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Abstracts

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Contents

Plenary Session I:
Genetics & Genomics, African Heritage & Changing Climates

OP1 Understanding African health through genetic diversity. M. Ramsay*, *Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, South Africa.*

Investigating genetic diversity in a population provides important insights into population history and also into the genetic contribution to complex traits, including susceptibility to disease. Human populations in Africa harbour greater genetic variation compared with other world populations as a result of a deep evolutionary history on the continent, from before the waves of out migration from Africa. Genomic studies in Africa are still hampered by a paucity of data and small cohort sample sizes, but provide unique opportunities. This talk will introduce an African population cohort from the H3Africa Consortium, referred to as the AWI-Gen (Africa Wits-INDEPTH partnership for genomic studies). AWI-Gen is a 4 country cohort of ~12,000 older adult men and women, from Ghana, Burkina Faso, Kenya and South Africa. Genetic association studies into the architecture of quantitative traits such as lipid levels have identified novel associated loci and replicated known signals. We have examined polygenic risk score distributions and trans-ethnic transferability for cardiovascular disease-related traits, as well as integrated prediction models including classic risk factors and polygenic scores to enhance predictability. We have also studied subset of ~5000 participants from South Africa, identifying unanticipated population sub-structure and assessed its impact on genetic associations. African populations have the potential to contribute novel findings to health and disease that could benefit the world.

OP2 Experiences in genomic selection for improved animal health and adaptability in Africa. A. Djikeng*[1,2], E. Rege[3], N. Mapholi[4], E. Ibeagha Awemu[5], S. E. Aggrey[6], R. Mrode[1,2,7], and O. Mwai[1], [1]*The International Livestock Research Institute (ILRI), Nairobi, Kenya,* [2]*The University Edinburgh, Scotland,* [3]*Emerge Centre for Innovations-Africa (ECI-Africa), Kenya,* [4]*University of South Africa (UNISA), South Africa,* [5]*Agriculture and Agri-Food Canada, Canada,* [6]*University of Georgia, Athens, GA,* [7]*Scotland's Rural College (SRUC), Scotland.*

Recent advances in animal breeding, genetics and data science can now offer opportunities to drive genetic improvement in African livestock production systems. In Africa and in other regions of the global south, livestock production systems are segmented and categorised into intensive (similar or close to systems in the global north), smallholder (generally mixed crops-livestock) and pastoral systems. The imperative to transform and sustain these livestock systems to urgently respond to the food systems' needs for transformation and resilience, to the environment and climate crisis and to socio-economic development needs, requires innovative approaches to deliver genetic gains. These innovative approaches to go beyond the research, innovation and adoption continuum to substantively include strategic partnerships, effective policies and investment mechanisms. Over the past decade, collective efforts and progress made in Africa offer opportunities for genetic improvement to address key systems' constraints including productivity, adaptation and health resilience. For this keynote presentation, practical examples of genetic improvement and opportunities for future consideration will be presented and discussed. Some reflections for definition of tropical adaptation and resilience will also be presented and discussed.

Key Words: genomic selection, livestock production, adaptability, Africa

OP3 Genetics and genomics for genetic improvement and sustainability of animals—A world perspective. C. Baes*, *Department of Animal Biosciences at the University Guelph, Guelph, Ontario, Canada.*

The world population is expected to double by 2050, and demands on land, water and energy needed for animal and crop agriculture are increasing at an increasing rate. Together with knowledge advancement in livestock nutrition, physiology, welfare, and veterinary science, application of genetic and genomic approaches offer meaningful tools for increasing the efficiency of both plant and animal agriculture. There are, however, still numerous challenges associated with applying genetic and genomic selection to breeding programs. The cost of technology and data management, societal concerns and values, integration of technologies with traditional breeding methods, regulation and policy aspects, as well as data privacy questions could, depending on the country in question, either slow or prohibit the use of these approaches in livestock selection programs altogether. Nevertheless, it is becoming increasingly apparent that genetics and genomics will play a critical role in meeting the changing demands of humanity. The widespread integration of genomic selection into breeding programs during the last decade has revolutionized animal agriculture in industrialized countries. Genomics have allowed accurate selection for low-heritability traits, development and integration of novel traits which are difficult and expensive to measure, and the ability to select animals with desired traits at an earlier age than was previously possible. This has led to increased accuracy and efficiency in animal breeding programs in some countries, but adoption and use of these tools has not been integrated into all production systems. In addition to advancement in genome editing and other technologies, there are more fundamental applications of genetics and genomics which have not yet been full harnessed globally. In terms of various aspects of sustainability, genetic and genomic approaches offer promising solutions. With growing awareness and concern for the environmental impact of animal agriculture, efficient breeding programs are becoming a priority, with goals ranging from simply increasing production, to breeding for traits such as feed efficiency and reduced methane emissions. In addition, management of genetic diversity is crucial for the long-term sustainability of animal populations under artificial selection. Intense direction selection reduces genetic variation by definition, but genomic technologies may also offer breeders additional tools to monitor and maintain genetic diversity. Understanding this balance is crucial to ensuring the health and viability of animal populations now and in the future. Despite numerous challenges associated with applying genetics and genomics to animal breeding programs (ethical and societal concerns, potential negative impacts on biodiversity, etc.), the benefits of these technologies must be thoroughly explored. A more global implementation of genetic and genomic tools in the context of animal breeding has the potential to contribute significantly to affordable, sustainable and nutritious animal protein for a growing world population.

Cattle Molecular Markers and Parentage Testing

OP4 ISAG Bursary Award: Population genomics of indigenous African cattle inferred from 537 whole-genome sequencing. A. Tijjani[1,2], S. Kambal*[3,4], K. Marshall[5], O. Hanotte[1,3,6], and on behalf of the African Cattle Genomics Consortium[1], [1]*Centre for Livestock Genetics and Health (CTLGH), International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [2]*The Jackson Laboratory, Bar Harbor, ME,* [3]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [4]*University of Khartoum, Khartoum, Sudan,* [5]*Inter-*

national Livestock Research Institute (ILRI), Nairobi, Kenya, [6]School of Life Sciences, University of Nottingham, University Park Campus, Nottingham, UK.

The escalating climate change crisis challenges cattle well-being and sustainable production across Africa. However, the ability of indigenous cattle to thrive in stressful conditions (e.g., high temperature, high altitude, high infectious disease prevalence), as well as limited feed and water availability, provide opportunities to mitigate the consequence of climate change. Here, a large data set of cattle populations was collated for genome-wide SNP discovery and population genomic analysis. We analyzed 537 whole-genome sequences representing 41 indigenous breeds from 12 sub-Saharan African countries, comprising newly generated and publicly available data. A total of ~33 million SNPs were discovered across all the cattle populations, with ~3.5 million of these being novel. Predicted non-synonymous mutations account for around 31% of annotated SNPs in coding genes. Furthermore, we found 31,677 SNPs potentially causing loss or gain of function compared with the cattle European taurine genome of reference (Hereford, ARS-UCD1.2), including 1,478 stop codon gain, 175 stop codon loss, and 258 start codon loss mutations. The population structure and admixture analyses separate non-admixed African taurine *Bos taurus taurus* from African indicine × taurine crosses. We observe a high genetic differentiation between longhorn and shorthorn taurine with a higher proportion of shared Muturu ancestry (shorthorn), compared with N'Dama (longhorn), among African zebu, sanga and zenga crossbreds. However, these proportions decrease from West to East. Our finding yields a comprehensive insight into African cattle population structure and variation profile, emphasizing the need for fine characterization to inform sustainable genetic improvement toward healthy and well-adapted cattle populations.

Key Words: African cattle, adaptation, single nucleotide polymorphism (SNP), population genomics

OP5 Low-density genotype panels performance for parentage verification in South African beef cattle breeds. Y. Sanarana*[1,2], D. Berry[1,3], A. Maiwashe[2], C. Banga[2,4], and E. Van Marle-Köster[1], [1]*University of Pretoria, University of Pretoria, Hatfield, Pretoria, Gauteng, South Africa,* [2]*Agricultural Research Council, Irene, Pretoria, Gauteng, South Africa,* [3]*Teagasc, Fermoy, County Cork, Ireland,* [4]*Botswana University of Agriculture and Natural Resources, Gaborone, Botswana.*

The available ISAG SNP marker panel has some limitations for parentage verification in local breeds. In this study, the information content and efficiency of 2 (multi-breed and breed population-specific) low-density genotype panels were tested in South African Bonsmara (BON) and Drakensberger (DRB) cattle breeds. SNPs for the multi-breed and population-specific panels were selected across and within the 2 breeds. The number of SNPs chosen per chromosome for the development of these panels was directly proportional to the chromosome length, which was measured from the first to the last SNP's genomic position. The block method dividing each chromosome into equal-length blocks was used to predetermine the number of SNPs required per chromosome. All SNPs were ranked based on the minor allele frequency (MAF) and a SNP with the highest MAF, good clustering quality and high call rate per block was selected. To minimize linkage disequilibrium (LD) among selected SNPs, the selected SNPs had to be at least 1 Mb apart. A total of 200 informative SNPs was compiled for each low-density genotype parentage panel. The panels were tested per breed of already validated 45 BON and 74 DRB sire-offspring pairs. Parentage exclusion was considered whenever the genotype of the sire was discordant with that of the offspring for more than once per SNP. The multi-breed panel performed comparatively lower than the population-specific panel. Five and two discordant SNPs were observed in the BON and DRB, respectively, when the multi-breed panel was tested. The multi-breed panel had MAF values of 0.38 and 0.40 while the breed-specific panel had 0.49 and 0.48 MAF values in the BON and DRB, respectively. The multi-breed panel falsely excluded 3 (6.6%) sire–offspring relationships of the BON whereas population-specific panels were free from false-negatives. These results suggest that breed-specific panels are better than a set of markers selected across breeds.

Key Words: pedigree, Sanga cattle, opposing homozygous, parentage testing

OP6 Genetic diagnosis of sex chromosome aberrations in cattle based on parentage test by microsatellite DNA, X- and Y-linked markers. L. Borreguero*[1], M. R. Maya[2], A. Trigo[2], I. Bonet[2], and J. A. Bouzada[1], [1]*Laboratorio Central de Veterinária, Algete, Madrid, Spain,* [2]*Tecnologias y Servicios Agrarios S.A., Madrid, Spain.*

Chromosomal abnormalities may result in a substantial loss of animal production or infertility, especially in the case of sex chromosome alterations, which are not often phenotypically visible for breeders. Through the routine DNA genotyping of animals, it is possible to identify profiles that are indicative of chromosome abnormalities by including additional DNA markers in usual panels for pedigree and parentage verification. Abnormal profiles of genetic markers located on sex chromosomes can help identify animals with chromosomal defects. Markers panel used for cattle DNA testing by Laboratorio Central de Veterinária of Algete (Madrid) consisting of 20 autosomal microsatellite markers (BM1818, BM1824, BM2113, CSRM60, ETH10, ETH152, ETH225, ETH3, ILTST006, INRA023, MGTG7, SPS115, TGLA122, TGLA126, TGLA227, TGL48, TGLA53, TGLA57), 3 microsatellite markers linked to sex chromosomes (BM6017, BM4604 and BYM) and the Amelogenin marker, a gene with distinct X and Y alleles, has proved been very useful for genealogical control and detection of chromosomal abnormalities, presenting a 99.99999% of exclusion probability. A new panel with additional sex-linked markers (BM6017, BM4604, HEL14, BMC6021, IDVGA82, TGLA325, BM861 and BYM) was implemented 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 cattle kept for breeding.

Key Words: cattle, genetic identification, genotyping, sex determination

Comparative and Functional Genomics

OP7 Exploring tissue-specificity in the regulatory landscape of bovine genome. G. Costa Monteiro Moreira*[1], C. Yuan[1], S. Dupont[1], L. Tang[1], Y. Lee[1], D. Becker[2], M. Salavati[3], R. Clark[4], E. Clark[3], G. Plastow[5], C. Kühn[2,6], C. Charlier[1], and BovReg consortium[7], [1]*Unit of Animal Genomics, GIGA Institute, University of Liège, Liège, Belgium,* [2]*Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany,* [3]*The Roslin Institute, University of Edinburgh, Edinburgh, UK,* [4]*Genetics Core, Edinburgh Clinical Research Facility, The University of Edinburgh, Edinburgh, UK,* [5]*Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada,* [6]*Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [7]*https://www.bovreg.eu/project/consortium/.*

Transcriptomic (mRNA-, totalRNA- and small-RNA-Seq), ATAC- and ChIP-Seq assays were compiled in a catalog of 129 tissue samples collected from 6 individuals of both sexes, different ages, kept in different environments and from 3 divergent dairy and beef cattle breeds/crosses: Holstein, Kinsella composite and Charolais × Holstein F_2 crossbred. From the de novo transcriptome assembly, 43,117 gene

models including ≥15k potentially novel transcripts were assembled; BovReg expanded the catalog of bovine non-coding RNAs by including non-polyadenylated transcripts (totalRNA assay). Long-read mRNA sequencing (ONT-Seq) is being performed to support predicted isoforms. A total of 1,265 (638 known and 627 novel) miRNAs were detected and, for ≥90%, potential primary transcripts (pri-miRNA) were identified. Interestingly, ~39.71% of the novel miRNAs overlapped with repeats with a strong enrichment for an ancient DNA transposon (Mariner). On average, 105,245 (ATAC), 28,187 (H3K4me3), 152,646 (H3K4me1), 127,855 (H3K27me3), 77,967 (H3K27ac) and 71,868 (CTCF) peaks (q-value ≥0.05) per sample were annotated. Investigating open chromatin regions in the same tissue across the different ages/environment/breeds, differentially accessible regions were identified. By applying nonnegative matrix factorization on regulatory elements × tissue samples, we detected tissue and/or organ-system (muscle, digestive system, etc.) specific components. Using WGS (>30×) from the 6 individuals, ~8.5k CNV and ~400 polymorphic long-terminal repeat transposons were annotated; we highlighted candidates potentially affecting gene expression based on their co-localization with regulatory elements. Tissue-specific unannotated genes, miRNAs/pri-miRNA and regulatory elements were detected contributing to the understanding of vital body functions in bovine. The results presented herein represent a substantial improvement on the regulatory landscape annotation in bovine. The BovReg project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 815668.

Key Words: cattle and related species, Functional Annotation of Animal Genomes (FAANG), functional assay, regulatory element

OP8 A multi-tissue porcine single-cell immune atlas: Resources for comparative and systems immunology. C. Tuggle*[1,2], L. Daharsh[1], M. Kapoor[1,2], P. Bk[2], S. Sivasankaran[3], K. Byrne[3], J. Herrera-Uribe[1], and C. Loving[3], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Bioinformatics and Computation Biology, Iowa State University, Ames, IA,* [3]*USDA-Agriculture Research Service, National Animal Disease Center, Food Safety and Enteric Pathogens Research Unit, Ames, IA.*

A single-cell level understanding of the porcine immune system will provide tools for improving both disease resistance and use of the pig in biomedical modeling. We performed scRNA-seq on bone marrow, lymph node, spleen, and thymus from healthy adult pigs. After quality control to remove duplicate cells and cells with high mitochondrial content, 50,559 cells and 18,673 genes were used for downstream analysis. Each tissue was individually mapped using nonlinear dimensional reduction, and distinct clusters were found and analyzed using the following computational tools: IKAP, ROGUE, pair-wise differential gene expression testing, and random forest models. Using a combination of model defined and canonical immune cell markers, we were able to annotate each cluster as part of a diverse set of immune cell types identified. We validated these annotations using publicly available human scRNA-seq data sets specific for each corresponding porcine immune tissue. We used our published porcine PBMC scRNA-seq data set to predict cells that may be tissue-resident or match circulating cells, and integrated all of the immune tissue data to compare all cell types across the 4 tissues, to identify shared and tissue-specific cell types. Finally, we created an online visualization tool for users to explore expression of individual genes and the annotation of clusters in each tissue as well as combined. We are currently using these data and SCENIC software to create a first-generation regulatory network across all tissues. These studies of immune tissues will be an important resource for improved annotation of porcine immune genes and cell types, including providing information for development of new reagents and as a tool for systems approaches in porcine immunity. Further, these data can inform human translational biomedical research using pigs as a biomedical model.

Key Words: scRNA-seq, porcine, immune tissue, atlas

OP9 ISAG Bursary Award: Single cell atlas of developing ovine tail tissue reveals multi-cellular origins contributing to fat deposition. J. Han*[1,2], [1]*Institute of Animal Science, Chinese Academy of Agriculture Science, Beijing, China,* [2]*School of Agriculture and Food Science, University College Dublin, Dublin, Ireland.*

Fat-tail sheep exhibit a unique trait whereby substantial adipose tissue accumulates in the tail, a phenotype that is advantageous in many agroecological environments. However, the genetic factors underpinning this phenotype are still not clear. Previous studies indicated that developing tail tissue is an ideal biomaterial to study adipogenesis and fat metabolism. Therefore, we collected embryonic tail tissues at 9 time points covering the stages before and after fat deposition to perform single cell transcriptome sequencing and single cell assay for transposase-accessible chromatin sequencing. Based on the expression of canonical markers, all cells obtained in 2 assays were assigned as 23 cell types, including progenitor cells with 10 subpopulations (progenitor and stem cell, PSC; connective tissue progenitor, CTP; myogenic progenitor), precursors (e.g., preadipocyte and preosteoblast), terminally differentiated cells (e.g., adipocyte, osteoblast, vascular smooth muscle cell [VSMC]) and several other cell types, suggesting high heterogeneity within tail tissue. Furthermore, we evaluated the specificity of markers identified by differently expressed genes analysis, which were divided into 3 categories, strong marker (A+), general marker (A) and highly expressed gene (A−), and the A+ markers were mainly identified in terminally differentiated cells, such as *TMEM120A*, *FASN* and *CYB5A* for adipocytes. We constructed the differentiation trajectories for all lineages, including adipogenic, myogenic, osteogenic, chondrogenic lineage and VSMC generation; importantly, cellular origins for preadipocyte have been identified, including one subset of PSC, one subset CTP and VSMC. Multi origins of adipocyte would be one vital reason that results in quick and massive fat deposition within ovine fat tail tissue. In our following work, we will focus on the cellular interaction across cell to reveal the influence of microenvironment on adipogenesis, and integrating the results of 2 assays to construct gene regulation networks governing fat deposition.

Key Words: single cell sequencing, adipogenesis, developmental biology, differentiation trajectory

OP10 A multi-omic approach to understanding genetic and phenotypic variation in mass-reared Black Soldier Flies (*Hermetia illucens*). C. Rhode*, K. Hull, and M. Greenwood, *Stellenbosch University, Stellenbosch, Western Cape, South Africa.*

The need for renewable, sustainable and environmentally friendly animal protein production has been intensified by a growing human population, adverse effects of climate change and diminishing natural resources. Black Soldier Fly (BSF) farming has been proposed as an alternative livestock production system that may meet the challenges for future food security, with low resource requirements, higher feed conversion ratios and similar nutritional value as conventional animal protein. BSF has also proven highly advantageous due to the dual potential of the larvae to act as a bioremedial agent, converting organic waste into usable biomass, creating a circular agricultural production system. Despite the industrial scale of BSF mass rearing, little is known about the drivers of genetic and phenotypic variation under these production conditions. This study, therefore used a multi-omic approach to assess the interplay between organismal genetics, functional genomics, the microbiome and feed-substrate on phenotypic development in BSF larvae. The population genomic assessment revealed that genetic drift is the major evolutionary force shaping genomic diversity, even in the presence of direct artificial selection for production traits. Additionally, few loci were significantly associated with these production traits, further illustrating the influence of stochastic evolutionary processes during the mass rearing period. The effects of selection on gene expression were also weak and differential transcriptomic profiles highlighted functional trade-offs between growth metabolism and immune function. Metagenomic analysis found significant associations been bacte-

rial taxa and protein-fat ratios in BSF, and that both feed-substrate and the interaction between feed- and host genetics played a significant role in the composition of larval gut microbiomes. The findings highlight the multidimensional and complex nature of BSF production and its impact on phenotypic development, with applications for future genetic management and improvement strategies for enhanced production.

Key Words: functional genomics, GWAS, insect farming, microbiome, population genomics

OP11 ISAG Bursary Award: Ribosome profiling reveals stage-specific translational regulation during muscle differentiation. A. Goldkamp*[1], L. Okamoto[2], K. Thornton[2], and D. Hagen[1], [1]*Oklahoma State University, Stillwater, OK,* [2]*Utah State University, Logan, UT.*

Myogenesis is an essential process, in which skeletal muscle fibers are formed. In most mammalian species, this process largely occurs prenatally as the number of muscle fibers is fixed close to birth. However, the differentiation of myogenic cells is crucial to support hypertrophy of skeletal muscle, in which the size of muscle fibers is increased postnatally resulting in enhanced muscle volume and mass. C2C12 cell lines are a widely used model for studying muscle myogenesis. Transcriptional changes during myogenesis have been well defined and differentially expressed transcripts throughout muscle development have been identified, yet transcriptome profiling often overlooks post-transcriptional events contributing to a phenotype. Thus, advancements in sequencing technologies have recently permitted the study of the translatome, which refers to the entire population of mRNA associated with ribosomes for protein synthesis and can be investigated through ribosome profiling. As skeletal muscle ultimately becomes meat in livestock animals, a deeper understanding of the regulatory factors involved in skeletal muscle growth is needed. In this study, we performed genome-wide ribosome profiling coupled with RNA sequencing in C2C12 cells in a temporal manner (0 h, 30 min, 1 h and 4 h) post-induction of differentiation. Through ribosome profiling, we have characterized key transcripts regulated at transcriptional and/or translational levels throughout myogenesis. In addition, we have identified codons contributing to stalling events across each development stage, which may be governed by alterations in tRNA expression. Overall, this work offers a snapshot of all active ribosomes at each time point of muscle development and can aid in our understanding of gene regulation in one of the most important economic traits in livestock, skeletal muscle.

Key Words: growth, muscle, myogenesis, differentiation

OP12 Chromosome conformation comparison in Piedmontese × Gaur F_1 fetal muscle tissue. M. R. Stegemiller*[1], K. L. Kuhn[2], T. P. Smith[2], B. D. Rosen[3], and B. M. Murdoch[1], [1]*Department of Animal, Veterinary, and Food Sciences, University of Idaho, Moscow, ID,* [2]*USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE,* [3]*USDA, ARS, Animal Genomics and Improvement Laboratory, Beltsville, MD.*

The 3-dimensional (3D) genome organization within the nucleus is vital for correct DNA interactions. The hierarchal 3D organization of the genome is comprised of chromosome territories, compartments, topologically associated domains (TADs), and loops. There are 2 chromosome compartments, compartment A represents open genomic regions and compartment B are closed or inactive regions of the genome. Chromatin conformation has been shown to be tissue specific and previous studies demonstrated as high as 80% conserved conformation in species including chicken, goats, and pigs, although little 3D organization research has been completed in cattle and very little information about haplotype-specific organization has been produced in any species. The present study uses a fetal F_1 cross (*Bos taurus* × *Bos gaurus*) harvested at 120 d gestation with muscle tissue subjected a variation of Hi-C called Micro-C, which uses a micrococcal nuclease to identify nucleosome positioning. The interspecies nature of the cross supports improved resolution of haplotype-specific variation within a single tissue type. Compartments and TADs at 50 kb resolution were identified independently for each parental haplotype by mapping sequence reads separately to "complete" telomere-to-telomere genome assemblies of each haplotype generated using trio binning approach with parental data to separate haplotypes. Comparison of 3D organization between the species shows 41 locations organized in opposite compartments in the 2 haplotypes, for example on chromosome 4 between 45 and 47.5 Mb the cattle haplotype is in the B compartment while the corresponding region of the gaur haplotype is in the A. There were 1613 TADs identified in the Piedmontese but only 1556 in the gaur haplotype, and differences in TAD conformation in the chromosome 4 region of compartment switch were observed. Defining compartment determination and 3D conformation during fetal development can show parts of the genome that are interacting, and which segments of DNA are active and identification of regions that diverge within the Bovidae family could indicate regions of biological significance between the species.

Key Words: Micro-C, 3D chromosome conformation

OP13 ISAG Bursary Award: DNA methylation dynamics regulating embryonic development in pig. J. de Vos*[1], M. Derks[1], H. Acloque[2], S. Djebali[3], S. Foissac[4], C. Guyomar[4], C. Kurylo[4], E. Giuffra[2], M. Groenen[1], and O. Madsen[1], [1]*Animal Breeding and Genomics, Wageningen University, Wageningen, the Netherlands,* [2]*Paris-Saclay University, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [3]*IRSD, Université de Toulouse, INSERM, INRA, ENVT, UPS, Toulouse, France,* [4]*GenPhySE, Université de Toulouse, INRAE, ENVT, Toulouse, France.*

A main aim of the Horizon 2020 GENE-SWitCH project (grant agreement no. 817998) is to generate exhaustive functional genomic annotations for several key tissues in both pigs and chickens across development. DNA methylation is an epigenetic modification which plays a crucial role in mammalian development, however there is still limited understanding of the overarching dynamics in the developing fetus. In this research we used both whole-genome and reduced representation bisulphite sequencing (WGBS and RRBS) data to evaluate the methylome dynamics during development at 30 d post fertilization (dpf), 70 dpf, and new born (NB) of 7 tissues (liver, kidney, brain, muscle, skin, small intestine and lung) in the pig. Developmental transitions were investigated using a 2-fold approach: 1) Dividing the methylome into unmethylated regions (UMR), indicative of promoters, and lowly methylated regions (LMR) indicating enhancers, and 2) performing differential methylation analyses. The number of UMRs across developmental stages within the various tissues ranged from 10,822 to 14,680 and from 35,073 to 75,477 for LMRs. The defined methylation states (UMRs, LMRs and fully methylated regions) were used to define the dynamic changes of the methylome and *cis*-regulatory elements during embryological development. The most notable finding was the shift of methylation states, from hypo- to hyper-methylation, in liver 70 dpf to NB. Lastly, differentially methylated regions were integrated and combined with differentially expressed genes from the same samples. The combined differentially methylated and expressed genes were involved in biological pathways related with general growth, e.g., regulation of developmental processes, during initial stages of development (30 dpf to 70 dpf), and with tissue-specific functions during maturation transition (70 dpf to NB). This trend was observed for most tissues, except in liver and brain which showed tissue-specific functions during early developmental stages like e.g., neurogenesis in brain.

Key Words: Functional Annotation of Animal Genomes (FAANG), integrative genomics, development, DNA methylation, epigenomics

OP14 Genomic and functional characterization of frequently used bovine cell lines. D. Becker*[1], G. C. M. Moreira[2], C. Mörke[1], M. Charles[3], F. Hadlich[1], C. Lopez-Roques[9], M. Schmicke[4], V. Blanchet[5], H. Taniguchi[6], E. Clark[7], C. Pfarrer[8], J. Vanselow[1], C. Charlier[2], D. Rocha[3], C. Kuehn[1,10], [1]*Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [2]*Unit of Animal Genomics, GIGA, Liege, Belgium,* [3]*INRAE, Jouy-en-Josas, France,* [4]*Veterinary Endocrinology and Laboratory Diagnostics, University of Veterinary Medicine*

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There is a strong demand for fully characterized cell lines from farmed animal species e.g., for functional genome, physiology or veterinary medicine. Particularly for validating potentially regulatory variants in non-coding regions of the genome, thoroughly described cell lines are essential for target tissues. However, while cell lines are heavily used as in vitro surrogates for in vivo experiments, most of them lack a comprehensive functional annotation of active genomic regions as well as a catalog of genetic variants. In our project, 4 cell lines frequently used in bovine research were characterized at functional and structural level: EBL (embryonic lung cell line), F3 (generated from bovine trophoblast cells), MAC-T (mammary gland epithelial cell line) and MDBK (kidney cell line). All cell lines were monitored for whole transcriptome (mRNA, total RNA, miRNA) and for their regulatory genomic landscape by epigenomic profiling of open chromatin (ATAC-seq) and histone modifications (chromatin marks H3K4me3, H3K4me1, H3K27me3, H3K27ac) and CCCTC-binding factor (CTCF) binding sites via ChIP-seq. Regarding the whole transcriptome level, we further explored the effect of different passages and differences between diverse sources of the cell lines. At genomic level, the cell lines were subjected to whole-genome short-read sequencing at high coverage (40–81×). We found on average 6.54 million single nucleotide polymorphisms, and 1.15 million small insertions/deletions were discovered. Comparing the transcriptomic profile of the cell lines to their tissue counterparts revealed profound differences e.g., in genes related to energy metabolisms in the MDBK cells or protein synthesis in MAC-T cells. Furthermore, we observed a substantial variation of the transcriptome associated with passage number and source of cell clones. An increased awareness of particular cell line-specific limitations is recommended when interpreting in vitro cell line experiments. This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 815668.

Key Words: cattle, cell line, transcriptome, epigenome, genetic variant

OP15 Competing endogenous RNA (ceRNA) in a non-model animal: Non-coding RNAs respond to heat stress in rainbow trout (*Oncorhynchus mykiss*) through ceRNA-regulated mechanisms. J. Quan*, *Gansu Agricultural University, Lanzhou, China.*

Background: Rainbow trout (*Oncorhynchus mykiss*) is a typical cold-water fish. With global warming and extreme heat, high summer temperatures are the biggest threat to rainbow trout farming. Rainbow trout initiate stress defense mechanisms in response to thermal stimuli, and competing endogenous RNA (ceRNA) regulation of target genes (mRNAs) mediated by non-coding RNAs (microRNAs [miRNAs], long non-coding RNAs) may be the main strategy for responding to thermal stimuli and enhancing adaptation. Results: We screened the LOC110485411–novel-m0007–5p–*hsp90ab1* ceRNA relationship pairs for affect heat stress in rainbow trout and validated their targeting relationships and functions based on preliminary high-throughput sequencing analysis results. The transfection of exogenous novel-m0007–5p mimics and inhibitors into primary rainbow trout hepatocytes effectively bound and inhibited the target genes *hsp90ab1* and LOC110485411 without significant effects on hepatocyte viability, proliferation, and apoptosis. The inhibitory effect of novel-m0007–5p overexpression on *hsp90ab1* and LOC110485411 under heat stress was time-efficient. Similarly, small interfering RNAs (siRNAs) affected *hsp90ab1* mRNA expression by silencing LOC110485411 expression time-efficiently. Conclusions: In conclusion, we found that in rainbow trout, LOC110485411 and *hsp90ab1* can bind competitively to novel-m0007–5p via 'sponge adsorption' and that interference with LOC110485411 affects *hsp90ab1* expression. These results provide potential for anti-stress drug screening in rainbow trout.

Key Words: rainbow trout, heat stress, ceRNA mechanism

OP16 ISAG Bursary Award: Functional variants associated with male fertility in reproductive tissues of Brown Swiss bulls. X. Mapel*, N. Kadri, Q. He, A. Leonard, A. Lloret-Villas, and H. Pausch, *ETH Zürich, Zürich, Switzerland.*

Male fertility is a crucial component of the beef and dairy industries, yet the impact of genotypic variation on gene expression in reproductive tissues has barely been explored. Here we employ whole genome and total RNA sequencing to characterize variants that influence gene expression in 3 male reproductive tissues. We sampled testis, epididymis, and vas deferens tissue from 117, 103, and 84 Brown Swiss (BS) bulls, respectively, and sought (i) to identify variants that effect gene expression and splicing in each tissue, (ii) explore similarities across tissues, and (iii) determine if these variants are associated with male fertility. We detected 21,847 expressed and 15,493 spliced genes across the 3 tissues and used over 21,000,000 sequence variants to conduct *cis* e/sQTL mapping. Testis had the most e/sQTL, with 15,642 eQTL in 11,164 genes (eGenes) and 11,450 sQTL in 7,000 genes (sGenes). We observed 4,768 eQTL (4,347 eGenes) and 3,165 sQTL (2,662 sGenes) in epididymis, and 4,211 eQTL (3,889 eGenes) and 1,920 sQTL (1,718 sGenes) in vas deferens. Gene expression, splicing, and e/sQTL effects were similar between epididymis and vas deferens. Testis had a unique functional profile; it contained over 1,000 tissue-specific expressed genes and 8,291 tissue-specific eQTL effects. We used PrediXcan to integrate expression and splicing phenotypes through transcriptome wide association studies (TWAS) with fertility phenotypes from 3,736 BS bulls and semen phenotypes from 902 BS bulls. We conducted genome wide association studies (GWAS) with these phenotypes and colocalized significant peaks with e/sQTL. TWAS revealed 14 genes associated with at least one fertility phenotype ($P < 1.0 \times 10^{-6}$), 5 of which had an e/sQTL that was colocalized with a GWAS peak (PP4 > 0.8). Our results validated previously described male fertility QTL (*WDR19*) and identified new possible causal genes (*KCTD19*). In conclusion, our results indicate that characterizing expression and splicing variation in male reproductive tissues may provide mechanistic insight into differential insemination success in bulls.

Key Words: functional genomics, system genetics (eQTL), cattle and related species, fertility

OP17 Transcriptome and histological analysis of skin of Brangus cattle under heat stress conditions. P. Alvarez Cecco*[1], M. Balbi[1], M. Bonamy[1], A. Rogberg-Muñoz[2], L. H. Olivera[1], G. Giovambattista[1], and M. E. Fernández[1], [1]*Intituto de Genética Veterinaria (IGEVET), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina,* [2]*Instituto de investigaciones en Producción Animal (INPA), Universidad de Buenos Aires, CONICET, Buenos Aires, Buenos Aires, Argentina.*

Heat stress is a major factor that negatively affects animal welfare and production systems. Livestock should adapt to tropical and subtropical areas, and to meet this, composite breeds have been developed. This work aimed to evaluate gene expression profiles in the skin of Brangus cattle under heat stress using a case-control design and to correlate this with skin histological characteristics. Two groups of bulls were set using rectal temperature as a criterion to define stress conditions: stressed (n = 5) and non-stressed (n = 5) groups. Skin transcriptomics was performed and correlations between breed composition, phenotypic and skin histological traits were evaluated. Gene expression results showed 4309 differentially expressed genes ($P_{adj} < 0.01$): 2113 were downregulated and 2196 upregulated. Enrichment and ontology analyses revealed 132 GO terms and 67 pathways ($P < 0.01$) including thermogenesis, glycolysis, gluconeogenesis, mitochondrial activity, antioxidant, immune response, and apoptosis. Terms and pathways identity indicated diversity in mechanisms directed to relieve the animals' suffering, acting from simple passive mechanisms (conduction, convection and radi-

ation) to more complex active ones (behavioral changes, evaporation, vasodilation and wheezing). Furthermore, significant differences between phenotypic and skin histological traits and correlations between pairs of traits suggested a direction toward heat dissipation processes. In this sense, number of vessels were positively correlated with number of sweat glands ($P < 0.001$) and both were positively correlated with indicus % ($P < 0.05$ and $P < 0.01$, respectively); gland size was positively correlated with epidermal thickness and negatively with hair length ($P < 0.05$); and epidermal thickness was negatively correlated with gland-epidermis distance ($P < 0.0005$). In conclusion, the present results support the notion that the response to heat stress is physiologically complex, producing significant changes in the expression of genes involved in different biological pathways, while animals ability to face it depends greatly on their skin features.

