wide association studies detected chromosome segments that explain up to 5% of the trait genetic variability, providing candidate regions for fine mapping. Low-density marker panels with 41 to 159 markers, based on informative tag-SNP identified within these regions, retain ~70% of the full panel r, and represent alternatives for lower cost predictions. Finally, selection indexes have been derived for optimizing selection of animals combining a desirable balance of economically relevant traits.

Key Words: adaptation, cattle, genome-wide association, genomic selection, meat production

WT100 Finding optimum levels of admixture in crossbred sheep populations in Ethiopia by use of ancestry informative genetic markers and phenotypes. T. Getachew[1,2], H. J. Huson[3], M. Wurzinger[1], J. Burgstaller[4], S. Gizaw[5], A. Haile[6], B. Rischkowsky[6], G. Brem[4], S. A. Boison[1], G. Mészáros[1], A. O. Mwai[7], and J. Sölkner*[1], [1]*University of Natural Resources and Life Sciences, Vienna, Austria;* [2]*Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia;* [3]*Cornell University, Ithaca, NY, USA;* [4]*University of Veterinary Medicine, Vienna, Austria;* [5]*International Livestock Research Institute, Addis Ababa, Ethiopia;* [6]*International Center for Agricultural Research in the Dry Areas, Addis Ababa, Ethiopia;* [7]*International Livestock Research Institute, International Livestock Research Institute, Nairobi, Kenya.*

Understanding the relationship between genetic admixture and performances is crucial for the success of crossbreeding programs. In this study we selected a small set of ancestry informative makers (AIMs) from Ovine 50KSNP data and validated their ability in estimating the contributions of parental breeds to get information about optimum admixture levels. Awassi × Ethiopian fat-tailed crossbred sheep populations kept in farmer villages in different districts were included in the study. AIMs were chosen based on differentiation (high Fst). We estimated admixture levels using ADMIXTURE software. Reducing the number of AIMs from 74 to 65, 55, 45, 35 and 25 did not substantially change the predicted admixture levels of individuals (r = 0.996–0.960). Association of admixture levels with lamb growth showed that Awassi level affected ($P < 0.05$) eight months weight in both farmer locations, lambs with higher Awassi levels were heavier. Lambing interval of ewes was longer as Awassi level increased, but this drawback was outweighed by the increased productivity of ewes in terms of eight months lamb weight per year. The results indicate that linking AIMs with on-farm phenotypic records provides a cheap and powerful tool for decision support for the optimum levels of crossbreeding under farmer conditions. Based on the results presented here, we were able to suggest optimum levels of breed composition for the two farmer environments investigated.

WT101 Matching breeds to production clusters using high-density SNP arrays: The case of East Africa. F. D. N. Mujibi*[1], E. K. Cheruiyot[2], T. Dusingizimana[3], M. Chagunda[4], J. Ojango[5], and R. Mrode[4,5], [1]*Nelson Mandela Africa Institution for Science and Technology (NMAIST), Arusha, Tanzania;* [2]*University of Nairobi, Nairobi, Kenya;* [3]*University of Rwanda, Kigali, Rwanda;* [4]*Scotland Rural University College, SRUC, Edinburgh, UK;* [5]*International Livestock Research Institute, Nairobi, Kenya.*

In East Africa, dairy farming is dominated by smallholder operations, typified by less than ten crossbred dairy cattle on small land holdings. Predominantly, most of the animals are Holstein-Friesian - local indicus breed crosses, reflecting farmer desire for higher milk yield and adaptation to local environments. However, due to lack of pedigree and breed information, performance evaluation is often impossible, and farmers end up with poor yielding cattle. Application of high density SNP arrays for breed composition determination would allow performance evaluation in different production systems to be undertaken. This study evaluated breed composition and milk yield for ~3,000 crossbred dairy cattle in Kenya, Uganda, Tanzania and Rwanda. These cows were genotyped at 150,000 – 700,000 SNP loci, using high density (HD) SNP arrays. The proportion of genes from international dairy breeds was estimated using admixture analysis with Holstein, Friesian, Canadian Ayrshire, Norwegian Red, Jersey, Guernsey, Ndama, Gir and Zebu as references. Production clusters were defined based on several factors including supplementary feeding, milk productivity and household wealth status. A fixed regression model using the G matrix was used to analyse milk test day yield accounting for year-month-test-date, parity, age, and breed type. Production clusters were fitted as fixed effects, while animals and permanent environment effects were considered random effects. Admixture results indicated large within-population genetic diversity ranging from less than 30% to 100% exotic dairy percentage. In all but feed intensive commercially oriented production clusters, the best performing animals had higher proportion of Norwegian Red-Friesian genes. In the feed-intensive systems, animals with a Holstein genetic background with at least 75% dairy composition were the best performing. These results indicate that matching breed type to production cluster is central to maximizing productivity and will be critical in shaping breed development. Additionally, SNP arrays will increasingly play a major role in providing information to guide breed improvement and decision making in smallholder production systems.

Key Words: breed/population identification, crossbreeding, single-nucleotide polymorphism (SNP), genotyping

WT102 Genomic selection based on current status in developing countries. R. Mrode*[1], J. Ojango[1], O. Mwai[1], and

J. M. Mwacharo[2], [1]*Animal Biosciences, International Livestock Research Institute, Nairobi, Kenya;* [2]*Small Ruminant Genetics and Genomics Group, International Centre for Agricultural Research in the Dry Areas, Addis Ababa, Ethiopia.*

Genomic selection (GS) has opened new opportunities in animal breeding in developed countries resulting in rapid rates of genetic progress and detection of genomic regions associated with QTLs. The current status of GS and use of molecular tools in breeding programs of developing countries, where smallholder systems predominate and the basic components for conventional breeding are lacking excepting for a few established breeds, is examined. Genotypic data in smallholder systems offer quick wins in terms of parent verification, breed composition determination and genetic evaluation using G. The few studies on genomic prediction (GP) in developing countries are mostly in dairy and beef cattle and are characterised by small reference populations (≈1000 to 2000 animals). The small reference populations indicate the need for across regional GP that pull data across countries. The gains in accuracy from molecular information range from 0.33 to 0.45. The G matrix has allowed the estimation of breeding values (BVs) and parameters applying random regression models in populations lacking pedigree. Multi-trait single-step has been used to incorporate information on foreign bulls with deregressed proofs and genotypic data only in developed countries, into the BVs of bulls in developing countries. Thus GS in developing countries would benefit from collaborations with developed countries, especially in the dairy sector as a large number of sires used are from developed countries where they may have been genotyped. The development of next-generation sequencing tools such as SNP panels have allowed determination of breed composition for crossbreds in eastern Africa and parent verification for beef breeds and small ruminants (SR) in South Africa. The tools have been used to investigate genomic diversity and genome-wide selection sweeps. The most prominent selection sweeps found in breeds/populations of cattle and SR from across Africa represent candidate regions spanning genes of known major effects (coat and skin properties, high temperatures, muscle function, feed efficiency, etc.) associated with adaptation. This information is important for incorporation into breeding programs aiming to utilise GS in developing countries.

Key Words: genomic selection, developing country, QTL, accuracy, GWAS

WT104 Selection of SNP markers for a dromedary camel genotyping array. M. Al Abri*[1], H. M. Holl[2], D. Miller[3], S. Abdalla[4], B. Shykind[4], J. Malek[4], Y. Mohamoud[4], K. Pasha[5], A. Khalili[5], D. F. Antczak[3], and S. Brooks[1], [1]*Sultan Qaboos University, Department of Animal and Veterinary Sciences, Muscat, Sultanate of Oman;* [2]*University of Florida, Department of Animal Sciences, Gainesville, Florida, USA;* [3]*Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA;* [4]*Weill Cornell Medical College in Qatar, Cornell University, Doha, Qatar;* [5]*Tharb Veterinary Hospital, Doha, Qatar.*

Domesticated over 3000 years ago, dromedary camels play a vital role in livestock production systems, especially in arid and semi-arid regions. As problems of global warming and shortages of potable water worsen, camels could be the livestock of choice as they are better adapted than other species to high ambient temperatures and limited resources. However, to take advantage of the agricultural advantages of the camel, improved understanding of the genetics underlying their unique biology is needed. To this day, there are relatively few published studies in the area of camel genetics and genomics. This is due, in large part, to a lack of genomics tools to conduct such studies. Additionally, while genotyping arrays are commercially available for most livestock, such arrays have not yet been developed for camels. In this work, we describe the development of a set of ~80,000 Single Nucleotide Polymorphisms (SNPs) for the dromedary camel. These SNPs are selected from Whole Genome Sequencing (WGS) of 9 camels and Genotyping by sequencing (GBS) data of 244 dromedary camels. We also discuss the development and evaluation of a reduced set of 100–200 SNP markers for parentage testing, a service in high demand for the camel racing industry. The genotyping panels will assist in estimating diversity parameters and genomic relationships between camels. They will also enable estimation of heritabilities for economic quantitative traits, GWA studies, and searches for selection signatures of important production and health traits. Finally, since no pedigree records or registries exist for most camels, genomic improvement/selection through such a SNP panel may be the only feasible method to boost its contribution to meat and milk production and therefore, food security in the future.

Key Words: Old World camelids, breed/population identification, genetic improvement, parentage, single nucleotide polymorphism (SNP)

WT105 Delineating Indian native cattle specific allelic variants and haplotypes in lactoferrin gene: A potential candidate for disease resistance. A. Sharma*[1,2], M. Sodhi[2], P. Jain[1], M. Kumar[2], and M. Mukesh[2], [1]*University Institute of Engineering & Technology, Kurukshetra, Haryana, India;* [2]*National Bureau of Animal Genetics Resources, Karnal, Haryana, India.*

Lactoferrin, a bioactive glycoprotein is member of transferrin family and plays an important role in immune defence, iron homeostasis, antioxidant and regulation of cell growth. The present investigation was undertaken to establish polymorphism data for Indian native cattle (INC) breeds in lactoferrin gene. Sequence data was generated for 2.3 kb comprising of 5′ flanking and untranslated, coding (17 exons) and 3′- untranslated regions across 72 animals representing 12 cattle breeds from different agro-climatic regions of India. Comparative data analysis across INC and taurine animals revealed a total of 19 SNPs with distribution of 3 in 5′-flanking region, 2 in 5′-untranslated region, 13 in CDS and one novel SNP in 3′-untranslated region. Out of 13 CDS SNPs, 6 were identified as non-synonymous - I145V, S538T, T546N, T596S, K627E, and H632R. Among these, I145V showed complete fixation with frequency of (1.0) and was found to be specific for INC. SNPs in 5 UTR occurred within transcription factor binding sites (AP-2α, SP1) could affect the transcription rate and novel SNPs in 3′UTR might alter the stability of gene. These SNPs in UTRs might be responsible for differential expression of lactoferrin gene in Indian cattle. The haplotype and LD analysis revealed 12 haplotypes and 3 haploblocks specific to INC breeds. The highest frequency (0.546) was observed for haplotype-1 (ACT) and LD occurred with Lodsq >2 indicated lower recombination rate for observed haploblocks. In addition, different physiochemical parameters of 6 nsSNPs were predicted by ProtPram Server and further anaysed by SIFT, PROVEAN, I Mutant and PloyPhen-2 tools revealed their non-deleterious nature. The study is first to report on nucleotide substitutions in lactoferrin gene among various Indian native cattle breeds. The data presented here provides baseline information to carry out the functional aspect of these identified variants and depicts the evolutionary differences from taurine cattle that could correlate the higher disease tolerance ability of Indian cattle breeds.

Key Words: lactoferrin, cattle, DNA sequencing, haplotypes, allelic variants

Author Index

Numbers following names refer to abstract numbers. A number alone indicates an oral presentation; an MT preceding the number indicates a Monday/Tuesday poster (Poster Session I) and a WT indicates a Wednesday/Thursday poster (Poster Session II) poster. Orals are listed first, followed by posters in session and numeric order.

The author index is created directly and automatically from the submitted abstracts. If an author's name is entered differently on multiple abstracts, the entries in this index will reflect those discrepancies. Efforts have been made to make this index consistent; however, error from author entry contributes to inaccuracies.

A

Abanikannda, F. H., WT73
Abanikannda, O. T., WT73
Abdalla, S., 11, MT22, WT104
Abdel-Azim, G., MT144
Abdollahi-Arpanahi, R., MT339
Abebe, A., 154, WT30
Abendaño, N., WT51
Abo-Ismail, M., MT78
Abo-Ismail, M. K., MT71, MT76
Abrahamsen, M., 42, MT55, MT156
Abrahante, J., MT45
Acloque, H., 19, 20, 144, 147, MT127, MT128, MT289, WT69
Acuna, G., MT231
Acutis, P., MT35
Acutis, P. L., MT43
Adam, E. N., MT133
Adeola, A. C., MT259
Adjei-Fremah, S., MT143
Affolter, V., 41, MT213
Afonso, J., MT297, MT337, MT353, MT354
Agaba, M., MT310
Ahbara, A., MT38, WT87
Ahmad, S. F., WT71
Ahmed, A., 58, MT22, MT117
Ahmed, A. S., MT303
Ahn, B. Y., MT6
Aida, Y., MT148, MT150
Ainslie, M. H., MT155
Aitnazarov, R., MT347
Ajmone-Marsan, P., 106, MT363
Akanno, E., MT78
Akanno, E. C., MT76
Aken, B., 149, MT287
Akiyama, T., MT305, MT308, MT309
Akkurt, M., 120, MT104
Al Abri, M., 11, WT104
Alabart, J. L., WT15
Alatiyat, R., MT38
Albayati, S., MT313
Al-Bayatti, S., MT38
Alborch, L., 77, MT160
Albuquerque, L. G., WT89
Alejo, J., 110, MT16
Alim, M. A., MT72
Aljumaah, R., MT38
Al-Jumaili, A., MT38
Allain, D., 153, WT27
Allais-Bonnet, A., MT7
Allanson, E., 26, MT66
Allen, A., 4, MT24, MT325
Allred, A., 116, 151, MT84, MT98
Almeida, J., MT281
Alshawi, A., MT313
Altet, L., 6, WT6
Álvarez-Rodríguez, J., MT277
Alves, E., 98, MT254, MT294
Alyan, M., MT223
Alykhan, M., MT190
Amaral, A., MT281
Amills, M., MT265, MT267, MT268
Amirina, S., WT20
Ammermüller, S., MT328
Anacleto, O., 71, MT166
Anadón, E., 67, MT216
Anderson, R., 101, MT365
Andersson, G., 65, 126, MT102, MT108
Andersson, L., 177, MT179, WT86
Ando, A., 57, MT109
Andrade, B., MT354
Antczak, D., 36, 58, MT22, MT117, MT210
Antczak, D. F., 11, WT104
Antolin, R., MT88
Appel, A. K., MT251
Arabi, S., 3, MT86
Arango, J., 73, MT161
Archibald, A., 104, 148, MT125, MT340, MT373, WT66
Archibald, A. L., 149, MT287, MT324
Ardesjö-Lundgren, B., 126, MT102
Arends, D., MT303
Argyle, D., 121, 127, MT99, MT105
Arranz, J., WT37
Arranz, J. J., 97, WT13, WT26
Arruda, A. M., 90, MT234
Arsic, N., MT346, MT348
Arsuaga, J. L., MT228
Artesi, M., 99, MT346, MT348, MT368
Ashley, E., WT46
Ashraf, B., WT25
Asín, J., WT51
Astiz, S., WT42
Atxaerandio, R., 131, MT242
Auffret, M., 130, MT240
Auvil, L., 163, WT61
Awosanya, L. A., WT73
Awusinu, K. Z., WT73
Ayaz, S., MT136
Ayuso, M., MT245