Key Words: cattle, functional genomics, RNA-seq, adaptation, animal welfare

Horse Genetics and Genomics

OP18 ISAG Bursary Award: The epigenetic landscape of the satellite-free centromere of horse chromosome 11. E. Cappelletti*[1], F. Piras[1], L. Sola[1], S. Peng[2], A. Barber[3], M. Santagostino[1], J. Petersen[3], R. Bellone[2,4], C. Finno[2], T. Kalbfleisch[5], E. Bailey[5], S. Nergadze[1], and E. Giulotto[1], [1]*Department of Biology and Biotechnology, University of Pavia, Pavia, Italy,* [2]*School of Veterinary Medicine, Department of Population Health and Reproduction, University of California–Davis, Davis, CA,* [3]*Department of Animal Science, University of Nebraska–Lincoln, Lincoln, NE,* [4]*School of Veterinary Medicine, Veterinary Genetics Laboratory, University of California–Davis, Davis, CA,* [5]*Gluck Equine Research Center, University of Kentucky, Lexington, KY.*

Centromeres are essential chromosomal loci which are epigenetically specified by the histone H3 variant CENP-A. Although mammalian centromeres are typically associated with satellite DNA, the centromere of horse chromosome 11 is satellite-free. We previously demonstrated that the position of its CENP-A binding domain is not fixed but slides within an about 500 kb region in different individuals, giving rise to positional alleles. These epialleles are inherited as Mendelian traits but their position can slide in a generation. As members of the equine FAANG community, we recently proved that the ECA11 CENP-A binding domain is located in the same region in tissues from different embryonic origin from the same individual, suggesting that the position of the centromeric domain is maintained during development. By performing ChIP-seq experiments with an anti-H3K9me3 antibody on chromatin extracted from horse fibroblasts, we proved that the ECA11 centromeric domain is contained in an about 3 Mb domain of constitutive heterochromatin. Using RNA-seq, ChIP-seq and miRNA-seq data sets from different tissues of 4 horses produced by the FAANG equine consortium, we evaluated the transcriptional profile and the histone marks associated with active/permissive chromatin or facultative heterochromatin in the ECA11 centromeric region. Our findings showed that, in the majority of tissues, the ECA11 centromere is contained within a transcriptionally silent domain corresponding to the heterochromatic region previously identified in fibroblasts. However, transcription of the Carbonic Anhydrase 10 gene, which is partially overlapping the centromeric locus, can be detected in the nervous system. Pilot ChIP-seq experiments with an anti-H3K9me3 antibody on brain chromatin from the FAANG stallions revealed that constitutive heterochromatin is shifted from the centromeric domain, suggesting that that CA10 gene expression may represent a boundary for centromeric function. We are currently performing H3K9me3 ChIP-seq experiments on other tissues to investigate the interplay between centromeric function and chromatin remodeling.

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

OP19 Genomics of Thoroughbred stallion subfertility. C. Castaneda, R. Juras, B. W. Davis, and T. Raudsepp*, *School of Veterinary Medicine, Texas A&M University, College Station, TX.*

An idiopathic form of subfertility in Thoroughbred (TB) stallions, with normal physical and semen parameters, has been attributed to impaired acrosome exocytosis (IAE). According to a genome-wide association study, the latter is significantly associated with a certain double-homozygous A/A-A/A genotype in *FKBP6* exon5. The association was recently confirmed by comparison of breeding records of 150 TB stallions with their *FKBP6* genotype. The molecular causes of this association are unknown. Development of a TaqMan assay for *FKBP6* genotyping determined that the frequency of the A/A-A/A genotype in global horse breeds/populations and TBs separately is 4%. While this genotype is present in other breeds, it is associated with subfertility only in TBs, suggesting that *FKBP6* is only tagging a TB-specific haplotype and not the cause. The aim of this study was to identify this haplotype and search for candidate genes and variants for IAE. By TaqMan assay, we detected the *FKBP6* A/A-A/A genotype in 22 subfertile TB stallions, of which 14 have confirmed IAE. We generated short-read whole-genome sequence data for 9 case TBs and aligned the data with the Equine Genome Variant Database (EGVD) comprised of 428 horses from 46 breeds, including 55 TBs. We found *FKBP6* A/A-A/A genotype in 21 horses (9 TB cases, 1 EGVD TB, and 11 horses of other breeds) and showed that despite the same genotype, the sequence variant landscape in a 110 kb region around *FKBP6* is the same only across TBs and is different in other breeds. We inspected 8,447 single nucleotide variants (SNVs) in all TBs (10 A/A-A/A and 54 other), determined a 171 kb haplotype block specific to A/A-A/A TBs only, and identified 38 implicated SNVs in 5 genes for further investigation. Of these, a variant in one candidate gene is homozygous only in case TBs and of low allele frequency (6%) in EGVD horses. These findings strongly support our hypothesis that the A/A-A/A genotype in *FKBP6* exon5 is tagging TB- and case-specific haplotypes which are expected to contain genetic variants responsible for the subfertility phenotype and IAE. Ongoing studies involve PacBio and RNA sequencing for the discovery of candidate structural and/or regulatory variants.

Key Words: IAE, *FKBP6*, haplotype, TaqMan

OP20 Whole-genome trio sequencing to reveal the genetics of equine microphthalmia. I. Shutava[1], B. Ekesten[1], C.-J. Rubin[2], S. Mäkeläinen[2], T. Bergström[1], J. Tetens[3], and S. Mikko*[1], [1]*Swedish University of Agricultural Sciences, Uppsala, Sweden,* [2]*Uppsala University, Uppsala, Sweden,* [3]*University of Göttingen, Göttingen, Germany.*

Microphthalmia is characterized by abnormally small eyes. It presents as a heterogeneous disorder that can be uni- or bilateral with variable penetrance. In humans, variants in at least 29 genes, are proposed to cause microphthalmia. Some of them are believed to be inherited as autosomal recessive, others autosomal dominant, and yet others as X-linked disorders. Environmental factors have been suggested as part of the etiology creating an even more complex picture. In horses, the knowledge about microphthalmia is poor, but there is an increasing number of reported cases. In this study, more than 50 equine microphthalmia cases were identified. More than 80% of them were females, although uni- and bilateral cases were distributed evenly between females and males. In all unilateral cases, the left eye was affected with the right eye being normal. Enucleated eyes from 3 cases were examined by light-microscopy and diagnosed with severe microphthalmia. Pedigree analysis identified potential inheritance patterns, and ancestral founders in specific sire family lineages. Eight cases, and 12 of their carrier parents were whole-genome sequenced at 20–60X. Using the GATK best practices workflow for cohort analysis, we developed a general pipeline for genome variant detection and prediction of their

effect. Bioinformatic tools predicted autosomal recessive, and X-linked variant effects, as well as putative protein functions. Candidate genes were examined, but so far, no conclusive variant common to the 8 cases was yet discovered.

Key Words: horse, genome sequencing, functional genomics, bioinformatics, animal health

OP21 Changes in the gene expression profile of equine mesenchymal stem cells (MSC) after their allogeneic administration in horses matched or mismatched for the major histocompatibility complex (MHC). A. Cequier[1,2], E. Bernad[1], M. García-Martínez[1], B. Serrano[1], F. Vázquez[1,2], A. Romero[1,2], A. Vitoria[1,2], L. Barrachina[1,2], and C. Rodellar*[1], [1]*Laboratorio de Genética Bioquímica LAGENBIO–Instituto Agroalimentario de Aragón–IA2 (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.*

Musculoskeletal injuries have a great impact in equine industry. Conventional treatments have limitations that can be overcome by the anti-inflammatory and regulatory effects of MSC. Allogeneic MSC administration is advantageous but require further knowledge on the interactions with the immune system in vivo. Such interactions can be influenced by donor-receptor MHC-matching/mismatching and MHC expression level, which changes upon MSC inflammatory exposure and differentiation. This study evaluated the expression of genes related to the immunomodulatory and immunogenic profiles of equine MSC after their in vivo administration under different conditions. Allogeneic MSC in basal conditions (MSC-B), inflammatory primed (MSC-P) or differentiated into chondrocytes (MSC-C) were administered to 18 MHC-matched/mismatched horses. MSC were encapsulated in alginate and 3 scaffolds/recipient placed subcutaneously and retrieved after 1, 3 and 6 weeks. The procedure was repeated to assess the effect of a second administration. After retrieval of each scaffold, the expression of genes related to immunomodulation (*VCAM1*, *IL6*, *COX2*, *iNOS*, *IDO*) and immunogenicity (*CD40*, *CD80*, *MHCI*, *MHCII*) of MSCs was assessed by RT-qPCR. MSC-C showed the highest immunomodulatory profile and their administration in MHC-mismatched horses did not increase their immunogenic profile. In contrast, MSC-P showed lower immunomodulatory and higher immunogenic profile in all recipients regardless of the MHC haplotype. MSC-B administered in MHC-mismatched horses showed a higher immunogenic profile and a lower immunomodulatory profile. MHC haplotype, inflammatory exposure and chondrogenic differentiation of equine MSC affect their immune profile in terms of gene expression. Interestingly, MSC-C may offer advantages for allogeneic cell therapy, contrary to previous in vitro findings. The role of MHC haplotype and its expression level in the interactions of MSC with the immune system needs to be further addressed in vivo to optimize cell therapies.

Key Words: horse and related species, cell biology, immunology, microsatellite, qPCR

OP22 A missense mutation of BCHE promotes the butyrylcholinesterase activity in Chinese horses. Y. Zhang*[1], X. Liu[2,1], and l. Jiang[1], [1]*Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China,* [2]*Centre d'Anthropobiologie et de Génomique de Toulouse, Toulouse, France.*

It is widely accepted that cholinesterase inhibition is the primary mechanism for acute organophosphorus (OP) toxicity. Butyrylcholinesterase (BChE) is an enzyme which displays a high degree of structural homology to AChE. Due to its high affinity for binding chemical warfare nerve agents (CWNAs), and that null-BChE yields no apparent health effects, exogenous BChE has been explored as a candidate therapeutic for OP intoxication. Animal models are imperative for evaluating the efficacy of OP medical countermeasures, and a thorough characterization of available animal models is important for translating results to humans. We detected the basal cholinesterase activity levels in the circulation of 663 blood samples from 10 different horse farms and pasture, and sequenced the genomes of 409 Chinese native horses from 8 breeds at an average depth-of-coverage of ~11.1×. genome-wide association study revealed top-association between variation at the butyrylcholinesterase (BCHE) and the cholinesterase activity levels. Among them, a missense mutation (Asn104Lys) made BCHE protein more stable and significantly increased the cholinesterase activity (P = 1.55E-11). The BCHE overexpression results of wild type and mutant type also confirmed that the missense mutation increased the cholinesterase activity of horse, but reduced in human. Fine-scale analysis across an extended population of 729 individuals came from 24 breeds showed that the mutation is widely distributed in Chinese horse breeds. Re-analysis of ancient DNA data showed that the C allele (mutation), first occurred some ~5,000 years ago, and rose in frequency since. Thus, the objective of this study was to compare the circulating levels of each of the cholinesterases of Chinese native horses, as well as to explore the mutation Asn104Lys found in Chinese horses was promoted the biological activity of BChE derived from horse and inhibited the biological activity of BChE derived from human. This mutation, along with corresponding future efforts, may finally lead to a novel therapeutic and source to combat organophosphorus intoxication.

Key Words: Chinese native horse, butyrylcholinesterase, organophosphate, biological modeling

OP23 Genomics of gaits in Icelandic horses is more complex than DMRT3. H. Sigurdardottir*[1,3], E. Albertsdottir[2], T. Kristjansson[3], M. Rhodin[4], G. Lindgren[1,5], and S. Eriksson[1], [1]*Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics, Uppsala, Sweden,* [2]*The Icelandic Agricultural Advisory Centre, Reykjavik, Iceland,* [3]*Agricultural University of Iceland, Faculty of Agricultural Sciences, Hvanneyri, Borgarbyggð, Iceland,* [4]*Swedish University of Agricultural Sciences, Department of Anatomy, Physiology and Biochemistry, Uppsala, Sweden,* [5]*KU Leuven, Livestock Genetics, Department of Biosystems, Leuven, Belgium.*

The Icelandic horse has captured interest in many countries largely because of its unique ability to perform 5 gaits, including the lateral gaits *tölt* and *flying pace*. Despite the gait versatility characterizing the breed, the ability to perform flying pace varies widely between individuals and some seem to lack the ability to pace altogether. The discovery of a single base change in the DMRT3 gene had a key role in understanding the variability, as this single mutation alters the pattern of locomotion and in a homozygous form, enables the flying pace. However, there is a sizable ratio of homozygous horses that do not perform pace, despite a favorable DMRT3 genotype. The quality of pace is assessed based on various features such as clarity of beat, speed, stride length and suspension phase. It is therefore likely that pacing ability and quality of the gait is influenced not only by the DMRT3 gene, but also other genetic and environmental factors. Hence, the aim of the present study was to further investigate the genetic background of pace in Icelandic horses by using a genome-wide association study. A total of 362 Icelandic horses with phenotypic records from breeding field tests were genotyped with the 670 K+ Axiom Equine Genotyping Array. Several SNPs on chromosomes 4 and 9 reached the suggestive genome-wide significance level ($P < 1.0 \times 10^{-5}$) and were identified to associate with the breeding score for pace. A haplotype analysis further revealed 2 opposite haplotypes on each chromosome having positive or negative effects on the pace score. The most frequent haplotype on chromosome 4 had favorable effect on pace score but unfavorable effects on breeding scores for tölt, trot, gallop, and canter. Likewise, the most frequent haplotype on chromosome 9 had favorable effect on pace score but unfavorable effect on scores for trot and gallop. This study revealed that there appear to be multiple regions of interest in relation to ability and quality of flying pace in Icelandic horses. Further studies of these regions are needed to better understand the genetic control of the gait.

Key Words: horse, animal breeding, genome-wide association, complex trait, quantitative trait locus (QTL)

OP24 ISAG Bursary Award: Identification of personality-related genes associated with tractability of handling in Thoroughbred horses. T. Yokomori*[1], A. Ohnuma[2], T. Tozaki[2], M. Ishimaru[3], F. Sato[3], Y. Hori[4], T. Segawa[1], and I. Takuya[1], [1]*Nihon University, Fujisawa, Kanagawa, Japan,* [2]*Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan,* [3]*Japan Racing Association, Urakawa, Hokkaido, Japan,* [4]*The University of Tokyo, Meguro, Tokyo, Japan.*

Recently, the focus in the horse racing industry has been on securing a second career for retired racehorses for the purposes of improving animal welfare, and personality is expected to be an important trait. Previously, we discovered 15 novel candidate genes associated with personality in horses based on human personality-related genes and polymorphisms in the Thoroughbred population. The aim of this study was to investigate the association between behavior data and 5 of the candidates: *CDH13*, *HSD11B1*, *ANKK1*, *SLC6A4*, and *GABRA6*. Behavioral investigation was conducted using a 3-point-scale questionnaire consisting of 17 items to evaluate tractability by a consensus of 3 caretakers for 169 one-year-old Thoroughbred horses (80 colts and 89 fillies) between 2011 and 2013. The data tables of 3-point scores were transformed into a polychoric correlation coefficient matrix. Using the matrix, singular regression analysis was conducted by sex. One genotype per gene was used as explanatory variables and a column of principal component (PC) scores for each PC as objective variables. Additionally, the genotypes were analyzed with 3 genetic models: dominant, recessive, and additive. The results of PC analysis indicated that PC3 represented "tolerance for movement restrictions due to harnesses" and PC4 represented "tolerance for human and external changes." For PC3, colts with recessive homozygote on *CDH13* showed low scores (dominant model, $P < 0.05$). For PC4, fillies with heterozygote on *SLC6A4* showed high scores (additive model, $P < 0.01$), and fillies with at least one minor allele on *SLC6A4* also showed high scores (recessive model, $P < 0.05$). Among potential factors related to tractability of horses, these results suggested that *CDH13* is involved in reactivity to movement restriction of a part of the body in colts and *SLC6A4* in reactivity to movement restriction and changes in surroundings in fillies. These 2 genes are expected to be useful as one of the parameters for evaluating psychological tolerance in Thoroughbred horses.

Key Words: horse, statistical genetics, genotyping, behavior, animal welfare

OP25 A resource for documenting and tracking genetic diversity in US Thoroughbred horses. J. L. Petersen*[2], T. S. Kalbfleisch[1], J. N. Cullen[3], and E. F. Bailey[1], [1]*University of Kentucky, Lexington, KY,* [2]*University of Nebraska–Lincoln, Lincoln, NE,* [3]*University of Minnesota, Minneapolis, MN.*

Genetic diversity in US Thoroughbreds has recently gained interest. Currently, there are no public resources that allow the equine research community to measure genetic diversity of the US Thoroughbred population or track changes in diversity as a function of time. For this project, whole-genome shotgun sequence data sets have been generated for nearly 100 animals born between 1965 and 1986, and more than 100 animals born from 2000 to 2020. The horses born between 2000 and 2020 were selected to represent the diversity of the breed based upon pedigree relationships to result in a catalog of genetic variants present in this breed. The data also allow for genomic estimates inbreeding within each horse, providing information to evaluate changes in genomic diversity in recent decades. Twenty-fold coverage of the genome was targeted for each horse. The sequence was mapped to the EquCab3.0 reference genome, and variants called utilizing the Burrows-Wheeler Aligner, and GATK Best Practices, respectively. Across all samples, 16.7 million variants (indels and SNPs) were identified. Estimates of individual inbreeding based upon runs of homozygosity (F_{ROH}) utilizing over 10 million bi-allelic variants were orders of magnitude (20- to 40-fold, on average) greater than estimates for the same horses based upon their 5-generation pedigree. Further, pedigree- and genomic-based estimates were weakly correlated ($R^2 < 0.3$). Individual inbreeding coefficients (F_{ROH}) of horses born between 2000 and 2020 was significantly greater than that of the horses born between 1965 and 1984/5; however, the rate of change in inbreeding did not differ between groups ($P = 0.80$). Ongoing analyses will further examine both genomic and pedigree-based estimates of diversity. Additionally, future data collection will involve genotyping of ~900 additional US Thoroughbreds utilizing low-pass sequencing, allowing for a more in-depth evaluation of genetic diversity of the breed. We anticipate releasing this initial data set to public sequence repositories in late 2023.

Key Words: genetic diversity, US Thoroughbreds

OP26 Construction of genome-wide INDEL database, application to a parentage test using INDELs for horse registration, and a gene-editing test for doping control. T. Tozaki*, A. Ohnuma, M. Kikuchi, T. Ishige, H. Kakoi, K.-I. Hirota, and S.-I. Nagata, *Genetic Analysis Department, Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan.*

Thoroughbreds are the most famous breed of racehorses worldwide and are currently of high economic value. To understand genomic variability in Thoroughbreds, we identified genome-wide insertions and deletions (INDELs) and obtained their allele frequencies in this study. INDELs were obtained from whole-genome sequencing data of 101 thoroughbred racehorses by mapping sequence reads to the reference genome. By integrating individual data, 1,453,349, 113,047, and 18 INDELs were identified in the autosomal (1–31) chromosomes, X chromosome, and mitochondrial genome, respectively, for a total of 1,566,414 INDELs. Diallelic INDEL sizes ranged from −286 to +476 bp, with the majority, 717,736 (52.14%) and 220,672 (16.03%), being 1-bp and 2-bp variants, respectively. INDELs have the advantage of having lower occurrence rates than SNPs and STRs. Therefore, from the identified INDELs, we selected 39 diallelic loci to construct a parentage and identification panel in horses. The panel showed 0.9994, 0.9855, and >0.9999 for PE1, PE2, and PE3, respectively, when analyzed by multiplex PCR of DNA from 67 Thoroughbred horses. Registration of Thoroughbred racehorses requires parentage testing using the STRs recommended by ISAG. While parentage testing using SNPs is under development, this INDEL panel may serve as a complementary panel. The racing industry prohibits the generation of genetically modified racehorses for fair competition. We recently developed a gene-editing test to detect such illicitly modified racehorses. This test uses the following criteria to identify artificial editing: identifying INDELs with homozygote of alternative alleles not found in current Thoroughbred populations. As this study analyzed and validated the types, sizes, locations, and frequencies of INDELs in the current Thoroughbred population, these results will contribute to improving the gene-editing test.

Key Words: horse and related species, genome sequencing, genotyping, sequence variation, sport

Microbiomes

OP27 Analysis of the gut microbiome sheds insights into breed resilience to challenges of antimicrobial resistance in Dohne Merino sheep. A. Khwela*[1,2], E. F. Dzomba[2], R. Pierneef[1], and F. C. Muchadeyi[1], [1]*Agricultural Research Council, Biotechnology Platform, Onderstepoort, Gauteng, South Africa,* [2]*Discipline of Genetics, School of Life Sciences, University of KwaZulu-Natal, Scottsville, KwaZulu-Natal, South Africa.*

Dohne Merino is one of South Africa's leading sheep breeds which is also reared in Australia, New Zealand, and other European countries. In South Africa, sheep and other livestock populations are ex-

posed to multiple diseases and parasites. The efforts to manage diseases and infections while maintaining high productivity has led to a high usage of antimicrobials in sheep production. This has resulted in a high prevalence of antimicrobial resistance (AMR), which is a major global concern that demands surveillance and action. The gut microbiome is of importance to the well-being of ruminant livestock by contributing to nutrition and health of the animals. The goal of this study was to investigate the gut microbial environment of South African Dohne Merino sheep by metagenomic sequencing of the rumen, reticulum, omasum, and abomasum of ewes (n = 6). We assessed relationships between microbiome composition and AMR prevalence across the 4 gut compartments. The members of the microbial population were fully characterized and the resistome of the gut was analyzed. The microbial population was analyzed at phylum, class, order, genus, and species level. A total of 18 phyla were detected with *Bacteroidetes* (54%) and *Firmicutes* (25%) being the most abundant. Members of the archaeal domain made up 16.7% of the overall population. A total of 1,769 species were detected in all the samples, with uncultured species dominating. A total of 12 AMR genes were identified in the gut and were found to confer resistance to 15 antimicrobials. A high prevalence of resistance to Tetracycline, Macrolide, Nitroimidazole and Lincosamide was observed across all 4 compartments. Tetracycline genes were most abundant making up 49% of the total AMR genes. The findings reveal that microbial population is influenced by each compartments' physiological conditions and function. The observed antimicrobial resistance profiles reveal breed resilience and are likely selected for by the usage of antimicrobials as feed additives and in the treatment of diseases.

Key Words: Dohne Merino, gut microbiome, AMR, metagenomics

OP28 Using a Snakemake workflow for metagenomic analysis of sheep rumen microbiome divergently selected for methane emissions. B. Perry, A. Kim, H. Henry, T. Bilton, A. McCulloch, K. McRae, S. Clarke*, P. Janssen, J. McEwan, and S. Rowe, *AgResearch Limited, Lincoln, Canterbury, New Zealand.*

The objective of this project was to develop a bioinformatic pipeline for the analysis of metagenomic sequencing data generated from sheep rumen content. The pipeline was built using the popular Snakemake workflow language. Raw data in the form of 250 bp paired-end reads in fastq.gz format were trimmed, quality filtered and profiled in terms of taxonomy and function using open source software kraken2, bracken and humann3. Rumen microbiomes were compared between sheep of high and low methane selection lines. It was observed that biodiversity of the rumen microbiome was significantly higher in the high methane line, possibly due to a high prevalence of archaeal genera. Methanogens were highly associated with the high methane line while the low methane line were associated with producers of butyrate and propionate, which are products of the fermentation pathway that are not converted to methane. Future work should consider adapting this pipeline to handle and analyze metatranscriptomic data and running the functional profiling step of the pipeline against alternative reference databases and additional functional ontologies.

Key Words: microbiome rumen methane sheep metagenomics

OP29 ISAG Bursary Award: Study of gut microbes and body metabolism function between Dorper and Tan sheep. Y. Ma*[1], X. Yang[1], G. Hua[1], G. Cai[1], X. Li[2], D. Feng[2], and X. Deng[1], [1]*Key Laboratory of Animal Genetics, Breeding, and Reproduction of the Ministry of Agriculture and Beijing Key Laboratory of Animal Genetic Improvement, China Agricultural University, Beijing, China,* [2]*Department of Animal Science and College of Agriculture, Ningxia University, Ningxia Hui Autonomous Region, China.*

Gut microbes interact with peripheral organs in the form of axis, affecting the growth of the host. Dorper and Tan sheep are known for their excellent growth performance and higher fat content, respectively. This study aims to establish microbial and host metabolic networks that provide for breeding against the gut microbiota. We collected 8-mo-old Dorper and Tan sheep (4 male, 4 female) rumen, cecum, colon intestinal contents and liver, hindgut organs tissues reared in the same environment. We then used 16S rRNA sequencing methods to obtain microbial signatures and combined with metabolomic analysis to mine the intestinal metabolic network. RNA-Seq technology was used to identify differentially expressed genes and pathways in liver and intestinal tissues. In our study, Dorper intestinal tissue has strong glycine serine metabolism (*PHGDH*, *PAST*), and through the one-carbon cycle, the production of NAD^+ is increased, and more nucleotides are produced (cytosine, uracil). *Lactobacillus*, *Pseudomonas*, and other bacteria are involved in the nucleotide metabolism process. Meanwhile, the kynurenine pathway in the tryptophan metabolism (*ACMSD*, *KYNU*) increased the production of quinoline, indole and xanthine, which mediated the increase of NAD^+ in the intestinal of Dorper. In Tan sheep, the decrease of bile acids in the intestine is related to the process of bile transport in the liver. *NTCP* and *ABCB11* genes promote the reabsorption and transport of intestinal bile acids, decrease the content of bile acids in the intestine, and reduce the digestion of fat in the intestine. In contrast, the lipid oxidation gene (*PPARGC1B*, *LPL*) decreased lipid accumulation in Dorper. In conclusion, our study confirms that increased nucleotide and NAD^+ in Dorper promotes intestinal and host growth. Enterohepatic circulation and fat genes promote lipid accumulation in Tan sheep. This work was supported by China Ningxia Agricultural Breeding Project (NXNYYZ20150103).

Key Words: nucleotide metabolism, enterohepatic circulation, lipid metabolism; NAD^+

OP30 Comparative metagenomic along the gut biogeography of indigenous chicken. A. Tangomo Ngnintedem*[1,2], E. Machuka[3], B. Waweru[3], J.-B. Domelevo Entfellner[3], M. Gitau Gicheha[4], J. Maina Kagira[4], R. Pelle[3], A. Djikeng[5], and C. Keambou Tiambo[6], [1]*Biotechnology and Bioinformatics Research and Training Unit, Department of Anim. Sci, FASA, University of Dschang, Dschang, Cameroon,* [2]*Department of Molecular Biology and Biotechnology, Pan-African University Institute of Basic Sciences, Technology and Innovation, Nairobi, Kenya,* [3]*Biosciences Eastern and Central Africa–International Livestock Research Institute (BecA–ILRI) Hub, Nairobi, Kenya,* [4]*Department of Animal Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya,* [5]*Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Easter Bush Campus, Edinburgh, UK,* [6]*Centre for Tropical Livestock Genetics and Health (CTLGH), ILRI Kenya, Nairobi, Kenya.*

The gut microbiome modulates the host immune system, metabolism, and adaptability and affects food safety. Unfortunately, information on the gut microbiome of African indigenous chicken remains scanty despite the contribution of this genetic resource to food and nutrition security in Africa. Therefore, our capacity to accurately quantify gut microbiome diversity is extremely relevant to formulate host-specific nutrition requirements, novel veterinary therapy, adaptability and productivity strategies. In the current study, 16S rRNA gene high-throughput Illumina sequencing was used to explore the metagenomic diversity of 4 gastrointestinal tract regions (crop, gizzard, jejunum, cecum) and liver in chicken. Eighty biopsies were collected from 16 individual chickens fed on the same diet and reared under an intensive farming system for a 14-week period. The α diversity indexes exhibited similar pattern distribution within-sample and tend to increase along the GIT, with a pic noted in the jejunum. The β diversity showed that the cecum microbiota forms a distinct cluster from other GIT microbiota. Analysis of the GIT content and liver revealed heterogeneous microbiota taxonomy dynamic along the GIT organ and liver. Venn diagram and heatmap also demonstrate that each organ sections form a unique ecosystem on its own, each associated with a particular physiological function. These results confirm the suitability of using the 16S rRNA gene high-throughput Illumina sequencing pipeline to accurately quantify gut metagenome. Further, the non-invasive sampling strategies from a single gut biogeography cannot give a clear map of the gut microbiome diversity because each organ section forms a particular ecosystem on its own.

Key Words: gut biogeography, comparative metagenomic, indigenous chicken, 16S rRNA gene, Illumina MiSeq

OP31 Bacterial diversity associated with feeding Boschveld chicken with the South African red sorghum variety. N. Nemukondeni*[1], C. A. Mbajiorgu[1], A. N. Sebola[1], O. M. Letsoalo[1], T. Mafuna[2], and M. Mabelebele[1], [1]*University of South Africa, Florida, South Africa,* [2]*University of Johannesburg, Auckland Park, South Africa.*

The objective of this study was to investigate the effect of feeding diets formulated with red sorghum variety containing low tannins grown in South Africa on the gut microbes of Boschveld indigenous chickens. A trial was conducted for 90 d using unsexed Boschveld indigenous chicken fed diets formulated with the inclusion of the red sorghum type at 5 inclusion levels (0, 25, 50, 75, 100%) replicated 4 times. Sample collections were done on d 60 and 90 of the trial, where ceca from 2 chickens per treatment per replicate were collected and instantly stored in a tube containing 90% ethanol and kept in ice for further analysis. All collected samples were sent for sequencing at Inqaba Biotechnical Industries in Pretoria, where Genomic DNA samples were PCR amplified using a universal primer pair 341F and 805R, targeting the V3 and V4 region of the bacterial 16S rRNA gene. The resulting amplicons were purified, end-repaired and Illumina-specific adapter sequences were ligated to each amplicon (NEBNext Ultra II DNA library prep kit). The amplicons were sequenced on Illumina's MiSeq platform using a MiSeq v3 (600 cycles) kit. Analysis was done using in-house python scripts version 3.6.1. kronaTools and the Rstudio software following phyloseq package R version 3.5.0. The findings of this study revealed different bacterial communities present in the gut of the studied indigenous chickens and varied more as chickens grew. However, common bacteria found regardless of the inclusion levels were *Firmicutes*, *Bacilli*, *Lactobacilli*, *Lactobacillus*, *Proteobacteria*, *Betaproteobacteria*, and *Streptococcaceae*. Although, *Burkholderiales* bacteria were only present in the gut of chickens that were offered red sorghum diet at a 50% inclusion level. It can be concluded that feeding Boschveld chickens diets formulated with red sorghum at any inclusion level up to 100% does not have any adverse effects on gut health.

Key Words: microbiota, unisex, amplified, indigenous chicken

OP32 Bacterial metagenomics sequencing of chickens fed tannins. T. Manyelo*, E. Malematja, N. Sebola, S. Kolobe, and M. Mabelebele, *University of South Africa, Gauteng, South Africa.*

Alternatives to antibiotics have been receiving attention in poultry production. A study was conducted to investigate the potential utilization of tannins as an alternative natural feed additive in broiler chickens. A total number of 600-d-old Ross 308 broiler chicks with an initial live weight of 40 ± 1.6 g/bird were assigned to 2 dietary treatment levels in a completely randomized design, replicated 6 times with 10 chicks per replicate. Tannin inclusion levels were at 0 and 0.5 g/kg DM. Gut microbiome were measured and commercial DNA extraction kit was used to isolate the DNA from the collected ceca samples from Ross 308 broiler chickens. Thereafter, the genomic DNA samples were PCR amplified using a universal primer pair 341F and 805R, targeting the V3 and V4 region of the bacterial 16S rRNA gene. Resulting amplicons were purified, end-repaired and Illumina-specific adapter sequences were ligated to each amplicon. Following quantification, the samples were individually indexed (NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1), and another AMPure XP bead-based purification step was performed. Amplicons were then sequenced on Illumina's MiSeq platform, using a MiSeq v3 (600 cycle) kit. Then, 20 Mb of data (2 × 300 bp long paired-end reads) were produced for each sample. The BLAST-based data analysis was performed using an Inqaba in-house developed data analysis pipeline. For cecal samples, the dominant phyla were in order: *Firmicutes*, *Proteobacteria* and *Bacteroidota*. Chickens supplemented with 0.5% tannins were dominant with phylum *Firmicutes* followed by those that are on treatments 0. On contrary, chickens on the control diet were dominant with *Proteobacteria* and the lowest found in cecum of the chickens fed with 0.5% tannins. In conclusion, tannins play significant role in enhancing gut microbes of chickens.

Key Words: chicken, tannin, gut microbe, DNA, caeca

OP33 High-throughput metagenomic characterization of the fecal microbiota of peste des petits ruminants–infected West African Dwarf goats. I. Muritala*[1], B. O. Sodimu[1], M. N. Bemji[1], M. A. Busari[1], G. F. Farayola[1], S. Saleem[2], N. Kumari[3], S. Jaiswal[3], M. A. Iquebal[3], S. M. Ahmad[2], A. O. Sonibare[4], M. Wheto[1], and E. M. Ibeagha-Awemu[5], [1]*Department of Animal Breeding and Genetics, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria,* [2]*Division of Animal Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Jammu and Kashmir, India,* [3]*Division of Agricultural Bioinformatics, ICAR-Indian Agricultural Statistics Research Institute, New Delhi, India,* [4]*Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria,* [5]*Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Québec, Canada.*

The gut microbiota (GM) is known to play vital roles in digestion, immunity and health maintenance in livestock and human. Microbiota dysbiosis has been associated with several human and livestock diseases but its role in the pathogenesis of peste des petits ruminants (PPR) disease in goat is not known. Thus, this study characterized the microbiota of feces of PPR infected (PPR-inf) (n = 19) and non-infected (CTL) (n = 14) West African Dwarf (WAD) goats. Next-generation amplicon sequencing of the 16S rRNA gene in fecal microbial DNA from PPR-inf and CTL goats was accomplished with Illumina MiSeq system. Bioinformatics analysis of generated microbial sequences was accomplished with QIIME 2 and other standard tools. Microbiota diversity was significantly ($P < 0.05$) higher in CTL goats with predominant bacterial genera like *Ruminococcaceae UCG-010* and *UCG-005*, *Akkemansia*, *Prevotella* and *Fusobacterium*, etc., compared with PPR-inf group. Meanwhile, *Campylobacter*, *Treponema*, *Moraxella*, *Bacteroidetes* and *Bacillus* were more abundant ($P < 0.05$) in PPR-inf goats compared with CTL goats. *Campylobacter* and *Moraxella* have been implicated in campylobacteriosis and lower respiratory tract infections, respectively in human and livestock. Functional prediction indicated that genes associated with transport system, substrate-binding, and multiple antibiotic resistance were prominent in PPR-inf goats while genes associated with general secretion pathway and GntR family transcriptional regulators were prominent in PPR-inf and CTL groups ($P < 0.05$). In conclusion, there were marked differences in the bacterial composition between PPR-inf and CTL goats. Our data suggest that microbiota alterations or dysbiosis could be responsible for diarrheic symptoms observed in PPR disease, and thus implicate the microbiota in PPR pathogenesis.

Key Words: fecal microbiota, microbial richness, goat, *Campylobacter*/*Treponema*/*Moraxella*

OP34 ISAG Bursary Award: Nasal microbiome diversity in West African Dwarf goats with peste des petits ruminants viral infection. I. Muritala*[1], M. N. Bemji[1], M. A. Busari[1], B. O. Sodimu[1], S. M. Ahmad[2], A. Negi[3], S. Jaiswal[3], M. A. Iquebal[3], B. Bhat[2], M. O. Ozoje[1], O. L. Ajayi[4], and E. M. Ibeagha-Awemu[5], [1]*Department of Animal Breeding and Genetics, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria,* [2]*Division of Animal Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Jammu and Kashmir, India,* [3]*Division of Agricultural Bioinformatics, ICAR-Indian Agricultural Statistics Research Institute, New Delhi, India,* [4]*Department of Pathology, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria,* [5]*Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Québec, Canada.*

Peste des petits ruminants (PPR), regarded as an economically important disease of small ruminants (especially goat), is a highly contagious disease characterized by occulo-nasal discharge and diarrhea, among others. Diarrheic episodes indicate microbiota changes during PPR and even though the microbiota has been implicated in the etiology of many infectious diseases, little is known about its role in PPR in goat. Thus, this study characterized the nasal microbiome in PPR

infected West African Dwarf (WAD) goats using 16S rRNA metagenomics sequencing. A total of 33, 8 to 12 mo old WAD goats, consisting of 19 PPR infected (PPRG) and 14 non-infected (CTLG) goats were studied. Microbial DNA extracted from nasal samples was subjected to high-throughput next-generation 16S-rRNA amplicon sequencing using Illumina MiSeq system. Bioinformatics analyses of generated sequences were done with QIIME 2 and other standard tools. The mean reads in CTLG (27472.50) was significantly ($P < 0.05$) lower than in PPRG (45520.13). Microbial diversity was higher ($P < 0.01$) in CTLG than in PPRG. The core bacterial genera *Corynebacterium 1*, *Dietzia*, *Brevibacterium*, *Brachybacterium*, *Kocuria*, *Micrococcaceae*, *Rikenellaceae* RC9 gut group, *Salinicoccus*, *Staphylococcus*, *Christensenellaceae R-7 group*, *Ruminococcaceae UCG-010* and *Akkermansia* were significantly more abundant ($P < 0.01$) in CTLG than in PPRG. Meanwhile, *Haemophilus*, *Mannheimia*, *Moraxella* and *Mycoplasma* were more abundant ($P < 0.05$) in PPRG than in CTLG, suggesting roles in PPR. Many species of *Haemophilus*, *Mannheimia*, *Moraxella* and *Mycoplasma* are known to cause several human and livestock diseases. Our data suggests that susceptibility to PPR could be associated with a shift in the normal nasal microbiome composition, which should be further studied for the development of management strategies for PPR control.

Key Words: metagenomics, infectious disease, microbial diversity, *Haemophilus*/*Mannheimia*/*Moraxella*/*Mycoplasma*, goat

OP35 Optimising metagenomic sequencing: A comparative study of ONT Adaptive Sampling strategies to improve microbial DNA recovery. E. L. Reinoso-Peláez*[1,2], M. Saura[1], C. González[1], F. Puente-Sánchez[3], and M. Serrano[1], [1]*INIA-CSIC, Madrid, Spain,* [2]*ET-SIAAB, Universidad Politécnica de Madrid, Madrid, Spain,* [3]*Swedish University of Agricultural Sciences, Uppsala, Sweden.*

One important constraint in metagenome studies is the large amount of host DNA recovered when extracting microbial DNA. To improve this limitation, Oxford Nanopore Technology (ONT) has developed a method called Adaptive Sampling (AS), which is based on the depletion of sequences with respect to a user-specified genome. The aim of this study was to compare the efficiency of AS versus conventional sequencing (CS) to determine the most efficient strategy for retaining the highest amount and quality of microbial DNA. For that, we sequenced microbial DNA from vaginal exudates of 12 ewes in a GridION device, under both sequencing strategies (AS and CS) and different quality thresholds (Q) for the basecalling: (i) AS and fast basecalling (AS-fast, Q = 8), (ii) AS and high accuracy basecalling (AS-high, Q = 9), (iii) AS and super accuracy basecalling (AS-sup, Q = 10), and (iv) CS and high accuracy basecalling (CS-high, Q = 9). During AS, sequences were mapped against the sheep genome (GCA_002742125.1) to reject host sequences from the nanopores. After basecalling, *minimap2* software was additionally used to remove sequences assigned to the host. The quality filtering was assessed using NanoPlot software. The remaining sequences were processed with SqueezeMeta v. 1.6.0 software to evaluate the potential to recover microbiota reads. The number (and total size) of reads obtained with the different strategies were 211.9 K (94.3 Mb), 98.1 K (47.5 Mb), 91.4 K (42.5 Mb), and 28.4 K (13.9 Mb) for AS-fast, AS-high, AS-sup and CS-high, respectively. AS-fast yielded the highest recovery but also the highest number of unclassified reads (68.4%). Regarding the reads assigned to bacteria, AS-fast, AS-high, and AS-sup showed the highest recovery, being 3.30, 3.38, and 3.74 times more than the CS-high, respectively. In summary, the strategy providing the highest bacterial enrichment was AS-sup, with 36.5 K reads (compared with 9.8 K for CS-high), thus proposing this strategy as the most efficient approach to recover the greatest amount of microbial sequences from biological samples contaminated with host DNA.

Key Words: Adaptive Sampling, metagenome, DNA recovery, ONT

OP36 Possible coevolution of balanced polymorphisms in the pig host and its intestinal microbiome. C. Hupperts*[1], M. Mni[1], W. Coppieters[1,2], C. Charlier[1], and M. Georges[1], [1]*Unit of Animal Genomics, GIGA-R and Faculty of Veterinary Medicine, Liège, Liège, Belgium,* [2]*GIGA–Genomics Platform, University of Liège, Liège, Liège, Belgium.*

A major effect of ABO genotype on the abundance of a genus of *Erisypelotrichaceae* (p.75.a5) in colon and feces was recently reported in the pig: animals with a functional ABO galactosyltransferase (A allele) have higher abundance of p.75.a5 than animals with a loss-of-function mutation (O allele) in this gene (Huang, 2022). The porcine O allele was shown to be at least 3.5 million years old and to likely be maintained by balancing selection. It was shown that this microbiome QTL was mediated by differences in colonic GalNAc concentrations between AA, AO and OO animals. It was also shown that the affected bacteria were capable of using GalNAc as carbon source. Members of the p.75.a5 genus are not the only intestinal bacteria that use GalNAc. Why then do members of this genus appear to be the only bacteria affected by ABO genotype in the pig? A possible hint came from comparing the organization of the GalNAc operon in ABO sensitive versus insensitive bacterial species. It appears that the GalNAc operon is not or less inducible in ABO sensitive bacteria when compared with ABO insensitive bacteria (including *E. coli*), and that this may be due to the absence of a operon repressor in the former. Our aim is to understand the molecular mechanisms that underpin the sensitivity of the p.75.a5 genus to the ABO genotype of the host. Preliminary analyses suggest (i) that the p.75.a5 genus harbors a balanced polymorphism in the GalNAc operon that conditions the sensitivity to ABO, and (ii) that this polymorphisms is in linkage disequilibrium with other segregating variants supporting extensive sexual exchange within the genus. We are exploring the possibility to use single cell DNA sequencing with ONT to further explore the population genomics of p.75.a5 and other intestinal bacteria, and to test the hypothesis of the coevolution of balanced polymorphisms in the host and its intestinal microbiota. Latest results will be presented.

Key Words: pig and related species, metagenomics, other method, linkage disequilibrium, animal health

OP37 Genetic selection of the host drives gut microbiota enterotypes across generations. J. Estellé*[1], C. Larzul[2], M. Borey[1], F. Blanc[1], G. Lemonnier[1], Y. Billon[3], M. Thiam[4], B. Quinquis[4], N. Galleron[4], D. Jardet[3], J. Lecardonnel[3], F. Plaza-Oñate[4], and C. Rogel-Gaillard[1], [1]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [2]*Université de Toulouse, INRAE, ENVT, GenPhySE, Castanet-Tolosan, France,* [3]*INRAE, GenESI, Surgères, France,* [4]*Université Paris-Saclay, INRAE, MGP, Jouy-en-Josas, France.*

The gut microbiota associated to animals has been linked to many phenotypes essential for livestock production. In parallel, an increasing number of studies aims at understanding how the host genetics influences the microbiota composition and its physiology. Indeed, gut microbiota displays inter-individual variability even in highly controlled and homogeneous environments, and there is a lack of knowledge on the role of host genetics. We previously showed that the gut microbiota of 60-d-old pigs raised in similar conditions could be structured into 2 enterotypes, enriched either in *Prevotella* and *Mitsuokella* genera ("PM" enterotype), or *Ruminococcus* and *Treponema* ("RT" enterotype). To assess the host genetics influence on gut microbiota composition, we are studying 2 divergent pig lines, named HPM and HRT, selected for gut microbiota enriched in genera pairs specifying each enterotype. Response to selection over 3 generations revealed, per line, an increase in the prevalence of the selected enterotype and average relative abundances of directly and indirectly selected bacterial genera. Estimated heritabilities were significant for 62 genera abundances. Whole metagenome sequencing refined differences between enterotypes at bacteria species levels, illustrating different functional potentials also. Overall, we experimentally demonstrated the influence of host genetics on gut microbiota, highlighting holobionts as units of selection and pigs as important biological models. The HPM and HRT divergent pig lines will potentially contribute to better understand the combined impact of host genetics and gut microbiota on a range of phenotypes relevant for

sustainable livestock systems, from growth and feed efficiency to health and welfare.

Key Words: microbiota, gut, host genetics, pig, selection

OP38 Differential miRNA profile in response to dietary treatment and their possible impact in the host-microbiota genetic regulation. T. Porto[1], T. Cardoso[2], J. Bruscadin[1], L. Conteville[2], P. Oliveira[1], G. Mourao[3], L. Coutinho[3], A. Zerlotini[4], J. Reecy[5], and L. Regitano*[2], [1]*Post-graduation Program of Evolutionary Genetics and Molecular Biology, Federal University of São Carlos, Sao Carlos, SP, Brazil,* [2]*Embrapa Southeast Livestock Research Center, Sao Carlos, SP, Brazil,* [3]*Department of Animal Science, University of São Paulo, Piracicaba, SP, Brazil,* [4]*Embrapa Digital Agriculture, Campinas, SP, Brazil,* [5]*Department of Animal Science, Iowa State University, Ames, IA.*

MicroRNAs (miRNAs) are key post-transcriptional regulators of gene expression of both host and microbiota, and thus have the potential to influence microbiota composition and functionality. This project aims to identify the expression profile of miRNAs expressed in the rumen wall from Nelore (*Bos indicus*) bulls under nutritional intervention and to study the interaction between host miRNAs and ruminal microbiota. Two groups of Nelore bulls were submitted to different diets, i.e., conventional high-grain diet (n = 26) and agricultural co-products diet (n = 22). Rumen wall samples were collected and total RNA was extracted. The sequencing of the miRNA libraries was performed on an Illumina Hiseq 2500 platform and yielded an average of 3.42 million reads per sample. Reads were mapped to the *Bos taurus* genome ARS-UCD1.2 with the software miRDeep2, and a differential expression analysis was performed using DESeq2. A total of 528 miRNAs were identified, 9 of them differentially expressed (DE) (FDR ≤ 0.1); 7 miRNAs were upregulated and 2 were downregulated in the group fed agricultural co-products. To investigate evidence of microbiota regulation by host miRNAs, we analyzed *in silico* the potential interaction between the DE miRNAs and ribosome binding sites (RBS) annotated in 913 publicly available Metagenome Assembled Genomes (MAGs) from bovine ruminal contents. All 9 DE miRNAs had predicted targets in MAGs genes. A total of 35 bacterial MAGs (25 of them classified as *Clostridiales*) presented predicted targets for the DE bta-miR-223, affecting 13 annotated bacterial genes. This miRNA has been previously identified as DE in bovines divergent for residual feed intake and thus has the potential to be an important regulator of this trait. The results from both analyses suggest that host miRNAs are affected by diet and may regulate genes of microorganisms found in the bovine rumen. Further analysis may indicate which metabolic pathways are influenced in this process, as well as whether this regulation can be modulated in different nutritional interventions.

Key Words: cattle and related species, genome regulation, non-coding RNA, MicroRNA, RNA-seq

OP39 Host genomic regions associated with ewes' vaginal microbiota. M. Ramon*[1], E. Reinoso-Pelaez[2], M. Saura[2], O. González-Recio[2], C. Gonzalez[2], R. Arias[1], M. Pérez-Guzman[1], I. Beltrán de Heredia[3], J. Calvo[4], and M. Serrano[2], [1]*CERSYRA-IRIAF, Valdepeñas, Ciudad Real, Spain,* [2]*INIA-CSIC, Madrid, Spain,* [3]*NEIKER, Arkaute, Spain,* [4]*CITA-ARAID-IA2, Zaragoza, Aragón, Spain.*

Fertility is a trait of great economic importance in livestock breeding programs. The outcome of artificial insemination (AI) depends on several factors, making it difficult to identify the causes of low fertility. One of the factors known to influence fertility is the composition of the vaginal microbiota at the time of AI. The characterization of the microbiota can help to identify which microorganisms may be in overabundance in sub-fertile animals so appropriate treatments can be considered. In addition, this characterization can be used to study the role that the host genome may play in the composition of its vaginal microbiome. This work aims to conduct an initial exploration of the relationships between host genotype and vaginal microbiota in sheep. For that, vaginal samples from 288 ewes were collected before IA. From them, DNA was extracted and bacterial 16S ribosomal RNA hypervariable regions V3 and V4 were sequenced. Bioinformatics pipelines using qiime2 and SILVA nr99_v138.1 database for taxonomic annotation were used to obtain feature abundance matrices. In addition, genomic data (Illumina ovina HD 680K AgResearch chip) from the same 288 ewes was available, and a QC analysis removing samples with a call rate below 95% and markers with a call rate below 95% and a MAF < 0.01 was carried previous to the analysis. A genome-wide association study (GWAS) was conducted using the mixed linear model approach in GCTA to look for associations between host genome and the microbial abundance for some important families known to affect fertility, such as *Actinomycetaceae*, *Fusobacteriaceae* and *Mycobacteriaceae*. Within the regions of the genome found to be significantly associated with the abundance for these families, we found genes involved in the immune response such as interleukins (ILs), FSHB and FSHR, GNRHR related to the follicle-stimulating and gonadotropin hormones, OXT and OXTR related to the oxytocin hormone, and THRA, THRB, TSHR and TRHR related to thyroid hormone. Further study of the role of these and many other identified genes will help us to learn more about the relationships between the host genome and the vaginal microbiota of ewes and its role in the fertility outcome

Key Words: microbiomics, genome-wide association, sheep and related species, fertility

OP40 Links between gut microbiome functions and feed efficiency in growing pigs fed a conventional or a high-fiber diet. A. Cazals[1], O. Zemb[2], V. Déru[2,3], J. Bidanel[4], H. Gilbert[2], and J. Estellé*[1], [1]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [2]*Université de Toulouse, INRAE, ENVT, GenPhySE, Castanet-Tolosan, France,* [3]*France Génétique Porc, Le Rheu, France,* [4]*IFIP-Institut du Porc, Le Rheu, France.*

The gut microbiota plays a major role in the digestive process in pigs and has been associated with feed efficiency, a major economic trait for pig industry. At the same time, pig production has to address social and environmental challenges, such as the choice of sharing land and natural resources between production for animal feed or for human consumption, and the environmental impact of meat production. Increasing the fiber content in the diets of pigs is an alternative to explore. It is therefore important to study the impacts of diet modulations on both feed efficiency traits and the composition and function of the microbiota. In this study, we predicted the relative abundances of the KEGG Orthologs (KO) functions from 16S sequencing data using PICRUSt2 software from 1170 fecal samples of Large White pigs, fed with conventional (CO) or a high-fiber (HF) diet. On a subset of 48 samples, a whole meta-genome sequencing (WMS) was performed. Increasing the fiber content did not change the functional richness of the microbiota, but 189 KO functions were differentially abundant between the diets. Since over 6000 KO functions were detected in the samples, the impact of HF diet on gut microbiota functional potential was relatively modest. Combining animals from both diets, in a model including "diet" as a fixed co-factor, residual feed intake (RFI) values for each pig were used to analyze the correlation between microbiota functions abundances and feed efficiency. With this approach, differential analysis revealed over 1600 KO differentially correlated to RFI value, and 24 enriched pathways. In the WMS data set containing much fewer individuals, no significant DA KO were found but 68 enriched pathways were identified by using the "gage" algorithm. Interestingly, some KO pathways such as "flagellar assembly" or "ribosome" were highlighted by using both 16S and WMS data sets. Overall, this study provides new insights to better understand how feed impacts the gut microbiome functions, which in turn contribute to the variability of feed efficiency in pigs.

Key Words: pig, gut microbiota, feed efficiency, residual feed intake, microbiota function

OP41 Comparison of rumen microbial analysis pipelines based on 16S rRNA gene sequencing. X. Ye*, Z. Cai, and M. Lund, *Center*

for Quantitative Genetics and Genomics, Aarhus University, Aarhus, Denmark.

To investigate complex rumen microbial communities, 16S ribosomal RNA (rRNA) sequencing is widely used. Here, we evaluated the impact of bioinformatics pipelines on the observation of OTUs and taxonomic classification of 750 cattle rumen microbial samples by comparing commonly used pipelines (LotuS and QIIME) with Usearch. In LotuS-based analyses, 189 archaeal and 3894 bacterial OTUs were observed. The observed OTUs for the Usearch analysis were significantly larger than the LotuS results. We discovered 1495 OTUs for archaea and 92,665 OTUs for bacteria using Usearch analysis. In addition, taxonomic annotations were made for the rumen microbial samples. All pipelines had consistent taxonomic annotations from the phylum to the genus level. Differences in top relative abundance were calculated for all microbial levels, including *Bacteroidetes* (QIIME: 72.2%, Usearch: 74.09%), *Firmicutes* (QIIME: 18.3%, Usearch: 20.20%) for the bacterial phylum; *Methanobacteriales* (QIIME: 64.2%, Usearch: 45.7%) for the archaeal class, *Methanobacteriaceae* (QIIME: 35%, Usearch: 45.7%) and *Methanomassiliicoccaceae* (QIIME: 35%, Usearch: 31.13%) for archaeal family. However, the predominant archaeal class varied between these 2 annotation pipelines. The *Thermoplasmata* was the top archaeal class according to the QIIME annotation, whereas *Methanobacteria* was the top archaeal class according to Usearch. Consequently, the various bioinformatics pipelines may influence the estimation of the relative abundance of the rumen microbial community. Even when using the same data, it is not possible to directly compare studies utilizing different pipelines.

Key Words: rumen microbial, 16S rRNA gene sequencing, bioinformatics pipeline

OP42 Exploring links between porcine genome copy number variants, the diversity and composition of pig gut eukaryote and prokaryote microbial communities. M. Ballester*[1], D. Crespo-Piazuelo[1], J. Morata[2], L. Ramírez[1], O. González-Rodríguez[1], C. Sebastià[3,4], A. Castelló[3,4], A. Dalmau[5], S. E. Ramos-Onsins[3], K. Alexiou[3], J. M. Folch[3,4], R. Quintanilla[1], and Y. Ramayo-Caldas[1], [1]*IRTA, Torre Marimon, Caldes de Montbui, Spain,* [2]*CNAG-CRG, Baldiri i Reixac 4, Barcelona, Spain,* [3]*CRAG, Campus UAB, Bellaterra, Spain,* [4]*UAB, Bellaterra, Spain,* [5]*IRTA, Monells, Girona, Spain.*

Recent evidence suggests that genetic variation in the pig genome partially controls the composition of porcine gut microbiota. However, since previous studies have been focused on the contribution of single nucleotide polymorphisms, little is known about the putative links between other sources of genetic variation like copy number variants (CNVs). The main goal of this study was to assess the association between porcine genome CNVs, the diversity and composition of pig gut prokaryotic and eukaryotic microbial communities. For this purpose, we used whole-genome sequencing data to undertake a comprehensive identification of CNVs followed by a genome-wide association analysis between the estimated CNV status and 52 microbial traits including 3 diversity indexes, and the relative abundance of 43 bacterial, 5 protists, and 1 yeast genus. We identified associations between CNVs and the relative abundance of 3 bacterial genera (*Faecalibacterium*, *Oscillospira*, and *Phascolarctobacterium*), one eukaryotic (*Kazachstania*), the richness and Shannon α-diversity of the bacterial communities. The CNV linked to the diversity index partially harbor *ABCC2-DNMBP* loci and was *in silico* predicted as gain. We validated by real-time quantitative PCR with a precision of 95.83% the gain of copies of this CNV. Further, its segregation and positive association with bacterial diversity was confirmed in an unrelated F_1 (Duroc × Iberian) cross. In summary, we report the first study exploring associations between porcine CNV and gut microbial traits. These results advise the relevance of considering the role of structural variants as host-genetic factors modulating porcine gut microbial communities and open the possibility of including CNVs in selection programs to simultaneously improve microbial traits and gut health.

Key Words: pig, genome-wide association, microbiomics, copy number variation (CNV), qPCR

OP43 Impact of the vaginal microbiota on the pregnancy rate by artificial insemination in three Spanish sheep breeds. E. L. Reinoso[1,2], F. Puente-Sánchez[3], C. González[1], J. H. Calvo[4], M. Serrano[1], and M. Saura*[1], [1]*INIA-CSIC, Madrid, Spain,* [2]*ETSIAAB Universidad Politécnica de Madrid, Madrid, Spain,* [3]*Swedish University of Agricultural Sciences, Uppsala, Sweden,* [4]*CITA-IA2, Zaragoza, Spain.*

Artificial insemination (AI) is an essential tool in ruminant breeding programs for dairy aptitude. Notwithstanding, its efficiency in sheep is low, which results in economic losses and delays selection efficiency. Recent studies in humans, suggest that microbial communities residing in the female reproductive tract seem to be involved in reproductive failure and pregnancy complications. In this study, we have analyzed the composition and abundance of the reproductive tract microbiota in sheep to investigate its relationship with fertility. For that, vaginal exudate samples were taken from 332 ewes from 4 different flocks belonging to 3 breeds (Latxa, Manchega with 2 flocks, and Rasa Aragonesa). Microbial DNA was extracted from the samples and the V3-V4 regions of the microbial 16S ribosomal RNA gene were sequenced using Illumina MiSeq technology. The sequences were analyzed by identifying the Amplicon Sequence Variant (ASV) with the Dada2 package of the R software (35,577 ASVs identified). Beta diversity studies were carried out using principal components (PCA) and PERMANOVA to determine the factors associated with the composition of the microbiota. Finally, differential abundance analysis was performed between pregnant and non-pregnant ewes. A linear model corrected by flock and linear models for each independent flock were fitted. Differentially abundant ASVs were found for the different models fitted, but no ASVs common to all breeds were detected. However, when grouping the ASVs at the taxonomic level of phylum/class, most of the significantly differential abundant taxa in pregnant ewes belonged to *Proteobacteria*/*Gammaproteobacteria* groups and those in non-pregnant belonged to *Fusobacteria*/*Fusobacteriia*. These results agree with previous studies in cattle and humans, and suggest that the composition of the vaginal microbiota depends mainly on the flock and can be greatly affected by elements related to management, rather than the genetics of the breed.

Key Words: ASV, fertility, microbiome, ribosomal RNA 16S, sheep

OP44 Preliminary results: Bacterial abundance in the microbiome from South African beef faecal samples through 16S rRNA targeted sequencing. O. P. Monchusi[1,2], K. P. Montso[2], C. N. Ateba[2], A. A. Zwane[1], and M. M. Makgahlela*[1], [1]*Agricultural Research Council, Old Olifantsfonteing, Irene, Centurion, Gauteng, South Africa,* [2]*North-West University, Mahikeng, South Africa.*

The gastrointestinal tract (GIT) of cattle harbours a complex microbial community. These microbes are crucial in animal nutrition, physiology, and health. Therefore a better understanding of microbial diversity in the GIT of beef cattle is imperative. This study aims to determine the microbial communities in different cattle breeds using 16S rRNA metagenomics sequence analysis. The Divisive Amplicon Denoising Algorithm 2 (DADA2) software package was used on RStudio for metagenomics analysis, and SILVA at 99% full-length trained classifier was used as a reference tool for taxonomic classification. A total of 40 fecal samples were collected from 4 free-grazing breeds (Tuli, Nguni, Afrikaner, and Holstein) comprising male and lactating cows from different provinces of South Africa. The results revealed that operational taxonomic units at the phyla level showed a similar distribution (approximately 50%) of *Firmicutes* across each breed. Followed by the Tuli breed, 34% of *Proteobacteria*, 30% of *Bacteroidota* in the Holstein, and 35% of *Planctomycetota* in both the Afrikaner and Nguni breed. From observations, there were similarities in the distribution of family, with an increased portion of *Planococcaceae*, *Clostridiaceae*, followed by *Ruminococcaeae*. However, female fecal samples were observed to have an increased number of *Lactobacillaceae* and *Carnobacteriaceae* with a variety of minimal abundance that was not identified in other breeds. In conclusion, cattle rumen fecal samples have an equal distribution in *Firmicutes* despite ecological region but exhibit a distinctive abundance of *Proteobacteria*, *Bacteroidota*, and *Planctomycetota*. These preliminary results show consistency in certain phyla

and suggest a significant difference within certain abundant microbes in a specific breed. Most female breeds have an increased number of *Lactobacillaceae*, a diverse family of lactic acid bacteria found in the gut microbiota of animals. Since 16s rRNA is a targeted metagenomics analysis, more studies should be conducted investigating the rumen microbiota at the species level.

Key Words: 16s rRNA, gastrointestinal tract, microbiome, faecal

Pig Genetics and Genomics

OP45 Initiative for African Indigenous Pig Genome Project. A. C. Adeola*[1,2], X. Shi[1], X. Liu[3], O. F. Olaniyan[4], C. A. M. S. Djagoun[5], G. Msalya[6], D. H. Mauki[7], N. K. Wanzie[8], G. Niba[9], P. D. Luka[10], S. C. Olaogun[11], V. M. O. Okoro[12], S. Zhao[13], J.-L. Han[14], M.-S. Peng[1,2], Y.-P. Zhang[1,2], [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, Yunnan, China,* [2]*Sino-Africa Joint Research Centre, Chinese Academy of Sciences, Kunming, Yunnan, China,* [3]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China,* [4]*West Africa Livestock Innovation Centre, Banjul, The Gambia,* [5]*Laboratory of Applied Ecology, Faculty of Agronomic Sciences, University of Abomey-Calavi, Cotonou, Benin,* [6]*Sokoine University of Agriculture, Morogoro, Tanzania,* [7]*Center for Cancer Immunology, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences (CAS), Shenzhen, China,* [8]*Department of Zoology, University of Douala, Douala, Cameroon,* [9]*National Centre for Animal Husbandry, Veterinary and Halieutic Training, Jakiri, Cameroon,* [10]*National Veterinary Research Institute, Vom, Nigeria,* [11]*Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria,* [12]*Department of Animal Science and Technology, School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Nigeria,* [13]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China,* [14]*International Livestock Research Institute, Nairobi, Kenya.*

African indigenous pigs' history and adaptation to environmental and human selection pressure underlies their remarkable diversity. Assessing this diversity is an important step toward understanding the genomic basis of productivity and adaptation fitness under the African farming systems. At the Kunming Institute of Zoology–Chinese Academy of Sciences, we have commenced international collaborative projects to improve our knowledge of African indigenous pig genomics. We collected about 1000 African indigenous pig samples and wild suids from 6 countries in sub-Saharan Africa and sequenced the genomes of 454 pigs. Population genomic analyses revealed large differences between East and West African populations, and gene flow from Eurasian populations was detected in African indigenous pigs. As this project progresses, we will focus on exploring the possibilities of introgression from wild suids into African indigenous pig genomes which may provide insights into local adaptation including candidate genes associated with adaptation to diverse African local environments. We also plan to incorporate additional samples from other parts of Africa. Through the generation of African indigenous pig genomic data combined with the existing multi-omics data for subsequent analyses, this collaborative project aims to gain in-depth insights into the demographic history and adaptive evolution of African indigenous pigs. Therefore, we hope to establish a collaborative network of African scientists from multidisciplinary fields to work together and contribute to this important project to conserve and rationalize animal genetic resources in Africa

Key Words: African indigenous pig, population genomics, population structure, adaptation, genomic selection

OP46 Identification of new transcription factors using eGWAS in four porcine tissues. S. Hosseini[1], M. Gòdia[1], M. Derks[1], B. Harlizius[2], O. Madsen[1], and M. Groenen*[1], [1]*Wageningen University & Research, Wageningen, the Netherlands,* [2]*Topigs Norsvin Research Center, Beuningen, the Netherlands.*

Efforts on porcine eGWAS have been mostly focused on a single tissue type, and although new meta-studies are now available, they use several porcine breeds coming from different genetic backgrounds, thus still masking regulatory regions of interest. In this study, we performed RNA-seq on 100 sows from a cross between 2 commercial pig breeding lines. RNA-seq was performed for 4 different tissues: liver, spleen, lung and muscle, selected for their key roles in metabolism and immune response. The same animals were genotyped with the high-density (660K markers) Axiom Porcine Genotyping Array. With an average of 44 M reads per sample, we identified 12,680, 12,650, 13,310, 12,595 genes in liver, spleen, lung and muscle expressed with at least 1 CPM. After filtering the genotype data, 535,896 SNPs were kept for the eGWAS analysis. We identified several eQTL regions that included 2 or more significant SNP associations. We found 4,293, 10,630, 4,533 and 6,871 eQTLs for liver, lung, spleen and muscle, respectively. As expected, a minority, 12, 6, 18 and 5% respectively, were classified as *cis*-eQTLs (<1 Mbp of their associated gene). Some of the most significant eGWAS peaks included *RDH16* in liver, involved in vitamin A metabolism and playing an important role in the regulation of feed efficiency in pigs by affecting energy metabolism, *TF* in lung, which plays a role in acute respiratory distress syndrome, and *OPLAH* in muscle associated with the regulation of energy metabolism in skeletal muscle. Interestingly, some *cis*-eQTL also had many *trans*-eQTL effects and these *cis*-eQTL were often associated with transcription factors, indicating likely target genes. That is the case for *ZNF577* in liver, that despite its unknown function, belongs to a zinc finger protein family that is associated with adipogenesis and hepatic lipogenesis. Another example is *Erf* in spleen, that has been found to be required throughout hematopoietic (blood cells) development. In conclusion, our results show that eGWAS can help annotating new transcription factors involved in complex phenotypes of interest such as behavior, health, and robustness.

Key Words: pig, eQTL, RNA-seq, gene expression

OP47 ISAG Bursary Award: Comprehensive identification of functional DNA elements and 3D chromatin interaction map in the pig genome. D. Wang*[1], M. Hu[1], Y. Guo[1], R. Kuang[1], H. Zhou[1], R. Ma[1], Z. Han[1], L. Li[1], H. Peng[1], Z. Xu[1], Y. Zhang[1], M. Zhu[1,3], C. K. Tuggle[4], Y. Zhao[1], S. Zhao[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding, and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei, China,* [2]*Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan, Hubei, China,* [3]*The Cooperative Innovation Center for Sustainable Pig Production, Wuhan, Hubei, China,* [4]*Department of Animal Science, Iowa State University, Ames, IA.*

As an important species in the livestock industry and biology research, the continuous development of pig genome studies provided a valuable resource to fundamentally enrich *cis*-regulatory element annotation and extend ENCODE and Roadmap Epigenomics projects in the research field of large animals. However, in contrast to the human and mouse ENCODE phase III, which further revealed landscapes of the 3-dimensional (3D) organization of chromatin and expanded into different developmental stages. On bias of that, we performed RNA-seq, ATAC-seq, ChIP-seq (H3K27ac and H3K4me3), in situ Hi-C and in situ ChIA-PET (RNA polymerase II and CTCF) in 11 diverse tissues of 2-week and 180-d Large White (LW) pigs (biological repeats n = 2),

including longissimus muscle, backfat, heart, liver, spleen, lung, kidney, duodenum, pancreas, cerebrum and cerebellum. In total, 137 data sets were generated at present. For Hi-C experiments, in total, more than 19.45 billion paired-end reads were sequenced across all samples, providing more than 343 × coverage of the pig genome. Approximately 720 million paired-end reads sequenced for each sample. After filtering potentially artificial reads, we obtained about 5,318 million unique and valid contact reads, among which 3, 970 million reads were *cis*-contacts. We further explored the 3D structure of the pig genome and identified an average of 2,358 topologically associating domains (TADs) and 22,412 loops in each tissue of adult LW pigs. Based on chromatin interaction analysis of ChIA-PET data, we detected 31,719–70,939 long-range interactions between chromatin loci in the heart, spleen, muscle, and other tissues of 180-d LW pigs. Our research explored and revealed a comprehensive epigenomics map of gene expression, chromatin accessibility, *cis*-regulatory elements and chromatin interactions in 11 diverse tissues of LW pigs at different developmental stages, providing high-quality reference information for pig genome research.

Key Words: pig, regulatory element, 3D chromatin interaction, epigenetics

OP48 Multi-breed, multi-tissue, and multi-omics aiding the quest for key porcine regulators. D. Crespo-Piazuelo[1], A. Reverter[2], Y. Ramayo-Caldas[1], R. Quintanilla[1], H. Acloque[3], M.-J. Mercat[4], M. C. A. M. Bink[5], A. E. Huisman[5], and M. Ballester*[1], [1]*Animal Breeding and Genetics Program, Institute of Agrifood Research and Technology (IRTA), Torre Marimon, Caldes de Montbui, Spain,* [2]*CSIRO Agriculture and Food, St. Lucia, Brisbane, Queensland, Australia,* [3]*INRAE GABI, Domaine de Vilvert, Jouy-en-Josas, France,* [4]*IFIP-Institut du Porc and Alliance R&D, La Motte au Vicomte, Le Rheu, France,* [5]*Hendrix Genetics, Boxmeer, the Netherlands.*

This study aims at identifying breed and tissue-specific key regulators in pigs by using co-expression and co-association gene network analysis. For that purpose, duodenum, liver, and muscle samples were obtained at slaughter from 300 pigs of 3 different breeds: Duroc, Landrace, and Large White (n = 100 each). Whole-genome sequencing and RNA-seq were performed on the Illumina NovaSeq6000 platform. RNA counts were quantified by RSEM/1.3.0 and normalized by TMM (trimmed mean of M-values). Lowly expressed genes and those missing in more than 20% of the animals were removed, remaining 13,891 genes expressed in duodenum, 12,748 genes in liver and 11,617 genes in muscle. Genetic variant calling, conducted with GATK/4.1.8.0 HaplotypeCaller, resulted in 44,127,400 polymorphisms (SNPs and indels) among all the individuals. After removing those variants with a minor allele frequency below 5% and more than 10% missing genotype data on each breed, 25,224,146 polymorphisms were kept among the 3 breeds. eGWAS were conducted using the fastGWA tool from GCTA/1.93.2. Gene co-expression networks were inferred using the PCIT algorithm and the list of genes encoding transcription factors (n = 1,109) and co-factors (n = 869), and the 100 most expressed genes in each tissue. Thus, a total of 2,248 genes remained to generate regulatory gene networks. Across breeds the transcription factors *CTCF*, *SP3* and *TOX4* and the cofactors *CHTOP*, *ELOB* and *NCL* were identified as the highest connected regulators in the duodenum, liver and muscle co-expression gene networks, respectively. Within breeds, *KHSRP*, *TOX4* and *CSDE1* transcription factors and *HNRNPU*, *CRK* and *CRK* cofactors showed the highest connectivity in Duroc, Landrace and Large White, respectively. Our results identified putative key regulatory genes that will be reassessed by the eGWAS analysis. These findings bring us closer to understanding gene expression regulation in porcine key tissues and will allow us to improve genomic evaluation procedures by considering this functional information. This study is part of the H2020 GENE-SWitCH project (grant agreement n° 817998).

Key Words: pig, genome regulation, network analysis, system genetics, RNA-Seq

OP49 ISAG Bursary Award: Allele-specific expression in pig genomic makeup and phenotypic implications. W.-Y. Yao*[1,2], L. Bai[2], K. Li[2], L. Fang[3], M. A. M. Groenen[1], and O. Madsen[1], [1]*Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands,* [2]*Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China,* [3]*Center for Quantitative Genetics and Genomics (QGG), Aarhus University, Aarhus, Denmark.*

Allele-specific expression (ASE) is the imbalance in expression between parental alleles at the same locus, which can be identified and quantified by RNA sequencing. ASE is often associated with the *cis*-regulation of expression quantitative trait loci (eQTL). Therefore, identifying ASE variants can provide insight into the transcriptomic control of complex traits in pigs. This work, based on PigGTEx, has 3 objectives: (1) Establish an atlas of ASE profiles among different breeds and tissues. (2) study the correlation between ASE and pig-eQTL and, (3) evaluate functional implications for specific sites and traits (e.g., introgression sites and GWAS). We selected 6,655 RNA samples, consisting of 23 pig breeds and 42 pig tissues from the Pig-GTEx RNA sampling. First, we developed a standardized computational pipeline to reduce reference mapping bias. The pipeline is based on the WASP method and provides accurate ASE profiling for large and complex RNA-seq data sets. We then used phASER software to identify the vast profile of ASE in different breeds and tissues as well as the haplotype level based on phASER Gene AE 1.2.0. The magnitude of the imbalance was quantified by allelic fold change (aFC), and the statistical significance of the imbalance was evaluated using binomial-based statistics. We detected a median number of 11,846 detectable expressed sites and 504 significant ASE sites for these RNA samples. We found many variations in ASE across tissues and breeds, ranging from 2.04% to 34.48%, suggesting complex genomic regulation of allelic expression. The highest ASE diversity was observed in the embryo and reproduction-related tissues. For future analyses, we will correlate the ASE with eQTL to decipher the effect of ASE. The identified ASE profiles in the breeds or tissues studied provide a valuable foundation for identifying the molecular regulatory codes driving complex traits and improving genomic prediction in pig breeding programs.