B

Babar, M., WT8
Badigian, B., MT318
Badigian, T. M., 158, WT28
Badiola, J., 110, MT16
Baes, C., MT356
Bagnato, A., 43, MT44, MT61, MT335
Bagnicka, E., MT5, MT341, MT343, MT344
Bahbahani, H., WT87
Bai, H., 50, MT59
Bai, L., MT150
Bailey, E., MT201
Baird, H. J., MT140
Bakhtin, M., MT190, MT223
Balasuriya, U. B. R., MT201
Baldo, A., 165, WT56
Balic, A., 30, MT177
Ballester, M., 136, MT238, MT252, MT253, MT267
Ballingal, K., 56, MT115
Bâlteanu, V., MT358
Bán, B., MT312
Bangulzai, N., WT7
Banos, G., MT325
Bao, M., MT145
Bao, W., MT2
Barbato, M., 106, MT363
Barrachina, L., MT181
Barro, M., 41, MT213
Bartens, M., 85, MT163
Bartunek, P., 46, MT57
Basarab, J., MT76, MT78
Basnayake, V., 8, MT26
Bastanlar, E. K., 188, MT232
Bates, R., MT258
Bauck, S., MT338
Baudoin, M., WT93
Bauer, J., MT74, MT192
Beatty, J., 122, MT100
Beck, J., MT336
Bed'Hom, B., 66, 78
Beeson, S., MT131, MT187
Beijerink, N., MT199
Beiki, H., 142, MT164, MT275
Belay, G., MT107
Belo, A., MT281
Beltramo, C., MT35
Benitez, R., MT245
Benítez, R., 141, MT269, WT35, WT42, WT53
Ben-Meir, Y., MT357

Berenos, C., WT25
Bergström, T. F., 65, MT108
Bergvall, K., 126, MT102
Berhane, Y. N., MT18
Bermingham, M., 121, MT105
Bernardes, P. A., WT48
Berri, M., 134, MT239, MT284
Berry, D. P., 76, 91, 166, MT76, MT173, MT233, MT325 WT55
Berthold, A., 75, MT157
Bertocchi, L., MT352
Bertolini, F., 43, 109, 137, 176, MT44, MT61, MT246, MT272, MT291, MT335, MT362, WT83
Bessa, R., MT281
Bettencourt, C., MT281
Bhushan, B., MT248
Biagetti, M., WT18
Bickhart, D., 23, MT154, MT159
Biek, R., 4, MT24
Bilgen, N., 120, MT104
Billis, K., 149, MT287
Billon, Y., 79, 134, MT168, MT239, MT262, MT284, WT45
Biolatti, C., MT35, MT43
Birkhart, D., 148, MT125
Birungi, J., MT311
Bishop, S., MT325
Bitterman, P. B., MT93
Bizelis, I., MT355
Bjelka, M., MT151
Bjorkman, P. J., 64, MT162
Blake, D., MT156
Blanc, F., 79, MT168
Blanco, A., 173, WT78
Blecha, F., MT146
Bleyer, M., MT79
Block, H., MT4
Blott, S., 33, MT182, MT211
Blott, S. C., MT90, MT206
Bodin, L., 155, WT31
Boichard, D., MT118, MT120
Boison, S. A., 185, WT100
Boladuro, B. A., MT259
Bolea, R., 110, MT16
Bolívar, H., MT228
Bonet, I., 67, MT216
Bongioni, G., 3, MT86, MT282, MT352
Bontrop, R., 56, MT115
Booth, R., 129, MT96
Borchel, A., 178, WT82
Bordbari, M., 41, MT213
Borkova, M., WT22
Borne, R., MT315
Borowicz, P. P., 112, MT8, WT34
Borowska, A., MT130
Bosch, L., 140, MT277, MT292
Boswood, A., WT46
Bottema, C. D. K., MT77
Bouahom, B., MT189
Bouguyon, E., 79, MT168
Boulton, K., MT156
Bourdillon, Y., 153, WT27
Boutin, M., 53, MT49, MT56
Bouzada, J. A., 67, MT216
Bovenhuis, H., MT145, MT281
Bovo, S., 43, 137, 176, MT61, MT272, MT291, WT83
Bowman, P., 100, MT129
Bowser, J., MT207
Bozzi, R., 98, MT294
Bradley, D., 166, MT325, WT55
Brajkovic, D., MT87
Brajkovic, V., MT87, MT355
Brantsæter, M., MT1
Braschi, B., 169, WT59
Brauning, R., 25, 101, MT140, MT340, MT365, MT372
Brem, G., 15, 32, 92, 185, MT23, MT214, WT33, WT100
Brenet, M., MT49
Brenig, B., MT79, MT336
Bressan, M., MT281
Brockmann, G. A., MT30, MT303
Brooks, S., 11, 58, MT22, MT117, WT104
Brown, P., 27, MT176
Brown-Brandl, T. M., MT279
Browne, J., 111, MT9
Browne, J. A., 76, 166, MT173, WT47, WT52, WT55
Bruce, H., MT4
Bruford, E., 169, WT59
Bryan, K., MT202
Brzakova, M., MT331
Buggiotti, L., 160, WT54
Bugno-Poniewierska, M., 7, 70, 146, MT15, MT184, MT194, MT195, MT198, MT220, MT330, WT4
Buitkamp, J., 59, MT110
Bulut, Z., 188, MT232
Bunevski, G., MT355
Bunikis, I., 177, WT86
Burgstaller, J., 185, WT100
Burkard, C., 28, MT175
Burns, B., MT121
Burns, E., 41, MT213
Burny, A., MT346, MT348
Burrell, A., 116, 151, MT84, MT98
Burt, D., 54, MT41, MT47, MT62
Burt, D. W., MT40
Bush, S., 104, MT373
Bush, S. J., MT324
Buss, C., MT297, MT353, MT354
Buttazzoni, L., MT153
Buus, S., 63, MT113
Buys, N., 33, MT182, MT211
Byrne, A., 4, MT24
Byrne, C., MT334
Byrne, M., 27, MT176

C

Cabaleiro, S., 173, WT78
Cabau, C., 20, 144, MT128, WT69
Caetano, A. R., 180, WT99
Cai, W., WT67
Cai, Y., MT119
Caldas, Y. R., MT265
Calvo, J. H., WT15
Calvo, L., MT269
Camargo, G. M. F., WT89
Cambisano, N., 99, MT368
Cambuli, C., MT352
Cameron, A., 101, MT365
Campia, V., MT35
Campos, C. B. N., MT204
Campos, G. S., 180, WT99
Canario, L., WT45
Candek-Potokar, M., 98, MT294
Canibe, N., 135, MT241
Caniglia, R., 152, WT5
Cañon, J., MT224
Cañón, J., MT82
Canovas, A., MT265
Cantrell, B., MT3
Cao, J., MT73
Cao, X.-H., WT11
Capitanu, B., 163, WT61
Capoferri, R., 3, MT86
Cappelletti, E., 36, MT210
Capra, E., 18, MT14
Caralheiro, R., WT72
Cardoso, A., WT71
Cardoso, F. F., 180, WT99
Cardoso, T. F., MT265, MT267, MT268
Carlsson, J., 91, MT233
Carvalheiro, R., WT89
Casas, E., MT142
Case, A., 58, MT117
Casiró, S., MT258
Castaneda, C., MT229
Castaneda, V., MT231
Castello, A., 77, MT160, MT265
Castelló, A., MT252, MT253, MT285
Castillo, F., 67, MT216
Castle, K., MT205
Caton, J. S., 112, MT8, WT34
Ceccobelli, S., WT18
Cerolini, S., 43, MT44, MT61
Cesar, A., MT297
Chagunda, M., 184, WT101
Chai, H.-H., 115, MT13, MT42
Chamberlain, A., MT321
Chantepie, L., 155, WT31
Chanthavixay, K., 17, 159, MT10, WT58
Charlier, C., 99, MT348, MT368
Chase, C., 103, MT370
Chen, J. L., 50, MT59
Chen, T., MT119
Chen, Y., MT222
Cheng, H., 42, 159, MT55, WT58
Cheng, H. H., 114, MT11
Cheruiyot, E. K., 184, WT101
Chevaleyre, C., MT284
Chilla, A., MT336
Chimonyo, M., WT21
Chitneedi, P. K., 97, WT13, WT26
Cho, G., MT132
Cho, I. C., MT188
Choate, L., 38, MT208
Choi, B.-H., 115, MT13, MT42
Choi, J.-W., MT180, MT188

Choi, M.-K., MT6, MT266
Choi, N., MT46
Choi, N.-R., 9, MT65
Choi, Y.-H., MT42
Choudhury, A., MT124
Chow, W., 148, MT125
Chowdhary, B. P., MT123
Christmann, A., 35, MT219
Chu, M.-X., WT11
Chu, T., 38, MT208
Chung, W.-H., MT180, MT188
Ciani, E., WT87
Cichero, D., 175, WT81
Cieslak, J., MT130, MT197
Cigliano, R. A., 77, MT160
Cildir, O., 120, MT104
Cinar, M. U., MT106
Ciullo, M., WT18
Clark, E., MT340
Clark, E. L., MT324
Clarke, L., WT66
Clarke, S., 25, 101, MT340, MT365, MT372
Clarke, S. M., MT140
Claude, A., MT207
Clements, D., 127, MT99
Clements, D. N., MT90
Clop, A., 77, MT160
Clop, À., MT285
Cochrane, G., WT66
Cociancich, V., MT323
Cockett, N., 25, MT340, MT372, WT23
Cockett, N. E., MT316
Coffey, M., MT295, MT325
Cohen-Zinder, M., MT357
Coizet, B., 152, WT5
Colazo, M., MT4
Collin, A., 53, MT56
Colussi, S., MT35, MT43
Consortium, B., MT230
Cooksey, A., 22, MT126
Cooley, A., MT207
Cooper, C., 27, MT176
Coppieters, W., 99, MT368
Corbo, M., 36, MT210
Cormican, P., 132, MT237, MT334, MT350
Correia, C. N., 76, 166, MT173, WT47, WT52, WT55
Cortes, O., MT203
Cortés, O., MT82, MT224
Costa, M., 68, MT134
Costa, M. R. T., MT203
Costa, R. B., WT89
Cothran, E. G., MT130, MT229
Coulombe, R., MT45
Courcier, E., 4, MT24
Coutinho, L., MT1, MT297, MT337, MT353, MT354
Cozzi, M. C., MT44, MT335
Creighton, E., 123, MT103
Crepaldi, P., 109, 152, MT362, WT1, WT5
Crespo-Piazuelo, D., 136, MT238, MT252, MT253
Criado-Mesas, L., 136, MT238, MT253
Crisà, A., WT43
Crisci, E., 144, MT128
Crisman, R., MT199
Crooijmans, R., MT345, WT88
Cross, A. J., MT279
Crouse, M. S., 112, MT8, WT34
Crovetti, A., 98, MT294
Crowe, M., MT153
Crowley, J., MT76
Cubric-Curik, V., MT87, MT355
Cunningham, H. L., MT206
Curik, I., MT87
Curry, J., WT19
Cuscó, A., 6, WT6
Cushman, R. A., 112, MT8, WT34
Czopowicz, M., MT341, MT343

D

Daetwyler, H., 25, 100, MT129, MT321, MT372
Dahinten, G., MT261
Dahlen, C. R., 112, MT8, WT34
Dall'Olio, S., MT272
Dalrymple, B., MT340
Dalrymple, B. P., MT316
Daly, K., 93, MT374
Dalziel, B., 21, MT167
Damas, J., 26, 44, MT66, MT67
Dammann, I., MT336
Danelli, F., 152, WT1, WT5
Danko, C., 38, MT208
Darfour-Oduro, K. A., 138, MT286
Das, P. J., MT123
Davassi, A., MT329
Davenport, K., MT318
Davenport, K. M., 158, WT28
Davey, M., 121, MT105
Davila, K. M. S., 84
Davila, K. M. Sarlo, MT367
D'Avino, N., WT18
Davis, B., MT123
Davis, S., 90, MT234
Davoli, R., MT280
Dawuda, P. M., MT259
De, A. K., 138, MT286
de Andrade, F. M., WT2
de Andrés, D., WT51
de Groot, M., 10, 151, MT63, MT98
de Groot, N., 56, MT115
De Keyser, K., 33, MT211
De Koning, D. J., MT118
de Lima, A., MT337
De Mercado, E., 141, WT53
de Oliveira, P., MT337
de Souza, M., MT337
de Torre, A., WT40
Deckers, M., 99, MT368
Deeb, N., 143
Dehareng, F., MT153
Dekkers, J., 16, 73, 143, MT161, MT275, WT70
Del Corvo, M., 18, 106, MT14, MT363
Delaney, M., 42, MT55
Delany, M., 159, WT58
Delany, M. E., 114, MT11
Demasius, W., MT138
Demyda-Peyras, S., MT326
Denis, C., 134, MT239
Deniskova, T., 92, WT33
Denny, P., 169, WT59
DePriest, J. S., MT201
DePriest, M., 39, MT209
DePriest, M. S., 40, MT221
Der Sarkissian, C., 87, MT235
Derrien, T., 20, 144, MT128, WT69
Désert, C., 53, MT49, MT56
Dessie, T., 154, MT107, MT311, WT30
Detry, C., 90, MT234
Dettleff, P., 179, WT75, WT76, WT77
Devos, J., MT4
Di, R., WT11
Di Battista, P., MT280
Di Palma, F., MT19
Dietrich, J., 119, MT94
Dietschi, E., 119, 128, MT94, MT95
Diniz, C. T. F. S., MT204
Diniz, W., MT297, MT353, MT354
Distl, O., 35, MT183, MT219
Djebali, S., 19, 20, 144, 147, MT127, MT128, MT289, WT69
Djikeng, A., MT311
Dobrowolski, M., MT20, MT21
Dodds, K., 101, MT365
Doeschl-Wilson, A., 71, 143, 173, MT166, WT78
Dogliero, A., MT35
Dong, K., 157, WT32
Dong, Y., WT65
Doran, T., 27, MT176
Doré, J., 134, MT239
Dotsev, A., 15, 92, MT23, WT33
DovČP., MT355
Downey, E., 103, MT370
Doyle, D., MT350
Dreger, D. L., 152, WT5
Drobik-Czwarno, W., 73, MT161
Drögemüller, C., MT323
Drögemüller, M., 121, 128, MT95, MT105
Drouilhet, L., 153, WT27
Dry, I., 21, MT167
Duan, Z. Y., 113, MT12
Ducro, B., 33, MT182, MT211
Dugarjaviin, M., MT202
Duguma, G., 154, WT30
Dunner, S., MT82, MT224
Durand, H. Morales, MT326
Durkin, K., 99, MT346, MT348, MT368
Dusel, G., MT333
Dusingizimana, T., 184, WT101
Dyomin, A., 44, MT50, MT67
Dzama, K., 81, MT169
Dzomba, E., MT306, WT21, WT92

E

Eberth, J., MT201
Eddy, A., MT207
Edgren, H., WT49

Edin, H., 78, MT165
Ehlers, K., WT94
Ehsani, A., 49, MT64
Ekaya, W., MT107
Ekine-Dzivenu, C., MT78
Elguero, B., 68, MT134
Elleder, D., 46, MT57
Ellis, K., MT92
Elsik, C., 168, WT57
Eltanany, M., WT17
Ema, P. J. N., 182, WT96
Emmerling, R., 100, MT129
Emrani, H., WT20
Engelmann, S., 75, MT138, MT157
Engle, B. N., MT317
English, A. M., MT334
Ensing, D., 4, MT24
Eory, L., 149, MT287, WT66
Ernst, C., 17, 114, 159, MT10, MT11, MT258, WT58
Ertugrul, O., 120, MT104
Escobar, S., WT75
Eshghabadi, M. A., WT20
Espinosa, J., 110, MT16
Esquerre, D., MT49
Esquerré, D., 20, 53, 147, MT56, MT284, MT289, WT69
Essa, A., MT313
Essa, A. A., MT38
Estany, J., 140, MT257, MT277, MT292
Esteban-Blanco, C., 97, WT26
Estelle, J., 79, MT168
Estellé, J., 134, 136, MT238, MT239, MT252, MT284
Esteve-Codina, A., 77, MT160
Etherington, G., MT19
Eusebi, P., MT82
Eusebi, P. G., MT224
Evans, B., 171, WT74, WT85
Evans, J., MT350
Ezoe, H., MT190