Key Words: pig, genome regulation, allele-specific expression, system genetics (eQTL), complex trait

OP50 Combined targeted and untargeted metabolomics in pigs coupled with genomic information: Towards a comprehensive genetic characterization of the pig metabolome. S. Bovo[1], G. Schiavo[1], F. Fanelli[2], A. Ribani[1], F. Bertolini*[1], M. Gallo[3], G. Galimberti[4], S. Dall'Olio[1], P. Martelli[5], R. Casadio[5], U. Pagotto[2], and L. Fontanesi[1], [1]*Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Bologna, Italy,* [2]*Department of Surgical and Medical Sciences, Endocrinology Unit, University of Bologna, Bologna, Italy,* [3]*Associazione Nazionale Allevatori Suini, Roma, Italy,* [4]*Department of Statistical Sciences "Paolo Fortunati," University of Bologna, Bologna, Italy,* [5]*Biocomputing Group, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy.*

Understanding the biological mechanisms governing the pig metabolism is fundamental for the development of new applications aimed at improving pig production efficiency. Production and reproduction traits are the final result of the molecular mechanisms taking place in an organism, that is the interplay within and between the biological layers encompassing the genome, proteome and metabolome spaces. Thus, the study of each layer allows to deconstruct such complex phenotypes in their small components providing new insights into their biology and additional new simpler phenotypes. Here, we characterized the genome and metabolome spaces to understand the genetic architecture governing the pig metabolism. To this purpose, targeted and untargeted metabolomic platforms were combined to analyze the abundance of more than 1000 plasma metabolites in about 1300 heavy pigs, including 900 Italian Large White and 400 Italian Duroc pigs, that were genotyped with a high-density SNP panel. For each breed, metabolomics profiles

were used to study the metabolite-metabolite relationships via a network approach. The networks reconstructed for both breeds resulted similar, though differences emerged, with poorly interconnected modules. Then, metabolomics and genomics data were coupled to study the effect of genome variability over the metabolome via genome-wide association studies (GWAS). Different genomic scans were carried out, including single-marker and haplotype-based analysis of both single metabolites and metabolite ratios. Moreover, whole-genome sequencing data were used for the identification of putative causative mutations. GWAS analyses allowed to detect several quantitative trait loci, most of them including genomic regions carrying enzyme-encoding genes known to control the analyzed metabolites. Overall, we obtained for the first time a comprehensive catalog of genes and variants linked to the pig metabolism, opening new scenarios for the improvement of pig production systems.

Key Words: functional genomics, genome-wide association, metabolomics, pig and related species

OP51 ISAG Bursary Award: Enhancer-promoter interaction map in the maternal-fetal interface during implantation reveals important regulatory regions and variations in pigs. Y. Sun*[1,2], R. Liu[1,2], H. Liang[1,2], K. Han[1,2], F. Wang[1,2], J. Cao[1,2], and M. Yu[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei, China,* [2]*College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China.*

Litter size, one of the most important reproductive traits of pigs, has pronounced effects on the profit of husbandry enterprises' enthusiasm of breeders. In addition, the litter size of pigs was directly affected by embryo implantation failure. Genome-wide association studies (GWAS) have successfully identified genetic variants associated with complex traits and diseases in the past decade. However, ~88% of those variants from GWAS studies are in non-coding regions of the genome and have been challenging to interpret. This study conducted RNA-Seq, ChIP-Seq, BL-HiC, and BL-HiChIP on luminal epithelium cells (LE) and endometrial tissue at d 12 (GD12) and d 15 of gestation (GD15). First, the differentially expressed genes (DEGs) were identified. Then, we identified active promoter regions and potential enhancer regions on the genome by H3K4me3 and H3K27ac modification. Next, the enhancer-promoter interactions were defined by combining Hi-C and Hi-ChIP data. The genes regulated by enhancer-promoter interactions were identified, including 642 DEGs between GD12 and GD15. Subsequently, we used HOMER to identify TF motif enrichment at the loop anchors within the 642 DEGs. Key TFs, such as C/EBP-β and NR4A1, were highly enriched in the loop anchors in the upregulated genes. Finally, we identified SNPs that locate in the transcription factor motifs and affect gene expression by altering the transcription factor binding. In conclusion, the high-resolution enhancer-promoter interaction map of pig endometrial tissue was constructed, and the key regulatory elements were identified. These findings provide insights in identifying the mechanisms of litter size in pigs.

Key Words: pig, litter size, embryo implantation, *cis*-regulatory element, SNP

OP52 ISAG Bursary Award: On the genetic basis of porcine semen traits: A large-scale genome-wide study on a synthetic line. P. Sá*[1], R. Godinho[2], M. Gòdia[1], C. Sevillano[2], B. Harlizius[2], O. Madsen[1], and H. Bovenhuis[1], [1]*Wageningen University and Research, Wageningen, the Netherlands,* [2]*Topigs Norsvin Research Center, Beuningen, the Netherlands.*

The pig industry is highly dependent on the production and commercialization of high-quality pig semen. Commercial boars are subject to regular collections and routine evaluations of semen quality, resulting in massive phenotypic data sets. The combination of automated phenotyping (CASA) and genomic tools allows for novel and unique opportunities to study the genetic background of boar semen characteristics. In this study, we estimated variance components for 14 semen traits to determine the extent to which these characteristics are influenced by systematic environmental factors and conducted a large-scale genome-wide association study to identify variants associated with these traits. Among others, total number of sperm cells and single morphological abnormalities were considered. Ejaculates were collected between 2007 and 2022 and evaluated using CASA system. The complete phenotypic data set included records from a total of 465,598 ejaculates collected from 5,758 commercial synthetic line boars, averaging 80 ejaculates per boar. Pedigree information included 17,701 animals spanning across 24 generations. Genetic parameters were estimated following a repeatability model. Genotype data included a total of 3,010 boars, with genotypes imputed to 660K SNP data. Preliminary results indicate moderate heritabilities; 0.20 for total number of cells, 0.18 for total motility and total abnormality rate. Repeatability estimates were 0.41 for total number of cells, 0.56 for total motility and 0.48 for total abnormality rate. The age of the boar at collection and the interval between collections were the most striking environmental effects. AI Station – Year – Season (AYS) of collection and temperature at collection, among others, were also found to be significant. The GWAS revealed several highly significant genomic regions that contained genes related to spermatogenesis and embryo development. Our results introduce new insight into the genetic nature of semen traits in pigs.

Key Words: pig and related species, genome-wide association, single nucleotide polymorphism (SNP), heritability, environment

OP53 Towards identification of new genetic determinants for post-weaning diarrhea in piglets. E. Ibragimov, E. Ø. Eriksen, J. P. Nielsen, C. B. Jørgensen, M. Fredholm, and P. Karlskov-Mortensen*, *University of Copenhagen, Frederiksberg, Denmark.*

Post-weaning diarrhea in pigs is a considerable challenge in pig farming industry due to its effect on animal welfare and production costs, and also due to the large volumes of antibiotics, which are used to treat diarrhea in pigs after weaning. Previous studies have revealed loci on SSC6 and SSC13 associated with susceptibility to specific diarrhea causing pathogens. However, post weaning diarrhea is a complex syndrome, which can be caused by a multitude of pathogens and, additionally, inherent factors in the pig may affect its overall susceptibility to diarrhea. Hence, this study aimed to identify new genetic loci for resistance to diarrhea based on phenotypic data. In-depth clinical characterization of diarrhea was performed in 258 pigs belonging to 2 herds during 14 d post weaning. The daily diarrhea assessments were used for classification of pigs into case and control groups. Genome-wide association studies (GWAS) and metabolomics association analysis were performed to identify new biological determinants for diarrhea susceptibility. With the present work we have revealed a new locus for diarrhea resistance on SSC16 specific to one of the studied herds. Furthermore, studies of metabolomics in the same pigs revealed one metabolite associated with diarrhea.

Key Words: pig, post-weaning diarrhea, AMR, GWAS

OP54 Identification of genomic regions associated with fatty acid metabolism across four tissues in pigs. J. Liu*[1,2], C. Sebastià[1,2], T. Jové-Juncà[3], R. Quintanilla[3], O. González-Rodríguez[3], M. Passols[1,2], A. Castelló[1,2], A. Sánchez[1,2], M. Ballester[3], and J. M. Folch[1,2], [1]*Plant and Animal Genomics, Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB Consortium, Bellaterra, Spain,* [2]*Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain,* [3]*Animal Breeding and Genetics Program, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Torre Marimon, Caldes de Montbui, Spain.*

Fatty acids (FAs) are components of lipids and have important roles such as structural components of cell membranes, cellular fuel sources, and precursors of signaling mediators. This study aims at iden-

tifying potential genomic regions associated with FA profiles in several tissues and explores their role on whole body metabolism in pigs. A total of 432 commercial Duroc pigs were employed in the present work. Samples of blood were collected at 60 ± 8 d of age to extract the plasma. In addition, samples of adipose tissue (backfat), liver, and gluteus medius muscle were collected after slaughter (180–200 d of age). All animals were genotyped with the GGP Porcine HD Array (Illumina). Genotypes were imputed from the whole-genome sequences of 100 animals and SNPs with MAF <5% or missing genotypes >10% were removed. GWAS was performed between the 9,751,141 resulting SNPs and FA composition traits by the *fastGWA* tool of GCTA v1.94.0. The genomic regions containing at least 3 significant consecutive SNPs with distances <1 Mb were selected for gene annotation. The GWAS results showed a common interval at SSC2: 7.56–14.92 Mb associated with the desaturase 5 activity in liver, backfat and muscle. Another interval located at SSC14: 103.81–115.64 Mb was identified for backfat and muscle FA composition, including UFA, SFA, C18:0, C18:1n7, C16:1n7/C16:0, and C18:1n9/C18:0. In addition, backfat-specific intervals were identified at SSC6: 146.07–148.36 Mb for MUFA, SFA, UFA, C18:1n9, and C18:1n9/C18:0 and at SSC4: 2.53–14.55 Mb for C14:0, C20:1n9, C20:2n6, C20:3n3, and C16:0/C14:0. In SSC15, a region at 86.99–101.29 Mb was associated with liver C18:4n3/C18:3n3. Finally, for plasma, the specific region SSC14: 118.92–124.75 Mb was associated with C18:0/C16:0. The current results increase our knowledge of the genetic architecture of FA-metabolism traits and will be useful in selection programs to improve health and energy metabolism in pigs. This study is part of the METAPIGEN (PID2020-112677RB-C21-22) and H2020 GENE-SWitCH (grant agreement no. 817998) projects.

Key Words: pig, genome-wide association, lipid, genetic marker, candidate gene

OP55 ISAG Bursary Award: Integrated analysis of genome-wide association studies and 3D epigenomic characteristics reveal the *BMP2* gene regulating loin muscle depth in Yorkshire pigs. S. Wan*[1], Y. Miao[2], Y. Zhao[1], S. Zhao[1], X. Xu[1], and T. Xiang[1], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture, Huazhong Agricultural University, Wuhan, Hubei Province, China,* [2]*Research Institute of Agricultural Biotechnology, Jingchu University of Technology, Jingmen, Hubei Province, China.*

The lack of integrated analysis of genome-wide association studies (GWAS) and 3D epigenomics restricts the deep understanding of genetic mechanisms of meat-related traits. With the application of such techniques as ChIP-seq and Hi-C, the annotations of *cis*-regulatory elements in the pig genome have been enriched, which offers a new opportunity to elucidate the genetic mechanisms and identify major genetic variants and candidate genes that are significantly associated with the important economic traits. Among these traits, loin muscle depth (LMD) is an important one as it largely affects the lean meat content. In this study, we integrated *cis*-regulatory elements and genome-wide association studies (GWAS) to identify the key gene and genetic variants regulating LMD. Five single nucleotide polymorphisms (SNPs) located on porcine chromosome 17 were identified to be significantly associated with LMD in Yorkshire pigs by GWAS. A 10 kb quantitative trait locus (QTL) was identified as a functionally important region by integrating linkage disequilibrium and linkage analysis (LDLA) and high-throughput chromosome conformation capture (Hi-C) analysis. Based on the results of GWAS, Hi-C, and *cis*-regulatory elements, the *BMP2* gene was identified as the major gene regulating variation in LMD. Furthermore, through using dual-luciferase assays and electrophoretic mobility shift assay (EMSA), 2 SNPs, SNPs rs321846600 and rs1111440035 were identified as candidate SNPs that may be functionally related to the LMD QTL in Yorkshire pigs. Our results shed light on the advantage of integrating GWAS with 3D epigenomics in identifying major genes for quantitative trait. This study is the pioneering work to identify the major genes and related genetic variants regulating one key production trait (LMD) in pigs by integrating genome-wide association studies and 3D epigenomics.

Key Words: GWAS, epigenomics, integrated analysis, loin muscle depth, pig

OP56 ISAG Bursary Award: Sequence-based GWAS identifies novel loci influencing growth and reproduction traits in pigs. A. Boshove*[1], M. F. L. Derks[1,2], B. Harlizius[1], E. F. Knol[1], M. S. Lopes[3], M. van Son[4], and C. A. Sevillano[1], [1]*Topigs Norsvin Research Center, Beuningen, the Netherlands,* [2]*Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands,* [3]*Topigs Norsvin, Curitiba, Brazil,* [4]*Norsvin SA, Hamar, Norway.*

Genome-wide association studies (GWAS) based on large-scale sequence data provide opportunities to map recessive deleterious variants in livestock populations using a non-additive model. Most deleterious variants segregate at relatively low frequency and therefore high sample sizes are required to identify these variants. In this study we report one of the largest sequence-resolution screens in pigs to date, with a total of 117,000 Large White animals imputed to sequence using a reference population of approximately 1,100 whole-genome sequenced pigs. We imputed a total of 22,000,000 SNPs with high accuracies (R^2 > 0.9) even for low frequency variants (>1–5% minor allele frequency). Using this sequence data we performed both an additive and non-additive GWAS for several production and reproduction traits. We observe a clear difference in the QTLs found by the additive and non-additive models. We fine mapped known QTLs to identify causal variants using the additive model and revealed a new, relatively low frequent variant on chromosome 2 with a large effect on back fat. The non-additive model especially yielded novel low frequency variants affecting the fitness of animals (i.e., reduced growth, smaller litter size). One of the most notable deleterious variants we found is located on chromosome 2 with major impact on both growth and back fat in homozygous individuals. Additionally, we identified several independently segregating haplotypes with strong effects around the *MC4R* locus on chromosome 1, spanning only 3 MB in size (1:59–62) and affecting both growth and back fat. Together we present a large-scale sequence-based association study that provides a key resource to identify novel variants for breeding and to further reduce the frequency of deleterious alleles.

Key Words: pig and related species, genome-wide association, imputation, quantitative trait locus (QTL)

OP57 Methods to predict lameness in sows. G. A. Rohrer*[1], L. Ostrand[2], L. A. Rempel[1], T. Schmidt[2], and B. Mote[2], [1]*USDA-ARS US Meat Animal Research Center, Clay Center, NE,* [2]*University of Nebraska, Lincoln, NE.*

The objective was to identify traits recorded on gilts at 5 mo of age predictive of future lameness. Mobility was measured using a pressure-sensing mat (GAIT4) and 7 d of video recorded daily activity (NUtrack). Gilts (n = 3659) were assigned codes to describe their lifetime soundness. Animals retained for breeding and never detected with mobility issues were recorded as sound (SND), while retained animals that became lame were coded as lame sow (LSW). Culled gilts were in 3 categories: culled for leg structure (STR), visibly lame gilt (LGT) and other reasons (CLL). GAIT4 system creates a series of measurements for each foot related to pressure, duration and step length of each foot and a lameness score for each foot. Traits used to predict an animal's mobility status summarized values for all 4 feet: average step length, average stance time, standard deviation of stance time, variance of lameness score and variance of total scaled pressure. NUtrack measurements were rotations, velocity, distance walked and times spent eating, sitting, standing, lying sternal and lying lateral. Mixed model analyses were conducted in R fitting fixed effects of breed of sire, contemporary group and soundness score, with animal fit as a random effect. Heri-

tability was estimated using animal effects from R models as phenotypes in WOMBAT, with 3 generations of pedigree. Analyses of GAIT4 measures found LGT and STR gilts had longer average stance time, greater variance of lameness scores and took shorter steps; estimates of heritability ranged from 0.23 to 0.28. NUtrack measurements predictive of soundness score were time eating, time standing, time lying lateral, distance walked and rotations. LGT and STR were less active and spent more time lying lateral than other animals. In addition, SND animals had more rotations and tended to have greater distance than LSW. Estimates of heritability for NUtrack measurements ranged from 0.21 to 0.31. Overall, NUtrack traits at 5 mo of age predicted soundness beyond gilt status and were heritable providing producers with traits to select gilts and improve mobility of future generations of pigs. USDA is an equal opportunity employer.

Key Words: pig, lameness, prediction, heritability

Plenary Session II: Exploring Genomic "Big" Data

OP58 Big data integration in the era of animal omics: Current and future challenges. L. Fang*, *QGG, Aarhus University, Aarhus, Denmark.*

Understanding how the central dogma works from genome to phenome and how it evolves within and across species remains a fundamental challenge in biology, genetics, and evolution. In farm animals like cattle, pig, and chicken, phenotypes of economic and environmental value (e.g., milk production and feed efficiency) lie predominantly in polygenic or even omnigenic traits. The vast majority (>90%) of genomic variants associated with such phenotypes are non-coding with minor effects and act by modulating intermediate molecular phenotypes (e.g., gene expression and protein abundance). Therefore, the systematically functional characterization of genomic variants (i.e., understanding the central dogma) through integrating large and complex multi-omics data (e.g., epigenome, transcriptome, proteome, metabolome and microbiome) is essential for illustrating the molecular mechanisms underlying these complex phenotypes, understanding adaptation/domestication, optimizing current genetic improvement programs, facilitating the future precision breeding, and allowing comparative genomics at functional level (i.e., evolution). Although the recent availability of large omics data in farm animals such as those from FAANG and FarmGTEx projects allows some pilot integrative genomics studies, there are still big challenges lying in this field, including data generation, data sharing, data preprocessing, missing data imputation, statistical modeling, results displaying and customized interactive reanalyzing. In the presentation, I will use the FarmGTEx project, which aims to provide a public resource of functional variants in distinct biological contexts across farmed species, as an example to discuss the current and future challenges in this promising field of integrative genomics in animals.

OP59 Microbiome solutions for improving the sustainability of cattle production. L. Guan*, *Department of Agricultural, Food and Nutritional Science, University of Alberta, Alberta, Canada.*

There is growing evidence that microbes within the rumen have both beneficial and detrimental impacts on cattle productivity and GHG emissions. The rumen microbiome consists of diverse and abundant microorganisms including bacteria, archaea, protozoa, fungi and viruses that work collectively to break down plant fiber and the subsequent microbial fermentation produces nutrients and energy substrates used by the cattle host. The changes in the presence/absence or abundance of select microbes within the rumen microbiome can alter the overall nutrient support to the animals and together with their metabolites, it can directly affect many economically important traits such as feed efficiency, methane emission, milk/meat yield and quality, and health. Global beef and milk production is projected to increase by 1.2% and 1.1% annually to 2050, highlighting the urgent need to develop novel tools and technologies targeting the rumen microbiome to reduce CH_4 emissions and improve feed efficiency in both beef and dairy cattle. Therefore, this presentation will highlight rumen microbiome research in beef and dairy cattle to date, and how the information generated can be applied to future strategies to improve production efficiency and environment-friendliness in cattle production.

Key Words: rumen microbiome, feed efficiency, methane emission

International Goat Genome (IGGC)

OP60 Combining ATAC-Seq and RNA-Seq data to investigate the molecular basis of lactation in goats. A. Noce*[1], M. Luigi-Sierra[1], A. Martínez[2], M. Wang[1], M. Macri[2], J. Delgado[2], A. Salama[3], X. Such[3], J. Jordana[3], and M. Amills[1,3], [1]*Centre de Recerca Agrigenòmica (CRAG), Campus Universitat Autònoma de Barcelona, Bellaterra, Spain,* [2]*Departamento de Genética, Universidad de Córdoba, Córdoba, Spain,* [3]*Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Spain.*

The goal of this study is to investigate the molecular mechanisms involved in milk synthesis in goats. Total RNA has been extracted from the mammary gland of lactating (n = 3) and dry (n = 3) goats and sequenced in a NovaSeq6000 (Illumina) platform. Sequencing quality was evaluated with the FastQC software v0.11.7. Raw reads were trimmed to remove adaptors and low-quality reads, using TrimGalore 0.5.0 tool. Trimmed reads were then aligned with the goat ARS1 reference genome using HISAT2 software and the total count of mapped reads was obtained using the featureCounts tool. In parallel, we obtained mammary gland tissue from lactating (n = 3) and dry (n = 3) goats, and it was submitted to the Active Motif company (https://www.activemotif.com/) to carry out ATAC-seq. ATAC-Seq reads quality was also evaluated with FastQC, aligned to the ARS1 goat genome using BWA-MEM tool, and post-alignment quality control was performed with the ATAQseqQC software. Peak calling was performed using the MACS2 software. Consensus peaks between replicates were obtained with DIffbind. To increase statistical power and provide a more reliable differential accessibility regions (DARs) analysis, only the peaks overlapping in at least 2 of the samples (consensus = 58,826) have been used. Both differential gene expression (DGE) and DARs analyses have been performed with DESeq2. Differential expression analysis revealed 1,342 downregulated and 1,034 upregulated genes (|FoldChange| > 1.5 and q-value < 0.05) in the milking condition. On the other hand, DAR analysis showed 3,867 significant DAR. Among these, 2,392 regions were enriched in milking goats while 1,475 were enriched in the dry condition (|FoldChange| > 1.0, q-value < 0.05). The integration of ATAC-Seq and RNA-Seq is currently underway. Genes that are DE and map close to DARs will be functionally characterized. Additionally, a genome annotation of the DARs will be carried out to identify which regulatory elements correspond to accessible regions co-localizing with DE genes. The results will shed light on the role of epigenomics in the regulation of gene expression in the mammary gland of lactating goats.

Key Words: ATAC-seq, RNA-seq, goat and related species

OP61 ISAG Bursary Award: Identification of long non-coding RNAs differentially expressed in the mammary gland of lactating and dry goats. M. Wang*[1], E. Varela-Martínez[1], M. Luigi-Sierra[1], A. Noce[1], A. Martínez[2], J. Delgado[2], A. Salama[3], X. Such[3], J. Jordana[3], and M. Amills[1,3], [1]*Centre de Recerca Agrigenòmica (CRAG), Campus Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain,* [2]*Departamento de Genética, Universidad de Córdoba, Córdoba, Córdoba, Spain,* [3]*Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

Long non-coding RNAs (lncRNAs) have recently attracted attention due to their role as potential regulators of gene expression. In this work, we aimed to characterize the catalog of caprine mammary lncRNAs in lactating and dry individuals and to compare their levels of expression. To achieve this goal, we have used one data set including 7 Murciano-Granadina goats sampled at 3 time points (early lactation, late lactation, and dry period) that was reported in a previous study. Moreover, we have generated a second independent data set consisting of 5 lactating and 5 dry goats to identify lncRNAs with consistent differential expression in both data sets. Total RNA preparations have been sequenced with an Illumina Hiseq 4000 machine. After quality control using Trimmomatic, reads aligned to SILVA rRNA database (release 138.1) were removed with the bbduk software. Then, clean reads were aligned to the goat ARS1 reference genome with the STAR software, and the transcriptome was assembled with Stringtie. We obtained an average of 65 and 78 million reads for the first and second data sets, respectively. After classifying transcripts with the gffcompare software, lncRNAs were screened according to class code ('u', 'i', 'x', 'o') and length. Then, CPC2, CPAT, LGC software and PFAM database were used to detect the coding potential of transcripts as well as the presence of protein domains. Subsequently, candidate lncRNAs were quantified with Kallisto to calculate the gene level of gene expression, and the edgeR software was used to perform differential expression analysis. By doing so, we detected 3,974 lncRNAs transcripts expressed in the goat mammary gland. Moreover, 950 and 546 lncRNA genes were differentially expressed between the lactation and dry stages in the first and second data sets, respectively. Noteworthy, 412 lncRNA genes were consistently identified as differentially expressed in both data sets.

Key Words: LncRNA, RNA-seq, goat, lactation

OP62 Genomic improvement in dairy goats using DNA sequencing. A. Caulton*[1], M. Wheeler[2], S. Clarke[1], R. Brauning[1], T. Van Stijn[1], H. Baird[1], R. Anderson[1], B. Foote[3], J. Foote[3], S. Cameron[4], T. Blichfeldt[5], J. Jakobsen[5], K. Dodds[1], and J. McEwan[1], [1]*AgResearch, Mosgiel, Otago, New Zealand,* [2]*AgResearch, Hamilton, Waikato, New Zealand,* [3]*Footes, Hikurangi, Northland, New Zealand,* [4]*Meredith Dairy, Meredith, Victoria, Australia,* [5]*NSG, As, Norway.*

In dairy goats, industry uptake of genomic technologies has been slow due to the small size of the industries coupled with the limited market for SNP array-based technologies. However, the potential benefits of genomic selection in dairy goats are large, because key traits are sex limited, recorded post selection and pedigree recording in large dairy goat herds is problematic. This has led our laboratory to utilize 2 low-cost genotyping strategies based on sequencing: RE-RRS and GT-seq. Historically, these approaches have suffered, in part because they are subject to missing or probabilistic genotyping calls. This made them difficult to integrate with existing genetic evaluation software. The sequencing-based technologies described above are currently used for separate genetic evaluations in Australia, New Zealand and Norway, with more than 87,000 samples genotyped to date using RE-RRS with more than 56K SNPs reported. Evaluation methodology and results for an example herd genetic trend will be presented, which for a 290 lactation length lactation show improvements of 26 L/doe/year and 4.51 kg cheese/doe/year over the last 5 year period.

Key Words: goat, genomic selection, sequencing, dairy, genotyping-by-sequencing

OP63 Heritability estimates of hematological, serological, morphological and productive traits in Murciano-Granadina goats, using a univariate animal model. M. Macrì[1,2], M. Amills[3,4], J. León Jurado[5], L. Gama[6], M. Luigi-Sierra[3], J. Delgado[2], J. Fernández[7], and A. Martínez Martínez*[2], [1]*Animal Breeding Consulting, Córdoba, Spain,* [2]*Universidad de Córdoba, Córdoba, Spain,* [3]*CRAG, CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, Bellaterra, Spain,* [4]*Universitat Autònoma de Barcelona, Bellaterra, Spain,* [5]*Diputación Provincial de Córdoba, Córdoba, Spain,* [6]*Universidad de Lisboa, Lisboa, Portugal,* [7]*Asociación Nacional de Criadores de Caprino de Raza Murciano-Granadina (CAPRIGRAN), Granada, Spain.*

Eighteen variables corresponding to hematological, serological, morphological and productive traits from 3254 Murciano-Granadina goats, collected during 2016–2018, were analyzed to estimate variance components and heritability using a restricted maximum likelihood (REML) approach. The MTDFREML set of programs was used to obtain restricted maximum likelihood estimations of genetic parameters, with a relationship matrix including 64,424 animals. A single-trait animal model was used to carry out the above task, using either single-records animal models (hematology, serological and morphology traits) or animal models with repeated measures (milk yield and components). Heritability estimates for hematological traits were 0.23 ± 0.08, 0.17 ± 0.07, 0.22 ± 0.08 and 0.25 ± 0.09 for red blood cells, hemoglobin, hematocrit and leucocytes, respectively. Heritability estimates for the various morphological traits were 0.18 ± 0.09, 0.20 ± 0.07, 0.12 ± 0.07, 0.08 ± 0.08 and 0.07 ± 0.07 for total classification, structure, dairyness, mammary system and feet and legs scores, respectively. For milk production and composition traits, the heritability estimates were 0.19 ± 0.05 for milk yield; 0.15 ± 0.05 and 0.27 ± 0.06 for fat yield and percentage; 0.21 ± 0.06 and 0.41 ± 0.08 for protein yield and percentage; and 0.17 ± 0.05 and 0.31 ± 0.06 for dry matter yield and percentage, respectively. Finally, the heritability estimates for serological traits were 0.02 ± 0.13 for agalactia and 0.05 ± 0.10 for CAEV (caprine arthritis encephalitis virus). The results of this study show that in Murciano-Granadina goats heritability estimates for dairy, morphology and hematology traits are moderate, while those for serology phenotypes appear to be really low.

Key Words: goat and related species, animal breeding, bioinformatics tools, heritability, milk production

OP64 Ascertaining the variability and demographic history of the Canarian goat breeds through the use of genome-wide SNPs data. G. Senczuk*[1], M. Macrì[2,3], S. Mastrangelo[4], M. Di Civita[1], M. del Rosario Fresno[5], J. Capote[5], F. Pilla[1], J. V. Delgado[3], M. Amills[6], and A. Martínez[3], [1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy,* [2]*Animal Breeding Consulting S.L, Córdoba, Spain,* [3]*Universidad de Córdoba, Córdoba, Spain,* [4]*Department of Agricultural, Food and Forest Sciences, University of Palermo, Palermo, Italy,* [5]*Instituto Canario de Investigaciones Científicas, Tenerife, Spain,* [6]*CRAG, CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, Bellaterra, Spain.*

The Canary Islands are home to more than 320,000 goats representing one of the most important economic resources, in terms of milk production and cheese industry. The presence of goats in the Canary Islands might trace back to the early 1st millennium BC, when settlers of Berber origin colonized the archipelago. Considering the relevance of goat genetic resources for the economy of the Canary Islands and the susceptibility of local insular populations to the loss of genetic diversity, we aimed to assess the genetic variability and origins of Canarian local breeds. To do so, we have genotyped, with the Goat SNP50 BeadChip (Illumina), 224 individuals belonging to 4 Canarian breeds (Palmera, Mejorera, South Tinerfeña and North Tinerfeña). Moreover, we have retrieved SNP data from 1,007 individuals from Africa and Southern Europe that were genotyped in the AdaptMap project. After filtering for missing call rate and minor allele frequency, we obtained a final data set of 45,149 SNPs. Diversity indices of the Majorera (Ho = 0.38, He = 0.385, F_{ROH} = 0.03), North Tinerfeña (Ho = 0.363, He =

0.364, F_{ROH} = 0.052) and South Tinerfeña (Ho = 0.36, He = 0.364, F_{ROH} = 0.034) breeds showed values in line with those of other breeds, while the Palmera breed displayed lower levels of genetic variation (Ho = 0.307, He = 0.309, F_{ROH} = 0.103). The strong genetic differentiation of the Canarian breeds resulted evident in all the analyses we performed, confirming a relationship with Northern African breeds. The Admixture and the TreeMix analyses did not suggest the existence of gene flow between Canarian goats and other continental breeds. This result is fairly unexpected, especially when considering that during the Age of Exploration the Canary Islands were an important maritime port of call, a circumstance that might have favored the exchanges of livestock genetic resources. This work represents a first step toward the genetic characterization of the Canarian goat breeds, paving the way for conservation of these invaluable insular genetic resources.

Key Words: goat and related species, conservation genomics, population genomics, biodiversity, breed diversity

OP65 The extreme genotypes of *CSN1S1* gene have a significant effect on milk composition and cheese yield in Carpathian goat. V. A. Balteanu*[1], R. K. Sigartau[2], D. Nadolu[3], and A. H. Anghel[4], [1]*University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Institute of Life Sciences, Cluj-Napoca, Cluj, Romania,* [2]*Babes-Bolyai University, Faculty of Mathematics and Computer Science, Cluj-Napoca, Cluj, Romania,* [3]*ICDCOC Palas, Constanta, Constanta, Romania,* [4]*Ovidius University, Constanta, Constanta, Romania.*

Goat milk represents in many European countries a valuable raw material for cheese industry due to an increased demand for cheeses, which are produced mainly from specialized breeds, i.e., Alpine, Saanen or Murciana. Carpathian goat population from Romania is over 1.5 million heads, occupying the third place in Europe. They are raised on extremely diverse natural pastures from Transylvanian hilly areas to southern Danubian plains. This imprints to milk a specific flavor and a high nutritional value. However, a high variability in cheese yield was reported by breeders. In goat *CSN1S1* locus a wide variety of mutational events were characterized, accounting for 20 alleles. They are classified according to the expression levels (3.5 to 0 g/L) in strong (A, B), medium (E), weak (F) or null (0) alleles. The defective F allele is characterized by a cytosine deletion at the 9th exon, which causes a drop in α_{S1}-casein synthesis from 3.5 to 0.45 g/L. Although *CSN1S1* gene polymorphism might be a major determinant of goat milk composition variability, this effect might vary depending on the breed. To determine nationwide the frequency of F allele we genotyped over 3000 Carpathian goats. Additionally, we tested in 150 goats, exhibiting extreme *CSN1S1* genotypes (ex. AA, AF and FF), the associations with milk composition (ex. whole protein, casein, fat, nonfat solids, lactose) and cheese yield. We found a high frequency of the defective F allele (0.27). The association tests highlighted significant differences between investigated genotypes, particularly for whole casein (AA: 3.00 ± 0.10^{a+}; AF: 2.64 ± 0.13^{b}; FF: 2.58 ± 0.11^{b+}) and whole protein (AA: 4.07 ± 0.13^{a+}; AF: 3.58 ± 0.17^{b}; FF: 3.50 ± 0.14^{b+}) contents and for liters of milk needed/kg of cheese (AA: 6.62 ± 0.47^{a} AF: 7.58 ± 0.47^{a}; FF: 8.11 ± 0.47^{b}). If we extrapolate nationwide these results, we can assume that more than 405,000 individuals carry at least one copy of the defective F allele. This could be translated in important economic losses. We concluded that selection for strong alleles could represent a valuable tool to improve these traits in Carpathian goat and to fulfill European market demands for goat cheeses.

Key Words: goat, milk, *CSN1S1*, cheese

Animal Epigenetics

OP66 Annotation of functional variations in four livestock genomes utilizing *cis*-regulatory elements datasets. R. Ma*[1], R. Kuang[1], M. Hu[1], Y. Guo[1], D. Wang[1], H. Zhou[1], Z. Han[1], L. Li[1], Z. Xu[1], Y. Zhang[1], Y. Zhao[1], X. Li[1,2], and S. Zhao[1,2], [1]*Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education and Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China,* [2]*Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan, China.*

With the widespread application of high-throughput sequencing in livestock, numerous variations were identified, particularly single nucleotide polymorphisms (SNPs) and small insertions/deletions (InDels), which are useful for improving the production and reproduction of agricultural animals. However, effective methods for screening countless genome variations in livestock research are still lacking, posing abundant challenges to genome breeding and production. Therefore, this research collected phenotypic data of 4 species and identified 6 categories of genome functional elements, including nucleosome-free regions (NFRs), open chromatin regions (OCRs), transcription factor binding sites (TF binding sites), footprints, motifs, and narrow peaks of H3 lysine 27 acetylation (H3K27ac). By classifying variations in pig, cow, sheep, and chicken based on functional regions and the likelihood of transcription factor (TF) binding, a new research approach and direction for effectively identifying important SNPs and small InDels was proposed. Approximately 64 million, 97 million, 63 million, and 23 million variations were collected in pig, cattle, sheep, and chicken, respectively. Of these, around 19 million, 39 million, 8 million, and 11 million variations were identified as potential functional mutations. The researchers developed a database called IFmutants, which confirms the necessity of SNPs and small InDels in the genomes of pig, cow, sheep, and chicken. SNPs and small InDels were categorized into 1–5 levels using their ranking scoring system in IFmutants and provided relevant motif lists and images of the variations simultaneously. Moreover, variations located in the topologically associating domains (TADs) were linked to the ISwine database (http://iswine.iomics.pro/). Ultimately, the results indicate that the classification of variations could effectively assist in screening advantageous variations of pig, cow, sheep, and chicken.

Key Words: livestock, SNP, small InDel, database, variation annotation

OP67 DNA methylation alteration patterns in repeat elements are similar during subclinical mastitis caused by *Staphylococcus chromogenes* and *Staphylococcus aureus*. M. Wang[1,2], N. Bissonnette[1], M. Laterrière[3], D. Gagné[3], and E. M. Ibeagha-Awemu*[1], [1]*Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Québec, Canada,* [2]*Département des Sciences Animales, Université Laval, Québec, Québec, Canada,* [3]*3Quebec Research and Development Centre, Agriculture and Agri-Food Canada, Québec, Québec, Canada.*

DNA methylation alterations in repeat elements (REs) may lead to abnormal RE activity which may influence genome stability and gene function during infections. Therefore, this study profiled the DNA methylation patterns of REs of milk somatic cells from dairy cows with *Staphylococcus chromogenes* (SC, n = 4), *Staphylococcus aureus* (SA, n = 16) subclinical mastitis and healthy cows (HC, n = 10) using whole-genome DNA methylation sequencing and bioinformatics analyses. Abundant differentially methylated cytosines (DMCs, 641197) occurred in REs (reDMCs) between SC and HC, accounting for 30% of total DMCs. Similarly, 33.8% (964,144) of total DMCs identified between SA and HC occurred in REs. Three-quarters of SC (72.8%) and SA (79.8%) reDMCs were hypermethylated in SC and SA groups compared with HC group. The majority of reDMCs were found in retrotransposons, including SINE, LINE and LTR. Interestingly, 339,772 reDMCs were common to SA and SC, accounting for 53% of SC-reD-

MCs and 35.2% of SA-reDMCs. More than 99% of common reDMCs showed methylation changes in the same direction, and ~75% and 25% were hyper- and hypo-methylated in SA and SC groups, respectively, suggesting similar roles in host responses to SC and SA mastitis. The common reDMCs were concentrated in SINEs (136,733), LINEs (120,813) and LTRs (48,806). BovB, Bov-tA2, BOV-A2, Bov-tA1, Bov-tA3, SL2a, L2b, MIR and MIRb were the REs harboring the most common reDMCs. Moreover, 107,499 common reDMCs were found within 10,314 genes. These genes showed significant involvement in pathways related to mammary gland homeostasis and health, such as *Leukocyte transendothelial migration*, *T cell receptor signaling pathway*, *Th17 cell differentiation*, *Chemokine signaling pathway*, *Leishmaniasis*, etc. suggesting possible regulatory roles of DNA methylation changes in REs during subclinical mastitis. In summary, this study revealed abundant and common DNA methylation changes in REs related to SC and SA subclinical mastitis, which suggests their possible involvement in similar mechanisms in the regulation of mammary gland health.

Key Words: differentially methylated cytosine, LINE, SINE, LTR, mastitis

OP68 Extending Ensembl regulatory annotation to farmed animals. G. R. Ilsley*, G. A. Merino, P. R. Branco Lins, M. Perry, D. Urbina-Gomez, and P. Harrison, *European Molecular Biology Laboratory, European Bioinformatics Institute, Hinxton, Cambridge, UK.*

Ensembl (https://www.ensembl.org/) is a widely used genome browser that has assisted the scientific community in interpreting the genome for more than 20 years. Ensembl's regulatory annotation identifies regions in the genome that might regulate and control the expression of nearby genes. These regulatory features can be used to filter and identify regions of the genome where non-coding variation or targeted mutations could have important consequences for gene expression and hence phenotype. Ensembl has well-established regulatory builds for human and mouse, which grew out of work on the ENCODE and BLUEPRINT projects. Now for the first time, we are extending this analysis beyond human and mouse. In collaboration with the GENE-SWitCH and AQUA-FAANG consortia, Ensembl is making regulatory annotation available for farmed animals including Pig, Chicken, Atlantic salmon and Turbot. EMBL's European Bioinformatics Institute also develops the FAANG Data Portal (https://data.faang.org/), which together with its rich and standardised metadata enables a wide range of experimental data sets for farmed animals to be discovered. Ensembl Regulation's computational workflow relies on this standardised metadata to retrieve and process primary data sets to identify open chromatin (ATAC-seq) and histone marks (ChIP-seq). These are then combined to produce genomic-level regulatory annotation. The analysis process will be described, along with examples of the resulting annotation and how it might be interpreted to guide further studies. Work is ongoing to add additional capabilities and further farmed animal species, and we are seeking feedback and collaborations with the community. Ensembl is primarily funded by the Wellcome Trust (WT222155/Z/20/Z), and the GENE-SWitCH and AQUA-FAANG projects have received funding from the European Union's Horizon 2020 Research and Innovation Programme under the grant agreement nos. 817998 and 817923, respectively.

Key Words: functional genomics, genome annotation, ATAC-seq, pig and related species, fish

OP69 Genome-wide acetylation modification of H3K27ac in bovine rumen cell following butyrate exposure. X. Kang[1,2], C. Li[2], R. L. Baldwin[1], G. Liu[1], and C. Li*[1], [1]*ARS, USDA, Beltsville, MD,* [2]*Ningxia University, Yinchuan, Ningxia, China.*

Butyrate contributes epigenetically to cellular function and rumen development in ruminant animals, which might be achieved by its genetic or epigenetic regulation of gene expression. To explore the role of butyrate on bovine rumen epithelial function and development, this study characterized genome-wide H3K27ac modification changes and super-enhancer profiles in rumen epithelial primary cell (REPC) induced with butyrate by ChIP-seq and analyzed its effects on the genes expression and functional pathways by integrating RNA-seq data. The results showed that the genome-wide acetylation modification (H3K-27ac) was observed in the REPC with 94,675 and 48,688 peaks in the butyrate treatment and control group, respectively. Totally, 9,750 and 5,020 genes with increased modification (H3K27ac-gain) and decreased modification (H3K27ac-loss) were detected in the treatment group. The super-enhancers associated genes in the butyrate-induction group were involved in the AMPK signaling pathway, MAPK signaling pathway, and ECM-receptor interaction. Finally, the upregulated genes (PLCG1, CLEC3B, IGSF23, OTOP3, ADTRP) with H3K27ac gain modification by butyrate were involved in cholesterol metabolism, lysosome, cell adhesion molecules, and PI3K-Akt signaling pathway. Butyrate treatment has the role of genome-wide H3K27ac acetylation on bovine REPC and affects the changes in gene expression. The effect of butyrate on gene expression correlates with the acetylation of the H3K27ac level. Identifying genome-wide acetylation modifications and expressed genes of butyrate in bovine REPC cells will expand the understanding of the biological role of butyrate and its acetylation.

Key Words: bovine, epigenetics, histone acetylation, transcription, gene regulation

OP70 Long-term selection impacts the rewiring of chromatin structure in chickens. D. Guan[1], Y. Wang[1], S. Aggrey[2], R. Okimoto[3], R. Hawken[3], and H. Zhou*[1], [1]*University of California, Davis, Davis, CA,* [2]*University of Georgia, Athens, GA,* [3]*Cobb-Vantress Inc., Siloam Springs, AR.*

Linear DNA molecules are often coupled with histones, further forming a twisted and compact structure in the 3-dimensional space in the nucleus. Such organization results in the miscellaneous regulation of gene expression, DNA replication, and repair, as well as recombination. Despite extensive research focusing on the dynamics of chromatin structure across species, development stages, and physiological states, how chromatin structure responds to long-term intensive selection remains unexplored. Herein, we generated 8 Hi-C data sets on 3 primary tissues (muscle, liver, and testis) from 2 different broiler genetic lines (Cobb700, a modern commercial meat-type line with outstanding breast meat yield, and ACRB, a randomly mated meat-type line) and 2 cell lines (DF1 and DT40) by the Micro-C technology. As expected, we found chromatin interaction were tissue/cell-specific, and for instance, a muscle-specific chromatin interacting region overlapped with the *MYOG* gene. This gene encodes myogenin, a muscle-specific transcription factor that can induce myogenesis. With the focus of interest on genetic line difference, we identified topologically associated domains (TADs) specifically in Cobb700 by comparing to ACRB in primary tissues. As a result, we found 80, 248 and 362 TADs specifically in muscle, liver, and testis, respectively, of Cobb700 at the 5-kb resolution, overlapping with 774, 1,961, and 3,422 genes, respectively. These genes were functionally enriched in olfactory receptor activity, type I interferon receptor binding, signaling receptor activity, RNA splicing, etc. Future works will further integrate gene expression, chromatin accessibility, gene regulatory elements, and signatures of selection to demonstrate the functional and biological impact of chromatin structure rewiring due to long-term intensive selection and breeding in poultry.

Key Words: chromatin structure, chicken, genetic selection, Micro-C, TADs

OP71 M6A demethylase ALKBH5 regulates PRRSV replication by manipulating host immune response. Q. Su*[1], X. Meng[1], B. Liu[1,2], and X. Zhou[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China,* [2]*Hubei Hongshan Laboratory, Wuhan, China.*

Porcine reproductive and respiratory syndrome (PRRS) caused by PRRS virus (PRRSV) is one of the most important health concerns

for the swine industry. PRRSV has evolved multiple strategies to regulate host innate immune response and facilitate its replication. Recent mounting evidence has indicated that N6-methyladenine (m6A) mRNA modification plays important roles in virus replication and host immune response. However, the potential relationship between m6A modification and PRRSV replication remains unclear. In this study, we demonstrated that PRRSV infection downregulated overall m6A level and upregulated the expression of m6A demethylase ALKBH5 in vivo. Knockout of ALKBH5 in PK-15CD163 cells inhibited PRRSV replication, while the complement expression of ALKBH5 reversed PRRSV replication. Furthermore, RIG-I encoded by DDX58 was predicted as the demethylated target of ALKBH5 by SRAMP and RMBasev2.0, which can reverse the effect of ALKBH5 to inhibit PRRSV replication. MeRIP-qPCR showed that ALKBH5 can downregulate the m6A level of RIG-I and promote its degradation during PRRSV infection. Moreover, we also demonstrated that ALKBH5 promoted PRRSV replication by inhibiting IFNβ expression through inhibiting RIG-I/IRF3 pathway. In summary, our findings explore the important role of m6A modification in PRRSV replication and provide new insights into the prevention and control of PRRS.

Key Words: PRRSV, m6A, ALKBH5, RIG-I, IFNβ

OP72 ISAG Bursary Award: Relationship between spleen and uterus gene expression and DNA methylation according to developmental stages of pigs. B. Ahn*[1], M. Kang[1], M. Choi[1,2], L. Rund[3], L. Shook[3], and C. Park[1], [1]*Department of Stem Cell and Regenerative Biotechnology, Konkuk University, Seoul, Korea,* [2]*Living Systems Institute, University of Exeter, Exeter, United Kingdom,* [3]*Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL.*

Understanding the patters of gene expression changes during development is critical to decipher development mechanisms. To understand the changes of gene expression and associated epigenetic regulation on immune and female reproductive tissues in pigs, we collected spleens and uteri from three 90-d-old fetuses (E90), three 28-d postnatal pigs (P28), and four 6-mo-old pigs (P180) and conducted RNA-seq and reduced representation bisulfite sequencing (RRBS). A total of 29,677 transcribed genes were detected and differentially expressed genes (DEGs, q-value <0.01 and fold change >1.5) between different developmental stages were determined. In spleens, 3.71% and 3.08% of the genes were up- and downregulated, respectively, in P28 than E90. In P180, 3.85% of the genes were downregulated compared with P28, while only 1.17% were upregulated. In uteri, 0.77% of the genes showed increased expression and 0.71% were decreased in P28 than E90. In P180 uteri, 1.84% of the genes were downregulated and 0.58% were upregulated comparing to P28. Gene enrichment analysis showed that the DEGs showing a gradual decrease in expression during spleen development were highly enriched in genes associated with mitotic sister chromatid segregation and DNA metabolism, indicating a decrease in cell proliferation according to the maturation of the immune system. In contrast, no consistent patterns of gene enrichment were observed among DEGs across different developmental stages in uteri. The analysis of DNA methylation showed that the spleen showed ~4% higher level of CpG methylation than that of the uterus regardless of different developmental stages. In addition, DNA methylation increases with age, and thus P28 showed ~10% higher level of DNA methylation than E90. Further analyses on differentially methylated regions (DMRs) associated with gene expression changes are in progress. Our study contributes to understanding the relationship between gene regulation and DNA methylation during porcine organ development.

Key Words: pig, organ development, RRBS, DNA methylation, differentially methylated region

OP73 RNA methylation as a mechanistic link between epigenotype and phenotype. S. Xie[1], B. Murdoch[1], and S. McKay*[2,3], [1]*University of Idaho, Moscow, ID,* [2]*University of Vermont, Burlington, VT,* [3]*University of Missouri, Columbia, MO.*

Determining the extent of epigenetic effects upon phenotypic variation involves characterization of both the epigenome and the epitranscriptome. Both DNA 5-methylcytosine (5mC) and RNA N6-methyladenosine (m6A) are common epigenetic modifications that play a role in transcription and post-transcriptional regulation, respectively. Recent evidence suggests the existence of a potential mechanism of coordinated transcriptional (5mC) and post-transcriptional (m6A) regulation in various biological processes, further emphasizing the need for epitranscriptomic annotation. Therefore, with the aim of elucidating the effects of DNA and RNA methylation on gene expression, we have performed Whole Genome Bisulfite Sequencing (WGBS), Methylated RNA Immunoprecipitation Sequencing (MeRIP-Seq) and RNA-Seq in the caruncle, spleen, and mammary gland from each of 4 cattle and 4 sheep. The results indicate that the density (5mC/C) of DNA 5mC were similar in 3 tissues (caruncle, spleen, and mammary gland) of sheep (2.04%, 2.62% and 2.53%) and cattle (2.52%, 2.77% and 2.61%), with spleen having the highest 5mC density in both species. RNA 6mA modifications identified from MeRIP data in the same 3 tissues yielded a total of 19,931, 26,463 and 11,018 peaks were identified in sheep, and 20,123, 19,467 and 17,774 peaks were identified in cattle. The average length of identified peaks are 2,063–7,213 bp and 8,270–9,036 bp in sheep and cattle, respectively. Moreover, the DNA and RNA modifications of long non-coding RNAs were simultaneously resolved in the relationship with gene expression for sheep and cattle. Subsequent analysis includes genome/transcriptome-wide associations between 5mC and m6A modifications as well as expression levels. While epigenetic annotation of modifications like DNA methylation and histone modifications has been accomplished through functional annotation of animal genomes initiatives, further epigenomic annotation is necessary to fully realize the effect of both DNA and RNA methylation on phenotypic variation. This work supports the annotation of animal epigenomes and epitranscriptomes while exploring a potential mechanistic link between epigenotype and regulation of gene expression.

OP74 Super-accessible chromatin regions are associated with increased gene transcription and regulation of cell differentiation in mammals. M. Hu*[1], Y. Zhao[1], X. Qi[1], H. Zhou[1], Y. Guo[1], L. Li[1], R. Kuang[1], R. Ma[1], G. Sun[4], L. Li[4], M. Zhu[1,3], X. Li[1,3], and S. Zhao[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education and Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China,* [2]*Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan, Hubei, China,* [3]*The Cooperative Innovation Center for Sustainable Pig Production, Wuhan, Hubei, China,* [4]*College of Biomedicine and Health, Huazhong Agricultural University, Wuhan, Hubei, China.*

Simultaneous binding of multiple transcription factors (TFs) within open chromatin regions is widespread in mammalian cells, but the mechanisms by which cooperation between TFs regulates gene expression is poorly understood. Here, through analysis of bulk and single-cell ATAC-seq data from 10 cell lines and 5 tissues from humans, pigs, and mice, we discovered open chromatin regions in promoters or enhancers that are strongly bound simultaneously by multiple lineage-specific TFs, which we refer as super accessibility elements (SAEs). Moreover, the SAEs are associated with exceptionally high expression of cell identity genes and enhanced the stability of both topologically associating domains and loops. To confirm the difference in gene expression activation between SAEs and TAEs, 16 gene-distal SAEs, 13 gene-distal TAEs, 8 gene-proximal SAEs and 8 gene-proximal TAEs in myoblast cells was randomly selected for validation using the Dual-Luciferase reporter assay. The gene-distal and -proximal SAEs all resulted in significantly higher reporter activity than corresponding TAEs, indicating that SAEs indeed activate gene expression to a greater extent than TAEs. Using differentiating myoblast cells as a model,

we found that the gain or loss of footprints for muscle lineage-specific TFs (e.g., MyoD/MyoG, Cebpb, and Max) in SAEs can affect chromatin looping and regulate cell differentiation. Moreover, the Cebpb and MyoG were discovered interaction with MyoD and enhance its binding through the localization analysis between TFs in SAEs. Together, our findings increase our understanding of how the interplay between the TFs is involved in critical biological processes in mammals.

Key Words: transcription factor, gene regulation, 3D genome structure, cell differentiation, epigenetics

OP75 Beyond the genome: Establishing molecular phenotypes to accelerate adaptation to a changing environment. A. Caulton*[1], R. Brauning[1], K. M. McRae[1], K. G. Dodds[1], C. Couldrey[2], P. L. Johnson[1], and S. M. Clarke[1], [1]*AgResearch, Invermay Agricultural Centre, Mosgiel, Otago, New Zealand,* [2]*Livestock Improvement Corporation, Hamilton, New Zealand.*

Breeding livestock that are resilient to environmental stresses is of critical importance considering the changing global climate. Genetic adaptation occurs through the selection of advantageous genetic variants over time, however adapting to environmental challenges often requires rapid biological responses that occur through changes in gene expression. Epigenetic modifications, including DNA methylation, alter gene expression without changing the DNA sequence, allowing for immediate and reversible modulation of physiological responses to environmental perturbation. The "Beyond the Genome" research program aims to exploit this phenomenon through the development and application of emerging DNA methylation profiling assays and high-throughput techniques to provide transformative industry-applicable tools. These will be used to select animals that are resilient against biotic/abiotic stresses that are increasing in prevalence with the changing global climate. A range of biological resources (including disease challenges and multigenerational families in ruminant species, sheep, cattle, deer) have been established to investigate the role of the methylome in adaptation to stress and to assess transgenerational inheritance of methylation patterns. In parallel, a focus on methylome profiling tools, tailored to enable core research through to industry application, have been investigated and will be presented. These include a mammalian methylation array that has been utilized to develop epigenetic clocks for livestock species, restriction enzyme DNA sequencing methods, with and without the use of deamination of cytosines, and comparisons to whole-genome methylome profiles with examples from both bisulphite sequencing and nanopore sequencing. The application of these tools to profile methylome changes in response to disease stress in sheep with also be presented. Establishing the methylome as a molecular phenotype to accelerate adaptation to a changing environment will facilitate the breeding of animals that are fit for the future.

Key Words: epigenomics, sheep and related species, animal breeding, adaptation, methyl-seq

OP76 African swine fever infection enhances the host transcriptional regulation of membrane protein-encoding genes mediated by changes in chromatin state. X. Qi*[1], Y. Xiang[1], L. Sun[3,4], L. Xing[3], S. Zhang[1], Q. Zhao[1], L. Zhang[1], J. Li[1], P. Zhou[1], Z. Zheng[1], X. Li[1], L. Fu[1,2], G. Peng[3,4], and S. Zhao[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education and Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China,* [2]*The Cooperative Innovation Center for Sustainable Pig Production, Wuhan, China,* [3]*State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China,* [4]*State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China.*

African swine fever virus (ASFV) is a virulent infectious virus with an extreme ability to infect primary porcine alveolar macrophages (PAMs). However, nothing is known about the host membrane proteins involved in ASFV infection. Here, we present a multi-omic epigenetic atlas of ASFV-exposed PAMs through profiling of 3D chromatin architecture and single-nucleus chromatin accessibility landscapes (snATAC). ASFV infection leads to a rearrangement of active chromatin signaling in the *cis*-regulatory region of the host genome, which is associated with the activation of immune cells and transcription of membrane protein-encoding genes in the macrophage activation pathway. Specifically, the host genome employs histone H3 lysine 27 acetylation-mediated enhancer-promoter interactions to boost the transcriptional activity of membrane protein-encoding genes, thereby contributing to macrophage activation. Moreover, comparing the macrophages carrying viral DNA identified by snATAC-seq with wild-type macrophages provides a more reliable collection of membrane protein-encoding genes associated with infected macrophage activation. Different dimensions data indicates a co-occurrence of these membrane protein expression with susceptibility, for instance, inhibiting the expression of membrane proteins such as *CD244* and *CD206* can significantly decrease the host's susceptibility to ASFV. Collectively, the data provide a new insight to the regulation of host gene expression during ASFV infection and highlighted the genes encoding membrane proteins associated with macrophage activation.

Key Words: African swine fever virus, primary porcine alveolar macrophage, 3D chromatin architecture, single-nucleus chromatin accessibility landscape, gene expression regulation

Applied Genetics and Genomics in Other Species of Economic Importance

OP77 ISAG Bursary Award: The development of a 61K Illumina SNP chip for dromedaries under the frame of the 2019 Agricultural Greater Good (AGG) initiative. M. Di Civita*[1], G. Senczuk[1], S. Bruno[2], V. Landi[3], S. Brooks[4], F. Almathen[5,6], B. Faye[7], S. B. S. Gaouar[8], M. Piro[9], K. S. Kim[10], H. Dadi[11], P. C. Iglesias[12], H. Al-Haddad[13], M. Al-Abri[14], F. Pilla[1], X. David[15], A. Eggen[15], P. Burger[16], and E. Ciani[2], [1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy,* [2]*Department of Biosciences, Biotechnologies and Environment, University of Bari "Aldo Moro," Bari, Italy,* [3]*Department of Veterinary Medicine, University of Bari "Aldo Moro," Valenzano, Bari, Italy,* [4]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [5]*Department of Public Health, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia,* [6]*Camel Research Center, King Faisal University, Al-Ahsa, Saudi Arabia,* [7]*CIRAD-ES, UMR SELMET, Montpellier, France,* [8]*Department of Biology, Abou Bakr Belkaid University of Tlemcen, Tlemcen, Algeria,* [9]*Department of Medicine, Surgery and Reproduction, Institut Agronomique et Vétérinaire Hassan II, Rabat BP, Morocco,* [10]*Department of Animal Sciences, Chungbuk National University, Chungbuk, Korea,* [11]*Ethiopian Biotechnology Institute (EBTi), Addis Ababa, Etiopia,* [12]*Department of Genetics, Faculty of Veterinary Sciences, University of Córdoba, Córdoba, Spain,* [13]*Department of Biological Sciences, Kuwait University, Kuwait City, Kuwait,* [14]*Department of Animal and Veterinary Sciences, Sultan Qaboos University, Muscat, Oman,* [15]*Illumina, Agrigenomics, Evry, France,* [16]*Research Institute of Wildlife Ecology, Vetmeduni, Vienna, Austria.*

Dromedary camels represent one of the most important domestic animals in terms of meat, milk, leather and transpiration in many low-income countries also contributing to many social and cultural as-

pects. In addition, dromedaries are so fascinating also from a biological point of view, being among the few big mammals to have evolved specific adaptations to extreme environmental conditions. Under the frame of the 2019 Illumina Agricultural Greater Good (AGG) initiative program which is aimed at supporting studies on sustainability, productivity, and nutritional density of agriculturally important crop and livestock species, a total of 179 dromedaries from the entire geographic distribution range were whole-genome sequenced (WGS). Raw reads were mapped against the dromedary reference genome (CamDro3) and the variants were called using the Illumina Dragen germline platform. From a total of 13,560,911 biallelic SNPs, after a multistep filtering approach aimed at ensuring SNPs evenness across chromosomes and removing SNPs with less than 0.05 minor allele frequency and 0.1 missing call rate, a subset of 61,208 SNPs was selected. The panel included 59,069 autosomic SNPs with an average distance of 32 kb, 1,230 SNPs on X chromosome and 77 mitochondrial SNPs. In addition, about 1,000 of loci from 47 genes with known functional relevance were enriched. The linkage-disequilibrium (LD) decay graph indicated that at r^2 value ranging from 0.3 to 0.5 we found pairs of loci separated 50 kb apart. This value resulted higher than that reported in other cattle commercial breeds and even higher than that observed in sheep breeds. This result confirms that the selection of SNPs with an average distance of 32 kb will perform well in linkage disequilibrium mapping approaches such as in looking for selection signatures or in genome-wide association studies. The panel is currently being validated and we are confident that will represent a further relevant step toward the understanding of dromedary genomics.

Key Words: Old World camelid, genome sequencing, genotyping, single nucleotide polymorphism (SNP)

OP78 Selection of an ovine SNP parentage panel for consideration as the ISAG comparison test panel. R. Ferretti*[1], K. Schutt[2], M. Dowling[2], J. Qiu[1], and R. Tait Jr.[1], [1]*Neogen GeneSeek Operations, Lincoln, NE,* [2]*Neogen Australasia, Ipswitch, QLD, Australia.*

Advances in medium- and high-throughput genotyping platforms have allowed for significant reduction in genotyping costs. A decade ago, this was a prohibiting factor for transitioning away from microsatellites over to single nucleotide polymorphism (SNP) technologies. At the 33rd International Society for Animal Genomics (ISAG) conference in 2012, this topic was raised and initial work was done by the International Sheep Genomics Consortium (ISGC) to adopt an official Ovine SNP comparison test (CT) panel using 88 autosomal SNPs and one male specific SNP. Here we propose an expanded panel of 201 SNP markers for consideration as the accepted ISAG Ovine Parentage CT panel. The aim for this parentage panel was to build off the original ISGC 89 SNP panel by incorporating SNP markers from newer iterations of academic and commercial parentage panels. Furthermore, to foster greater adoption we have considered attributes of a SNP panel including: 1) backward compatibility to multiple historic genotyping platforms; 2) global relevance across populations; and 3) the ability to be platform agnostic. To achieve this, we used a 3-step approach for SNP selection. First, the candidate SNPs should be available in the public domain. Second, SNPs should be represented on at minimum 2 genotyping platforms: Agena (Sequenom), KASP, Illumina, Affymetrix, GBS/NGS. Lastly, final SNP selection was made using SNPs displaying high Minor Allele Frequency (MAF) and highest average call rate across data sets and platforms. A total of >200,000 animals consisting of more than 20 breeds and sample representation from 6 different geographic regions were evaluated. From this data a subset of 200 highly informative SNPs from a candidate pool of 857 SNPs were selected.

Key Words: sheep and related species, animal breeding, genotyping, parentage

OP79 High-throughput detection of single nucleotide polymorphisms with flexible content panels. S. Camiolo[1], J. Yeakley[1], E. Clark[2], B. Seligmann[1], and J. McComb*[1], [1]*BioSpyder Technologies Inc., Carlsbad, CA,* [2]*Zoetis Inc., Kalamazoo, MI.*

Detection of single nuclear polymorphisms (SNPs) is a powerful tool for genetic selection and maximization of the breeding potential of farm animals. It can also be used to estimate disease susceptibility or for pathogen detection. Most approaches to SNP calling, however, have significant limitations. Microarrays can measure many SNPs simultaneously but come with fixed content that cannot be customized or easily expanded without distorting original performance. Due to high costs of creating and qualifying each production lot, microarrays are usually available only for a subset of species, and often suffer from significant levels of lot-to-lot variability. qPCR detection is work intensive and severely limited in the number of samples and gene targets that can be evaluated simultaneously. Direct sequencing is expensive and produces data that is difficult to interpret correctly. TempO-SNP is a novel targeted assay capable of inexpensive high-throughput and high-plexity detection of SNPs from any species. It relies on direct hybridization of 2 adjacent barcoded oligomers to the target DNA, which are ligated into the reporter probe only if the correct SNP base is present. The content of such SNP panels is flexible as new probes can be added to the mixture easily and without affecting prior content. The assay does not require specialized instrumentation and the TempO-SeqR software pipeline makes SNP calling and report creation straightforward and painless. In partnership with Zoetis, we demonstrate TempO-SNP detection of hundreds of targets from hundreds of samples simultaneously, across multiple species. We also show that the assay can measure SNPs from crude tissue lysates or without need for DNA extraction, as well as from hair and blood. TempO-SNP shows excellent call and accuracy rates in a side-by-side comparison of data from the same samples produced by Zoetis' current microarray approaches. Additionally, TempO-SNP can be combined with existing commercial TempO-Seq technology to obtain RNA expression data from the same tissue lysates. Robust samples like dried blood spots on paper can also be used for both RNA and DNA readouts.

Key Words: SNP, genotyping, genetics, parentage, RNA

OP80 Genetic differentiation of *Camelus bactrianus* from Kazakhstan. K. Dossybayev*[1,2], D. Ualiyeva[1], M. Amandykova[1,2], T. Kapasuly[1,2], A. Mussayeva[1], Z. Orazymbetova[1], G. Shaltenbay[1,2], and B. Bekmanov[1,2], [1]*Laboratory of Genetics and Cytogenetics, Institute of Genetics and Physiology, Almaty, Kazakhstan,* [2]*Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan.*

The Bactrian camel represents an Old World camel that is well adapted to the cold and dry deserts of Middle and Central Asia. It is used to be the main source of food and logistics for the nomadic tribes of people since ancient times. Nowadays camels are bred worldwide for meat and dairy products. Recently, in Kazakhstan camel farming has been growing rapidly, particularly, in 2022 there registered around 272 thousand camels. The successful development of animal husbandry mainly depended on the genetic characteristics of farm breeds. Mitochondrial DNA is an optimal molecular marker which due to the high mutation rate allows for tracing the evolutionary history of matrilines as well as determining the speciation process. Nowadays, the genetic differentiation of local camels is poorly understood. Thus, to investigate the evolutionary relationships of domesticated Bactrian camels from Kazakhstan with extant populations spread worldwide, we determined the sequences of mitochondrial D-loop region from 13 camels, of the Almaty population. Totally, the analysis involved 50 samples including the sequences from GenBank. The targeted mitochondrial region consisted of a total length of 321 bp, which was analyzed by the Sanger method. The phylogenetic analysis recovered 2 main clusters representing the basal position of the monophyletic clade of Kazakhstani Bactrian camels with Arabian Dromedary camels, and a polyphyletic clade of *Camelus ferus* and *Camelus bactrianus* from Eastern Central Asia (Chi-

na, Mongolia). These results were supported by the haplotype network analysis as well with detection of 3 haplogroups. The obtained results suggest the possible past admixture and origin of a common ancestral form of the Central Asian population from the Arabian Peninsula. The current research may play a crucial role in the future investigations of the evolutionary history of the species. This research was funded by grant AP14870678 of the Ministry of Sciences and Higher Education of the Republic of Kazakhstan.

Key Words: *Camelus bactrianus*, Kazakhstan, mtDNA, phylogeny

OP81 Genetic diversity and population structure among Central European native sheep breeds using microsatellite markers. Z. Sztankoova, M. Milerski, M. Brzáková, J. Rychtárová, and J. Kyselova*, *Institute of Animal Science, Praha-Uhrineves, Czech Republic.*

Analysis of microsatellite loci is highly informative in reconstructing the historical processes underlying the evolution and differentiation of animal populations. This study used 13 polymorphic microsatellite markers recommended by FAO and ISAG to analyze the genetic diversity, genetic structure, variation, and phylogenetic relationship of 6 Central European sheep breeds (Czech Wallachian, CWA, n = 36, Sumava, S = 46, Slovak Wallachian, SWA, n = 59, Improved Wallachian, IPW, n = 59, Swiniarka, SWI, n = 35, and Uhruska sheep UHR, n = 19). The 172 alleles were observed in 254 animals. The number of observed alleles per locus varied from 7 to 17 per locus (average of 13.23). The mean number of effective alleles per locus was 5.77, with PIC ranging from 0.613–0.907 (equal to 0.77). Fst within subpopulations showed a low level of inbreeding. Nei's genetic distances between breeds were calculated, and results showed that the smallest distance was recorded between CWA and SWA (0.108). The largest was between the polish SWI and UHR sheep breeds (0.283). Principal component analysis showed that Czech and Slovak sheep breeds are closely related compared with Polish sheep breeds, specially SWI. Analysis of molecular variance showed a 6% variance among breeds and a 94% variance within populations. The ΔK value indicated that the most suitable group number was K = 4. These results showed genetic diversity, which is essential for future selection, animal breeding, and keeping the genetic diversity of native breeds. On the other hand, these results could help preserve genes in these breeds, thereby ensuring their preservation in the Czech and Slovak Republic and Poland. Therefore, future study is recommended to screen other middle European sheep breeds for comparison purposes.

Key Words: native sheep, gene resource, gene diversity, population structure, microsatellite

OP82 Genome-wide association study between copy number variations and economically important traits in American mink. P. Davoudi*[1], D. Ngoc Do[1], B. Rathgeber[1], S. Colombo[1], M. Sargolzaei[2,3], G. Plastow[4], Z. Wang[4], G. Hu[1], S. Valipour[1], and Y. Miar[1], [1]*Department of Animal Science and Aquaculture, Dalhousie University, Truro, NS, Canada,* [2]*Department of Pathobiology, University of Guelph, Guelph, ON, Canada,* [3]*Select Sires Inc., Plain City, OH,* [4]*Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

Copy number variations (CNVs) are structural variants consisting of duplications and deletions of DNA segments, which are known to play important roles in the genetics of complex traits in livestock species. However, CNV-based genome-wide association studies (GWAS) have not been reported in American mink. Therefore, the purpose of the current study was to investigate the association between CNVs and complex traits in American mink. A CNV-based GWAS were performed with the ParseCNV software program using deregressed estimated breeding values of 27 traits as pseudophenotypes, categorized into traits of growth and feed efficiency, reproduction, pelt quality, and Aleutian disease tests. The study identified a total of 10,137 CNVs (6,968 duplications and 3,169 deletions) using the Affymetrix Mink 70K single nucleotide polymorphism (SNP) array in 2,986 American mink. The association analyses identified 353 CNV regions (CNVRs) associated with at least one of the studied traits. These CNVRs overlapped with a total of 321 potential candidate genes, and among them several genes have been known to be related to the traits such as *ARID1B*, *APPL1*, *TOX*, *CXCL12*, and *PHYHIPL* (growth and feed efficiency traits); *DLGAP2*, *UNC5D*, *GRM1*, *SYCP2L*, *ARF1*, *RNASE9*, *RNASE10*, *WNT3*, *WNT3A*, and *WNT9B* (reproduction traits); *MYO10*, and *LIMS1* (pelt quality traits); and *IFNGR2*, *APEX1*, *UBE3A*, and *STX11* (Aleutian disease tests). Overall, the results of the study provide potential candidate genes that may regulate economically important traits and therefore may be used as genetic markers in mink genomic breeding programs.