F

Fabre, S., 155, WT31
Fages, A., 87, MT235
Faherty-O'Donnell, S. L., WT47
Fair, S., MT334, WT39
Fang, J., MT33
Fang, S., MT249
Fang, X., MT298
Farahi, M., 49, MT64
Farias, F. F., 126, MT102
Farkasova, H., 46, MT57
Farquhar, L., MT324
Farre, M., 26, 44, 163, MT66, MT67, WT61
Farrè, M., 160, WT54
Farries, G., 34, MT215
Farzan, V., MT155
Fathoni, A., MT69
Favaro, L., MT43
Fedorov, V., 15, MT23
Feeney, D. A., MT93
Fegraeus, K. J., MT179
Felkel, S., 32, MT214
Feng, C., 177, WT86
Feng, W., MT255
Ferenčaković, M., MT355
Fernandes, I., 90, MT234
Fernandez, A., MT245
Fernandez, M., WT93
Fernández, A., 98, 141, 173, MT269, MT276, MT294, WT35, WT53, WT78
Fernández, A. I., 136, MT238, MT252, MT253
Fernández, J., 173, WT78
Fernández, M., 165, WT56
Fernández-Barroso, M. A., MT254
Fernández-León, J., 67, MT216
Ferretti, R., 116, MT84, MT338
Fève, K., MT262
Finlay, E., WT39
Fitzsimmons, C., MT4, MT78, MT300, MT327
Flach, E., WT46
Fleming, A., MT356
Fleming, D. S., MT146, MT256
Flicek, P., 56, 149, MT115, MT287, WT66
Flores, E. B., WT90
Flossmann, G., MT261
Flynn, P., 91, 117, MT80, MT83, MT233
Fogarty, U., 4, MT24
Foissac, S., 19, 20, 144, 147, MT127, MT128, MT289, WT69
Folch, J., MT276, WT15
Folch, J. M., 136, MT238, MT252, MT253
Follett, M., MT318
Follett, M. A., 158, WT28
Fontanesi, L., 43, 98, 137, 176, MT19, MT44, MT61, MT272, MT291, MT294, MT335, WT83
Foote, B., 101, MT365
Fornal, A., 70, MT81, MT194, MT195, MT196, MT220
Foster, J., MT329
Fourdraine, R., MT144
Fowler, K. E., 26, MT66
FR-AgENCODE Consortium, 19, MT127
Francino, O., 6, WT6
François, L., 33, MT182, MT211
Frantz, L., MT259
Fraser, R. S., MT158
Frattini, S., 152, WT1, WT5
Fredholm, M., 135, MT91, MT241
Freitas, L. A., WT48
Friedrich, J., MT88
Fries, R., 100, MT129, MT328
Frieten, D., MT333
Fritz-Waters, E., 103, MT370
Fu, W., WT44
Fu, Y., MT250
Fukazawa, K., MT304
Fukushima, M., MT305, MT308, MT309
Fulton, J., 42, 54, 73, MT41, MT47, MT55, MT62, MT161
Fulton, J. E., 66, MT116
Funston, R., MT3
Fusi, F., MT352

G

G+E Genotype Plus Environment Consortium, WT38
GplusE Consortium, MT153
Gaba, R. C., 138, MT286
Gabler, N., 16, MT275, WT70
Gaginskaya, E., 44, MT50, MT67
Galaverni, M., 152, WT5
Galimberti, G., MT272
Galindo-Pellicena, M. A., MT228
Galkina, S., 44, MT50, MT67
Gallardo, R., 55, MT52
Galli, A., 3, MT86, MT282, MT352
Gálvez, F., WT50
Gama, L., MT281
Gama-Carvalho, M., MT281
Gamba, C., 87, MT235
Gamba, R., 36, MT210
Gandini, G., 152, WT1, WT5
Gandolfi, B., 123, MT103
Gangsei, L. E., MT278
Gao, H., MT222
Gao, J., MT70
Gao, S., WT44
Gao, X., MT222
Gao, Y., 139, MT288
Garcia, S., WT50
García, F., 98, MT294
García, P. P., WT93
García Girón, C., 149
García-Casco, J., 98, 141, MT294, WT53
García-Casco, J. M., MT254
Garcia-Contreras, C., MT245
García-Contreras, C., WT35, WT42
García-Rodriguez, A., 131, MT242
Garrayo, J., MT245
Gaunitz, C., 87, MT235
Gaur, U., MT51
Geigl, E.-M., 125, MT97
Geistlinger, L., MT297, MT354
Genho, J., MT338
Genova, F., 123, MT89, MT103
Georges, M., 99, MT346, MT348, MT368
Geraci, C., 98, MT294
Gerber, V., MT185
Gerbert, C., MT333
Getachew, T., 185, WT100
Ghosh, S., MT123
Gianino, F., 41, MT213
Gianino, G., 41, MT213
Gibson, A., 85, MT163
Giegl, E.-M., 105, MT360
Gil, M., MT268
Gilbert, H., MT262
Gill, C., 51, MT53
Gill, C. A., 84, MT317, MT367, WT68
Ginja, C., 90, MT228, MT234
Giorgi, M., MT262
Giovambattista, G., 165, MT326, WT56
Girardi, M., MT87
Girón, C. García, MT287
Giuffra, E., 19, 20, 144, MT127, MT128, WT69

Giulotto, E., 36, 41, MT210, MT213
Giwa, A. O., WT73
Gizaw, S., 154, 185, WT30, WT100
Gladyr, E., 92, WT33
Glass, E., MT325
Goddard, M., 100, MT129, MT321
Gòdia, M., MT285
Gokhale, S., 104, MT373
Gol, S., 140, MT257, MT292
Goldammer, T., 178, WT82
Golzalez-Bulnes, A., MT245
Gombac, M., MT323
Gómez Izquierdo, E., 141
Gomez-Garrido, J., 77, MT160
Gondro, C., WT90
Gonzalez, M. V., WT14
González-Bulnes, A., WT42
Gónzalez-Bulnes, A., WT35
Gonzalez-Prendes, R., MT265
González-Prendes, R., MT267, MT268
Gonzalez-Recio, O., 131, MT242
González-Rodríguez, O., MT267, MT268
Gordon, S., 111, MT9
Gordon, S. V., 76, 166, MT173, WT47, WT52, WT55
Gorkhali, N., 157, WT32
Gorla, E., MT44, MT335
Gormley, E., 76, 166, MT173, WT47, WT55
Gorzynski, J., WT46
Goszczynski, D., MT326
Goszczynski, D. E., WT93
Götherström, A., 90, MT234
Goto, R. M., 64, MT162
Goto, T., MT40
Götz, K.-U., MT261
Gough, K. F., 34, MT215
Gourdine, J., MT262
Gourichon, D., 53, MT49, MT56
Gow, A., 121, MT105
Gozzo, F., 36, MT210
Grahn, J., WT3
Grahn, R., WT3
Grange, T., 105, 125, MT97, MT360
Graves, T., 42, MT55
Gray, K., 169, WT59
Gredler-Grandl, B., 100, MT129
Grelet, C., MT153
Gresham, C., 22, MT126
Griebel, P., MT346, MT348
Griffin, D., 44, MT67
Griffin, D. K., 26, MT66
Grimholt, U., 56, MT115
Grindflek, E., MT278
Grobler, J. P., WT94
Groenen, M., 147, MT289, MT345, WT42
Groenen, M. A. M., 138, MT286
Gromboni, C., MT353
Große-Brinkhaus, C., 74, MT137, MT170, MT251
Groth, D., 60, MT111
Gu, L., MT34
Guan, D., MT299
Guan, L., 167, WT60
Guan, L. L., MT307
Guenther, J., MT138
Guerrero, J., 4, MT24
Guerrero-Bosagna, C., MT1
Guethlein, L., 56, MT115
Gugiu, G., 64, MT162
Guillocheau, G., 102, MT369
Guimaraes, S., 105, MT360
Gul, S., MT136
Guldbrandtsen, B., MT118
Gulias-Gomes, C. C., 180, WT99
Günther, J., 75, MT157
Guo, G., MT73
Guo, X.-F., WT11
Gurgul, A., 146, MT5, MT15, MT184, MT330
Gurtner, C., 119, MT94
Gutierrez, L., WT93
Gutierrez-Gil, B., 97, WT13, WT26, WT37

H

Haase, B., 122, MT100, MT205
Hadfield, T., MT340, WT23
Hadlich, F., 114, MT11, MT138, MT333
Hagen, D., 168, WT57
Haggerty, L., 149, MT287
Hahaut, V., MT346, MT348
Haile, A., 154, 185, WT30, WT100
Häkkinen, S., WT49
Hall, R., 148, MT125
Hall, T., 111, 166, MT9, WT55
Hall, T. J., 76, MT173, WT39
Halliday, C., MT199
Halstead, M., 159, WT58
Hameed, A., WT7
Hamill, R. M., MT270, MT271
Hamilton, N., MT205
Hamilton, N. A., MT199
Hammer, S., 57, MT109
Hammermüller, J. D., MT158
Hammon, H. M., MT333
Hammond, J., 23, 56, MT115, MT159
Hammond, J. A., MT154, MT315
Han, H., MT202
Han, X., 139, MT288
Han, Y., MT316, MT339
Hanghøj, K., 87, MT235
Hanotte, O., 94, 108, 183, MT38, MT40, MT107, MT313, MT361, WT87, WT95, WT98
Hanzawa, K., MT39, MT319
Hara, H., MT39, MT319
Harburg, S., MT322
Harland, C., 99, MT368
Harlizius, B., MT278
Harman, K., MT156
Harrington, R., 121, 127, 128, MT95, MT99, MT105
Harris, R. A., MT316
Harrison, P., WT66
Hartatik, T., MT69, WT24
Haskell, M. J., MT90
Hawken, R., 42, 78, MT55, MT156, MT165
Hayes, B., MT121, MT321
He, P., MT150
He, X.-Y., WT11
Heath, S., 77, MT160
Heaton, M., 8, MT26
Hecht, J., MT285
Hedhammar, Å., 126, MT102
Hehl, A., MT138
Hehl, J., 75, MT157
Heimeier, D., 23, MT154, MT159
Heimes, A., MT138
Heldmann, A., MT328
Heller, R., 24, MT364
Hendrick, S., 167, WT60
Henke, D., 119, MT94
Henne, H., MT251
Henry, S., 27, MT176
Henryon, M., 172, WT79
Hermida, M., 173, WT78
Hermine, O., MT348
Herndon, M. K., MT106
Herrera, J. R. V., WT90
Herring, A. D., 84, MT317, MT367
Hess, A., 143
Hess, M., 143
Heuß, E., MT251
Hewicker-Trautwein, M., MT183
Hickey, J., MT88
Highland, M. A., MT106, WT14
Hilburger, K., 41, MT213
Hill, A., 166, WT55
Hill, E., MT202
Hill, E. W., 34, MT215
Hillestad, B., 172, WT79
Hillier, L., 42, MT55
Hirano, T., MT39, MT319
Hirota, K., 69, MT135
Hirota, K.-I., MT178
Hlongwane, N., WT92
Ho, C., 56, MT115
Ho, S., 57, MT109
Hoban, R., MT205
Hocking, P. M., MT40
Hoedemaker, M., MT138
Hofmanova, B., MT193
Högler, S., 119, MT94
Hoj-Edwards, S., MT88
Holgado, F. D., WT93
Hölker, M., 74, MT137, MT170
Holl, H., 58, MT22, MT117
Holl, H. M., 11, WT104
Hollmann, A. K., MT79, MT336
Hom, B. Bed, MT116, MT165
Honda, T., MT305, MT309
Horbanczuk, K., MT341, MT343
Horin, P., 31, MT218
Horodyska, J., MT270, MT271
Hoskens, H., MT182
Hou, Y., MT250
Hourlier, T., 149, MT287
Hron, T., 46, MT57
Hu, A., MT250
Hu, W.-P., WT11
Hu, X., 29, MT174, MT244, MT249
Huang, H., MT73
Huang, J., 16, WT70

Huang, L., 145, MT70, MT290, WT9
Huang, Y., MT299
Huau, C., 153, WT27
Hume, D., 104, 148, MT125, MT156, MT373
Hume, D. A., MT324
Hurtado, A., 131, MT242
Huson, H. J., 185, MT92, WT100
Hussain, T., WT8
Hwang, J., MT132
Hwang, N.-H., MT180
Hwangbo, J., MT42

I

Iannuccelli, N., WT45
Ibañez-Deler, A., WT15
Ibe, U. K., MT31
Ihler, C. F., MT179
Ilska, J., MT90
Isabel, B., 141, MT245, WT35, WT42, WT53
Isaja, V., MT43
Ishengoma, E., MT310
Ishige, T., MT39
Islam, M. A., 74, MT137, MT170
Iwamoto, E., MT305, MT308, MT309
Izquierdo, E. Gómez, WT53
Izuogu, O., 149, MT287

J

Jaeger, H., MT318
Jaeger, H. K., 158, WT28
Jafarikia, M., MT155
Jagannathan, V., 32, MT185, MT214, MT323
Jain, P., 86, WT105
Jameel, M., MT33
Jammes, H., MT7
Janczak, A., MT1
Janecka, J., MT123
Jang, A., MT180
Jang, G.-W., 115, MT13
Jankowski, T., 73, MT161
Janssens, S., 33, MT182, MT211
Jarczak, J., MT341, MT343
Jarmuz, W., MT341, MT343, MT344
Jasielczuk, I., MT330
Jaures, K. S., MT311
Jehangir, M., WT71
Jehl, F., 53, MT56
Jennings, R., 26, MT66
Jensen, P., MT1
Jevons, L., MT329
Jiang, L., 157, WT32, WT65
Jiang, Y., 24, MT119, MT364, WT44
Jin, L., 51, MT53
Jin, S., 9, MT46, MT65
Jin, S. Y., MT70
Johansson, A., WT10
Johnson, C., 121, MT105
Johnson, E., 121, MT105
Johnson, M., MT207
Johnson, P. L., MT140
Johnson, S., WT25
Jonas, E., WT10
Joost, S., 109, MT362
Joseph, S., 26, MT66
Joshi, V., WT19
Jouneau, L., MT7
Józsa, C., MT312
Juga, J., 182, WT96
Jugo, B. M., WT51
Jung, K., MT183
Jung'a, J., MT107
Juras, R., MT229
Just, F., MT283

K

Kaba, J., MT341, MT343
Kaiser, M., 82, MT172
Kakoi, H., 69, MT135, MT178
Kalashnikov, A., MT151
Kalbfleisch, T., 8, 39, 41, MT26, MT201, MT209, MT213
Kalbfleisch, T. S., 40, MT221
Kamboh, A. A., MT33
Kanemaki, M., MT189
Kanis, E., WT88
Karberg, M., 151, MT98
Karim, L., 99, MT368
Karimi, K., 14, MT25
Karlskov-Mortensen, P., 135, MT91, MT241
Karrow, N., 80, MT171
Kasarda, R., MT193
Katz, L. M., 34, MT215
Kaufman, J., 56, MT115
Kause, A., 143
Kaushikkar, S., MT329
Kawaguchi, F., MT304, MT305, MT309
Kawecka, A., MT152
Kawecka, E., MT344
Kazymbet, P., MT190, MT223
Kean, M., 117, MT83
Keane, O. M., MT149
Kearney, F., MT80
Kearney, J., 117, MT83
Kebede, A., MT107
Keel, B. N., MT279
Keliang, W., MT226
Kelly, A. K., 132, MT237
Kelly, G., 4, MT24
Kelly, M., MT121
Kelly, T., 55, MT52
Kemper, A., MT186
Kenny, D., MT350, MT351
Kenny, D. A., 132, MT237, MT302, MT334
Keogh, K., MT302, MT351
Keonouchanh, S., MT189
Kern, C., 17, 114, 159, MT10, MT11, WT58
Kerr, D., MT139
Khalili, A., 11, MT22, WT104
Khan, M., MT296
Khan, N., 87, MT235
Khanshour, A. M., MT130
Kharzinova, V., 15, 92, MT23, WT33
Khatib, H., 18, MT14
Khatkar, M. S., MT314
Khatkar, M.-S., 129, MT96
Khattak, I., MT136
Khosa, A. N., WT7
Kiazim, L., 26, MT66
Kiefer, H., MT7
Kierczak, M., 126, MT102
Kigoshi, H., MT305, MT309
Kiilerich, P., 135, MT241
Kijas, J., 25, 171, MT316, MT340, MT372, WT74, WT85
Kikuchi, M., 69, MT135, MT178
Killick, K. E., 76, 166, MT173, WT55
Kim, D., MT6
Kim, D. C., MT180, MT188
Kim, H., 183, WT98
Kim, H.-Y., 9, MT65
Kim, J., 163, 183, WT61, WT98
Kim, J.-B., MT180
Kim, J.-D., MT180
Kim, J.-M., MT42
Kim, K., 148, MT125,
Kim, K.-S., MT147
Kim, N. Y., MT188
Kim, N.-Y., MT180
Kim, S., MT132
Kim, S.-W., MT147
Kim, W.-I., MT147
King, H., 171, WT74, WT85
Kleiter, M., 119, MT94
Klopp, C., 20, 53, 144, MT49, MT56, MT128, WT69
Klukowska-Rötzler, J., MT195
Klumplerova, M., 31, MT218
Knol, E. F., MT278
Knowles, D. P., MT106, WT14
Kobayashi, E., MT305, MT308, MT309
Koch, C., MT194, MT195, MT333
Kochan, J., 7, WT4
Kohama, N., MT305, MT308, MT309
Kohara, J., MT150
Komissarov, A., 44, MT67
Kongsro, J., MT278
Koren, S., 148, MT125
Korf, I., 17, 114, 159, MT10, MT11, WT58
Korkmaz, F., MT139
Koscielny, M., MT152
Kosciuczuk, E., MT344
Koshel, E., MT50
Koshinsky, H., WT19
Kott, T., WT22
Koufariotis, L., MT121
Kounnavongsa, B., MT189
Kovarík, A., WT50
Kowalska, K., MT184
Koy, M., 75, MT157
Kozyrev, S., 126, MT102
Kraft, M., MT185
Kramer, L., 103, MT370
Kranjcevicova, A., MT74
Kranjcevicová, A., MT192
Krebs, S., MT87, MT355
Kremitski, M., 42, MT55
Kristiansen, K., 135, MT241
Kroezen, V., MT356
Kube, P., 171, WT74, WT85