Key Words: copy number variation (CNV), genome-wide association, complex trait, candidate gene, animal breeding

Domestic Animal Sequencing and Annotation

OP83 Invited Workshop Presentation: The human genome is finally complete, now what? S. Koren*, *National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.*

Since its initial release in 2000, the human reference genome has covered only the euchromatic fraction of the genome, leaving important heterochromatic regions unfinished. Addressing the remaining 8% of the genome, the Telomere-to-Telomere (T2T) Consortium recently completed the 3.055 billion–base pair sequence of a human genome, T2T-CHM13. The completed regions include all centromeric satellite arrays, recent segmental duplications, and the short arms of all 5 acrocentric chromosomes, unlocking these complex regions of the genome to variational and functional studies. Building on this largely manual effort, we have since improved and automated this strategy in Verkko, an iterative, graph-based pipeline for assembling complete, diploid genomes. Verkko begins with a multiplex de Bruijn graph built from long, accurate reads and progressively simplifies this graph by integrating ultra-long reads and haplotype-specific markers. The result is a phased, diploid assembly of both haplotypes, with many chromosomes automatically assembled from telomere to telomere. Verkko has been used to generate multiple draft T2T genomes, including human as well as important agricultural species, such as tomato. The complete assembly of diploid genomes is a critical step toward the construction of comprehensive pangenome databases and chromosome-scale comparative genomics.

OP84 ISAG Bursary Award: An organism-wide ATAC-Seq peak catalogue for the bovine and its use to identify regulatory variants. C. Yuan*[1], L. Tang[1], T. Lopdell[2], C. Oget-Ebrad[1], G. Costa Monteiro Moreira[1], J. L. Gualdron[1], Z. Cheng[3], M. Salavati[3], D. C. Wathes[3], M. A. Crowe[4], W. Coppieters[1], C. Charlier[1], T. Druet[1], M. Georges[1], H. Takeda[1], [1]*GIGA Institute, University of Liège, Liège, Belgium,* [2]*Livestock Improvement Corporation, Hamilton, New Zealand,* [3]*Royal Veterinary College, Herts, UK,* [4]*School of Veterinary Medicine, University College Dublin, Dublin, Ireland.*

We herein report the generation of an organism-wide catalog of 976,813 *cis*-acting regulatory elements detected by ATAC-Seq. We regroup these regulatory elements in 15 tissue-specific and one tissue-shared components by nonnegative matrix factorization. Correlation between the genome-wide density of peaks and transcription start sites, between peak accessibility and expression of neighboring genes, and enrichment in transcription factor binding motifs supports their regulatory potential. Using a previously established catalog of

12,736,643 variants, we show that the proportion of single nucleotide polymorphisms mapping to ATAC-Seq peaks is higher than expected and that this is due to an ~1.3-fold higher mutation rate within than outside peaks. Their site frequency spectrum indicates that variants in ATAC-Seq peaks are subject to purifying selection. We generate eQTL data sets for liver and blood and show that variants that drive eQTL fall into liver and blood-specific ATAC-Seq peaks more often than expected by chance. We combine ATAC-Seq and eQTL data to estimate that the proportion of regulatory variants mapping to ATAC-Seq peaks is approximately 1 in 3, and that the proportion of variants mapping to ATAC-Seq peaks that are regulatory is approximately 1 in 25. We discuss the implication of these findings on the utility of ATAC-Seq information to improve the accuracy of genomic selection.

Key Words: cattle and related species, epigenomics, ATAC-seq, regulatory element, genomic selection

OP85 Development of genomic tools for American mink (*Neogale vison*). Y. Miar*, *Dalhousie University, Truro, Nova Scotia, Canada.*

Mink Genome Sequencing Project is established on long-term goal of developing novel genomics tools to study the genome biology and evolution of mink. The research project agenda is organized around 3 major goals: (1) development of first chromosome-level genome assembly with high quality for American mink to help understanding the biology and evolution of the order Carnivora, (2) designing the first mink single nucleotide polymorphism (SNP) genotyping array (70K) for genomic discovery in mink, and (3) using genomics to understand the genetic architecture of economically important traits. The genome assembly is generated using PacBio long reads (2,884,047) with Hi-C data resulting in 183 scaffolds and scaffold N50 of 220 Mb. This genome assembly (ASM_NN_V1) is gap-free with a length of 2.68 Gb and 98.6% of the genome is covered by 15 chromosomes. Genome contiguity, the number of scaffolds, and annotation has significant improvement compared with the first draft (NNQGG.v01). A custom genotyping array is developed using the developed mink reference genome and DNA panel composed of 100 whole-genome sequences data of American mink from 5 color-types. This Affymetrix Mink 70KSNP array created 63,375 SNPs, which is sufficient for conducting genomic studies in mink research program and beyond. This work represents a long-term investment in understanding the genome biology and evolution of mink to enhance the developments of superior, healthy, and highly efficient mink. This project would facilitate the discovery of genomic regions, markers and genetic networks underlying economically important traits in American mink, which will contribute novel insights into the genetic architecture of key traits and is a critical step to implement genomic selection in mink breeding programs. The results of this research have the potential to impact the mink industry and improve the overall performance of the mink industry, which is in difficulty now due to several economic factors such as declining fur prices, disease prevalence and high feed costs.

Key Words: American mink, genome assembly, SNP array

OP86 ISAG Bursary Award: Identification and comparison of plant-derived miRNAs based on massive public data. H. Liu*[1], P. Xu[1], Y. Liao[1], C. Li[1], J. Dou[1], Y. Wang[1], Z. Tang[1], J. Xu[1], D. Yin[1], S. Zhu[1], L. Yin[1,2], M. Yu[1], S. Zhao[1,2], X. Liu[1,2], Y. Fu[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei, China,* [2]*Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, Hubei, China.*

Numerous studies have reported that plant miRNAs could be absorbed into the circulatory system and organs of animals, and these studies have broadened our view of cross-kingdom communication. However, limited by the scale of data, the relationship between plant-derived miRNAs and animal diets, and the distribution of plant-derived miRNAs in various tissues are lack of in-depth exploration. In this study, we collected 5,834 miRNA sequencing data involving 46 tissues from 6 common animals including pig, cow, sheep, chicken, mouse and monkey. After bioinformatics analysis, we identified a total of 5,430 plant-derived miRNAs in 6 species, which is distinctly higher than 7 in carnivores (crocodiles, seals) and 3 in yeast. It suggests that these miRNAs are indeed related to the diet of animals. To investigate the potential dietary sources of these miRNAs, we mapped them to miRBase and found most of them were from crops or grasses, such as *Zea mays*, *Glycine max* and *Medicago truncatula*. However, we also found that miRNAs, which have higher expression level in each species, mainly from the miR166, miR167, miR168, miR159, miR396, miR482 families but not affected by the dietary source of animals. It indicates that the absorption of the dietary miRNAs in the recipient may be affected by their stability, and most of the plant miRNAs are difficult to be absorbed. Our study also demonstrates that plant-derived miRNAs widely exist in various tissues, with an average detection rate of 38.06%, among which the detection rates in oral cavity, body fluid (blood, milk) and excreta (urine and feces) is higher than 50%, which may be related to their absorption and transportation pathways. In summary, our study confirmed that plant-derived miRNAs are derived from diet based on large-scale sequencing data, and their expression level in various tissues is highly associated with their absorption and transport pathways. It will advance the understanding of plant miRNAs regulation across kingdoms and provides new theoretical insights into the effects of diet on animal breeding.

Key Words: miRNA, plant-derived miRNA, tissue expression profile, cross-kingdom regulation

OP87 Overview of Ruminant T2T Consortium. B. M. Murdoch*[1], S. D. McKay[2], B. D. Rosen[3], and T. P. L. Smith[4], [1]*University of Idaho, Moscow, ID,* [2]*University of Missouri, Columbia, MO,* [3]*USDA, Agricultural Research Service, Animal Genomics and Improvement Laboratory, Beltsville Agricultural Research Center, Beltsville, MD,* [4]*USDA, Agricultural Research Service, Genetics and Animal Breeding, Clay Center, NE.*

The first draft human genome assembly was released over 20 years ago, but a gapless telomere to telomere (T2T) "complete" assembly was elusive until last year. The highly repetitive nature of the pericentromeric, subtelomeric and duplicated gene families such as the rRNA arrays made them impossible to assemble until advances in long read sequencing technologies, coupled with new bioinformatic tools, resolved these structures. We recently proposed application of these new resources, tools and knowledge in support of a "Ruminant T2T Consortium" with the goal of generating complete genomes for the ruminant evolutionary lineage. The ruminant suborder is represented by 6 families and 66 living genera, found in geographically dispersed areas, adapted to a wide variety of environments, and subjected to both natural and artificial selection. Our hypothesis is that generating T2T assemblies of ruminant species with a resolution from closely related (e.g., capable of interbreeding, even if only generating sterile offspring) to higher evolutionary distance (up to the estimated 25 million years ago last common ancestor) will inform our understanding of the underpinnings of ruminant evolution, shed light on the genomic consequences of domestication, and enhance our knowledge of the functional roles of heterochromatin and other repeat regions of the genome. A ruminant T2T workshop, held in February at the USDA Meat and Animal Research Center in Nebraska, featured presentations from the founders of the human T2T effort and leaders of the vertebrate genomes and earth biogenome projects to provide details of their experience and lend assistance in designing/optimizing the project. The consortium developed working groups to conduct orthogonal data production and analyses. The working groups include Chromosome Evolution, 3D Genome Architecture, Comparative Methylome, Assemblers and Curation, Annotation, Variant Discovery and population sequencing, Immunome analyses, Cytogenetic. This update is intended to share the outcomes of the ruminant T2T workshop and provide an opportunity for interested members of the International Genomes community to participate.

Key Words: Ruminant T2T, genome assembly, pangenome

OP88 Discovering the missing structural variation in the bovine genome. A. Chamberlain*[1,2], T. Nguyen[1], J. Wang[1], and I. Macleod[1,2], [1]*Agriculture Victoria, Bundoora, Victoria, Australia,* [2]*La Trobe University, Bundoora, Victoria, Australia.*

SV often show large and sometimes deleterious effects on phenotypes and remain largely unexplored in livestock. The Bovine Long Read Consortium (BovineLRC) aims to use long read sequencing technologies to sequence cattle at population scale to characterize the structural variation of the bovine genome for downstream applications. As a pilot study 41 animals from 2 breeds were sequenced. In an analysis of 2 parent-offspring trios we show that between 10× to 20× coverage resulted in some reduction in the SV discovery rate versus higher read depth, but this may be an acceptable compromise for population scale studies to spread sequencing costs over a larger number of animals. However, if the purpose is to discover a deleterious Mendelian mutation among a small group of known affected or carrier animals, the results here suggest that at least 30× would be preferable. SNP and INDEL called from various read depths were compared with calls from short read sequences. At read depths from 5 to 60× recall and precision of SNP was considerably higher than for INDEL. At ≥10× coverage, SNP recall was 0.86 and reached 0.99 at 60×. The precision for both SNP and particularly INDEL suggested that the long-read variant calls include a relatively high, but likely overestimated proportion of false positives. Using sequences from all animals a total of 76,572 SVs were detected across all samples, one-third of which were segregating in only one breed. Insertions and deletions tended to be smaller and duplications larger. Insertions and deletions more often segregated across both breeds, while inversions were more often breed specific. Few duplications were detected but they tended to be slightly more likely to be breed specific. The results highlight that it would be beneficial to have a data set with large numbers of animals and breeds to understand the structural variation that exists and explore the impact of SVs on traits of interest.

Key Words: bovine, structural variation, long read sequencing

OP89 Discovery of deleterious genetic variants in farmed animals. X. R. Arias[1], J. L. Petersen[2], B. M. Murdoch[3], F. M. McCarthy[4], and T. S. Kalbfleisch*[1], [1]*University of Kentucky, Lexington, KY,* [2]*University of Nebraska–Lincoln, Lincoln, NE,* [3]*University of Idaho, Moscow, ID,* [4]*University of Arizona, Tucson, AZ.*

Data and samples are often siloed at the diagnostic labs where they are generated and collected, respectively. In this project we aim to provide an integrative service to genetically characterize samples and couple these data with their respective pathology reports to identify and publish putative deleterious alleles found in farmed animals. This project has linked scientists from 5 different research institutions, and 4 different veterinary diagnostic labs. The tools and service we have created will provide an interface that will allow diagnosticians to submit samples and reports for genetic analysis, and subsequent distribution of derived information to producers, and the research community. Recessive lethal alleles exist benignly in breeding populations, until a sire and dam carrying them are mated. One-quarter of the resulting pregnancies will be homozygous for the lethal allele and will result in an aborted pregnancy, or death soon after birth. In cattle, sheep, and horses, abortions are often necropsied. Although many have a known cause, such as being the result of a viral or bacterial infection, many do not. Those that do not may harbor a homozygous genotype for a lethal recessive allele. We currently have and are building sequence data sets for on the order of 100 healthy animals from each of these species. This project is collecting pathology reports for and will sequence 40 abortions, up to 15 each from cattle, sheep, and horses to look for alleles that are homozygous in these samples, but not in the larger population. Here we present preliminary sequencing results, and methods we have developed to distinguish potential lethal alleles from variants whose genotypes have no homozygous variant genotypes due to collapsed duplications or other assembly artifacts.

Key Words: bioinformatics, reference genome, deleterious allele

OP90 Assessment of different enrichment methods to characterize bovine circRNAs. Y. Wang[1,2], J. Wang[3], R. J. Gruninger[4], T. A. McAllister[4], and L. L. Guan*[1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada,* [2]*Institute of Animal Genetics and Breeding, College of Animal Science and Technology, Sichuan Agricultural University, Chengdu, Sichuan, China,* [3]*State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Animal Science and Technology, Guangxi University, Nanning, Guangxi, China,* [4]*Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada.*

Circular RNAs (circRNAs) characterization using sequencing data is more challenging than linear RNAs because the back-splicing junction reads are the only feature for circRNAs identification. To date, the knowledge of bovine circRNAs is limited. In this study, the total RNA of liver and rumen epithelium from three 15-mo-old Kinsella Composite beef were extracted, and the circRNAs were enriched using 6 approaches before RNA-sequencing, namely, ribosomal RNAs removal using Ribo-zero (Ribo); linear RNAs degradation using Ribonuclease R (R); linear RNAs and RNAs with structured 3′ ends degradation using Ribonuclease R then tailing and poly $(A)^+$ RNA depletion (RTP); ribosomal RNAs coupled with linear RNAs elimination using Ribo-zero and Ribonuclease R (Ribo-R); ribosomal RNA, linear RNAs and RNAs with poly (A) tailing elimination using Ribo-zero, Ribonuclease R and poly $(A)^+$ RNA depletion (Ribo-RP); and ribosomal RNA, linear RNAs and RNAs with structured 3′ ends elimination using Ribo-zero, Ribonuclease R, tailing and poly $(A)^+$ RNA depletion (Ribo-RTP), respectively. RNA-sequencing analysis showed that different approaches resulted in varied number of circRNAs per million clean reads ($P_{adj} < 0.05$) and false positive rate of identifying circRNAs. Totally, 4,051 of 50,837 circRNAs were defined as high confident circRNAs (back-splicing junction reads ≥2 and detected in at least all replicates of one method in one tissue), and most of them could be identified with Ribo-RTP method. Besides, 308 of 2,285 and 260 of 2,939 high confident circRNAs were commonly identified from 5 methods in liver and rumen tissues, respectively. Conservation analysis revealed that 507 bovine high confident circRNAs had shared splicing sites with human circRNAs. The present study provides valuable information for researchers to choose the best circRNAs enrichment methods when studying circRNAs from tissue samples which will enable researchers to study their regulatory function in cattle and other livestock animals.

Key Words: bovine circRNA, enrichment approach, RNA-seq, circRNA conservation

Genetics of Immune Response and Disease Resistance

OP91 Invited Workshop Presentation: Uncovering the basis of natural tolerance to African cattle diseases using integrative omics. J. Prendergast*, *Roslin Institute, University of Edinburgh, Scotland, UK.*

Infectious diseases are one of the largest constraints on African livestock production and consequently the ability to improve the livelihoods of smallholder farmers on the continent. Although it has been known for decades that African breeds have developed natural tolerances to a range of these diseases, the biological mechanisms underlying this tolerance remain poorly understood. If we can understand the basis of this tolerance the hope is we can develop more productive animals better suited to the African environment. In this talk I will discuss our

ongoing work to map the basis of natural disease resistance in African cattle. Using East Coast fever as an exemplar disease I will show how we are integrating a range of traditional and modern omics approaches, from genetic linkage studies to nascent RNA sequencing and genome-wide massively parallel reporter assays, to uncover the biological mechanisms underlying the observed natural tolerance to this economically important disease.

OP92 ISAG Bursary Award: IUIS-VIC Travel Award 1: Transcriptomic signatures of peripheral immune cells associated with immune competence traits in Australian Angus cattle. A. Wilson*[1], P. Alexandre[2], T. Legrand[2], S. Denman[2], T. Reverter[2], C. Stewart[1], and R. Farr[1], [1]*Commonwealth Scientific and Industrial Research Organization, East Geelong, VIC, Australia,* [2]*Commonwealth Scientific and Industrial Research Organization, St Lucia, QLD, Australia.*

Infectious disease incurs considerable cost for Australian beef cattle producers through loss in productivity, monitoring, and treatment. Immune competence (IC), a combined measure of cell-mediated (cell-IR) and antibody-mediated (Ab-IR) immune responses, is a generalized disease resilience trait that can be combined with production traits into a weighted selection index for the aim of breeding high-producing and disease resilient animals. In this study we characterized the molecular responses that are associated with IC trait in livestock, with a particular emphasis on the coding and non-coding transcriptome. In this study, 51 Australian Angus cattle were evaluated for cell-IR and Ab-IR at weaning. This was performed by measuring delayed-type hypersensitivity and antibody titers following intradermal and intramuscular vaccination with a multivalent clostridial vaccine, respectively. Total RNA isolated from peripheral blood mononuclear cells (PBMCs) collected before vaccination was profiled using next-generation sequencing. Whole transcriptome analysis was performed using the nf-core/RNa-seq pipeline. Animals were ranked by their Ab-IR, Cell-IR and IC scores and split into quartiles. The lowest and highest quartiles were classified as low and high responders, respectively. Machine learning demonstrated that, for each of the 3 traits, a distinct 5-gene signature could accurately (>95%) classify animals as high or low responders. Differential gene expression analysis with DESeq2 comparing low and high responders found that several genes were differentially expressed (>1.5 FC, *P*-value <0.05) for each trait. A total of 2014 long non-coding genes were found to be highly expressed in PBMCs, including 1350 novel lncRNAs. Machine learning also revealed a that distinct 4-lncRNA signature can also accurately (>95%) distinguish high and low responders. Our ongoing research is now focused on understanding the interplay between coding and non-coding RNAs and the functional role that this plays in IC with the goal of improving immune resilience in beef cattle.

Key Words: immune competence, Angus cattle, whole transcriptome, machine learning

OP93 Association of variants in antibacterial *TLR* genes with reproductive traits in Czech Simmental cattle. K. Novak*[1], K. Samake[2], and M. Bjelka[3], [1]*Institute of Animal Science, Prague-Uhrineves, Czech Republic,* [2]*Charles University, Prague, Czech Republic,* [3]*Breeding Company CHD Impuls, Bohdalec, Czech Republic.*

The Toll-like receptor functions are reflected in various phenotypic traits in addition to the immune response itself. Therefore, we tested the *TLR* gene diversity present in the Czech Simmental (Czech Red Pied) cattle population for association with not only the traits of infection resistance, but also with the female reproductive traits. The starting bull set comprised 164 animals representing complete gene pool. Hybrid resequencing with Illumina X-Ten WGS and PacBio amplicon sequencing yielded 7 and 9 SNPs in the *TLR4* and *TLR5* genes, respectively. The SNPs were validated with primer extension assays and characterized by the assignment to haplotypes and by function prediction. The breeding values for the reproductive traits, namely incidence of cystic ovaries, early reproductive disorders (ERD), calving ease, maternal calving ease, production longevity and calf vitality index were used for the association study. Nominal *P*-values < 0.05 for associations were detected in 18 combinations between 14 polymorphisms and 15 traits using one-way ANOVA. After Benjamini-Hochberg test, the *TLR4* variants g.610C > T (rs43578094) and g.10310T > G (rs8193072) in the reference AC000135.1 remained strictly associated with the index of ERD and maternal calving ease, respectively, at FDR < 0.05. The *TLR4* variant g.9422T > C (rs8193060) was associated with as many as 4 traits. The permissive FDR interpretation for the *TLR5* variants indicated associations with cyst incidence, ERD and maternal calving ease. Positional matches with known QTLs for calving ease endorse the causative roles of *TLR4* and *TLR5*. The findings are consistent with the known effects of *TLR4* variation on reproduction in model species. Consequently, the Bayesian inference supports the role of bovine *TLR4* and *TLR5* in the formation of female reproductive traits. However, additional experiments discriminating between different mechanisms of action are necessary. The project was supported by the Institutional Research Concept MZE-RO0723 and by the grant QK22020280 of the Ministry of Agriculture of the CR.

Key Words: cattle and related species, immunogenomics, genotyping, innate immunity, disease resilience

OP94 ISAG Bursary Award: CRISPR-SpRY-mediated base-editing screening identifies TMEM41B amino acids that are critical for transmissible gastroenteritis virus replication in pig. Y. Zhou*[1], J. Zhang[1], Y. Zhang[1], X. Li[1,3], S. Xie[1,2], C. Zhao[1,2], and S. Zhao[1,3], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, Hu Bei, China,* [2]*Hubei Hongshan Laboratory, Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, Hu Bei, China,* [3]*The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan, Hu Bei, China.*

Combating and controlling epidemic and endemic diseases is a considerable cost of livestock production. Genome editing in pigs could greatly accelerate the progress of breeding programs, thereby increasing disease resistance. However, many infectious diseases still lack effective targets for genome editing. To solve this issue, we performed genome-wide CRISPR screens in porcine cells with transmissible gastroenteritis virus (TGEV). We identified numerous candidate host factors for TGEV infection. Transmembrane protein 41B (TMEM41B) was found to be a *bona fide* host factor involved in TGEV infection, knockout of TMEM41B completely inhibit TGEV replication. Further studies have shown that TMEM41B is also essential for the replication of other RNA viruses. Unfortunately, complete knockout of TMEM41B can lead to embryonic death in mice. Therefore, CRISPR-SpRY-mediated base-editing screens were developed to identify the key amino acid residues of the TMEM41B protein that involve in TGEV replication. We found that several previously unreported residues required for TGEV infection are highly enriched post-TGEV selection. Finally, we found that 4 amino acids in TMEM41B are prerequisites for the virulence of TGEV in porcine cells. In the future we aim to generate TMEM41B single-base edited disease-resistant pigs.

Key Words: pig, TMEM41B, disease resistance, base-editing, CRISPR screen

OP95 Genome-scale CRISPR screen identifies TRIM2 and SLC35A1 associated with porcine epidemic diarrhea virus infection. H. Liu[1], J. Wang[2], Z. Guo[1], X. Zeng[2], Y. Yang[1], S. Li[1], X. Li[1,4], S. Zhao[1,3], C. Wang[2], and S. Xie*[1,3], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, Hubei, P. R. China,* [2]*Key Laboratory of Pig Molecular Quantitative Genetics of Anhui Academy of Agricultural Sciences, Livestock and Poultry Epidemic Diseases Research Center of Anhui Province, Anhui Provincial Key Laboratory of Livestock and Poultry Product Safety Engineering, Hefei, Anhui, P. R. China,* [3]*Hubei Hongshan Laboratory, Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, Hubei, P. R. China,* [4]*The Cooperative Innovation*

Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan, Hubei, P. R. China.

CRISPR/Cas9 has been used extensively in pigs as one of the tools in disease resistance research. Porcine epidemic diarrhea (PED) is the most devastating disease in the global pig industry due to its high fatality rate in piglets. Existing vaccines have become ineffective against the epidemic PEDV variants. There is still a need to breed disease-resistant pigs to prevent and control these viruses. In this study, we designed a pooled African green monkey genome-scale CRISPR/Cas9 knockout (GeCKO) library containing 75,608 single guide RNAs targeting 18,993 protein-coding genes to identify key host factors facilitating PEDV infection and potential therapeutic targets in Vero E6 cells. We discovered several highly expressed but unreported genes associated with PEDV infection after challenging the Vero E6 cells with PEDV. For instance, knocking out the tripartite motif 2 (TRIM2) and the solute carrier family 35 member A1 (SLC35A1) significantly inhibited PEDV replication. Quantitative glycoproteomics showed that knocking out SLC35A1 interfered with glycoprotein expression, especially ADAM17, APNEP and ACE2, to reduce PEDV infection; rather than the entry-dependent cellular CMP-Sialic Acid. These findings provide a new perspective for a better understanding of host-pathogen interactions and candidate targets for the generation of gene-editing disease-resistant pigs. In addition, we also developed a deep learning strategy-based sgRNA activity prediction algorithm, and thus built an online sgRNA design platform, called sgRNAcas9-AI (http://123.57.239.141:8080/), which can help users to design highly active sgRNAs. In the future, we will use gene-editing technology to produce PEDV-resistant pigs.

Key Words: pig, CRISPR screening, PEDV, TRIM2, SLC35A1

OP96 ISAG Bursary Award: LncRNA446 regulates tight junctions by inhibiting the ubiquitinated degradation of Alix after porcine epidemic diarrhea virus infection. Y. Xiao*, W. Qin, H. Wang, and W. Bao, *Yangzhou University, Yangzhou, Jiangsu, China.*

Porcine epidemic diarrhea (PED) is a highly contagious disease, caused by porcine epidemic diarrhea virus (PEDV), which causes huge economic losses. Tight junction-associated proteins play an important role during virus infection; therefore, maintaining their integrity may be a new strategy for the prevention and treatment of PEDV. Long noncoding RNAs (lncRNAs) participate in numerous cellular functional activities, yet whether and how they regulate the intestinal barrier against viral infection remains to be elucidated. Here, we established a standard system for evaluating intestinal barrier integrity and then determined the differentially expressed lncRNAs between PEDV-infected and healthy piglets by lncRNA-seq. A total of 111 differentially expressed lncRNAs were screened, and lncRNA446 was identified due to significantly higher expression after PEDV infection. Using IPEC-J2 cells and intestinal organoids as in vitro models, we demonstrated that knockdown of lncRNA446 resulted in increased replication of PED, with further damage to intestinal permeability and tight junctions. Mechanistically, RNA pulldown and an RNA immunoprecipitation (RIP) assay showed that lncRNA446 directly binds to ALG-2-interacting protein X (Alix), and lncRNA446 inhibits ubiquitinated degradation of Alix mediated by TRIM25. Furthermore, Alix could bind to ZO1 and occludin and restore the expression level of the PEDV M gene and TJ proteins after lncRNA446 knockdown. Additionally, Alix knockdown and overexpression affects PEDV infection in IPEC-J2 cells. Collectively, our findings indicate that lncRNA446, by inhibiting the ubiquitinated degradation of Alix after PEDV infection, is involved in tight junction regulation. This study provides new insights into the mechanisms of intestinal barrier resistance and damage repair triggered by coronavirus.

Key Words: long noncoding RNA, porcine epidemic diarrhea virus, intestinal barrier, tight junction, Alix

OP97 ISAG Bursary Award: Multi-omics integration analysis deciphering genetic basis of host resistance to PRRSV. Q. Wu*[1], T. Zhang[1], X. Wu[1], X. Zhou[1,2], and B. Liu[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China,* [2]*Hubei Hongshan Laboratory, Wuhan, China.*

Porcine reproductive and respiratory syndrome (PRRS) is a globally prevalent and highly contagious disease that causes huge losses to pig industry. To gain insight into the genetic basis of host resistance to porcine reproductive and respiratory syndrome virus (PRRSV), we have conducted an artificial infection trial with 159 individuals from an advanced generation intercross population of PRRSV-resistant Tongcheng pigs and PRRSV-susceptible Large White pigs. Immune response traits were measured and samples were collected at 8 different time points during PRRSV infection. Genome-wide association analysis (GWAS) based on genome-wide sequencing data and 95 immune response traits have identified key genes and SNPs related to disease resistance. The indicator traits for disease resistance were strongly associated with SLA family genes on chromosome 7. Integrated analysis of genome and transcriptome data extracted from white blood cells at d 7 post PRRSV infection of 113 pigs identified 417,283 expression quantitative trait loci (eQTLs) and 213,449 splicing quantitative trait loci (sQTLs) affecting the immune response to PRRSV infection. eQTLs-specific genes and sQTLs-specific genes were respectively enriched in lipid metabolism and immune response pathways, while the genes were enriched in the antigen processing and presentation pathways. The colocalization of GWAS signals, eQTLs and sQTLs identified the causal SNPs responsible for differences in the white blood cell counts and lymphocyte counts in blood during PRRSV infection. The integration of transcriptome and metabolome revealed concerted molecular events triggered by the infection. Metabolomics data set from serum indicated the majority of metabolite levels were downregulated after PRRSV infection and were significantly positively correlated to the expression levels of marker genes in adaptive immune response.

Key Words: pig, QTL, multi-omics, SLA, PRRSV

OP98 Superior survivability of *GBP1* and *GBP5* heterozygous pigs undergoing porcine respiratory syndrome outbreaks. R. Pena*[1], K. Keutgens[2], and L. Fraile[1], [1]*Universitat de Lleida-AGROTECNIO Centre, Lleida, Spain,* [2]*PXL University of Applied Sciences and Arts, Hasselt, Belgium.*

The porcine respiratory disease complex causes respiratory signs leading to pneumonia in rearing pigs and failure to gain weight later in the finishing period. Although the etiology is complex, it usually involves coinfection of viral and bacterial agents. Typically, 30 to 70% of pigs will be affected during an outbreak, with a 4 to 6% mortality rate, depending on the secondary infections. In a screening experiment in production farms in Northeast Spain, we collected samples from transition and rearing farms with active respiratory outbreaks, with mortalities ranging from 13.8 to 71.4%. In each farm, samples were collected from 50 to 80 dead animals (CASES; outbreak) and 50–80 of the best growing pigs (CONTROLS; 2 weeks later). Microbiology and serology test confirmed that farms were infected with PRRSV and either *Streptococcus suis* or *Actinobacillus pleuropneumoniae*. Two SNP molecular markers at *GBP1* (rs80800372) and *GBP5* (rs340943904) genes were genotyped in an initial cohort of 478 samples (269 cases and 209 controls) from 3 farms undergoing PRRSV + *Streptococcus suis* coinfection. Results show that incidence of cases (pigs dead during the outbreak) was reduced by 7% in heterozygous pigs for either marker when compared with the incidence of cases in pigs homozygous for the major (susceptible) allele. Despite their close mapping on the pig chromosome 4, these 2 markers have a moderate linkage disequilibrium, with an r^2 of 0.8. We are currently testing other molecular markers previously associated with resilience to infections in a larger cohort of approximately 900 pigs, including farms infected with PRRSV and *Actinobacillus pleuropneumoniae.*

Key Words: pig and related species, disease resilience, infectious disease, genetic marker, animal health

OP99 IUIS-VIC Travel Award 2: Due to their improved immunity, disease-resistant common carp fish are also less infective. B. Dorfman*, E. Marcos-Hadad, R. Tadmor-Levi, and L. David, *Department of Animal Sciences, R.H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel.*

Common carp (*Cyprinus carpio*) is among the most widely produced aquaculture species. Outbreaks of a disease caused by cyprinid herpes virus type 3 (CyHV-3) have been significantly damaging its production worldwide. Our group has been breeding for CyHV-3 disease-resistant strains. When infected, resistant fish control better the viral replication while susceptible fish cannot and consequently succumb to the disease. This resistance relies on improved host immunity. In this study, experiments using infection-by-cohabitation tested the infectivity of disease-resistant and susceptible fish and how the combination of resistance and infectivity differences affect mortality and disease spread. Disease-resistant and susceptible fish played roles of shedders (infecting) and cohabitants (infected) in all 4 type-role combinations. Mortality rates were highest in groups of susceptible cohabitants infected by susceptible shedders and lowest in groups of resistant cohabitants infected by resistant shedders. However, fewer mortalities were found in susceptible cohabitants infected by resistant shedders compared with susceptible cohabitants infected by susceptible shedders. Additionally, viral loads in spleen of resistant cohabitants infected by resistant shedders were lower compared with the same resistant cohabitants infected by susceptible shedders. Finally, virus levels in water of tanks with susceptible shedders were higher than in tanks with resistant shedders. Taken together, we empirically and clearly demonstrated that since disease-resistant fish control better the virus replication they release less virus particles into the environment and hence, infect other fish less than disease susceptible fish. This study demonstrates that incorporating resistant fish benefits aquaculture production twice, by reducing both mortalities and disease spread.

Key Words: fish, immunology, qPCR, infectious disease, aquaculture

OP100 ISAG Bursary Award: Functional diversity of Toll signaling pathway in Czech Simmental cattle with respect to health and resilience traits. K. Samake*[1], T. Valcikova[2], M. Bjelka[3], and K. Novak[4], [1]*Charles University, Prague, Czech Republic,* [2]*Czech University of Life Sciences, Prague, Czech Republic,* [3]*Breeding Company CHD Impuls, Bohdalec, Czech Republic,* [4]*Institute of Animal Science, Prague-Uhrineves, Czech Republic.*

The work was aimed at the screening for and interpretation of functional polymorphism in the key members of the Toll signaling pathway in the population of the Czech Simmental cattle. Focus was on the transcription factor NFkappaB as the main pleiotropic factor for phenotypic traits and to MyD88 as an interactor insufficiently studied until now. Hybrid resequencing with Illumina X-Ten WGS and PacBio amplicon sequencing yielded 22 and 13 SNPs in *NFkappaB1* and *NFkappaB2* genes, respectively, while over 30 SNPs were found in the *MYD88* gene. The PacBio amplicons were used for haplotype determination in the given population, resulting in 7 haplotypes for *NFkappaB1*, 6 haplotypes for *NFkappaB2* and additional tens of SNPs in *MYD88*. Based on the functional prediction and haplotype assignment, a subset of candidate SNPs of interest was chosen for subsequent genotyping with the SNaPshot technique. The working sets of reactions included 8 and 11 SNPs in *NFkappaB1* and *-2* genes, respectively, and 18 SNPs in *MYD88*. The presence of a nonsynonymous mutation R474G in *NFkappaB1* allows to assume phenotypic effects due to the pronounced pleiotropy of this gene. The results from genotyping of individual animals were used for haplotype reconstruction in the given population and for the association study in the set of 164 bulls using haplotype data. The breeding values for milk production, health traits and female fertility were correlated with allelic forms of the 2 key genes along with the *TLR* gene series. The ongoing work includes the resilience trait of milk production as the indicator potentially affected by the Toll pathway members. The project was supported by the Institutional Research Concept no MZE-RO0723 of the Ministry of Agriculture of the CR.

Key Words: cattle and related species, immunogenomics, genotyping, innate immunity, animal health

OP101 ISAG Bursary Award: Genomic markers associated with immune traits in Sasso chickens raised in Ethiopia. M. Girma*[1,2], M. Katrina[3], S. Kate[3], W. Esatu[2], B. Solomon[2], T. Dessie[2], P. Androniki[3,4], V. Lonneke[3], H. Olivier[2,5], B. Georgios[3,6], and M. Dikeledi[1], [1]*Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Florida, South Africa,* [2]*CTLGH-LiveGene, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [3]*Centre for Tropical Livestock Genetics and Health, The Roslin Institute, University of Edinburgh, Easter Bush Campus, Midlothian, UK,* [4]*The Royal Veterinary College, Hawkshead Lane, Hatfield, Hertfordshire, UK,* [5]*Cells, Organisms and Molecular Genetics, School of Life Sciences, University of Nottingham, Nottingham, UK,* [6]*Scotland's Rural College (SRUC), Animal and Veterinary Sciences, Easter Bush, Midlothian, UK.*

Newcastle disease virus (NDV) is a highly contagious avian pathogen that threatens poultry production. It is endemic to many areas and responsible of many epidemic events. Selection for antibody (Ab) response has potential to effectively improve the resistance to disease in chickens. However, the origin of the variation among chickens in Ab response to NDV remains unclear. Here, we aimed to identify the genes modulating Ab response to a viral pathogen such as NDV while under outdoor conditions. A genome-wide association study (GWAS) was performed. After NDV vaccination, Sasso T451A chickens were naturally exposed to environmental challenges at the ILRI poultry research facility in Addis Ababa, Ethiopia. Phenotypic and immune data from 1022 chickens in 2 batches (batch 4 with 507 birds and batch 5 with 515 birds) were recorded. Genotyping information from low-pass sequencing was obtained from 935 chickens (2,676,181 SNPs). The results revealed chickens from batch 4 show a stronger Ab response at 56 d-old and a lower Ab response at 112 d-old compared with batch 5 chickens. We used BioMart data mining and variant effect predictor tools to annotate the SNPs and candidate genes, respectively. Five significant SNPs (rs316795557 (*FOXP2*) – chr 1, rs313761644 (*CEP170B*) – chr 5, rs733628728 – chr 13 and 2 unnamed SNPs – chr 30 and chr 33) were associated ($P < 3.92E-7$) with chicken antibody response to NDV. These SNPs are in genomes regions including several genes regulating the immune response. The results of this study pave the path for more investigation into the importance of the chicken immune response to NDV.