Kubickova, S., 31, MT218
Kuehn, C., MT138
Kuehn, D., 121, MT105
Kühn, C., 75, MT157, MT333
Kuisma, M., WT49
Kul, B. C., 120, MT104
Kulak, M., 44, MT50, MT67
Kumar, A., MT248
Kumar, M., 86, WT105
Kumar, P., MT248
Kumar, S., 104, MT248, MT373
Kunieda, T., MT189, MT190
Kunz, E., MT355
Kuo, R., WT66
Kustantinah, K., WT24
Kyalo, M., MT107
Kyriazakis, I., 143
Kyselova, J., WT22

L

Labrune, Y., MT262
Lachance, H., MT3
Lagarrigue, S., 19, 20, 53, 144, MT49, MT56, MT127, MT128, WT69
Lahbib-Mansais, Y., 147, MT289
Lahoz, B., WT15
Lamberigts, C., 33, MT182, MT211
Lamichhaney, S., 177, WT86
Lamont, S., 45, 82, MT54, MT172
Lamont, S. J., 55, MT52
Larkin, D., 44, 160, MT67, MT347, WT54
Larkin, D. M., 26, 163, MT66, WT61
Larzul, C., 134, MT239
Lasagna, E., WT18
Lascor, C., WT45
Lashmar, S. F., WT91
Latifah, L., WT24
Lavery, J., 4, MT24
Lawal, R. A., 94, MT40, WT95
Lawlor, P. G., MT270, MT271
Le, C. Q., MT266
Le, M. T., MT266
Le Danvic, C., MT7
Lebrero, M. E., WT40
Lee, D., MT46
Lee, H., MT266
Lee, J., MT6, MT266
Lee, J. H., 9, MT46, MT65
Lee, J.-H., 57, MT109
Lee, K.-T., 115, MT13, MT42
Lee, S. H., 9, MT46, MT65
Leeb, T., 32, 128, MT95, MT214
Lefevre, L., 104, MT373
Lehnert, S. A., MT322
Lemonnier, G., 79, 134, MT168, MT239, MT284
Leonardi, M., 87, MT235
Lepage, P., 134, MT239, MT284
Leplat, J., 79, MT168
Leplat, J. J., MT284
Leroux, S., 53, MT49, MT56
Leschnik, M., 119, MT94
Levenez, F., 134, MT239
Lewin, H. A., 163, WT61
Lhorente, J., WT72
Li, C., MT4, MT76, MT78, MT250, MT300, MT327, WT67
Li, D., MT51
Li, D. L., 50, MT59
Li, G., MT298
Li, H., MT338
Li, J., MT222, MT250, MT260, MT320, MT332
Li, L., MT250
Li, M., 51, MT53
Li, N., 29, MT174, MT244, MT249
Li, Q., 164, WT62
Li, R., WT44
Li, S., MT34
Li, W., 145, MT290
Li, X., 52, 139, MT58, MT73, MT146, MT288, WT36
Li, Y. L., 50, MT59
Liachko, I., 42, MT55
Liaubet, L., 147, MT289, WT45
Librado, P., 87, MT235
Lillico, S., 21, 28, MT167, MT175
Lillie, B. N., MT155, MT158
Lim, B., MT147
Lim, D., 115, MT13, MT42
Lim, K.-S., 115, MT13, MT42
Lima, A., MT297, MT353, MT354
Lima, R. M. G., MT204
Lin, D., MT36
Lin, L., MT72
Lin, Y. Q., WT9
Lindblad-Toh, K., 126, MT102
Lindgren, G., 33, MT179, MT182, MT211
Lipkin, E., 54, MT41, MT47, MT62, MT357
Lippi, Y., WT45
Liron, J., 165, WT56
Lirón, J. P., WT93
Liu, B., MT311
Liu, D., MT249
Liu, H., MT307
Liu, J., MT307, WT65
Liu, J.-F., MT255
Liu, L., 113, MT12
Liu, Q.-Y., WT11
Liu, S., WT67
Liu, W., MT342
Liu, X., WT36
Liu, Y., 51, 138, MT53, MT286, MT316
Liu, Y. F., 50, MT59
Liu, Z., MT260, MT320, MT332
Liuti, T., 121, 127, MT99, MT105
Lobo, R. N. B., 154, WT30
Lofgren, S. E., MT90
Longeri, M., 123, MT89, MT103
Lopes, F. B., MT338
Lopes, M. S., MT278
López-Bote, C., 141, MT245, MT269, WT53
López-Pérez, O., 110, MT16
Lough, G., 143
Loving, C. L., 142, MT164
Loyau, T., MT262
Lozano, J. M., 67, MT216
Lühken, G., WT17
Luján, L., WT51
Lund, M., MT120
Lund, M. S., MT118
Lunney, J., 57, 143, MT109
Luo, N., MT299
Luo, W., MT250, WT64
Lwin, M., MT227
Lyons, L., 122, 123, 124, MT100, MT101, MT103
Lyons, L. A., MT89
Lyons, R., MT121
Lyu, S., MT30

M

Ma, J., 52, 163, MT58, WT61
Ma, L., MT260
Ma, Y., 157, MT34, WT32, WT36
Maccari, G., 56, MT115
Mach, N., 134, MT239
MacHugh, D., 111, MT9
MacHugh, D. E., 76, 166, MT173, WT47, WT52, WT55
Mack, M., 41, MT213
Mack, S., MT207
Mackowski, M., MT130, MT197
MacLeod, I., 100, MT129
MacLeod, J., 39, 41, MT209, MT213
MacLeod, J. N., 40, MT133, MT221
MacNeil, M., MT78
MacNeil, M. D., MT76
Madsen, O., 138, 147, MT286, MT289, WT42
Magee, D., 111, MT9
Magee, D. A., 76, 166, MT173, WT47, WT52, WT55
Maharani, D., MT69
Mählmann, K., MT194, MT195
Maiwashe, A., MT124
Mäkeläinen, S., 138, MT286
Makgahlela, M. L., MT124
Malek, J., 11, 58, MT22, MT117, WT104
Malesa, M., MT306
Mallikarjunappa, S., 80, MT171
Malomane, D., 95, MT236
Mamiru, M., 154, WT30
Manafiazar, G., MT76
Mandawala, A., 26, MT66
Manjula, P., 9, MT65
Mannen, H., MT189, MT190, MT223, MT304, MT305, MT308, MT309
Mannnen, H., MT227
Manrique Osinaga, I. P., WT93
Mansour, T., MT207
Manunza, A., MT265
Mapholi, N. O., WT94
Marçais, A., MT348
Marchant, T., 121, 127, 128, MT95, MT99, MT105
Marchitelli, C., MT153, WT43
Marcon, D., 153, WT27
Marczak, S., MT344
Marelli, S. P., 152, WT1, WT5
Marete, A. G., MT120

Marghazani, I. B., WT7
Marie-Hélène, P.-v. d. L., MT165
Marikar, F., WT8
Marin-Cely, J., 129, MT96
Marioni-Henry, K., 127, MT99
Marleen, V., MT345
Mármol-Sánchez, E., MT267, MT268
Maroilley, T., MT284
Marsan, P. A., 18, MT14
Marsh, S., 56, MT115
Marshal, K., 182, WT96
Martell, H., 26, MT66
Marthey, S., MT7
Marti-Marimon, M., 147, MT289
Martin, F. J., 149, MT287
Martín-Burriel, I., 110, MT16
Martinez, A. B., 177
Martinez, A. Bario, WT86
Martinez, A. M., MT230
Martinez, M., 68, MT134
Martinez, V., 175, 179, WT75, WT76, WT77, WT81
Martínez, P., 173, WT78
Martínez-Montes, A., MT276
Martinez-Royo, A., WT15
Martínez-Valladares, M., WT13
Martins, C., WT71
Maruyama, S., MT304
Mason, B., MT321
Masoudi, A. A., 49, MT64, WT20
Massa, A., 161, MT340, WT63
Massilani, D., 105, MT360
Mastrangelo, S., WT87
Mateo, J., WT37
Mátlová, V., MT151
Matos, J., 90, MT234
Matsuhashi, T., MT304
Matsumoto, Y., MT305, MT309
Mauri, N., 119, MT94
Maya, M. R., 67, MT216
Mayer, F., 77, MT160
Mayes, M., 103, MT370
Mayoral, T., 67, MT216
Mazzoni, G., 137, MT272, MT291
Mbajiorgu, C. A., MT243
McBride, S., MT325
McCabe, M. S., 132, MT237
McCarthy, F., 22, MT126
McCarthy, J., 117, MT83
McClure, J., MT80
McClure, M., 117, MT80, MT83
McColl, K., 27, MT176
McCormick, C., 4, MT24
McCoy, A., MT186
McCue, M., 37, MT131, MT186, MT187, MT212
McCulloch, A., 25, MT372
McCulloch, M. E., MT324
McEwan, J., 101, MT365
McGivney, B. A., 34, MT215
McGovern, E., 132, MT237
McGrew, M., 30, MT177
McKay, S., MT3
Mckenna, C., MT351
McLean, K. J., 112, MT8, WT34
McLoughlin, K. E., 76, MT173, WT47, WT52
McQuillan, C., WT39
McRae, K. M., MT140
McTier, L., 121, MT105
McWilliam, S., 25, 171, MT372, WT74, WT85
McWilliam, S. M., MT322
Mdladla, K., WT92
Meade, K., 80, MT171
Meade, K. G., WT39
Medrano, J., 159, WT58
Medrano, J. F., MT154
Medugorac, I., MT87
Megens, H., MT345
Meiying, F., MT247
Mendoza, K., MT45
Meneses, C. L. P., MT204
Meng, Q., MT316
Mengmeng, Z., MT226
Menzies, M., WT74
Mercat, M., 98, MT294
Mercat, M. J., MT284
Mercat, M.-J., 134, MT239
Meripet, P., MT148
Mesbah-Uddin, M., MT118
Mészáros, G., 185, WT100
Metzger, J., 35, MT183, MT219
Meutchieye, F., MT311
Meyer, A., MT158
Meyerholz, M., MT138
Mi, L., MT260
Miar, Y., 14, MT25
Mickelson, J., 37, MT131, MT187, MT212
Mickiewicz, M., MT343
Miglior, F., MT356
Mikko, S., 65, MT108, WT10
Miksza-Cybulska, A., MT152
Mileham, A., 28, MT175
Millán, A., 173, WT78
Miller, D., 11, 36, 58, MT22, MT117, MT210, WT104
Miller, L., MT256
Miller, L. C., MT146
Miller, M., 42, MT55
Miller, M. M., 64, 66, MT116, MT162
Miller, S. P., MT71
Ming-Whitfield, B., 41, MT213
Minor, K. M., MT93
Minx, P., 42, MT55
Miracle, P. T., MT87
Mirkena, T., 154, WT30
Miron, J., MT357
Missirian, V., WT19
Missohou, A., 182, WT96
Miszczak, M., 5, MT17
Mochal, C., MT207
Modesto, P., MT35, MT43
Moe, H., MT189, MT227
Moe, K., MT189
Mohamed, A., 171, WT85
Mohamoud, Y., 11, MT22, WT104
Mohamoud, Y. A., 58
Mohamoud, Y. Ali, MT117
Mohsin, M., WT7
Moioli, B., WT43
Molaee, V., WT17
Molina, A., MT326
Mompart, F., 147, MT289
Monney, C., 119, MT94
Monson, M., 82, MT172
Montano, D. A., MT231
Montedoro, M., MT352
Montfort, M., MT285
Montserrat, S., 77, MT160
Moore, K., MT295
Moore, S., MT121
Moore, S. S., MT71
Moran, A., MT295
Moravcikova, N., MT193
Moreira, O., MT281
Moretti, E., WT1
Morgan, E., 60, MT111
Moro, D., 27, MT176
Morota, G., MT339
Morrice, D., MT20, MT21
Mortlock, S.-A., 129, MT96
Moska, M., MT20, MT21
Motycka, J., MT74
Mourão, G., MT353, MT354
Mousel, M., 161, MT340, WT63
Mousel, M. R., 156, MT106, WT14, WT29
Mrode, M., MT325
Mrode, R., 181, 183, 184, WT98, WT101, WT102
Mucha, A., MT20, MT21
Muchadeyi, F., 81, MT169, MT306, WT21, WT92
Muchadeyi, F. C., 186, WT91, WT94, WT97
Mujibi, F. D. N., 184, WT101
Mukesh, M., 86, WT105
Mukhanbetkaliyev, Y., MT75
Mukiibi, R., MT300, MT327, WT88
Mulder, H., 143
Mullaart, E., 99, MT368
Mullen, M., MT80
Mullen, M. P., 76, 166, MT173, WT55
Mullin, V., 107, MT366
Munari, D. P., WT48
Muñoz, M., 98, MT254, MT276, MT294
Munyard, K., 20, 60, 144, MT111, MT128, WT69
Munyard, K. A., 12, MT27
Murdoch, B., 161, MT316, MT318, MT340, WT63
Murdoch, B. M., 158, WT28
Muret, K., 20, 53, MT49, MT56, WT69
Murphy, D. N., 149, MT287
Murphy, M., MT149
Murphy, R. W., MT259
Musilova, P., 31, MT218
Musthafa, M., WT8
Mutai, C., MT311
Muzny, D. M., MT316
Mwacharo, J., MT38, MT311, WT87
Mwacharo, J. M., 181, WT102
Mwai, A. O., 185, WT100
Mwai, O., 154, 181, WT30, WT102
Mwai, O. A., MT311

N

Na, R., MT299
Naccache, F., 35, MT219
Nag, R., 149, MT287
Nagata, S., 69, MT135
Nagata, S.-I., MT178
Nai, R., MT260, MT332
Nakajima, A., MT309
Nakajima, T., MT308
Nalpas, N., 111, MT9
Nalpas, N. C., 76, 166, MT173, WT47, WT52, WT55
Namous, H., 18, MT14
Nanduri, B., 22, MT126
Napolitano, F., MT153
Naranbhai, V., 166, WT55
Nascimento, G. B., WT48
Nassar, M. K., MT30
Naval Sanchez, M., 25
Nayeri, S., MT71, MT356
Ncube, K., WT94
Nergadze, S. G., 36, MT210
Neßler, J. N., MT79
Neuditschko, M., 32, MT214
Neuhoff, C., 74, MT137, MT170
Neumann, K., 175, WT81
Neves, J., WT2
Ni, A. X., 50, MT59
Niciura, S., MT337
Nicolini, P., MT339
Niedziela, D., MT149
Nielsen, M., 63, MT113, MT123
Nieuwland, M. G. B., MT145
Ning, C., WT65
Nishibori, M., MT190, MT223
Nishimura, S., MT319
Nizamlioglu, M., 188, MT232
Nizanski, W., 7, WT4
Nneji, L. M., MT259
Nogueira, A., MT353
Nolan, M., MT156
Nomura, K., MT190
Nonnis, S., MT89
Nordgreen, J., MT1
Norton, E., 37, MT212
Novák, K., MT151
Novosel, D., MT355
Nowak, A., 7, WT4
Nunes, J. M. S., WT2
Nuñez, Y., MT245, MT269, WT35
Núñez, Y., 98, 141, MT276, MT294, WT42, WT53
Nussbaumer, M., 121, MT105
Nxumalo, K. S., WT94

O

Oatley, J., 28, MT175
Obi, O. C., MT31
O'Connell, K., 117, MT83
O'Connor, R. E., 26, MT66
Oczkowicz, M., MT264
O'Doherty, A., 111, MT9
Oevermann, A., 119, MT94
O'Farrelly, C., WT39
Ogah, D. M., MT32
Ojala, K., WT49
Ojango, J., 181, 184, WT101, WT102
Oke, U. K., MT31
O'Keefe, J., 4, MT24
Okimoto, N., MT150
Okoro, C. L., MT243
Okoro, V. M. O., MT243
Okuda, Y., MT189, MT190
Oladele-Bukola, M. O., MT259
Olaogun, S. C., MT259
Olivas, J., MT269
Oliveira, P., MT297, MT353
Oliveira, R. D., WT14
Olorungbounmi, , MT259
Olsson, C., MT179
Olsson, M., 126, MT102
Oluwole, O. O., MT259
O'Meara, D., 4, MT24
Omitogun, O. G., MT259
Oncu, M. D., 188, MT232
Öner, Y., MT18
Onzima, R. B., WT88
Oosterlinck, M., 33, MT211
Opiela, J., 146, MT15
Oppelt, J., 31, MT218
Orellana, J., WT93
Orlando, L., 31, 39, 87, MT209, MT218, MT235
Orlando, L. A. A., MT123
O'Rourke, B. A., MT314
Ortega Masague, M., WT93
Ortillés, A. L., WT40
Osama, S., MT311
Oster, M., MT283
Østerbye, T., 63, MT113
Ostrander, E. A., 152, WT5
O'Sullivan, N., 73, MT161
O'Sullivan, T., MT246
O'Toole, D., 119, MT94
Ottoni, C., 125, MT97
Ottun, O. N., WT73
Ovilo, C., MT245
Óvilo, C., 98, 136, 141, MT238, MT269, MT294, WT35, WT42, WT53
Oyama, K., MT304, MT305, MT308, MT309
Oyekanmi, N., 183, WT98
Ozmen, O., 120, MT104

P

Pabiou, T., MT80
Paces, J., 46, MT57
Pacholewska, A., MT185
Pagnacco, G., 152, WT1, WT5
Pajer, P., 46, MT57
Paller, T., MT323
Paniccià, M., WT18
Pant, S., 80, MT171
Paradis, F., MT4
Paria, N., MT123
Park, C., MT6, MT132, MT266
Park, C. K., MT147
Park, E.-W., 115, MT13
Park, J.-E., 115, MT13, MT42
Park, N. G., MT188
Park, S. H., MT188
Parmentier, H. K., MT145
Pasha, K., 11, MT22, WT104
Patel, A., 179, WT77
Patil, M., MT329, WT19
Patil, M. A., 118, MT85
Patterson, E. E., MT93
Pausch, H., 100, MT129, MT261, MT328
Pawlina, K., 146, MT5, MT15, MT194, MT195
Pedersen, A. Ø., 135, MT241
Peelman, L., WT41
Peeters, L., MT182
Peletto, S., MT35, MT43
Pelle, R., MT107
Pemberton, J., WT25
Pena, B., 153, WT27
Pena, R., 140, MT257, MT277, MT292
Peña, S., WT93
Peñagaricano, F., 18, MT14, MT339
Penedo, M. Torres, WT3
Peng, D., MT36
Peng, M.-S., MT259
Peng, X., MT33
Penso-Dolfin, L., MT19
Péntek, I., MT312
Periasamy, K., WT8
Perkins, T., MT338
Perrier, J., MT7
Pértille, F., MT1
Pessani, D., MT43
Petersen, J., 41, MT213
Petrini, J., MT354
Petterson, M., 177, WT86
Petzl, W., MT138
Phillippy, A., 148, MT125
Picchi, A., WT1
Pickering, L., 81, MT169
Piestrzynska-Kajtoch, A., 70, MT81, MT152, MT196, MT220
Piga, S., MT43
Pilkington, J., WT25
Pilla, F., WT87
Pinard-Van der Laan, M. H., 19, 78
Piórkowska, K., MT191, MT198, MT263, MT264
Pirani, A., 118, MT85, MT329, WT19
Piras, F. M., 36, MT210
Pires, A. E., 90, MT234
Pitarch, L. B., 67, MT216
Pitchford, W. S., MT77
Pitel, F., 53, MT49, MT56
Plastow, G., MT76, MT78
Plysnina, P., MT347
Podbielska, A., 5, 7, MT17, WT4
Podstawski, P., MT184
Polgar, Z., MT90
Polli, M., 152, WT1, WT5
Ponsuksili, S., 114, MT11, MT283
Porter, R., MT351
Porto-Neto, L. R., MT322

Posik, D. M., WT93
Powell, R., 121, MT105
Pozzi, A., 3, MT86
Prando, A., 165, WT56
Presho, E., 4, MT24
Previtali, C., 3, MT86, MT282
Pribyl, J., MT74, MT192
Priyadi, D. A., WT24
Pröll, M. J., 74, MT137, MT170, MT251
Proudfoot, C., 28, MT175
Prowse-Wilkins, C., MT321
Psifidi, A., MT156
Puglisi, R., MT352

Q

Qiao, X., MT247
Qin, X., MT316
Qin, Z., MT72
Qiu, Q. Q., 24, MT364
Qu, B. B., WT9
Queiroz, S., WT72
Quintanilla, R., MT265, MT267, MT268
Qureshi, S., MT248

R

Radko, A., 5, 7, MT17, MT81, MT196, MT330, WT4
Radovic, S., MT87
Raeber, A., 116, MT84
Rafati, N., 177, WT86
Rahmatalla, S. A., MT303
Raimondi, E., 36, MT210
Rakhshandeh, A., MT275
Ramayo-Caldas, Y., 134, 136, MT238, MT239, MT252
Ramírez, O., 6, WT6
Ramirez Alanis, C., 41, MT213
Ramos, S. B., WT48
Ran, X., MT226
Randlaht, I., MT229
Raney, N., MT258
Raphael, M., MT311
Rashidi, H., 143
Rasmussen, M., 63, MT113
Rattei, T., 32, MT214
Ratti, C., 68, MT134
Rau, A., 53, 144, MT7, MT49, MT56, MT128, MT252
Raudsepp, T., MT123, MT229
Rawat, C., MT248
Rebl, A., 178, WT82
Reczynska, D., MT341, MT343, MT344
Reecy, J., 103, MT370
Reed, K., MT45
Regitano, L., MT297, MT337, MT353, MT354
Reich, C., MT321
Reina, R., WT51
Reissmann, M., MT303
Reixach, J., 140, MT277, MT292
Remacha, A. R., MT181, WT40
Renaudeau, D., MT262
Ressaissi, Y., MT301
Reverter, A., MT322
Revilla, M., 136, MT238, MT252, MT253
Rey, A., MT245, WT42
Reyer, H., 15, 92, MT23, MT270, MT271, MT283, MT303, WT33
Reynolds, L. P., 112, MT8, WT34
Ribani, A., 43, 137, MT19, MT61, MT291
Ribeca, P., MT315
Ribeiro, C., MT203
Rice, E., 38, MT208
Richards, S., MT316
Richardson, I. W., 76, 166, MT173, WT55
Ridpath, J., 103, MT370
Ridpath, J. F., 84, MT367
Rieder, S., 32, MT214
Rigler, D., 32, MT214
Riina, M. V., MT43
Riley, D. G., MT317
Riquet, J., 98, MT262, MT294
Rischkowsky, B., 154, 185, WT30, WT100
Ristov, S., MT355
Riva, J., WT1
Robelin, D., 147, MT289
Robinson, J., 56, MT115
Rocha, D., 102, MT369
Rocha, M., MT297, MT337, MT353, MT354
Rochat, E., 109, MT362
Rochus, C., WT10
Rodellar, C., MT181, WT40
Rodriguez, A., MT318
Rodriguez, A. M., 158, WT28
Rodriguez, C., MT254
Rodriguez-Fernández, C., 90, MT234
Rodríguez-Gil, J.-E., MT285
Roehe, R., 130, MT240
Rogberg Muñoz, A., WT93
Rogel-Gaillard, C., 57, 79, 134, MT109, MT168, MT239, MT284
Rogers, J., MT316
Rohmeier, L., MT138
Rohrer, G. A., MT279
Roman-Ponce, S. I., 43, MT44, MT61
Romero, A., MT181
Rony, S., 74, MT137, MT170
Ropka-Molik, K., 70, MT184, MT191, MT198, MT220, MT264
Rosa, G. J. M., MT338
Rose, R., MT262
Rosen, B., 108, 148, MT125, MT361
Rosewick, N., MT346, MT348
Ros-Freixedes, R., MT277
Ross, P., 17, 114, 159, MT10, MT11, WT58
Rothammer, S., MT355
Rothschild, M. F., 109, 176, MT246, MT362, WT83
Rowe, S. J., MT140
Rowland, B., 143
Rowland, K., 55, MT52
Rubin, C.-J., 177, WT86
Rubio, Y. B., MT258
Rubis, D., MT81
Rue-Albrecht, K., 111, MT9, WT47, WT52
Ruiz, F. J., MT44
Rund, L., 148, MT125
Rund, L. A., 138, MT286
Russ, I., MT261
Rychtarova, J., WT16
Rytz, U., 128, MT95

S

Sá, J., MT281
Saelao, P., 17, 159, MT10, WT58
Safronova, O., MT190
Sahana, G., MT118
Sahoo, N. R., MT248
Saifitdinova, A., 44, MT50, MT67
Salamon, D., MT355
Salavati, M., WT38
Salim, B., 183, WT98
Salinas-Pena, M., 110, MT16
Samaha, G., 122, MT100
Samorè, A. B., MT272
San Cristobal, M., WT45
Sanchez, M. N., MT372, WT74
Sánchez, A., 6, MT285, WT6
Sánchez-Esquiliche, F., MT254
Sánchez-Molano, E., MT90
Sanders, J. O., MT317
Sang, H., 30, MT177
Sang, Y., MT146
Sanke, O. J., MT259
Santos-Silva, J., MT281
Sanz, A., 110, MT16
Sanz-Rubio, D., 110, MT16
Sargolzaei, M., 14, 80, MT25, MT71, MT171, MT356
Sarry, J., 155, WT31
Sarti, F., WT18
Sarto, P., WT15
Sasaki, S., MT148
Sasazaki, S., MT223, MT227, MT304, MT305, MT308, MT309
Saunders, G., 25, MT372
Saura, M., 173, WT78
Savegnago, R. P., WT48
Savelkoul, H., MT182
Sawyer, J. E., 84, MT317, MT367
Sawyer, R., MT318
Sawyer, R. J., 158, WT28
Sbardela, A. P., WT48
Scalvini, F. G., MT89
Schachtschneider, K. M., 138, MT286
Schaefer, R., 37, MT187, MT212
Schellander, K., 74, MT137, MT170, MT251
Schenkel, F., MT71, MT356
Schiavo, G., 43, 137, MT19, MT61, MT272, MT291
Schibler, L., MT7
Schlötterer, C., 32, MT214
Schmidova, J., MT193
Schmidt, C., 45, MT54
Schnabel, R., MT80
Schoenebeck, J., 127, 128, MT95, MT99
Schook, L., 57, 148, MT109, MT125
Schook, L. B., 138, MT286
Schroeder, S., 148, MT125

Schroyen, M., 16, 142, MT164, MT275, WT70
Schubert, M., 87, MT123, MT235
Schuberth, H.-J., 75, MT138, MT157
Schuller, S., 119, MT94
Schulz-Schaeffer, W. J., MT336
Schurink, A., 33, MT182, MT211
Schütz, E., MT79, MT336
Schwartz, J., 23, MT159
Schwartz, J. C., MT154
Schwarz, T., 127, MT99
Schwind, R. M., 138, MT286
Scienski, K., WT68
Scott, E., 41, MT213
Seal, R., 169, WT59
Sebastiani, C., WT18
Segura, J., MT269
Seichter, D., MT261
Selionova, M., 92, WT33
Sellem, E., MT7
Semik, E., MT5, MT194, MT195
Semmer, J., 59, MT110
Seo, D., 9, MT46, MT65
Seong, H., MT180, MT188
Seong, P. N., MT188
Sereno, F. T. P. S., MT203
Serrano, M., WT15
Servin, B., 109, MT362
Sest, K., MT323
Settar, P., 73, MT161
Seyfert, H.-M., 75, MT138, MT157
Shabtay, A., MT357
Shang, D., 51, MT53
Shang, P., 164, WT62
Sharma, A., 86, WT105
Shave, R., WT46
Shimogiri, T., MT189, MT227
Shin, D., MT273, MT274
Shin, S. M., MT188
Shiraigol, W., MT202
Shykind, B., 11, MT22, WT104
Sidadolog, J. H. P., MT69
Siddavatam, P., 116, 151, MT84, MT98
Siddiki, M., MT296
Siegismund, H. R., 24, MT364
Signorelli, F., MT153
Silió, L., MT254
Silva, J., MT353
Silva, V., MT345
Simcic, M., MT355
Simianer, H., 95, MT236
Simões, L., 90, MT234
Simpson, B., 8, MT26
Sinha, R., MT248
Sironi, G., MT89
Sistiaga-Poveda, M., WT51
Skrlep, M., 98, MT294
Skuce, R., 4, MT24, MT325
Slack, E. A., 26, MT66
Slate, J., WT25
Sloniewska, D., MT341, MT343, MT344
Smakova, A., MT75
Smith, J., 54, MT41, MT47, MT62
Smith, T., 8, 23, MT26, MT159, MT340
Smith, T. P., MT316
Smith, T. P. L., MT154
Smolucha, G., MT152
Snelling, T., 130, MT240
Snyman, H. N., MT158
Snyman, M., WT21
Soares, M., WT2
Sochorová, J., WT50
Sodhi, M., 86, WT105
Soh, P., 129, MT96
Solano-Gallego, L., 77, MT160
Solkner, J., 154, WT30
Sölkner, J., 185, MT355, WT100
Soller, M., 54, MT41, MT47, MT62
Sollero, B. P., 180, WT99
Soma, P., MT306, WT92
Son, J. K., MT188
Son, S., MT274
Song, K.-D., MT273, MT274
Song, Z., 29, MT174
Sonstegard, T., 106, 108, MT361, MT363
Souza, K. T., MT204
Souza, M., MT353
Soyeurt, H., MT153
Spangler, G., 108, MT361
Spark, N., MT107
Splichal, J., MT74
Splichalova, P., 31, MT218
Squires, J., MT356
SS, W., MT248
Stadtmueller, B. M., 64, MT162
Staric, J., MT323
Stear, M., 60, MT111
Steen-Jensen, D. B., 63, MT113
Stefaniuk-Szmukier, M., 70, MT191, MT198, MT220
Steffen, F., 119, MT94
Steibel, J., MT258
Stella, A., 18, 109, MT14, MT362
Stevens, J. C., MT201
Stewart, R., 130, MT240
Stinckens, A., 33, MT182, MT211
Stokar-Regenscheit, N., 119, MT94
Stortti, F., WT2
Stothard, P., MT71, MT76, MT300, MT327
Stout, T. A. E., MT123
Straathof, C., MT4
Strand, E., MT179
Strillacci, M. G., 43, MT44, MT61, MT335
Strive, T., 27, MT176
Stryhn, A., 63, MT113
Stuczynska, A., MT263
Su, R., MT320, MT332
Suarez-Vega, A., 97, WT13, WT26
Suárez-Vega, A., WT37
Sudrajad, P., MT46
Sugimoto, Y., MT148, MT319
Suleimenov, M., MT190, MT223
Sullivan, K., 36, MT210
Sullivan, S., 42, MT55
Sumadi, , MT69
Summers, K. M., MT324
Sun, D., MT72
Sun, H., MT307
Sun, Y. Y., 50, MT59
Sunarto, A., 27, MT176
Surlis, C., MT350
Svara, T., MT323
Svenson, K., 121, MT105
Svensson, E., 90, MT234
Svitakova, A., MT193, MT331, WT16, WT22
Swiderski, C., MT207
Symonová, R., WT50
Szmatola, T., 146, MT5, MT15, MT184, MT264, MT330
Sztankoova, Z., WT16, WT22