Key Words: antibody response, genome-wide linkage analysis, Newcastle disease, Sasso T451A, vaccine challenge

OP102 ISAG Bursary Award: Assessment of haemagglutination titre and serum lysozyme concentration in Nigerian indigenous chicken genotypes. U. Akpan*, A. S. Adenaike, M. I. Takeet, A. A. Bello-Ibiyemi, and C. O. N. Ikeobi, *Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.*

This research was conducted to assess the immune status of 3 indigenous Nigerian chicken genotypes, namely, normal feather, frizzled feather and naked neck. The chickens were inoculated with sheep red blood cell (SRBC), a multi-determinant antigen. Two hundred sixty-eight chickens from the 3 genotypes divergently selected for high and low response to SRBC and a randombred control line, originating from the same base population were used for the study. The birds (8 weeks of age) were inoculated with 1 mL of 1% suspension SRBC via the jugular vein and antibody responses at 5, 10 and 15 d post inoculation (dpi) were measured. Data collected on the haemagglutination (HA) titer and serum lysozyme concentration (SL) were analyzed using the Linear Model procedure of R version 4.0.2. Result showed that gen-

otype did not significantly ($P > 0.05$) affect HA titer. However, result obtained showed significant ($P < 0.05$) prevalence of antibody response at 5 dpi. There was significant ($P < 0.05$) interaction effect of genotype and sex for SL at 5 dpi, in which female frizzle feather had significant higher value when compared with female normal feather. The study therefore suggests that Nigerian indigenous female frizzle feather chicken genotype has a good potential for serum lysozyme response and could be selected to improve such trait. Also, selections for immunocompetent traits like HA titer and serum lysozyme concentration should be prioritized at 5 dpi.

Key Words: immune response, SRBC, haemagglutination, serum lysozyme, indigenous chicken

OP103 Exploring, evaluating, and quantifying the mammalian alveolar macrophage response to intracellular mycobacterial pathogens using an integrative multi-omics approach. T. J. Hall[1], M. Mittermite[2], J. A. Browne[1], G. P. McHugo[1], J. F. O'Grady[1], E. L. Clark[3], M. Salavati[3,4], S. V. Gordon[2,5], and D. E. MacHugh*[1,5], [1]*UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland,* [2]*UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland,* [3]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, Scotland, United Kingdom,* [4]*Dairy Research and Innovation Centre, SRUC South and West Faculty, Barony Campus, Parkgate, Dumfries, Scotland, United Kingdom,* [5]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland.*

Bovine tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium bovis*, which is responsible for significant economic losses in the livestock industry worldwide and can also cause TB disease in a range of other mammals, including humans. Alveolar macrophages are the primary cells targeted by the pathogen during early infection, and while they play a crucial role in controlling the infection, the exact nature of the host-pathogen interaction, and the genetic and epigenetic factors that drive host tropism are not fully understood. Here, we have used RNA-seq, miRNA-seq, ChIP-seq, and ATAC-seq to examine the effect of *M. bovis* infection on the bovine alveolar macrophage (bAM) epigenome and transcriptome. In addition to this, we have also challenged bAMs with *M. tuberculosis* (the primary cause of human TB), *M. bovis* BCG (the vaccine strain), and gamma-irradiated (killed) *M. bovis* to examine the bAM epigenomic and transcriptomic responses across multiple pathogenic insults. The results of this multi-omics comparison have shed new light on the function of pivotal response genes and support the hypothesis that pathogen-driven epigenetic reprogramming of the host macrophage is key to bacterial survival and host tropism for *M. bovis* and other TB-causing mycobacteria.

Key Words: cattle, tuberculosis, macrophage, transcriptome, epigenome

ISAG-FAO Genetic Diversity

OP104 Genomic tools for the monitoring of genetic diversity. P. Boettcher*[1], R. Baumung[1], P. Burger[2], L. Colli[3], I. Curik[4], G. Leroy[1], C. Looft[5], A. Manunza[6], G. Mészáros[7], D. Ouedraogo[8], B. Rosen[9], A. Stella[6], Y. Utsunomiya[10], J. Windig[11], J. Soelkner[7], [1]*Food and Agriculture Organization of the UN, Rome, RM, Italy,* [2]*University of Veterinary Medicine Vienna, Vienna, WI, Austria,* [3]*Università Cattolica del Sacro Cuore, Piacenza, PC, Italy,* [4]*University of Zagreb, Zagreb, Croatia,* [5]*University of Applied Science Neubrandenburg, Neubrandenburg, MV, Germany,* [6]*IBBA-CNR, Milan, MI, Italy,* [7]*BOKU, Vienna, WI, Austria,* [8]*Joseph KI-ZERBO University, Ouagadougou, KAD, Burkina Faso,* [9]*United States Department of Agriculture, Beltsville, MD,* [10]*São Paulo State University, São Paulo, SP, Brazil,* [11]*Wageningen University and Research, Wageningen, GE, the Netherlands.*

Census population size is a key factor influencing the risk of extinction of animal populations. For the past 30 years, FAO has used this metric for monitoring the state of animal genetic resources, by using data provided by countries in the Domestic Animal Diversity Information System (DAD-IS). Indicator 2.5.2 of the UN Sustainable Development Goals is based on this criterion. Genetic variation of a population has impacts on reproductive fitness and adaptation to environmental changes, and thus also influences its extinction risk. Maintenance of the genetic diversity within populations of wild and domesticated species is therefore among the goals of the recently-adopted Kunming-Montreal Global Biodiversity Framework. FAO has worked with international experts to review approaches for the measurement of within-population diversity, has proposed effective population size (Ne) as a suitable indicator. Ne is generally straightforward in its interpretation. An additional advantage is the possibility of estimation with demographic, pedigree or genomic data. Ne has furthermore been proposed as an indicator for implementation of the Biodiversity Framework. For monitoring the Ne of livestock breeds, genomic data may be the most feasible option for most countries. At the same time, methods for estimating historical Ne are improving, facilitating monitoring of recent diversity loss. From the economic point of view, the current genotyping costs for estimating Ne for most breeds and species based on 100 genotyped individuals would be around USD 2500/breed. Many countries have already demonstrated the technical capacity, scientific interest and political will to study their breeds on the molecular level. A literature review of 250 randomly selected breeds from a representative group of countries, revealed that genomic studies (SNP or whole-genome sequencing) have already been undertaken on 39% of breeds when considering in-country research and 68% when accounting for international studies on transboundary breeds. This proportion increases to 75% when all molecular genetic studies are considered. Member countries have requested FAO to continue to review, develop and refine indicators for genetic diversity and propose related data fields for incorporation into DAD-IS.

Key Words: genetic diversity, breed, monitoring, genomics, effective population size

OP105 Genetic characterization of deleterious alleles in traditional cattle populations in Europe and Africa. R. Crooijmans*[1], R. Gonzalez-Prendes[1], M. Derks[1], N. Ghanem[2], C. Ginja[3], D. Kugonza[4], L. Makgahlela[5], and K. Juha[6], [1]*Wageningen University and Research, Animal Breeding and Genomics, Wageningen, the Netherlands,* [2]*University of Cairo, Animal Reproduction Department, Cairo, Egypt,* [3]*University of Porto, Centro de Investigacão em Biodiversidade e Resursos Genéticos, Vairão, Portugal,* [4]*Makerere University, Animal Breeding and Genetics, Kampala, Uganda,* [5]*Agricultural Research Council, Animal Breeding and Genetics, Pretoria, South Africa,* [6]*Natural Resources Institute Finland, Jokioinen, Finland.*

Traditional local cattle breeds are under severe pressure of extinction worldwide due to their low production performance compared with commercial breeds, as well as their legislation. Breed replacement or crossbreeding with commercial transboundary cattle is a threat to these native breeds. One very big advantage of traditional local cattle breeds is the high adaptation to the ecosystems they have lived in. How did this adaptation shape the genome of these animals? Within the OPTIBOV-project, we have sampled and sequenced over 500 cattle from 26 traditional breeds. Additionally, we established standardized protocols (SOPs) for phenotype collection in 6 different countries (Finland, the Netherlands, Portugal, Egypt, Uganda, and South Africa) for both traditional cattle breeds and one commercial dairy breed. The derived phenotypes are used in combination with whole-genome sequence data of the same animals to try to find association to adaptation to different ecotypes. Each individual has a certain number of harmful mutations in its genome. These mutations can lower the fitness of the individual carrying them, dependent on their dominance and selection

coefficient. Effective population size, selection, and admixture are known to affect the occurrence of such mutations in a population. We detect breed-specific variations, including SNPs and structural variants (SVs). To improve the detection of SVs, we create breed-specific long read Nanopore reference genomes. The relative roles of demography and selection are key to understanding the process of adaptation. In this study we will investigate the number of deleterious alleles. We hypothesize that the series of events of bottlenecks, introgression, inbreeding and strong artificial selection associated with domestication increased mutational load in these traditional cattle breeds.

Key Words: traditional cattle breed, deleterious allele, WGS

OP106 Genetic structure of Criollo sheep populations with Iberian and African breeds. J. Cappello[1,2], M. Revidatti*[1,2], S. De la Rosa[1,2], V. Morales[1,2], E. Tejerina[1,2], BiOvis Consortium[2], and A. Martínez[2,3], [1]*Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste, Corrientes, Argentina,* [2]*Red CONBIAND, Córdoba, España,* [3]*Facultad de Veterinaria, Universidad de Córdoba, Córdoba, España.*

A set of 23 STR markers were analyzed in 1239 sheep belonging to 28 populations (America, Spain, and Africa): Criollo populations from Argentina (5), Mexico (1), Brazil (1), Paraguay (1), Ecuador (1), USA (2), Chile (1), Bolivia (1), Uruguay (1), Perú (1). We also included samples from 8 Spanish and 5 African breeds. The objective was to analyze interracial diversity between Criollo and Iberian/African breeds through the Bayesian clustering method. The genetic structure was determined by using Structure v. 2.3.4. The optimal K was estimated by using the Structure Harvester program, which turned out to be K = 26. When K = 4 was assumed, the 5 Argentinean, the Bolivian and the Paraguayan Criollo populations clustered together and the 4 local Spanish breeds was added to this group. Another group was represented by Nigerian and Pelibuey. The third cluster grouped the Uruguayan and the USA; and the last one formed by Brazilian, Mexico and Balearic Islands breeds. At K = 7 the Argentinian Criollo separated from the other groups, while Mallorquina and Ecuadorian Pelibuey; the Nigerian breeds and Spanish Pelibuey; the USA and Uruguayan sheep; Brazilian, Mexico and Menorquina population; and Roja Mallorquina and Canaria formed the other 6 different clusters. At K = 10 breeds in the Criollo group generally showed different ancestry relative to each other with very few exceptions. The influence of Spanish breeds was detected in Mexican (by Menorquina) and one Argentinean population (by Roja Mallorquina). No influence could be detected from the African populations. At K = 16 the Criollas are grouped among themselves as follows: 2 of the Argentine populations; 2 from the USA; and a third cluster formed by Mexico and Peru breeds. Criollo group did not show influence of Spanish or African breeds. Finally, at K = 26, each breed separated as a unique population, with a low percentage of admixture, except for the Nigerian ones, which remain in the same cluster. These results suggest that creole sheep constitute a different group, not necessarily composed of a high proportion of the supposed ancestral breeds. This work has been developed within the CONBIAND network.

Key Words: ewe, local breed, molecular

OP107 ISAG Bursary Award: An insight into whole-genome resequencing data of Indian native goats with global breeds reveals high within-breed genetic diversity and distinct population structure. N. Balasubramaniam*[1,2], S. Dixit[2], S. Singh[2], S. Koloi[1,2], and I. Ganguly[2], [1]*ICAR-National Dairy Research Institute, Karnal, Haryana, India,* [2]*ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana, India.*

Indian goats possess varied levels of adaptability, productivity and disease resistance. Genomic diversity of goats surviving in extreme conditions hold answers to building a sustainable goat production system. We studied diversity of 11 Indian breeds (n = 102) compared with worldwide goats from 30 breeds and 5 outgroups (n = 101, public domain). Indian goat samples were sequenced using Illumina NOVASEQ6000 platform. Sequences were combined to generate a VCF file; annotation was done using the *Capra hircus* reference. Nucleotide diversity (p), inbreeding coefficient (F), observed (Ho) and expected heterozygosity (He), proportion of polymorphic SNP (PN), average pairwise genetic distance (DST), effective population size (Ne), linkage disequilibrium (LD, r^2) and LD decay were obtained. NJ tree, PCA and Admixture were carried out to assess population structure. A total of 21.44 billion reads remained after quality control and alignment with ARS1. Average coverage was 99.2% and 17.2% duplication rate; depth of sequencing was ~9×. Kanni Adu (KAN) had 1.95 M SNP while Jharkhand Black (JB) had 30.77 K; average r^2 was highest in JB (0.859) and lowest in Jakhrana (JAK) (0.496). LD showed rapid decay ($r^2 < 0.2$) within 5 kb in all the breeds except JB, Sangamneri (SAN) and KAN. Changthangi (CHA) (0.379) and JAK (0.363) had the highest p, while KAN had the highest PN (0.340). Average Ho was lowest in JB (0.119) and highest in CHA (0.505); He was lowest in KAN (0.249) and highest in CHA (0.378). KAN, Tellicherry (T), SAN and JB were distinct in PCA. Admixture graph at k = 2–12 showed that k = 8 had minimum error (0.423). PCA revealed that other *Capra* species were distinct from *C. hircus*. Indian goats were widely distinct from exotic; 3 breeds from Pakistan clustered with Indian goats. Goats spread over the Indian subcontinent have high within-breed diversity (>94%), harboring substantial genetic variations to respond to future demands and climate change. Within-breed diversity, gene flow among indigenous animals and shared genomic regions are contributors for admixed nature of animals.

Key Words: Indian, goat, diversity, admixture, PCA

OP108 Differences in effective population sizes and breed contributions to genetic variation in Estonian farm animal breeds. E. Sild*, S. Värv, T. Põlluäär, H. Viinalass, and T. Kaart, *Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, Tartu, Estonia.*

Genome-wide SNP data were used to study effective population sizes and breed contributions to the total genetic variation in local horse and dairy cattle populations in Estonia. Due to the endangered risk status of local horse breeds, diversity assessments are of great importance—both the within-breed and between-breed components should be considered when implementing conservation plans. Five horse breeds (160 horse samples in total) and 2 dairy breeds (75 cattle samples in total) were included in the analyses. All of the studied local breeds have suffered loss of population size during recent decades. In addition, intensive dairy cattle selection, associated with limited genetic material worldwide, has influenced the local gene pool and decreased the effective population size (N_e). Genomic inbreeding estimates ranged for horse breeds F_{ROH} = 0.06–0.14 and for cattle breeds F_{ROH} = 0.04–0.11. The LD-based N_e analyses (Wier and Hill, 1980) showed that local horse breeds retain higher N_e than international breeds. The lowest N_e was found for the Trakehner breed, (N_e = 62) raised in Estonia, and the highest for the Tori Horse (N_e = 134). The admixed Tori has a partly closed population with rather similar N_e = 133. This closed population, based on Studbook division, showed the highest contribution (24.0%) to horses' genetic diversity while lower contributions were found for the Estonian Native (16.8%) and the Estonian Heavy Draft Horse (16.1%) as well as for Tori sport-type horses (18.1%). In cattle, N_e LD was almost twice as high in the Estonian Reds (249) than in the Estonian Holsteins (144), while the breed contributions were similar for the Estonian Red (42.4%) and Estonian Holstein breeds (57.6%), despite the higher number of Holsteins (10 thousand versus 72 thousand dairy cows, respectively). The study was supported by ETAG grant PRG554.

Key Words: animal breeding, horse and related species, cattle and related species

OP109 Genetic diversity of Clydesdale and Shire draft horses with implications for management. J. L. Petersen*, A. M. Barber, A. M. Fuller, and I. Grazian, *University of Nebraska–Lincoln, Lincoln, NE.*

The draft horses of the UK, the Scottish Clydesdale and British Shire, have been bred for their size and utility as work horses for over 200 years; formal breed registries were established in the late 1800s.

Thousands of these popular horses were exported to North America and elsewhere near the turn of the 20th century. In the 1930s, however, draft horse populations were greatly reduced. Each breed is now considered "at risk" by the Rare Breed Survival Trust. Using varying data sets, the purpose of this study was to characterize the diversity of and evaluate population structure within and between breeds. Clydesdales born between 2016 and 2018 in the US (n = 34), Canada (n = 29), or Scotland (n = 31) were genotyped at ~72,000 SNP markers. Microsatellite genotypes (12 loci) were compiled from over 3,500 Shire horses born between 1971 and 2021, all registered with the American Shire Association. Finally, whole-genome sequence (WGS) was generated on 29 horses for comparisons of diversity. The data demonstrate evidence of differentiation between Scottish and North American Clydesdales with pairwise F_{ST} values between the Scottish sample and either North American sample 5-fold greater than that between the Canadian and US samples. Greater diversity determined by expected heterozygosity and allelic richness was identified in the North American Clydesdales compared with the Scottish sample. Individual inbreeding was significantly ($P < 0.001$) higher in the Scottish Clydesdales (avg $F_{ROH} = 0.34$) compared with the Canadian and US samples (avg $F_{ROH} = 0.30$ in each). Microsatellite data from the Shires demonstrated that allelic richness decreased between 1971 and 2021, although the reduction was not significant ($P = 0.34$). A similar reduction in expected heterozygosity was observed. Two breeds were differentiated by WGS, which also revealed that the Clydesdales had individual inbreeding coefficients that were, on average, 1.1× that of the Shires. These data can help breeders determine the best means to continue to breed quality horses while being mindful of genetic diversity.

Key Words: single nucleotide polymorphism, microsatellite, population genomics, inbreeding, equine

OP110 History and genetic diversity of African sheep: Perpendicular contrasts of phenotypes and genomic diversity. A. Da Silva[1], A. Ahbara[2], S. Ben Jemaa[3], Y. Cao[4], E. Ciani[5], E. Dzomba[6], O. Hanotte[7], S. Mastrangelo[8], A. Missohou[9], A. Molotsi[10], A. Muchadeyi[11], J. Mwacharo[12], M.-L. Li[4], S. Hall[13], J. Lenstra*[14], [1]*PEREINE/E2LIM, Faculty of Science and Technics, Limoges, France,* [2]*Department of Zoology, Faculty of Sciences, Misurata University, Misurata, Libya,* [3]*Laboratoire des Productions Animales et Fourragères, Institut National de la Recherche Agronomique de Tunisie, Université de Carthage, Ariana, Tunisia,* [4]*CAS Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China,* [5]*Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari "Aldo Moro," Bari, Italy,* [6]*Discipline of Genetics, School of Life Sciences, University of KwaZulu-Natal, Scottsville, South Africa,* [7]*School of Life Sciences, University of Nottingham, Nottingham, UK,* [8]*Dipartimento Scienze Agrarie, Alimentari e Forestali, University of Palermo, Palermo, Italy,* [9]*Animal Production and Nutrition Unit, Inter-State School of Veterinary Science and Medicine (EISMV), Dakar, Senegal,* [10]*Department of Animal Sciences, University of Stellenbosch, Matieland, Stellenbosch, South Africa,* [11]*Agricultural Research Council, Biotechnology, Platform, Onderstepoort, South Africa,* [12]*International Centre for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia,* [13]*Department of Environmental Protection and Landscape, Estonian University of Life Sciences, Tartu, Estonia,* [14]*Faculty of Veterinary Medicine, Utrecht University, Utrecht, Utrecht, the Netherlands.*

Domestic sheep in Africa have adapted to contrasting and extreme climates and play an important role in the local community-based economies of several African countries. Here we review the Neolithic immigrations of thin-tailed sheep as deduced from archeological evidence, the later introduction of fat-tailed breeds and the more recent history of sheep in Egypt, the Maghreb, West and Central Africa, East Africa and Southern Africa, respectively. We present a comprehensive molecular survey on the basis of 50K SNP genotypes of African 55 breeds contributed by several laboratories. We propose that gene flow has a major influence of the relationships of breeds from different regions and has partially overwritten the diversity profile created by the initial migrations. A genetic contrast between sheep North and South of the Sahara is perpendicular to the West-East contrast of thin- and fat-tailed sheep. There are no close genetic relationships between African and Asian fat-tailed breeds, whereas we observe within Africa only a modest effect of the tail types on the breed relationships.

Key Words: sheep, population genomics, single nucleotide polymorphism (SNP), breed diversity, heritage

OP111 An archaeogenomics study of Iron Age cattle from Althiburos, Tunisia. C. Ginja*[1], S. Guimarães[1], R. da Fonseca[2], R. Rasteiro[3], R. Rodríguez-Varela[4], L. G. Simões[5], C. Sarmento[1], M. Carme Belarte[6], N. Kallala[7], J. Ramon Torres[8], J. Sanmartí[9], A. M. Arruda[10], C. Detry[10], S. Davis[11], J. Matos[12,13], A. Götherström[4], A. E. Pires[1,14], and S. Valenzuela-Lamas[10,15], [1]*BIOPOLIS-CIBIO-InBIO, Universidade do Porto, Vairão, Portugal,* [2]*Center for Global Mountain Biodiversity, GLOBE Institute, University of Copenhagen, Copenhagen, Denmark,* [3]*Bristol Medical School, University of Bristol, Bristol, UK,* [4]*CPG, The Centre for Palaeogenetics, Stockholm University, Stockholm, Sweden,* [5]*Human Evolution, Department of Organismal Biology, Uppsala University, Uppsala, Sweden,* [6]*ICREA, Institut Català de Recerca i Estudis Avançats, Barcelona, Spain, and ICAC, Institut Català d'Arqueologia Clàssica, Tarragona, Spain,* [7]*INP, Institute National du Patrimoine, Tunis, Tunisia,* [8]*Consell Balear d'Eivissa, Eivissa, Balearic Islands,-Spain,* [9]*Departament de Prehistòria, Història Antiga i Arqueologia, Universitat de Barcelona, Barcelona, Spain,* [10]*UNIARQ, Centro de Arqueologia da Universidade de Lisboa, Faculdade de Letras da Universidade de Lisboa, Lisboa, Portugal,* [11]*LARC/DGPC, Laboratório de Arqueociências, Direcção Geral do Património Cultural, Lisboa, Portugal,* [12]*Unidade Estratégica de Investigação e Serviços de Biotecnologia e Recursos Genéticos, Instituto Nacional de Investigação Agrária e Veterinária, I.P., Oeiras, Portugal,* [13]*CE3C, Centre for Ecology, Evolution and Environmental Changes, Universidade de Lisboa, Lisboa, Portugal,* [14]*Faculdade de Medicina Veterinária, Universidade Lusófona, Lisboa, Portugal,* [15]*Archaeology of Social Dynamics, Consejo Superior de Investigaciones Científicas-Institució Milà i Fontanals d'Humanitats (CSIC-IMF), Barcelona, Spain*

Cattle are one of the principal domesticated animals in Eurasia and Africa and, as attested by figurines, rock art and craftwork produced across many cultures, have high symbolic value. Genomic analyses of aurochs, the ancestors of cattle, and early domestic cattle confirmed independent events of introgression of wild stock. However, the origins of the divergent North African taurine cattle are still debated, and diachronic archaeogenomics data from this region are needed to test alternative demographic scenarios. The major aim of this study was to investigate the genomic composition of Iron Age cattle from the Maghreb, a key region for understanding the dynamics of cattle dispersal and admixture with local aurochs following their earliest domestication in the Fertile Crescent more than 10,000 years ago. We generated genome-wide high-throughput sequence data and mitogenomes for 4 archeological specimens of domestic cattle from the Eastern Maghreb, i.e., Althiburos, El Kef, Tunisia, a permanent settlement of local Numidian agropastoralists. We also obtained PCR-based sequence data for a fragment of the mitochondrial hypervariable D-loop region for these and a further 8 cattle specimens collected in Althiburos. Five specimens were directly radiocarbon dated to ~2,877–2,003 cal BP, i.e., 9th to 1st century cal BCE. Four of these yielded sufficient autosomal genome coverages (0.01× to 0.10×) for population genomic analyses. Principal component analysis and model-based clustering of autosomal data showed Althiburos cattle were genetically close to the pre-domestic Northwest African aurochs and shared ancestry with present-day N'Dama taurine cattle. Maternal lineages were assigned to the R and T1 haplogroups found in 2 and 10 Althiburos specimens, respectively. These Iron Age specimens from Althiburos are the oldest R-mitogenomes described so far in domestic cattle. Our results corroborate the introgression of auroch females into the domestic stock of cattle from Althiburos, and renew the debate on whether there was a third domestication event of taurine cattle in North Africa.

Key Words: domestic cattle, auroch, ancient DNA, population genomics, domestication

OP112 ISAG Bursary Award: Temporal changes in genomic diversity of the northernmost cattle populations in Europe. M. Weldenegodguad*[1], M. Kjetså[2], A. Blauer[3], A. M. Johansson[4], C. Sarmento[5], S. Guimarães[5], C. Ginja[5], M. Honkatukia[2], and J. Kantanen[1], [1]*Natural Resources Institute Finland, Jokioinen, Finland,* [2]*NordGen–Nordic Genetic Resource Center,* Ås, Norway, [3]*University of Turku, Turku, Finland,* [4]*Swedish University of Agricultural Sciences, Uppsala, Sweden,* [5]*BIOPOLIS-CIBIO-InBIO, Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, Portugal.*

In Northern Fennoscandian regions, cattle (*Bos taurus*) are one of the most important livestock species in terms of their economic, societal, and cultural values. Ancient DNA studies play a pivotal role in investigating the evolutionary and population history of domestic animals. Ancient DNA data can also help to examine temporal changes in the genetic diversity. To investigate the population history of Northern Fennoscandia cattle, we performed high-throughput sequencing and retrieved genome sequences of ancient cattle specimens from Finland, Norway and Sweden. The specimens were excavated in the northern regions of these countries. We used the existing whole-genome sequence data of the northern native cattle breeds and investigated genetic relationships between these and their ancient counterparts. We successfully extracted the DNA and constructed genomic libraries from 27 ancient specimens: 5 post-medieval samples (ca. 300 years old) from Finland; 10 medieval and post-medieval samples (ca. 700–300 years old) from Sweden; and 12 medieval and post-medieval samples (ca. 700–300 years old) from Norway. Of the total samples, 14 yielded sufficient autosomal genome coverages (0.01× to 0.29×) for population genomic analyses. Moreover, for 13 samples it was possible to collect mitogenomes (90% covered at over 3×) for a phylogenetic analysis. More than 1.6 million SNPs were detected in the ancient samples and that may represent the unique genetic diversity of the Medieval Fennoscandian cattle populations.

Key Words: ancient DNA, cattle, genetic diversity, palaeogenomic, population genomics

OP113 ISAG Bursary Award: Admixed ancestry or independent race: A phylogenetic meta-analysis on the phylogeography of Philippine chickens. C. Godinez*[1,2], J. Layos[2,3], Y. Yamamoto[2], T. Kunieda[4], and M. Nishibori[2,1], [1]*Department of Animal Science, College of Agriculture and Food Science, Visayas State University, Visca, Baybay City, Leyte, Philippines,* [2]*Laboratory of Animal Genetics, Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima, Japan,* [3]*College of Agriculture and Forestry, Capiz State University, Burias, Mambusao, Capiz, Philippines,* [4]*Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Ehime, Japan.*

An important unresolved question is whether the founding lineages of chickens introduced into the Philippines were descended from wild endemic populations or domestic chickens from mainland Southeast Asia (MSEA) that had become feral. A total of 562 complete mtDNA control region sequences of Southeast Asian chickens (n = 223, ISEA and n = 339, MSEA) were meta-analyzed in this study, along with 35 sequences of Pacific chickens, to elucidate the genetic relationship and population history of Philippine chickens with the rest of the chicken populations across Southeast Asia. The Bayesian phylogenetic tree revealed the distinct phylogenetic position of the diverse D-lineage, indicating that the Philippine-Pacific D1b subclade underwent in situ diversification in the Philippines with their founding population (sub-haplogroup D1a) before expanding eastward. Notably, red junglefowls from Palawan and Mindoro have been found to be related to the ancestral Hap-D2 lineage, implying that the Philippine red junglefowl descended from the MSEA founding population and became exoferal or admixed following colonization in the archipelago. There is also close genetic affinity between Philippine and Cambodian chickens, both of which belong to haplogroup D. Meanwhile, there is no discernible subgrouping of chicken populations in ISEA. This phylogenetic meta-analysis contributes to the general understanding of Philippine chicken phylogeography, allowing for better management of these genetic resources.

Key Words: evolutionary genomics, feralisation, mitochondrial DNA, phylogeny, poultry and related species

OP114 ISAG Bursary Award: Multiple origins and genetic diversity of Philippine native pigs. J. B. Banayo*[1,2], K. L. V. Manese[2], K. O. Furusho[2], A. J. Salces[2], and T. Yamagata[1], [1]*Nagoya University, Chikusa, Nagoya, Japan,* [2]*University of the Philippines Los Baños, Laguna, Philippines.*

The native pig has a niche among women-led smallholder farms in the Philippines. Considering this sociocultural importance and its crucial role in addressing the issue of household food security and livelihood in rural communities, this study was conducted to characterize the genetics and physical traits of native pigs in the Philippines (PhNP) to inform conservation management. We compared PhNP (n = 157 collected from 7 Philippine provinces in Luzon and Visayas regions) with other breeds using partial mtDNA D-loop sequence, 21 ISAG-FAO recommended microsatellite markers, and 18 physical traits. Phylogenetic analysis showed that PhNP have Asian wild boar (*Sus scrofa*) origins from multiple domestication centers, such as the East (D2), Southeast Asia (D7), and the Cordillera/Lanyu clade. We further show that D7 clustered within the general D2 clade, suggesting its D2 ancestry and formation due to a population bottleneck upon dispersal to Southeast Asia. Our results also dispel the local belief that the PhNP were domesticated from endemic wild pigs of the Philippines. Linear discriminant (LD) analysis of physical traits differentiated the pigs representing the D2 (North Luzon lowlands), and the D7 subclades (South Luzon and Visayan), but not the Cordillera clade (North Luzon highlands). Discriminating traits (coefficient of LD 27.0 to −46.0) were the ratios of tail to body length, ear to body length, and snout to head length, supporting the locally observed variation in ear and snout. On the other hand, microsatellite analysis showed pairwise *Fst* between 0.130 and 0.427, suggesting sufficient genetic differentiation between PhNP populations. The effective population size (*Ne*) was below 50, except Kalinga, which has 420. Kalinga is a predominantly indigenous community that uses wild pigs in PhNP production, highlighting hybridization benefits to PhNP. To mitigate low *Ne*, we propose to encourage breed use by incentivizing native pig farming as a conservation service. This study shows the diverse genetics of PhNP and contributes to our understanding of domestic animal diversity in an island country.

Key Words: pig and related species, breed diversity, phylogeny, effective population size, physical trait

OP115 ISAG Bursary Award: The first *Rangifer tarandus* Y chromosomal phylogeny. E. Bozlak*[1,2], K. Pokharel[3], M. Weldenegodguad[3], A. Paasivaara[3], J. Kantanen[3], and B. Wallner[1], [1]*Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Vienna, Austria,* [2]*Vienna Graduate School of Population Genetics, University of Veterinary Medicine Vienna, Vienna, Austria,* [3]*Natural Resources Institute Finland, Jokioinen, Finland.*

Reindeer (*Rangifer tarandus*) are one of the intriguing mammals known for their closeness to present indigenous human populations as an important source of meat and materials. Reindeer, called caribou in North America, has a circumpolar distribution and all extant populations belong to the same species. Studies based on autosomal microsatellites, mtDNA and genomic data have revealed insights into the species' population structure, history and the domestication process. Due to its uniparental inheritance without recombination, the Y chromosome is a potent locus for decoding the male population history of mammals. Here, we created the first *R. tarandus* Y phylogeny and draw conclusions on the male demography of species. We assembled 1,320 Y chromosomal contigs from whole-genome sequencing (WGS) data, representing in total 1.3 Mb of single-copy Y region, which were used as reference for variant calling with short-read data. We next mapped 55 WGS males representing semi-domestic and wild individuals from

Fennoscandian and Russian tundra reindeer, forest reindeer, Svalbard reindeer and caribou. A maximum parsimony tree was created based on 450 polymorphic sites. We observed 2 early separated clades: Clade A containing samples from Arctic Islands, Eurasia, and a few samples from North America (so-called Eurasia+ clade) and Clade B formed only by caribou from North America. Most caribou and samples collected from Arctic islands in the Eurasia+ clade (A) formed long private branches. This can be interpreted as a signature of early population movements before the northern land/sea boundaries were formed after the Last Glacial Maximum. Within Eurasia+, there were haplogroups specific for some geographic regions. Furthermore, we detected at least 3 recent expansion events, mainly represented by semi-domestic samples in different regions. These findings might be related to independent domestication events of reindeer, as previously suggested by mtDNA patterns. This study places *R. tarandus* onto the list of species with resolved Y phylogenies. It further builds the base for Y-chromosomal haplotype screening for studying diversity, migration and admixture among populations.

Key Words: reindeer, Y chromosome, sequence variation

OP116 ISAG Bursary Award: Adipose gene expression profiles of four cattle breeds highlight selective pressures and tissue functions. D. Ruvinskiy*[1], K. Pokharel[1], A. Amaral[2], M. Weldenegodguad[1], M. Honkatukia[1,3], H. Lindberg[1], J. Peippo[1,3], P. Soppela[4], P. Uimari[5], C. Ginja[6], and J. Kantanen[1], [1]*Natural Resources Institute Finland (Luke), Jokioinen, Finland,* [2]*CIISA–Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal,* [3]*Nordic Genetic Resources Center,* Ås, Norway*,* [4]*Arctic Centre, University of Lapland, Rovaniemi, Finland,* [5]*Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland,* [6]*BIOPOLIS-CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal.*

Less functional genomic characterization of cattle breeds has been applied in studies focusing on population genomics, adaptation genomics and cattle genetic resources. Adipose tissues are crucial in regulating metabolism and energy balance with their ability to re-structure based on external changes. We have sequenced RNA samples extracted from metacarpal, perirenal, tailhead and prescapular adipose tissues of 81 individuals of Yakutian cattle (Sakha Republic), Northern Finncattle (Finland), Mirandesa (Portugal), and the commercial Holstein breed, and compared differentially expressed genes (DEGs) between tissues, breeds, and sexes. In total, 20,714 genes were expressed in our data, and we found the highest number of tissue-specific expressed genes in the metacarpal adipose tissue (672). The gene with the highest mean abundance in the metacarpal adipose tissue was *HOXD13* associated with adipogenesis. Moreover, the principal component analysis of normalized expression profiles showed a separation of the metacarpal adipose tissue from the others. In breed comparisons, some upregulated genes in in Yakutian cattle are associated with energy metabolism and response to cold temperatures (*NR4A3*, *TEKT3*, and *FGGY*). In the Mirandesa cattle breed, the upregulated genes are related to immune response and inflammation (*AVPR2*, *CCN1*, and *IL6*), while in the Northern Finncattle the upregulated genes appear to be involved in various physiological processes, including energy metabolism (*IGFBP2*). In the sex-based comparisons, *TPRG1* was upregulated in 3 tissues in Yakutian cattle females, suggesting adaptation related to feed efficiency. Most DEGs were found between Yakutian cattle versus Holstein and several DEGs associated with immunity were upregulated in Yakutian cattle indicating potential differences in disease resistance and immunity between the 2 breeds. This study shows the vast difference in gene expression profiles in adipose tissues between breeds from different environments, most likely highlighting selective pressure and the potential significance of metacarpal adipose tissue in regulatory functions.