T

Tabata, R., MT223
Tadesse, T., 154, WT30
Tait, J., 8, MT26
Tait, R., MT370
Tait, R. J., MT338
Tait Jr., R., 103
Takahashi, Y., MT190
Takeshima, S., MT148
Takeshima, S.-N., MT150
Talbot, R., 148, MT125
Talenti, A., 152, WT1, WT5
Tamma, N., 99, MT368
Tammen, I., MT314
Tan, T., 29, MT174
Tan, T. Y. K., 12, MT27
Tan, W., 21, MT167
Tan, X., MT299
Tapio, M., 182, WT96
Tarekegn, G. M., MT311
Tatsuda, K., MT37
Taxis, T. M., MT142
Tayal, A., 168, WT57
Taylor, J., MT80
Taylor, J. B., MT106, WT14
Taylor, J. F., MT124
Teasdale, M., 150, MT371
Tebug, S. F., 182, WT96
Tedeschi, G., MT89
Telo da Gama, L., MT203
Telugu, B., 28, MT175
Tengvall, K., 126, MT102
Terakado, A. P. N., WT89
Tesfaye, D., 74, MT137, MT170
Tesfaye, K., MT107
Tholen, E., 74, MT137, MT170, MT251
Thomas, A., 123, MT103
Thomer, A., MT183
Tibau, J., MT267, MT268
Tijhaar, E., MT182
Tijjani, A., 183, WT98
Tinel, S., 33, MT182, MT211
Tipold, A., MT79, MT336
Tizard, M., 27, MT176
Tizioto, P., MT297, MT337, MT353, MT354
Toivonen, J., 110, MT16
Tombacz, K., 85, MT163
Tomley, F., MT156
Tomlinson, C., 42, MT55

Tor, M., 140, MT257, MT277, MT292
Toro, M., 173, WT78
Torres, J., 110, MT16
Torricelli, M., WT18
Tosser-Klopp, G., 20, WT69
Tozaki, T., 69, MT135, MT178
Trakooljul, N., 114, MT11, MT270, MT271
Trigo, A., 67, MT216
Trincas, E., MT43
Triviziaki, S., MT355
Tsairidou, S., 71, MT166
Tseng, E., 148, MT125
Tseng, J., 58, MT117
Tsimnadis, E. R., MT314
Tsuchimura, M., MT304
Tsunoda, K., MT190
Tuggle, C., 16, 159, MT275, WT58, WT70
Tuggle, C. K., 17, 142, MT10, MT164
Tullio, R., MT354
Tweedie, S., 169, WT59

U

Uddin, M. J., 74, MT137, MT170
Uemoto, Y., MT305, MT308, MT309
Unni, D., 168, WT57
Upadhyay, M., MT345
Upadhyay, M. R., WT88
Ureña, I., 90, MT228, MT234
Urnov, F., 28, MT175
Uskenov, R., MT75
Usman, T., MT136
Utzeri, V. J., 137, MT19, MT291

V

Vaccari, G., WT18
Vahala, J., 31, MT218
Valcíková, T., MT151
Valdez, F., WT93
Valdiosera, C., MT228
Valente, G. T., WT71
Van den Broeke, A., MT346, MT348
Van der Laan, M. H. Pinard, MT127
van der Poel, J. J., MT145
van der Sluis, R., 1, MT60
van der Werf, J. H., WT90
van der Zwan, H., 1, MT60
van Dreumel, T., MT246
Van Eenennaam, A., 159, WT58
van Haeringen, W., 151, MT98
Van Marle-Koster, E., MT124
Van Poucke, M., WT41
van Son, M., MT278
Van Stijn, T., 101, MT365
Van Tassell, C., 108, MT361
Vander Jagt, C., MT321
Vanlierde, A., MT153
Varela-Martínez, E., WT51
Vázquez, F. J., MT181
Vazquez-Gomez, M., MT245
Vázquez-Gómez, M., WT35, WT42
Vega-Murillo, V. E., MT44
Vega-Pla, J. L., MT203
Velez-Irizarry, D., MT258
Velie, B., 33, MT182, MT211
Velie, B. D., MT179
Vella, G., 142, MT164
Verbyla, K., 171, WT74, WT85
Verdugo, M., 89, MT359
Verleih, M., 178, WT82
Vernesi, C., MT87
Vernimmen, D., 111, MT9
Vesela, Z., MT193, MT331
Vevey, M., MT335
Viguera, J., MT269
Vilaró, F., MT277
Vilkki, J., MT118
Villanueva, B., 173, WT78
Villarreal-Ramos, B., 76, MT173, WT47, WT52
Villa-Vialaneix, N., 144, 147, MT128, MT289
Villegas Castagnasso, E. E., WT93
Viluma, A., 65, MT108
Vincent-Naulleau, S., MT284
Vinsky, M., MT300, MT327
Visser, C., 1, MT60, WT91
Vitoria, A., MT181
Vodicka, R., 31, MT218
Vogl, C., 32, MT214
Voillet, V., WT45
Volodkina, V., 44, MT67
Vordermeier, H. M., 76, MT173, WT47, WT52
Vostra-Vydrova, H., MT192, MT193
Vostry, L., MT74, MT192, MT193, MT331

W

Wade, C., 122, MT100
Wade, C. M., MT199
Walker, A., 130, MT240
Wallace, R., 130, MT240
Wallner, B., 32, MT214
Wang, D., 113, MT12, MT307
Wang, G., 51, MT53
Wang, H., MT2, WT65
Wang, J., 51, MT36, MT53, MT244, MT249
Wang, L., 52, 164, MT58, WT62
Wang, M., 81, MT169
Wang, O., MT307
Wang, R., MT320, MT332
Wang, W., 24, MT364
Wang, X., MT119
Wang, X.-Y., WT11
Wang, Y., 17, 159, MT10, MT73, MT320, WT9, WT58
Wang, Z., 38, 164, MT208, MT260, MT332, WT62
Ward, A. K., 112, MT8, WT34
Warr, A., 130, 148, MT125, MT240
Warren, W., 42, MT55
Waters, S., MT80, MT350, MT351
Waters, S. M., 132, MT237, MT300, MT302, MT327, MT334
Watson, M., 130, MT240, MT324
Weaber, R., MT3
Wei, Z., MT298
Weigend, A., 95, MT236
Weigend, S., 95, MT236
Weikard, R., MT138, MT333
Weinert, M., 75, MT157
Weld, R., 117, MT80, MT83
Wells, M., MT338
Wemheuer, W. E., MT79, MT336
Wemheuer, W. M., MT336
Werling, D., 85, MT163
Westberg-Sunesson, K., WT10
Wheeler, M., 101, MT365
White, S., 161, MT340, WT63
White, S. N., 156, MT106, MT316, WT14, WT29
Whitelaw, B., 28, MT175
Whitelaw, C. B., MT324
Whitelaw, C. B. A., 21, MT167
Wiedemar, N., MT323
Wiedmer, M., 119, MT94
Wiener, P., 97, MT88, MT90, WT26
Wierzbicki, H., MT20, MT21
Wilkinson, S., MT325
Willet, C. E., MT314
Williams, J., 18, 103, 104, MT14, MT370, MT373
Williamson, P., 129, MT96
Willis, C., 116, MT84
Wilson, G., 4, MT24
Wimmers, K., 15, 92, 114, MT11, MT23, MT270, MT271, MT283, MT303, WT33
Witarski, W., MT184
Witkowski, M., MT194
Wodas, L., MT130, MT197
Wolc, A., 55, 73, 82, MT52, MT161, MT172
Wolf, J., MT354
Woloszyn, F., 155, WT31
Womack, T., WT68
Won, K.-H., MT273
Wong, B., MT329
Wong, S., WT49
Woo, J. H., MT188
Woolley, S. A., MT314
Woolliams, J., MT325
Woolliams, J. A., 71, MT90, MT166
Woolums, A., 103, MT370
Worku, M., MT143
Worley, K., MT340
Worley, K. C., MT316
Wouobeng, P., MT311
Wu, C., 164, WT62
Wu, D.-D., MT40
Wu, J., MT320
Wu, N., MT51
Wu, S., MT2
Wu, X., MT338
Wu, Z., MT156
Wurmser, C., MT261, MT328
Wurzinger, M., 154, 185, WT30, WT100

X

Xiao, H., MT260, MT320
Xie, H.-B., MT259
Xie, S., 139, MT288
Xie, Y., MT260, MT332

Xing, Y., 29, MT174
Xu, G. Y., 113, MT12
Xu, J., MT338
Xu, L., MT222, MT225
Xu, S. S., 50, MT59
Xue, F. G., 50, MT59

Y

Yamagata, T., MT190
Yamagishi, J., MT150
Yamamoto, Y., MT189, MT190
Yamanaka, H., MT227
Yan, W., MT225
Yáñez, J., WT72
Yáñez, J. M., 170, WT80
Yang, G., 139, MT288
Yang, I.-C., MT180
Yang, L., MT34
Yang, M., 157, MT51, WT32
Yang, N., 113, MT12
Yang, R., MT249, MT298
Yang, S., 113, MT12
Yang, Y.-R., MT42
Yaro, M., 60, MT111
Yates, B., 169, WT59
Yechshzhanov, T., MT75
Yerle-Bouissou, M., 147, MT289
Yi, Z., MT72
Yildirim, I. S., 188, MT232
Yin, H., WT67
Yin, L., WT36
Yoshida, E., MT305, MT308, MT309
Yoshida, G., WT72
Young, R., 104, MT373
Younger, L., 58, MT117
Yu, H., MT298
Yu, X., MT298
Yu, Y., 113, MT12, MT73, WT65
Yuan, Z., MT72
Yudin, N. S., MT347
Yun, S., MT132
Yung, R., MT36
Yurchenko, A., MT347

Z

Zabek, T., MT5, MT194, MT195, MT330
Zalewska, M., MT344
Zambonelli, P., MT280
Zamorano, P., WT75
Zappaterra, M., MT280
Zaragoza, P., 110, MT16, MT181, WT40
Zaton-Dobrowolska, M., MT20, MT21
Zentek, J., MT30
Zerbe, H., MT138
Zerbino, D., WT66
Zerjal, T., 53, MT49, MT56
Zerlotini-Neto, A., MT337
Zhamaliyeva, S., MT75
Zhang, C., MT36, MT249
Zhang, G., 24, MT364
Zhang, H., 52, 164, MT58, WT62
Zhang, J., MT299
Zhang, L., MT222
Zhang, Q., MT73
Zhang, R., MT249
Zhang, S., MT73, WT67
Zhang, W., MT222, MT311
Zhang, X., MT48, WT64
Zhang, Y., 52, MT58, MT73, MT320, MT332
Zhang, Y.-P., MT40, MT259
Zhang, Z., 145, MT290
Zhao, C., 139, MT288
Zhao, K., 167, WT60
Zhao, M., MT260, MT332
Zhao, P., MT255, WT65
Zhao, S., 139, MT288, WT36
Zhao, Y., MT244, MT249, MT260, MT299, MT320, MT332, WT44
Zhao, Z., MT298, MT299
Zheng, X., MT255, WT65
Zheng, Y. C., MT70, WT9
Zheng, Z., MT119
Zhou, C., WT67
Zhou, H., 17, 55, 114, 159, MT10, MT11, MT52, WT58
Zhou, X., WT36
Zhou, Z., 145, MT290
Zhu, M., WT36
Zhu, Y., 145, MT290
Zi, X. D., WT9
Zidi, A., MT265
Zinovieva, N., 15, 92, MT23, WT33
Zou, C., MT250
Zou, H., MT244
Zubiria, I., 131, MT242
Zukowski, K., MT191, MT198, MT263
Zulkifli, N. A., MT77
Zurbrigg, K., MT246
Zwane, A. A., MT124
Zwierzchowski, L., MT341, MT343
Zytnicki, M., 144, 147, MT128, MT289

Key Word Index

Numbers following entries refer to abstract numbers. A number alone indicates an oral presentation; an MT preceding the number indicates a Monday/Tuesday poster (Poster Session I) and a WT indicates a Wednesday/Thursday poster (Poster Session II) poster. Orals are listed first, followed by posters in session and numeric order.

A

ACAT1, MT351
acclimation, MT42
accuracy, 181, WT102
ACLY, MT351
acute phase proteins, MT341, MT343
adaptation, 109, 180, MT49, MT262, MT362, WT44, WT99
adaptive immunity, MT138, MT313
adaptive introgression, 106, MT363
ADAPTmap, 109, MT362
adipogenesis, 51, MT53
adipose, MT351
admixture, 89, 108, MT76, MT193, MT222, MT224, MT359, MT361, WT88
adult tissues, 115, MT13
aflatoxin, MT45
aflatoxin B_1, MT33
Africa dairy zebu, 183, WT98
aging, MT5
AgriSeq, 116, MT84
algorithm, 163, WT61
allele frequency, 98, MT294
allele-specific expression, 179, MT264, MT337, WT1, WT77
allelic variants, 86, WT105
allergy, 126, MT102, MT182, MT358
amino acids, 129, MT96
ancestral chromosome reconstruction, 163, WT61
ancient DNA, 87, 89, 90, 93, 105, 107, 125, 150, MT87, MT97, MT228, MT234, MT235, MT359, MT360, MT366, MT371, MT374
Angora cat, 120, MT104
animal, WT50
animal breeding, 14, 71, 154, 172, MT25, MT74, MT75, MT166, MT249, MT277, MT295, MT328, WT2, WT9, WT17, WT30, WT79
animal domestication, 93, 94, 107, MT189, MT190, MT228, MT249, MT355, MT366, MT374, WT8, WT95
animal health, 12, 54, 59, 79, 82, 111, 123, 128, MT9, MT27, MT41, MT47, MT62, MT73, MT79, MT95, MT103, MT110, MT138, MT140, MT153, MT168, MT172, MT181, MT194, MT195, MT325, MT328, MT336, WT17, WT18, WT47, WT51, WT52
animal nutrition, MT267, MT268, MT334
animal welfare, MT1, WT1
annotation, 169, MT124, WT59
antibody response, 103, MT370
antimicrobial peptide, MT39
apoptosis, MT33
aquaculture, 173, 179, WT73, WT75, WT76, WT77, WT78
Arabian horse, MT191
Arabians, MT198
Arbocel, MT30
artificial selection, MT34
association, MT72, MT248
association analysis, MT339
asthma, MT185
ATAC-seq, 19, 144, MT127, MT128
athletic performance, 34, MT181, MT215
Atlantic salmon, 170, WT80
average daily gain, MT327
avian, 43, MT61
avian genomes, 26, MT66
avian genomics, 1, MT60

B

B chromosome, WT71
bacteria, MT344
badger, 4, MT24
basophil, MT272
beef cattle, MT76, MT78, MT300, MT317, MT327, MT338, WT89
beef marbling, MT308
beefalo, MT318
beta (β)-defensin, WT3
BHV-1, 21, MT167
big data, MT120
biochemical genetics, WT14
biodiversity, 90, 92, 95, MT20, MT203, MT230, MT234, MT236, WT33
bioinformatics, 1, 17, 56, 76, 102, 130, 159, 169, MT10, MT60, MT115, MT173, MT240, MT245, MT267, MT329, MT369, WT39, WT58, WT59
bioinformatics tools, 37, 149, 168, MT119, MT212, MT287, MT326, WT13, WT57, MT337
bioinformatics tools/data mining, 160, WT54
biomarker, 142, 167, MT164, MT185, MT277, WT47, WT60
biomedical model, 31, 138, MT93, MT218, MT286, WT14
bison, 8, 105, MT26, MT360
Bligon goat, WT24
blood, 53, MT56, MT198, WT45
blood leukocytes, MT341, MT343
body weight, MT178
Bos indicus, 84, MT317, MT367
bottleneck analysis, MT231
bovine, MT3, MT4, MT81
bovine 50K BeadChip, 81, MT169
bovine leukemia virus, MT142, MT148, MT150
bovine mastitis, 75, MT157
bovine parentage, 116, MT84
bovine respiratory disease complex, 103, MT370
bovine TLR2, 85, MT163
bovine tuberculosis, 166, WT55
BovineHD BeadChips, 15, MT23
BPI gene, MT2
brachycephaly, 121, MT105
Brahman cattle, MT69
brain, MT3
breed, 131, MT137, MT242
breed discrimination, 9, MT65
breed distribution, MT226
breed diversity, 91, MT70, MT130, MT233, MT303, WT87, WT88, WT91, WT93
breed diversity and composition, MT76
breed identification, 108, MT361
breed improvement, 104, 186, MT373, WT97
breed/population identification, 11, 32, 184, MT183, MT214, MT303, MT355, WT93, WT101, WT104
breeds, 156, WT29
brindle, WT3
Bubalus bubalis, MT312
bull fertility, MT339
bull sperm cell, MT7
Burmese cat, 122, MT100
BVDV, 84, MT367

C

CA, 70, MT220
CAEV, MT341
calf, MT333
calf death, MT319
camel, MT18
cancer, MT346, MT348
cancer/testis antigen, MT342
candidate gene, 35, 52, 94, 123, 128, MT58, MT77, MT95, MT103, MT147, MT183, MT219, MT253, MT254, MT283, MT298, MT323, MT331, MT336, WT14, WT15, WT67, WT95
canine, 5, 129, MT17, MT96, WT40
Capra hircus, MT299
caprine, WT23
carcass trait, MT37, MT304
cardiac defects, MT246
cardiomyopathy, WT46
cartilage, MT133
cashmere goat, MT320, MT332
cat, 125, MT97
cathelicidins, MT341, MT343
cats and related species, 123, MT103

cattle, 3, 23, 76, 80, 81, 86, 103, 106, 115, 165, 180, MT4, MT13, MT75, MT77, MT79, MT86, MT121, MT142, MT150, MT151, MT153, MT154, MT159, MT169, MT171, MT173, MT222, MT227, MT298, MT302, MT304, MT305, MT314, MT318, MT322, MT323, MT330, MT334, MT335, MT336, MT342, MT344, MT347, MT350, MT351, MT352, MT363, MT370, WT38, WT56, WT67, WT91, WT99, WT105
cattle and related species, 89, 91, 100, 107, 111, 112, 168, 182, MT8, MT9, MT129, MT138, MT233, MT295, MT297, MT315, MT326, MT328, MT329, MT331, MT337, MT353, MT355, MT359, MT366, WT34, WT47, WT52, WT57, WT93, WT96
cattle breeds, 85, MT163, MT231
CD4 gene, MT136
cell biology, MT181
cell culture, MT181
centromere, 36, MT210
characteristics, MT226
chicken, 26, 30, 42, 45, 49, 64, 73, 78, 95, 113, 114, MT1, MT11, MT12, MT30, MT34, MT37, MT40, MT48, MT51, MT54, MT55, MT59, MT64, MT66, MT107, MT145, MT161, MT162, MT165, MT177, MT226, MT236
chicken MHC, 66, MT116
China, MT226
Chinese Holstein, MT73
ChIP-seq, 17, 41, 77, 145, 159, 161, MT10, MT160, MT213, MT290, WT58, WT63
chondrocyte, MT133
chromosome evolution, 163, WT61
chromosome pairing, MT318
chromosome wide scan, MT243
climate, 109, MT362
c-Myc, WT64
CNV, MT44, MT48, MT59, MT335, MT345
coat colour, MT197, MT247, WT10
coat colour variation, WT20
coccidiosis, 78, MT165
coho salmon, 170, WT80
colonisation, 4, MT24
Columba livia domestica, 10, MT63
commercial native chicken, MT46
comparative, 169, WT59
comparative genomes, 26, MT66
comparative genomics, 20, 24, 31, 40, 91, 142, 160, 168, MT164, MT218, MT221, MT233, MT303, MT364, WT49, WT52, WT54, WT57, WT69
compensatory growth, MT302
complex trait, 33, 54, 100, 145, MT47, MT62, MT73, MT129, MT182, MT211, MT290, MT322, WT13
conservation, 91, MT130, MT229, MT233, WT41, WT94
conservation genetics, MT192, MT193
conservation genomics, MT130, MT189, MT190, WT8
contact lenses, WT40
control region, 120, MT104
convergent genetic changes, MT244
copy number variation, MT266, MT342
copy number variation (CNV), MT119
copy number variation region, 81, MT169
Coturnix coturnix japonica, 44, MT67
cow, MT296, WT65
craniofacial anomaly, 121, MT105
creole pigs, MT230
CRISPR/Cas9, 21, 27, 28, 30, MT167, MT175, MT176, MT177
crossbreeding, 182, 184, MT324, WT96, WT101
cross-validation, MT338
CTCF, 147, MT289
cumulus cell, WT11
custom chip, MT120
Cyt *b* gene, MT69
Cytochrome *b* gene, 120, MT104, WT7

D

dairy, MT143, MT321
dairy cattle, MT5, MT72, MT118
dairy cow, MT120, MT307
dairy goats, 101, MT365
data mining, 168, WT57
data sharing, WT66
databases/repositories, 56, 149, MT115, MT287, WT49
de novo genome, 24, MT364
de novo mutations, 99, MT368
de novo genome assembly, 1, MT60
death receptors, MT33
defect, MT80
deletion, MT358
developing country, 181, WT102
development, 112, MT8, WT34
diabetes mellitus, 122, MT100
diagnostic imaging, 127, MT99
diet, 141, MT49, WT53
dietary fat, MT269
different lactation points, WT65
differential expression, 77, MT160
differentiation, WT64
disease, 119, 124, MT80, MT94, MT101
disease genetics, 126, MT102
disease resilience, 55, 78, 173, MT41, MT52, MT165, WT78
disease resistance, 85, 137, MT163, MT272, MT291
disorder region, MT48
divergent alleles, WT44
divergent genetic changes, MT244
divergent selection, MT34
diversity, 66, 186, MT87, MT107, MT116, MT151, MT223, MT227, MT229, WT97
Djallonke, 60, MT111
DNA analysis, 125, MT97
DNA extraction, 132, MT237
DNA marker, MT304, MT305
DNA methylation, 114, MT1, MT2, MT5, MT6, MT11
DNA microsatellite marker, MT37
DNA sequencing, 31, 35, 86, 151, MT71, MT93, MT98, MT130, MT189, MT190, MT195, MT197, MT218, MT219, MT314, MT328, WT8, WT105
dog, 119, 121, 126, 128, 152, MT91, MT94, MT95, MT102, MT105, WT5
dogs and related species, 6, 127, 151, MT88, MT90, MT93, MT98, MT99, WT1, WT2, WT6
domestic cat, 124, MT101
domestic goat, 188, MT232
domestic ruminants, MT310
domestic sheep, 156, WT29
domestication, 87, MT19, MT235
dominant black, WT3
dominant white, MT205
donkey, MT132
doublesex and mab-3 related transcription factor 3, MT179
drift, MT345
dry matter intake, MT327
ducks, 52, MT58
duplication, 65, MT108
Duroc, MT257, MT280

E

E. coli, 75, MT157
E. coli mediated diarrhea, MT248
effective population *size*, WT90
eggshell coloration, MT40
ELISA, MT142
energy balance, WT35
enhancer, 145, 161, MT290, WT63
entropion, 156, WT29
entropion eye, WT23
environment, MT262
EPA, MT207
epigenetic marks, 36, MT210
epigenetics, 16, MT1, MT3, MT4, MT139, WT70
epigenome, 18, MT14
epigenomics, 17, 38, 110, 111, 112, 159, MT6, MT8, MT9, MT10, MT16, MT194, MT195, MT208, MT250, MT298, WT58, WT67
eQTL, MT158, MT253
equidae, MT201
equine, 39, MT131, MT178, MT207, MT209
equine amniotic membrane, WT40
Ethiopia, MT107
Europe, 105, MT360
European cattle, MT345
European Sea Bass, 176, WT83
evolution, 43, 65, 177, MT19, MT61, MT108, MT123, MT201, WT71, WT74, WT86
evolutionary adapatation, MT310
evolutionary algorithm, WT72
evolutionary genomics, 32, 89, MT214, MT355, MT359
exosome, MT260
expression, MT42
expression QTL, MT258, MT265, MT321
extracellular matrix protein, MT106

F

familial amiloidosis, MT89
fat acid, WT22
fat metabolism, MT300
fat/lipid, MT252, MT354, WT37
fatness, MT269, MT276
fat-tailed, 157, WT32
fatty acid composition, MT304, MT305
FecB, WT11
feed efficiency, MT77, MT78, MT270, MT271, MT275, MT300, MT354
felids, 124, MT101
feline, 7, 122, MT100, WT4
Felis Cat, MT89
ferret, MT19
fertility, 49, MT7, MT64, MT123, MT261, MT282, MT304, WT38, WT39
fetal development, 147, MT289
fetal maturity, WT45
FFPEs, MT89
fgf1, 178, WT82
fibrosis, 29, MT174
fish, 172, 175, 179, WT50, WT73, WT75, WT76, WT77, WT79, WT81
fluorescent in situ hybridization, 26, MT66
food safety, MT155
forensic parameters, 5, MT17
Fst, MT92
full genome sequencing, 183, WT98
functional annotation, 22, MT126
Functional Annotation of Animal Genomes (FAANG), 16, 17, 19, 20, 111, 144, 149, 159, MT9, MT10, MT127, MT128, MT287, MT324, MT340, WT58, WT69, WT70
functional assay, WT9
functional genomics, 34, 37, 38, 41, 104, 114, 126, 142, 165, 168, 175, MT11, MT41, MT49, MT102, MT164, MT197, MT208, MT212, MT213, MT215, MT275, MT336, MT373, WT35, WT42, WT47, WT51, WT56, WT57, WT66, WT81

G

gadd45a, 178, WT82
GBLUP, 101, MT365
GBS, 101, 116, MT84, MT365
gene, MT72, MT179
gene discovery, 21, MT167
gene doping, MT178
gene expression, 16, 38, 138, 175, MT2, MT6, MT208, MT252, MT253, MT268, MT271, MT286, MT297, MT315, MT321, MT324, MT334, MT344, MT353, WT20, WT35, WT37, WT52, WT68, WT70, WT81
gene introgression, MT121
gene network, MT302
gene ontology, 94, WT95
gene regulation, 114, MT11
gene sets, MT339
genetic, MT80
genetic admixture, MT311
genetic diseases, 70, MT220
genetic disorder, 12, MT27, MT75, MT199, MT314, MT323, MT328, WT2
genetic distance, MT21
genetic diversity, 188, MT32, MT39, MT44, MT46, MT196, MT202, MT231, MT232, MT335
genetic group, MT296
genetic identification, 6, MT282, WT1, WT6
genetic improvement, 11, 28, 55, 80, 154, 155, 158, 165, MT52, MT75, MT171, MT175, MT295, MT322, MT337, WT15, WT28, WT30, WT31, WT56, WT104
genetic introgression, 153, MT36, WT27
genetic marker, 6, 80, MT72, MT171, MT187, MT203, MT331, MT358, WT6, WT16, WT22
genetic polymorphism, WT22
genetic regulation, MT265
genetic trend, MT144
genetic variation, WT92, WT94
genetics, 121, MT105
genetics populations, MT231
genome, 25, 42, 108, MT19, MT55, MT361, MT372, WT71, WT74
genome annotation, 38, 40, 104, 149, MT118, MT208, MT221, MT287, MT340, MT373
genome assembly, 26, MT22, MT50, MT66
genome editing, 28, MT175
genome evolution, 36, MT210
genome regulation, 41, 102, 111, MT9, MT213, MT337, MT353, MT369
genome sequence, MT40
genome sequencing, 40, 94, 100, 123, MT87, MT91, MT103, MT129, MT180, MT221, MT261, MT278, MT298, MT322, MT323, WT75, WT95
genome-enabled breeding, 182, WT48, WT96
genome-wide, MT224
genome-wide association, 12, 33, 34, 37, 54, 55, 78, 80, 82, 100, 103, 123, 127, 128, 134, 153, 155, 156, 158, 173, 180, MT27, MT47, MT52, MT62, MT71, MT73, MT79, MT90, MT93, MT95, MT99, MT103, MT119, MT129, MT140, MT165, MT171, MT172, MT182, MT183, MT187, MT206, MT211, MT212, MT215, MT239, MT261, MT262, MT283, MT322, MT325, MT354, MT370, WT27, WT28, WT29, WT31, WT49, WT78, WT87, WT99
genome-wide association analysis, 170, MT356, WT80
genome-wide association study, 129, MT59, MT96
genomewide SNPs, WT21
genomic, 131, MT242
genomic DNA, WT7
genomic evaluations, MT144
genomic imprinting, 115, MT13
genomic prediction, MT71, MT74, MT250, MT277, MT329, MT338, WT36
genomic selection, 1, 14, 94, 101, 118, 173, 175, 180, 181, MT25, MT60, MT78, MT85, MT313, MT365, WT8, WT36, WT48, WT78, WT81, WT95, WT99, WT102
genomic selection signature, 157, WT32
genomics, 130, 150, MT240, MT371
genotype, MT37
genotyping, 6, 10, 12, 32, 55, 59, 82, 108, 118, 184, MT27, MT43, MT52, MT63, MT75, MT85, MT110, MT140, MT172, MT190, MT214, MT222, MT262, MT329, MT330, MT347, MT361, WT6, WT17, WT101
genotyping by sequencing, 101, MT365
genus *Equus*, 36, MT210
German Landrace, MT137
giraffe, MT310
goat, 109, MT154, MT223, MT311, MT341, MT343, MT358, MT362, WT88
goat and related species, WT18
goat colostrum, WT43
goat milk, WT43
goats and related species, 93, MT228, MT303, MT374
goose, 51, MT53
Greater flamingos, MT35
growth, MT276
growth and development, WT42
growth and fat deposition, MT30
gut, MT284
gut microbiome, 136, MT238
GWAS, 73, 122, 135, 171, 181, MT91, MT100, MT161, MT178, MT241, MT247, MT272, MT279, MT280, MT308, WT85, WT102

H

H3K27ac, 161, WT63
hair follicle, MT320, MT332
haplogroup, MT311
haplotype, 23, 69, MT36, MT69, MT87, MT132, MT135, MT159
haplotypes, 66, 86, MT116, MT311, WT105
harness racing, MT179
hatchability, MT31
HD SNP chip array, MT188
health, MT144
heat exposure, 53, MT56
heat stress, MT42, MT279
hematology, MT272
hepatocellular carcinoma, 138, MT286
HepG2, MT257
heritability, 134, MT31, MT90, MT239, MT251
heterozygosity, WT88
heterozygosity (Hp), MT299
Hi-C, 19, 144, MT127, MT128
Hi-C (high-throughput chromosome conformation capture), 147, MT289
highly pathogenic avian influenza, 73, MT161
high-throughput sequencing, MT355
high-throughput sequencing (HTS), 119, MT94, MT119

homozygosity, 35, MT219
horn, 24, MT364
horse, 39, 58, 65, 70, 87, MT108, MT117, MT123, MT131, MT133, MT178, MT184, MT185, MT187, MT192, MT193, MT194, MT196, MT199, MT203, MT205, MT206, MT209, MT220, MT229, MT235
horse genome, 36, MT210
horses and related species, 31, 32, 33, 34, 35, 37, 40, 41, MT130, MT181, MT182, MT183, MT186, MT189, MT190, MT195, MT197, MT211, MT212, MT213, MT214, MT215, MT218, MT219, MT221
HTS, MT186, MT336
human, 163, WT61
hybridization, MT201
hydroxymethylcytosine, MT3
hypothalamus, MT276

I

Iberian backrosses, MT276
Iberian cattle, 90, MT234
Iberian pig, 136, 141, MT238, MT254, MT269, WT35, WT42, WT53
identification, 7, WT4
IGF2R, MT4
Illumina sequencing, MT51
immune, 30, 113, MT12, MT177
immune profiling, MT251
immune response, 75, MT147, MT149, MT157
immune system, 79, MT168
immunity, MT256
immunogenetics, 23, 57, MT109, MT159
immunogenomics, 58, 59, 79, 142, MT110, MT117, MT138, MT143, MT164, MT168, MT266, MT315
immunology, MT139
imprinting, MT264
imputation, 80, 100, MT88, MT129, MT171, MT187
inbreeding, MT82, MT90, MT323, MT326, WT72, WT91
indel, MT227
indigenous, MT32
indigenous breeds, MT124
indigenous genetic resources, MT226
indigenous sheep, WT20, WT94
infectious disease, 71, 82, 110, MT16, MT47, MT158, MT166, MT172, WT47
inflammatory response, MT344
innate immunity, 85, MT151, MT155, MT158, MT163, MT315
integrative genomics, 52, 76, 145, 166, MT58, MT173, MT290, WT55
integrity, WT41
intestine transcriptome, MT333
intramuscular fat, 140, MT270, MT292
introgression, 8, MT26
invasiveness, MT184
Iraqi cattle, MT313
Ireland, 4, MT24
iTRAQ, 164, WT62

J

Japanese Black cattle, MT308, MT309, MT319
Japanese quail, MT39
Jeju crossbred, MT188
Jeju horse, MT180, MT188
Johne's disease, 167, WT60

K

K locus, WT3
Kebumen Ongole Grade Cattle, MT69
Kenana, 183, WT98
ketosis, MT356
KIR, MT154
KIT gene, MT205
Kit, MT345

L

lactoferrin, 86, WT105
lameness, MT144
Landrace, MT273
landscape genetics, 4, MT24
laying hens, 53, MT56
LEI0258 microsatellite, MT107
leishmaniasis, 77, MT160
leptin knockout, 29, MT174
leukocyte receptor complex, 23, MT159
LFS, 70, MT220
Lidia breed, MT82, MT224
linkage disequilibrium, 173, MT46, WT78, WT90
linkage phase, WT90
lipid deposition, 164, WT62
liver, 16, 53, MT56, MT327, WT70
livestock production systems, 186, WT97
lncRNA, MT255, WT64
local ancestry, 106, MT363
local breed, 98, MT294
long non-coding RNAs, 20, WT69
long read sequencing technology, 148, MT125
lovebird, 1, MT60
luciferase, MT257
lymphocytes, MT251
lyso-phospholipids, 64, MT162

M

machine learning, 38, MT208, WT36
major gene, 98, MT80, MT294
major histocompatibility complex, MT148
major histocompatibility complex class I-like molecules, 64, MT162
male fertility, MT342
Malopolski horse, MT196
mammary gland, MT307, WT65
mapping, MT124, MT319
mastitis, MT149
mastitis resistance, MT136
MAT, MT248
maternal nutrition, 18, MT14
maternal traits, MT78
mature body weight, MT106
MC1R, WT20
MC1R, MT259
meat pH, MT280
meat production, 180, MT245, MT298, MT353, WT37, WT99
meat quality, MT254, MT269, MT271
meiosis, MT318
melanocortin 4 receptor (MC4R), WT24
melatonin, MT320
Mendelian model, MT243
mesnchymal stem cells, WT40
metabolome, 45, MT54
metabolomics, 129, MT96, MT307
metadata, WT66
metagenome, MT143
metagenome prediction, 136, MT238
metagenomics, 135, 137, MT241, MT291
methylcytosine, MT3
MHC, 31, 42, 56, 57, 58, 59, 60, 65, MT55, MT108, MT109, MT110, MT111, MT115, MT117, MT150, MT218, MT266
microarray, 118, 178, MT85, MT330, WT45, WT82, WT93
microbiome, 130, 131, MT143, MT240, MT242
microbiomics, 134, MT239
microbiota, 132, MT237
microRNA, 110, MT7, MT16, MT51, MT142, MT194, MT260, MT267, MT285, MT352, WT67
microsatellite, 7, 58, MT35, MT70, MT82, MT117, MT193, MT225, MT229, MT230, MT282, MT312, WT4, WT76
microsatellite imputation, MT204
microsatellite marker, MT132, MT243
milk, MT260, MT277
milk β-hydroxybutyrate, MT356
milk production, 182, MT74, MT153, MT301, MT358, WT16, WT67, WT96
milk protein genes, MT18
milk somatic cells, MT343
milk traits, 183, WT98
miRNA, 74, MT170, WT64
miRNAome, 167, WT60
miRNAs, MT185
mitochondrial, MT32
mitochondrial DNA, MT223, MT259
mitochondrial function, MT77
mitochondrial sequencing, 90, MT234
mock communities, 132, MT237
molecular genetics, 119, MT94
Mongolian horse, MT202
monocyte-derived cells, MT146
monocytes, 84, MT367
monogenic trait, 155, WT31
morphometrics, 127, MT99
mosaicism, 99, MT368
motility, MT184
MRBLUP, WT36
mRNA-microRNA network, MT332
MSC, 146, MT15

mtDNA, 43, 120, MT61, MT87, MT104, MT132
MUFA, 140, MT292
multispecies, 19, 20, 160, MT127, WT41, WT54, WT69
muscle, MT198, MT250, MT253, WT34
muscle growth, 164, WT62
muscle tissue, MT191
Mustela, MT19
Mustelids, 14, MT25
mutation, MT306
mycobacterium tuberculosis, 166
Mycobacterium tuberculosis, WT55

N

native, 49, MT64
native breeds, MT347
native chicken, 9, MT46, MT65
native pigs, MT248
natural autoantibodies, MT145
natural killer cells, MT154
nervous system, 119, MT94
network analysis, 37, MT212, MT252, MT275, MT297
new chicken breeding stock, 9, MT65
New World camelids, 12, MT27
next-generation sequencing, 118, 132, MT85, MT237, WT71
NF1, 157, WT32
NGS, 116, 131, 137, 176, MT39, MT84, MT151, MT242, MT273, MT274, MT291, WT19, WT83
Nigerian indigenous pigs, MT259
nomenclature, 57, 169, MT109, WT59
NONCODE, MT255
non-coding RNA, MT268, MT333, WT51
noncoding RNAs, 22, MT126
non-synonymous SNP, MT273, MT274
North American Holstein dairy cattle, MT71
novel variants, MT124
nuclear architecture, 147, MT289
numt, 43
numt, MT61
nutrigenomics, MT30, MT269, WT34
nutrition, MT333

O

obesity model, 29, MT174
ocular diseases, WT40
Old World camelids, 11, MT22, WT104
oleic acid, 140, MT292
oleic acid percentage, MT309
oligosaccharide gene, WT43
oocyte, WT11
OpenArray, MT81
other application, MT197
other species, MT20, MT21
OTUs, 136, MT238
ovary, MT51
ovine, WT19, WT23
ovine MHC, MT106
ovine SNP50K, MT306
oxidative stress, 29, MT174

P

palaeogenomics, 107, MT366
Pan troglodytes, WT46
pan-genome, WT44
parentage, 6, 8, 11, 151, 152, MT26, MT35, MT43, MT98, MT282, WT5, WT6, WT19, WT104
parentage identification, MT312
parentage prediction, 117, MT83
parentage testing, MT81
parentage verification, 69, MT135
parvovirus, 137, MT291
pasture asthma, MT207
PBMCs, 74, MT137, MT170
pbMEC, 75, MT157
PCA, MT92
PCR, WT1, WT7, WT24, WT41
PCR-RFLP, MT106
pedigree, MT82
pedigree line, 49, MT64
Pekin duck, MT42
pelt color, MT306
performance traits, 14, MT25
Peyer's patches, MT284
PGC, 30, MT177
phenotypes, 124, MT101
phenotypic correlation, 14, MT25
phosphorus efficiency, MT283
phylogenetic character, MT132
phylogeny, MT70, MT201, WT73
phylogeography, MT223
pig, 16, 29, 74, 98, 135, 138, 140, 142, 146, 148, 164, MT2, MT15, MT125, MT137, MT164, MT170, MT174, MT241, MT245, MT246, MT253, MT255, MT258, MT263, MT265, MT266, MT275, MT279, MT282, MT283, MT284, MT285, MT286, MT292, MT294, WT62, WT70
pig breeds, WT92
pig domestication, MT244
pig muscle, 147, MT289
pigeon, 10, MT63
piglet mortality, MT251
pigs, 114, 143, MT6, MT11, MT154, MT158, MT250, MT252, MT261, MT264, MT277, MT278, MT280, WT45
pigs and related species, 17, 28, 79, 134, 144, 149, MT10, MT128, MT155, MT168, MT175, MT239, MT249, MT262, MT287
Piscirickettsia salmonis, 170, WT80
PLAG1, MT309
pleiotropy, MT278, MT354
Poly(I:C), 113, MT12
polymorphism, 56, 57, 64, MT18, MT109, MT115, MT162, MT245, MT248, MT312
polymorphisms, 102, MT369
polyphenol, MT143
population, MT32, WT87
population differentiation, WT92
population differentiation (Fst), MT299
population expansion, MT311
population genetics, MT118, MT222
population genomics, MT40, MT74, MT119, MT313, MT330, WT93
population identification, MT20, MT43, MT203
population structure, 182, MT90, MT192, MT193, WT87, WT88, WT96
porcine, MT260
PorcineSNP60, WT92
portal, WT66
positive selection, 183, WT98
poultry, 54, 82, MT33, MT41, MT44, MT47, MT49, MT62, MT172
poultry and related species, 55, MT36, MT50, MT52
PRA, MT91
PRDM9, MT131
predicted networks, MT146
prediction of complex traits, MT339
pregnancy, 112, MT8
prenatal nutrition, MT4
primates, 163, WT61
Primer3, WT7
PRM, 164, WT62
PRNP polymorphism, MT152
product traceability, 3, MT86
production systems, WT21
production traits, MT310
pro-inflammatory cytokines, MT251
protein modeling, MT273
proteome, WT65
proteomics, MT89
proviral load, MT150
PRRS, 143
PRRSV, 74, MT137, MT146, MT147, MT170, MT256
pseudogenes, 99, MT368
puberty, 165, WT56
PUFA, MT350
Punjab Urial, WT8
putative variants, MT120

Q

qPCR, WT9, WT35, WT39
qRT-PCR, MT51
QTL, 181, MT41, MT243, WT102
QTL-rich region, MT263
quails and humid tropics, MT31
quality control, 117, MT83
quantitative genetics, 54, MT62, MT301
quantitative trait locus, MT278
quantitative trait locus (QTL), 127, 128, 158, MT95, MT99, WT28
quantitative traits, MT338

R

racing, MT198
rainbow trout, 170, WT80
rDNA, WT50
rearrangement, 44, MT67
recombination, 158, MT131, MT318, WT28

red fox, MT20, MT21
reddening, WT68
reference genome, 39, MT209, MT316
reference genome sequence, 148, MT125
regression, MT243
regulatory element, 160, WT54
reindeer, 15, MT23
repeat, 44, MT67
reporter assay, 85, MT163
reproduction, 113, 155, 177, MT12, MT278, MT285, MT331, MT334, MT350, WT31, WT86
reproductive performance, MT296
Re-seq, 115, MT13
residual feed intake, MT77
resilience, 143
resistance, 73, 143, MT161
respiratory system, MT93
responsible mutation, MT305
restriction enzyme, WT24
retained heterozygosity, MT76
retrotransposon, 121, MT105
retrovirus, MT348
RFI, MT300, MT351
river buffalo, WT90
RNA sequencing, 126, MT102, MT271
RNA-seq, 19, 20, 40, 41, 53, 77, 110, 112, 115, 135, 141, 144, 165, 175, 179, MT6, MT8, MT13, MT16, MT45, MT49, MT56, MT127, MT128, MT133, MT138, MT139, MT146, MT160, MT183, MT184, MT198, MT213, MT221, MT241, MT245, MT250, MT258, MT264, MT267, MT268, MT275, MT284, MT297, MT300, MT307, MT315, MT316, MT321MT324, MT333, MT334, MT352, MT353, WT13, WT34, WT37, WT38, WT42, WT51, WT52, WT53, WT56, WT69, WT77, WT81
ROH, MT44, MT92, MT326
RORÇMT320
16S rRNA, 132, 136, MT237, MT238
rRNA cluster, 44, MT67
rumen, 24, 130, MT240, MT364
ruminant biology, 24, MT364
runs of homozygosity, MT224
Russia, MT347

S

S. aureus, 75, MT157
Sahelian, 60, MT111
salmon, 171, WT74, WT85
Salmonella, MT155
sarcoid, MT184
sarcoma, 138, MT286
scavenger protein, MT201
SCD, MT257
SCID, 70, MT220
scrapie, MT152
selection, 150, MT222, MT301, MT371
selection footprints, WT21
selection scan, 97, WT26
selection signature, 106, MT363
selective signature, MT299
selective sweep, MT273, MT274
selective sweeps, MT180
selenium, MT33
sequence variation, 56, MT115
sequencing, 102, MT124, MT142, MT369, WT24
serum, 167, WT60
sex, WT74
sex chromosome, 177, MT342, WT86
sex determination, 177, MT35, MT50, WT73, WT75, WT86
sex reversal, 68, MT134
sheep, 18, 25, 59, 60, 92, 108, 110, 153, 155, 157, 158, 161, MT14, MT16, MT110, MT111, MT140, MT152, MT225, MT316, MT361, MT372, WT7, WT9, WT10, WT27, WT28, WT31, WT32, WT33, WT39, WT51, WT63, WT87
sheep and goat, WT44
sheep and related species, 97, 154, MT324, MT340, WT13, WT14, WT15, WT16, WT17, WT26, WT30, WT37
sheep breeds, WT21
Siberian Husky, MT92
signature of selection, MT40
signatures of selection, MT347
single nucleotide polymorphism, 3, 92, MT86, MT136, MT202, WT33
single nucleotide polymorphism (SNP), 11, 91, 97, 184, MT74, MT187, MT233, MT303, MT313, MT330, WT18, WT26, WT91, WT101, WT104, WT48
single nucleotide variants (SNVs), MT155
single-cell RNA-sequencing, WT11
single-step, WT89
sire, MT31
skeletal muscle, MT48, MT258, WT64
skeletal system, MT186
SLA, 57, MT109
SLA-1, MT266
small RNA, MT256
SMRT sequencing, 177, WT86
SNP, 9, 15, 66, 69, 98, 152, MT23, MT30, MT59, MT65, MT69, MT72, MT81, MT82, MT116, MT135, MT180, MT247, MT294, , MT319, WT5, WT89
SNP array, 106, 186, MT338, MT363, WT97
SNP chip, MT254, WT90
SNP genotyping, 116, MT84, WT19, WT94
SNP haplotype, MT204
SNP markers, 95, MT236
SNP parentage, 117, MT83
soft sweep, MT34
somatic, MT346
somatic cell score, WT16, WT22
sostdc1, 178, WT82
SOX6, MT48
spatial autocorrelation, MT231
sperm motility, 49, MT64
spreading of science, WT2
sRNA-seq, MT185
SRY gene, MT223
SSC15, MT263
ST3GALT5, WT43
ST6GALT1, WT43
Staphylococcus aureus, MT149
statistical genetics, MT301
stayability, MT317
STR, 10, 69, MT63, MT135
STR profiling, 5, MT17
stray cat, 120, MT104
stress, MT1
stress response, 178, WT82
structural variants, MT118, MT346
structure, MT227
sub-Saharan Africa, 60, MT111
Swakara, MT306
swine heterogeneous stock, 145, MT290
systems biology, 76, 135, MT173, MT241, MT252, MT297

T

tandem repeats, 176, WT83
targeted enrichment of genomic DNA, MT263
targeted enrichment sequencing, 23, MT159
targeted gene sequencing, MT158
Thoroughbred, 69, MT135, MT180, MT188
tick resistance, 81, MT169
tissue composition, 141, WT53
tissue specificity, MT255
TLR1A, MT145
TLR2 stimulation, 77, MT160
TMT, WT65
tolerance, 143
trackhub, WT66
training, MT191
transciptomics porcine, MT270
transcription factor, MT354
transcription factor binding site, WT20
transcriptome, 18, 45, 51, 104, 141, 146, MT14, MT15, MT45, MT53, MT54, MT191, MT276, MT284, MT373, WT13, WT42, WT53
transcriptome analysis, MT256, MT327
transcriptomics, 102, MT369
transgenerational phenotypic and epigenetic inheritance, 113, MT12
transgenic, 30, MT177
transit loss, MT246
transposable elements, 99, MT368
trend validation, MT144
tribe Caprini, WT44
trichostatin A, 146, MT15
tropical adaptation, MT121
turkey, 43, MT32, MT45, MT61

U

uniformity of production, WT72
unmapped reads, 137, MT291
US sheep breeds, MT106

V

vaccination, 74, MT170

vaccine, MT150
variant calling, MT264
variants, 25, MT205, MT372, WT74
vitamin A, 140, MT292
VNN1, MT305

W

water buffalo, 104, MT373
website, 169, WT59
weight, WT89
weight gain, WT89
whole-genome association study, MT148
whole-genome screening, 21, MT167
whole-genome sequence, 99, MT118, MT121, MT356, MT368
whole-genome sequencing, 124, MT101, MT299, WT46
wild boar, MT230
wild goat, 188, MT232
wild ruminants, MT310
wild species, MT43
WNT signal, MT332
wool production, 97, WT26
wool shedding, 153, WT27
Wuzhishan pig, MT247

X

X chromosome, 68, MT134

Y

Y chromosome, 32, 68, MT123, MT134, MT214, MT225, MT259
yak, MT70
Yorkshire, MT274