Key Words: cattle and related species, adaptation

OP117 Reference genome of the native Finnhorse as a tool to study the adaptation of northern Eurasian horse breeds. K. Pokharel*[1], M. Honkatukia[1,2], C. Ginja[3], M. Weldenegodguad[1], J. Peippo[1,2], H. Lindeberg[4], T. Reilas[1], and J. Kantanen[1], [1]*Natural Resources Institute Finland, Jokioinen, Finland,* [2]*NordGen–Nordic Genetic Resource Center,* Ås, Norway*,* [3]*Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, Portugal,* [4]*Natural Resources Institute Finland, Maaninka, Finland.*

The Finnhorse is a native horse breed of Finland and has been an important part of Finnish culture, economy, and society in general. To better understand the genetic makeup of this breed and its evolution, we performed high-throughput sequencing and de novo assembly of the Finnhorse genome (a mare of the working horse breeding section of the Finnhorse herd book). We used a combination of Illumina and PacBio sequencing technologies to generate high-quality sequence data with an estimated coverage of 100×. Moreover, we sequenced RNA samples (n = 30) of neck (taken from the neck, below the mane), metacarpal, and tail head adipose tissues of the Finnhorse, the Siberian Yakutian horse and the Portuguese Garrano breed to investigate adaptation of horses to northern and southern Eurasian biogeographic regions. Adipose tissues are known to play crucial role in thermoregulation and insulation, which are important adaptations for animals living in cold environments. The final Finnhorse genome assembly consists of 2.37 Gb with an N50 scaffold of 83.77 Mb. We identified a total of 19,748 protein-coding genes in the Finnhorse genome using a combination of ab initio prediction and homology-based approaches. The high-quality genome assembly and annotation of Finnhorse will facilitate further investigation of the genetic basis of various phenotypic traits, as well as contribute to the development of new breeding strategies and management practices in conservation of horse genetic resources. Moreover, transcriptome study of adipose tissues provides valuable insights into how these tissues function and how they may have evolved to help horses adapt to the northern Eurasian environment.

Key Words: assembly, genetic resource, adipose tissue, gene expression

Applied Genetics of Companion Animals

OP118 Invited Workshop Presentation: Using dog genomic resources in museomics: An assessment of dog introgression into the Iberian wolf genome. R. Godinho*, *CIBIO-InBIO, Universidade do Porto, Campus de Vairao, Vairao, Portugal.*

Dogs and wolves share notable genomic similarities, despite marked morphological and behavioral differences driven by strong human-mediated selection acting on dogs. Hybridization between the 2 can lead to the introgression of new variants, which may disrupt their phenotypic differentiation. This can have either positive effects by enhancing their adaptive potential or negative effects by posing a serious conservation concern for the wild population. The Iberian wolf (*Canis lupus signatus*) is a gray wolf subspecies that inhabits the profoundly modified landscape shaped by humans and livestock in the Iberian Peninsula. Here, dogs and wolves have coexisted for thousands of years, creating ample opportunities for contact and historical hybridization. Genomic resources developed for dogs offer a wealth of information that might be used to investigate dog-wolf hybridization, thanks to the extremely well-annotated reference genome and the large panels of single nucleotide polymorphisms (SNPs) that have also been extensively used on wolves. However, while these resources are extremely useful for high-quality contemporary DNA samples, their use is limited for low-quality DNA samples, such as those from historical collections. Still, the comparable genotyping of both historical and contemporary

samples would represent a major methodological advance for temporal assessments of dog-wolf hybridization. In this presentation, I will discuss a new 100,000 SNP capture array that my team developed and validated for historical DNA, which is compatible with the Canine HD BeadChip. First, I will address the potential of this new tool to compare the performance of different tissues favored by traditional taxidermy practices in large mammals as a source of endogenous DNA. Then, I will present its application in 2 empirical case studies involving dog-wolf hybridization in the Iberian Peninsula: i) assessing the prevalence of dog-wolf hybridization and patterns of genetic diversity over a period of substantial demographical fluctuations of the wolf population in the 20th century; and ii) uncovering the geographical origin of an ancient hybridization event that has resulted in the introgression of dog variants that may be associated with the ability of Iberian wolves to persist in human-dominated landscapes. These case studies show the remarkable utility and potential of genomic resources developed for domestic animals in increasing our understanding of wild populations.

OP119 Obligatory testing in dogs: Input from breeders and organizations. E. Beckers*, N. Buys, and S. Janssens, *Center for Animal Breeding and Genetics, KU Leuven, Leuven, Belgium.*

Cats and dogs are burdened with many genetic diseases and several breeds have low genetic diversity. Breeding Healthy Pets was started in association with the Flemish government (Belgium) to create a sustainable breeding policy for all cat and dog breeds in Flanders. The latter aims to reduce the frequency of disease-causing variants while maintaining genetic diversity. One objective is to make several genetic tests obligatory. A list of relevant genetic diseases was compiled based on available scientific literature for 21 dog breeds (15 with low genetic diversity, 5 brachycephalic breeds and the most popular breed in Belgium). While studies exist on many genetic diseases, literature on allele frequencies and disease prevalences in some breeds can be scarce. Moreover, frequencies can change over time and geographic location. With this in mind, a survey was sent to dog breeders and breed organizations, inquiring about the occurrence of genetic disorders within the Flemish population of their dog breed. Forty replies contained minimal useful information: at least one breed and genetic disease was mentioned. The majority of the respondents were dog breeders (n = 30) and/or board members of a breed club (n = 18). Others were dog show judges (n = 7), board members for another kind of organization (n = 5), or future one-time hobby breeders (n = 2). Of the board members, 16 were authorized to represent their organization and were asked to answer according to their organization's official point of view. Information was gathered on 29 breeds, 13 of which fall under the 21 dog breeds selected for this study. Most of the answers were in line with what was found in the literature. Genetic conditions with little or no available prevalence information were added to the list of relevant genetic diseases when these emerged from the survey, like hemivertebra in the pug. The feedback, therefore, proved useful for fine-tuning the list. Other countries compiling a sustainable breeding policy could likewise benefit from the input of breeders and organizations. A final list will be confirmed after input from veterinarians with several clinical specializations.

Key Words: animal breeding, population genomics, animal health

OP120 AgriseqPI 1.0: Reporting utility for SNP-based parentage determination with targeted genotyping by sequencing panels. S. Chadaram*[1], A. Burrell[1], K. R. Gujjula[1], C. Carrasco[1], S. Daly[3], S. Udumudi[2], N. Anjuri[2], V. H. Kema[2], and A. Udumudi[2], [1]*Thermo Fisher Scientific, Austin, TX,* [2]*ATS GeneTech Pvt, Ltd., Hyderabad, Telangana, India,* [3]*Thermo Fisher Scientific, Lissieu, Lyon, France.*

The AgriSeqPI is a bulk report generation plugin primarily developed for SNP-based parentage and identity analysis using the AgriSeq Targeted Genotyping by Sequencing (T-GBS) parentage panels for canine, feline, bovine, and equine, run using the Torrent Suite Software (TSS). The reporting plugin is based on the ISAG rules and guidelines for parentage determination using SNP markers. End users can generate individual reports for each sample on a sequencing run using AgriSeqPI. AgriSeqPI can be run manually or automatically (via plan run) after a sequencing run is completed on Ion Torrent. The plugin retrieves the genotype data from Torrent Variant Caller (TVC) and AgriSum Toolkit and outputs a comprehensive report for each sample for all parentage markers in each panel. The report includes sample, reference, panel (test) details, and a summary of the results. AgriSeqPI allows users to export the reports in bulk mode per customer for ease of processing. Supported output formats include JSON and PDF. The report can be fully customized allowing users to add or remove the report section and even facilitating custom logos on the final report. AgriSeqPI enables veterinarians and veterinary service labs to enhance their product offerings related to the AgriSeq parentage panel by automating the report creation process. The plugin will support the users to adhere to the ISAG parentage determination guidelines. AgriSeqPI is publicly available and can be downloaded via Thermo Fisher Cloud and installed on TSS at no additional cost. For research use only. Not for use in diagnostic procedures.

Key Words: multispecies, genotyping, bioinformatics, breed/population identification, parentage

Populations and Polymorphism: Comparative MHC Genetics

OP121 MHC haplotype diversity in the main equine breeds of the Iberian Peninsula. M. García-Martínez[1], A. Cequier[1,2], E. Bernad[1], B. Serrano[1], A. Romero[2,1], F. Vázquez[2,1], A. Vitoria[2,1], S. Fuente[2,1], C. Cons[1], C. Rodellar*[1], and L. Barrachina[1,2], [1]*Laboratorio de Genética Bioquímica LAGENBIO–Instituto Agroalimentario de Aragón-IA2 (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.*

Major histocompatibility complex (MHC) genes are related to immune functions and can reflect genetic diversity, but furthermore, their study can have applications for cell therapy. The immunogenicity of mesenchymal stem cells (MSC) is key for safe and effective allogenic treatments and can vary upon the degree of MHC matching between donor and patient. Thus, establishing the most common equine MHC haplotypes provides critical information for biobanks aiming at providing allogenic cells for therapy in horses. The goal of this study was to enlarge our current knowledge on equine MHC diversity, focusing on Purebred Spanish (PRE), Purebred Arabian (PRá), Hispano-Arabian (Há) and Lusitano (PSL) as the most common breeds in the Iberian Peninsula. Using a validated panel of 10 microsatellite markers, MHC haplotypes were determined in related and/or homozygous animals and haplotype frequencies were calculated in unrelated animals. In PRE horses (n = 110 unrelated animals) the most common haplotype was HapPRE10 (20.45%), followed by HapPRE11 (9.54%) and HapPRE13 (7.72%). Haplotype frequencies in PRá, Há, PSL have not been calculated due to the limited number of unrelated horses currently available. Nevertheless, preliminary results showed that the most common haplotypes were HapPRA02 in PRá, HapPRA09 in Há and HapPRE06 in PSL. Provided the preference for homozygotes as MSC donors, we found 12 homozygous animals in the whole PRE population analyzed (n = 268): 7 for HapPRE10, 2 for HapPRE11. From the total PRá (n = 34), Há (n = 40) and PSL (n = 16) horses studied, only 2 PRá homozygotes were found (COR42 and HapPRA02). Finally, allelic frequencies showed 6–15 alleles with no marked predominance except for region UMNJH-38, where around 60% of animals of all breeds shared the allele 156. Moreover, one new allele (208) in ABGe9030 region was found in 5 horses (4 PRE, 1PSL) shared in all of them HapPRE36

haplotype. Although sample needs to be enlarged, this study provides relevant information for the MHC diversity in equine breeds in the Iberian Peninsula, which can have applications in genetic diversity and cell-based therapies in horses.

Key Words: MHC, horse and related species, haplotype, microsatellite, immunology

OP122 Successful reduction of proviral load by a novel bovine leukemia virus vaccine targeting cattle carrying susceptible bovine leukocyte antigen (BoLA)-*DRB3* allele. Y. Aida*[1,2], S.-N. Takeshima[2,3], L. Bai[2,4], J. Kim[2], Y. Matsumoto[2], R. Matsuura[1,2], and J. Kohara[5], [1]*Laboratory of Global Infectious Diseases Control Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan,* [2]*Viral Infectious Diseases Unit, RIKEN, Saitama, Japan,* [3]*Department of Food and Nutrition, Jumonji University, Saitama, Japan,* [4]*Graduate School of Science and Engineering, Iwate University, Iwate, Japan,* [5]*Animal Health Group, Animal Research Center, Hokkaido Research Organization, Hokkaido, Japan.*

Bovine leukemia virus (BLV), the etiological agent of the enzootic bovine leucosis, spread worldwide. Previously study demonstrated that the cattle with bovine major histocompatibility complex (*BoLA*)-*DRB3*016:01/*016:01* genotype tended to develop lymphoma and high BLV proviral load. One of problems to develop BLV vaccine is unstable effect of the vaccine due to the individual difference for disease susceptibility. We here tried to develop the BLV vaccine for susceptible cattle with *BoLA-DRB3*016:01/*016:01* genotype which are difficult to create vaccines and evaluated its vaccine effect in cattle. First, we determined the Th1 epitope in BLV against susceptible cattle using a total 118 peptides corresponding to GAG p15, p24 and p12, and ENV gp51 and gp30. Next, we optimized our determined Th1 epitopes by *in silico* peptide modeling to improve low affinity binding between peptide and susceptible BoLA DR molecule. To 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 subcutaneous and intradermal vaccination of p12–4R1/gp51R1 peptides-conjugated CO_3Ap. Finally, to demonstrate effect of this vaccine in cattle, we produced 6 susceptible cattle by fertilized ovum transplantation technique. All of 6 animals were infected by BLV and intradermally immunized 3 times with either vaccine or PBS as a control at 2 weeks post infection. The proviral load, lymphocyte count, and antibody titer were measured for 119–161 d. Among 6 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 suppresses proviral load in susceptible cattle.

Key Words: *BoLA-DRB3*, bovine leukemia virus, susceptible, vaccine

OP123 A multi-omics approach to provide complete genomic information on long-debated genes in birds. Q.-S. Zhao*[1], F. Zhu[1], Z.-T. Yin[1], Y.-X. Sun[1], Y.-C. Jie[1], J. Smith[2], L.-W. Shao[1], N. Yang[1], and Z.-C. Hou[1], [1]*National Engineering Laboratory for Animal Breeding and Key Laboratory of Animal Genetics, Breeding and Reproduction, MARA; College of Animal Science and Technology, China Agricultural University, Beijing, China,* [2]*The Roslin Institute & R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, UK.*

Chicken is an important model species for scientific discovery in developmental biology, genetics, virology, and immunology. Several important genetics-related issues in birds, such as the 'missing' gene hypothesis, including *leptin*, *ovocleidin-17 (OC17)*, *tumor-necrosis factor-α* (*TNF-α*) etc., and the complete structure of the major histocompatibility complex (MHC) have not yet been solved. The Silkie is a world-standard chicken breed, which is famous for a vast array of phenotypic variations not commonly seen in other domestic breeds of chicken. The genetic mechanism for its unique phenotype has been partially resolved, but its genomic signature remains unknown. To address these biological and evolutionary issues, we have de novo assembled a high-quality chromosome-level reference genome of the Silkie by incorporating Nanopore and PacBio High Fidelity (HiFi) single-molecule real-time long-read sequences as well as sequences from high-throughput chromatin conformation capture (Hi-C) technologies and applied multi-omics methods in our analyses. The new Silkie genome contains 39 pseudo-chromosomes and 39 unplaced scaffolds with an N50 length of 91.5 Mb. Our study first resolves the complete genomic sequences for genes (i.e., *leptin*, *TNF-α*, *CTL*, questioned as to whether they existed) whose existence in bird genomes, and we also provide has been long-debated, and also provides the 519 kg base full-length MHC genomic sequences containing 38 novel genes and manual annotations for chicken. Besides, the gene(s) encoding eggshell mineralization-specific C-type lectins are identified. Furthermore, we also provide whole-genome methylation and genetic variation maps and resolve the highly complex genetic causative mutation of fibromelanosis. Finally, we experimentally show *leptin* binding to the identified leptin receptor in chicken. In summary, the high quality Silkie genome assembly resolves several enigmas in avian biology and also provides the basis for further functional and genomic studies of these long-debated genes.

Key Words: Silkie, genome assembly, MHC, leptin, *TNF-α*

OP124 Analysis of the genetic diversity of swine leukocyte antigen 1-linked olfactory receptor genes and analysis of correlation with reported porcine testicular expression levels. M. Kang*, B. Ahn, S. Youk, and C. Park, *Department of Stem Cell And Regenerative Biotechnology Graduate School of Konkuk University, Seoul, Republic of Korea.*

A large number of olfactory receptor (OR) genes are highly polymorphic and present in multiple clusters in the mammalian genome. They are mainly expressed in the olfactory epithelium but also expressed in several other tissues. The major histocompatibility complex (MHC) consists of the fastest evolving genes in the genome and maintains the highest inter-individual genetic diversity for specific immune reactions against a large number of foreign antigens. Although OR gene clusters are widely spread across the genome, some OR clusters have close linkage associations to MHC genes in mammals. To study the effect of evolutionary impact of MHC diversity on the evolution of other linked genes, we compared the genetic diversity between 8 MHC linked and 21 unlinked OR genes together with their reported levels of expression in pig testes. Genetic diversity of 4 highly expressed ORs (OLF42–1, OLF42–3, LOC100156552, LOC100514111; FPKM >0.05), 4 low-expression ORs (LOC100516811, LOC100522686, LOC100157348, LOC100516618; FPKM <0.01), and swine leukocyte antigen 1 (SLA1) encoding a MHC classical class I gene were assessed using polymerase chain reaction-sequence based typing (PCR-SBT) for 32 pigs of 6 different breeds. A total of 73 alleles with an average of 9.13 alleles per locus were identified from 8 MHC-linked OR genes which is higher than those of 21 MHC unlinked OR genes with an average allele number of 6.33, suggesting the presence of the possible effects of the linkage disequilibrium (LD) with MHC. Interestingly, a highly expressed OR, OLF42–3, showed LD in all analyzed breeds (n = 5), and further analysis is ongoing with more individuals for other OR genes. MHC-linked OR might play a role in reproduction considering that MHC-linked OR genes are also expressed from seminiferous tubule, sperm, and oocyte cumulus cells although their functions are unclear. OR genes linked to the MHC region may also contribute to the maintenance of specific MHC haplotypes and MHC-OR relationships.

Key Words: pig and related species, DNA sequencing, linkage disequilibrium, olfactory receptor, MHC

OP125 Association of the *IRAK1* gene polymorphism with health, milk and exterior traits in cattle. L. Tichý*[1,2], V. Šteiger[1], L. Zavadilová[2], D. Schröffelová[1], J. Kyselová[2], M. Pribánová[1], L. Vostrý[2], J. Kucera[1], and Z. Sztankóová[2], [1]*Czech Moravian Breeders' Corpora-*

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Interleukin-1 receptor-associated kinase 1, encoded by the *IRAK1* gene, is an essential enzyme in the Toll-like receptor signaling pathway. Although there is a lack of information about the *IRAK1* gene in cattle, it is possible to estimate the effect of the *IRAK1* gene in other animal species. Polymorphism in its coding region may cause changes, not only in immunity but also in metabolic processes. The objective of the study was to investigate rs110533802 polymorphism of *IRAK1* in different cattle breeds and estimate its functional impact. DNA microarray technology (Illumina Infinium HTS method) was used to determine the rs110533802 polymorphism in 10,492 cows of the 6 cattle breeds. The same allelic frequency of 40% of the mutant allele T was found in Czech (n = 4954) and Hungarian (n = 4268) Holstein populations. Beef breeds were represented by Charolais (n = 261), Simmental (n = 281), Limousine (n = 365), and Aberdeen Angus (n = 289) populations. The allelic frequency of the mutant allele T varied between 0 and 10% in them. The next genotyped population was the Czech Fleckvieh dual-purpose cattle (n = 74), with the mutant allele frequency of 7%. Significantly higher occurrence of the mutant allele in dairy cattle leads to the hypothesis that it may be associated with milk yield. The GLM statistical model with fixed effects of herd, year and season of calving, age at calving, age at evaluation and genotype of polymorphism rs110533802 was applied to estimate effects of polymorphism on the investigated traits in 1016 Holstein dairy cows. Health, milk yield and exterior traits were selected as dependent variables. Statistically significant results were found for somatic cell score ($P = 0.0436$), udder depth ($P = 0.0001$), udder texture ($P = 0.0202$), angularity ($P = 0.0450$), body condition score ($P = 0.0047$), and stature ($P = 0.0134$). According to the obtained results, the rs110533802 polymorphism of *IRAK1* gene may be considered a genetic marker of some exterior and mammary gland traits in Holstein cattle.

Key Words: cattle, functional genomics, microarray, innate immunity, milk production

OP126 Integration of information from multiple gene expression and genome-wide association studies on host resistance of cattle to infestation with *Rhipicephalus microplus* ticks. K. Chooyoung*, B. Mable, and N. Jonsson, *School of Biodiversity, One Health and Veterinary Medicine College of Medical, Veterinary and Life Sciences University of Glasgow, Glasgow, United Kingdom.*

The cattle tick *Rhipicephalus microplus* causes massive damage to cattle throughout the tropics and subtropics. Resistance to tick is moderately heritable (h^2 ~0.4). Many studies have examined gene expression in the skin and blood of cattle of high and low resistance to ticks, and several genomic-wide association studies (GWAS) have been conducted. However, no genomics or phenotypic biomarkers for resistance are in commercial use. The objective of this study was to combine information from gene expression studies (GEXS) and genome-wide association studies (GWAS) on host resistance to infestation with *R. microplus*, to create a list of candidate biomarkers (genes or gene products) for which there were multiple sources of supporting evidence. From the literature, 16 GEXS (7 microsatellites studies, 9 NGS; 4 blood and 12 skin studies) and 12 GWAS (9 SNPs and 3 others) were identified that provided sufficient information to identify genes as significantly associated with tick resistance (as per authors' original declarations). This yielded 10,495 DEGs and 288 QTLs, which were then filtered to only those genes for which multiple studies showed consistent results. The final list included those QTLs significant in at least 2 independent GWAS (n = 11); DEGs significant in at least 4 skin (n = 6) or 2 blood (n = 10) GEXS; QTLs that were also significant DEG in at 1 blood or 2 skin GEXS (n = 10). The list of genes included 3 transcription factor genes, 12 genes associated with immune function, 3 genes with the extracellular matrix, 6 genes with the structural protein, and 13 genes in others. A total of 37 genes were identified for which multiple sources of evidence could be obtained and which are being further investigated for their value as biomarkers, either genomic or phenotypic.

Key Words: genome-wide association study, gene expression study, biomarker, cattle and related species

OP127 Investigating the role of β-globin in the response to mycotoxin exposure in sheep. K. McRae[1], E. Willems[2], A. Thomas[2], R. Clarke[1], J. Plowman[2], E. Maes[2], S. Clarke*[1], and P. Johnson[1], [1]*AgResearch Ltd., Mosgiel, New Zealand,* [2]*AgResearch Ltd., Lincoln, New Zealand.*

Facial eczema (FE) is an animal health challenge of great importance in ruminants in New Zealand. Ingestion of the mycotoxin sporidesmin leads to liver and bile duct damage, which can result in photosensitization and reduced production. In sheep, there is considerable genetic variation in tolerance to FE, and a quantitative trait locus (QTL) in the β-globin locus has been reported to explain 5% of the phenotypic variance in the response to FE. Mass spectrometry of hemoglobin from animals with differing genotypes at this locus indicated that the QTL is associated with different forms of adult β-globin, with haplotype A animals more tolerant to FE. Adult haplotype A sheep can switch from the synthesis of hemoglobin A (Hb-A; $\alpha_2\beta_2^A$) to the juvenile hemoglobin C (Hb-C; $\alpha_2\beta_2^C$) in response to hypoxia and anemia. To test the hypothesis that animals that are homozygous for haplotype A at the β-globin locus undergo a switch in hemoglobin type from Hb-A to Hb-C in response to exposure to the toxin sporidesmin, 10 animals homozygous for haplotype A and 10 animals homozygous for haplotype B were monitored through a controlled sporidesmin challenge. Blood samples were taken at d 0, 2, 7, 14 and 21 post-challenge for liver enzyme analysis, complete blood counts, and proteomic analyses to determine which forms of hemoglobin were present. In parallel, a DNA sample was taken from each animal at d 0, and the β-globin locus was sequenced using the Oxford Nanopore Technologies adaptive sampling method, which through targeted long-read sequencing of a region enables simultaneous capture of multiple sources of information including methylation and structural variants in a single run.

Key Words: sheep, animal health, disease resilience, proteomics, DNA sequencing

Genome Edited Animals

OP128 Evaluation of the resistance of Liang Guang Small Spotted pigs with partial deletion of the CD163 SRCR5 domain to porcine reproductive and respiratory syndrome virus 2 infection. Y. Wu*, X. Liu, Y. Chen, and Z. He, *School of Life Sciences, Sun Yat-sen University, Guangzhou, Guangdong, China.*

Porcine reproductive and respiratory syndrome viruses (PRRSVs) have posed a serious threat to the swine industry. CD163 has been identified as essential receptor for PRRSV infection mainly through the interaction of the scavenger receptor cysteine-rich domain 5 (SRCR5) region with virus. Therefore, we previously employed CRISPR/Cas9 to deleted a 41-aa fragment containing the ligand-binding pocket (LBP) in the SRCR5 domain of CD163 in Chinese indigenous pig breed Liang Guang Small Spotted pig. Here, we describe the evaluation of in vivo and in vitro viral challenge of gene-edited pigs. The porcine alveolar macrophages (PAMs) were isolated for PRRSV JXA1 strain challenge. Through cytopathic effect (CPE) analysis, immunofluorescent staining and Western blot analysis of viral protein expressions, and detection of viral nucleic acids, we found the PRRSV was absent in PAMs derived from gene-edited homozygotes at any time point, indicating that the homozygous PAMs were fully resistant to PRRSV infection in vitro. In contrast, PAMs derived from both the gene-edited heterozygous and wild type pigs were susceptible to PRRSV infection. Furthermore, we

found the heterozygotes are more susceptible to PRRSV infection, as reflected by pig death occurred first on d 5 after challenge, and all died on d 7, with high viremia and fever throughout the animal viral challenge. While the first death of wild type pig occurred on d 10 post challenge, and the survival rate was 66.7%. In contrast, the gene-edited homozygotes pigs did not present fever and viremia, and all survived after viral challenge. Finally, the necropsy showed that severe lesions were found in lungs of gene-edited heterozygous and wild-type pigs, while no obvious lesion was found in lungs of gene-edited homozygous pigs. Our results indicate the small deletion in SRCR5 of CD163 can confer fully resistance to PRRSV infection at homozygous state, whereas the gene-edited heterozygotes were more susceptible to viral infection. The underlying mechanisms will be further investigated.

Key Words: Liang Guang Small Spotted pig, anti-PRRSV, CD163 SRCR5

OP129 Withdrawn

OP130 Rethinking the genetic basis of pregnancy recognition in ruminants: Pregnancy in type I interferon receptor (*IFNAR2*) knockout sheep. C. J. Davies*[1,2], E. K. Peterson[1,2], M. J. Brothers[1,2], A. J. Thomas[1,2], H. M. Rutigliano[1], Y.-M. Lee[1], and I. A. Polejaeva[1], [1]*Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT,* [2]*Center for Integrated Biosystems, Utah State University, Logan, UT.*

Type I interferons (IFN) initiate immune responses to viruses. Their effects are mediated by the type I interferon receptor, IFNAR, a heterodimer of IFNAR1 and IFNAR2. One or both genes encoding the sheep IFNAR were disrupted in fetal fibroblast lines using CRISPR/Cas9 and lambs were produced by somatic cell nuclear transfer. Subsequently, a herd of about 30 *IFNAR2*$^{+/-}$ ewes was developed and bred to either *IFNAR2*$^{+/-}$ or *IFNAR2*$^{-/-}$ rams to produce *IFNAR2*$^{-/-}$ lambs. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) for IFN-stimulated gene (ISG) expression confirmed that IFNAR deficient sheep fail to respond to IFN-α (IFNA). The accepted mechanism for pregnancy recognition in ruminants involves a novel type I IFN, interferon-tau (IFNT), interacting with IFNAR. Because of the numerous publications on the role of IFNT in pregnancy recognition, we hypothesized that *IFNAR2*$^{-/-}$ ewes would be infertile. However, in December 2022 we bred 2 approximately 9-mo-old *IFNAR2*$^{-/-}$ ewes to an *IFNAR2*$^{+/-}$ ram and, to our surprise, both ewes became pregnant. Pregnancy in these ewes was confirmed by progesterone profiling, pregnancy specific protein B (PSPB) enzyme-linked immunosorbent assay (ELISA), and ultrasound exams. The ultrasound exams demonstrated the presence of a live fetus with a heartbeat in both ewes. At the time of their ultrasound exams, the ewes were at 46 and 50 d of gestation, which are well past the time of pregnancy recognition in sheep. The establishment of pregnancy in 2 *IFNAR2*$^{-/-}$ ewes demonstrates that the accepted mechanism of pregnancy recognition in ruminants, which involves IFNT interacting with IFNAR, is incorrect or is not required for establishment of pregnancy. Either there is another receptor for IFNT or an alternative, possibly redundant, mechanism for pregnancy recognition. Since *IFNAR1*$^{-/-}$ and *IFNAR2*$^{-/-}$ fetuses survive to term and are healthy at birth, and both *IFNAR2*$^{-/-}$ rams and ewes are fertile, unless the effects of IFNT are mediated by an alternative receptor, IFNT is not required for pregnancy recognition or the development of a healthy ruminant fetus.

Key Words: sheep, CRISPR-Cas9, pregnancy, fertility

OP131 Validation of the *PDGFD* gene function in sheep tail formation using base editing-induced start codon silencing. P. Kalds*[1,2], S. Zhou[1,3], S. Huang[1], K. Sun[1], Y. Gao[1], J. Han[4,5], Y. Chen[1], and X. Wang[1], [1]*Key Laboratory of Animal Genetics, Breeding and Reproduction of Shaanxi Province, College of Animal Science and Technology, Northwest A&F University, Yangling, China,* [2]*Department of Animal and Poultry Production, Faculty of Environmental Agricultural Sciences, Arish University, El-Arish, Egypt,* [3]*College of Veterinary Medicine, Northwest A&F University, Yangling, China,* [4]*CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China,* [5]*Livestock Genetics Program, International Livestock Research Institute (ILRI), Nairobi, Kenya.*

Sheep are one of the significant livestock species that have been used as a source of meat, milk, wool, and other byproducts. After domestication, the sheep tail phenotype has diverged into various morphological patterns. In modern breeding practices, the short and thin tail phenotype is most preferable. Recent genomic analyses highlighted the potential of the *PDGFD* gene as a significant candidate for the fat-tail phenotype. However, functional investigations are limited. In this research, we applied an adenine base editor (ABE)-induced start codon silencing to produce *PDGFD*-disrupted sheep. The editing efficiency at the cellular level was 56%. Using the microinjection approach, 14 lambs were generated, of which 9 (64.3%) were gene-edited. Targeted deep sequencing showed that the base conversion ranged from 35.06% to 98.30% in *PDGFD*-edited lambs. No bystander mutations were observed within the base editing window, and no off-target mutations were detected in the *PDGFD*-edited lambs. The phenotyping results showed a significant proportional reduction in tail volume of the *PDGFD*-edited group compared with the wild-type (WT) group ($P < 0.00$), but no significant difference between their tail lengths and body weights ($P > 0.05$). Hematoxylin and eosin staining of the tail adipose tissues did not support differences in adipocyte size between the 2 groups, suggesting a potential association of *PDGFD* with the adipocyte number rather than the adipocyte size. To further compare the transcriptomic profiles of the *PDGFD*-edited and WT groups, total RNAs were extracted from the tail adipose tissues. Among the 694 overlapped DEGs, 365 and 329 were up- and downregulated, respectively. The 329 downregulated DEGs were included in 49 significant GO terms and 40 significant

KEGG pathways (adjusted $P < 0.05$). The most significant GO terms and KEGG pathways were relevant to lipid regulation. These results showed that targeting the *PDGFD* gene in sheep could generate a proportional reduction in the tail fat volume and affect the transcriptomic profiles of tail adipose tissues.

Key Words: sheep, genome editing, base editing, RNA-seq, fat tail trait

OP132 ISAG Bursary Award: Field-deployable nucleic acid detection with RAVI-CRISPR. D. Tao[1], B. Xu[1], S. Li[1], C. Zhao[1], S. Zhao[1,3], X. Li[1,2], and S. Xie*[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, China,* [2]*Hubei Hongshan Laboratory, Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, China,* [3]*The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan, China.*

Rapid diagnosis based on naked-eye colorimetric detection remains challenging, but it could build new capacities for molecular point-of-care testing (POCT). By screening a range of candidate Cas12a nucleases, here we identify novel Cas12a homologous nucleases with high activity, which could facilitate the CRISPR-based nucleic acid detection. Next, we evaluated the performance of 16 types of single-stranded DNA-fluorophore-quencher (ssDNA-FQ) reporters for use with CRISPR/Cas12a based visual colorimetric assays. Among them, 9 ssDNA-FQ reporters were found to be suitable for direct visual colorimetric detection, with especially very strong performance using ROX-labeled reporters. In addition, we developed a convolutional neural network algorithm standardize and to automate the analytical colorimetric assessment of images and integrated this into the MagicEye mobile phone software. Subsequently, a field-deployable assay platform named RApid VIsual CRISPR (RAVI-CRISPR) based on a ROX-labeled reporter with isothermal amplification and novel Cas12a/crRNA targeting was established. We deployed RAVI-CRISPR in a single tube toward an instrument-less colorimetric POCT format that requires only a portable rechargeable hand warmer for incubation. Our study demonstrates this novel RAVI-CRISPR system for distinguishing different nucleic acid targets with high specificity and sensitivity as the simplest-to-date platform for rapid pen-side testing.

Key Words: RAVI-CRISPR, Cas12a, MagicEye, point-of-care testing

OP133 ISAG Bursary Award: sgRNAcas9-AI: A program for prediction of CRISPR/Cas9 and its variant sgRNA activity using deep learning. S. Li[1], X. Zhang*[2], S. Zhao[1,3], C. Zhao[1,4], and S. Xie[1,4], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, China,* [2]*Institute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover, Hannover, Germany,* [3]*Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan, China,* [4]*The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan, China.*

The clustered regularly interspaced short palindromic repeats and associated proteins 9 (CRISPR/Cas9) system-mediated genome modification has been widely used in various species and cell types. In recent years, several mutated and modified Cas9 variants have emerged with the advantages of low-target and broadened protospacer adjacent motif (PAM) loci compared with the wild type. However, there is a lack of algorithms to accurately predict the cleavage efficiency and off-target effects of sgRNAs in Cas9 and its variants, which are critical in determining the efficiency of gene editing. Here, we developed a new algorithm for sgRNA activity prediction (sgRscore) using deep learning strategies. Public sgRNA activity data sets of 9 Cas9 variants, including spCas9, eSpCas9(1.1), HypaCas9, evoCas9, SpCas9-VRQR, Sniper-Cas9, SpCas9-HF1, SpCas9-NG and xCas9, were collected to train our model. Compared with the other 6 mainstream models, sgRscore showed the best prediction performance. The attention mechanism and lightGBM algorithm were applied to explore the interpretability of sgRscore. By combining sgRscore with Crisflash, we developed a new software named sgRNAcas9-AI for the calculation of sgRNA on-target activity and off-target effect of Cas9 and its variants. It has been deployed to online website.

Key Words: sgRscore, sgRNA activity prediction, deep learning, sgRNAcas9-AI

Small Ruminant Genetics and Genomics

OP134 Gene expression profiling of the abomasum, duodenum, jejunum and ileum of resistant and susceptible Dohne Merino sheep naturally infected with *Haemonchus contortus*. T. M. Ramantswana*[1,2], D. P. Malatji[2], R. E. Pierneef[1], P. Soma[3], M. Van Der Nest[4], and F. C. Muchadeyi[1], [1]*Agricultural Research Council, Biotechnology Platform, Onderstepoort, Pretoria, South Africa,* [2]*University of South Africa, Florida, Gauteng, South Africa,* [3]*Agricultural Research Council, Animal Production Institute, Irene, Pretoria, South Africa,* [4]*University of Pretoria, Hatfield, Pretoria, South Africa.*

Gastrointestinal nematode (GIN) infections are a concern that is affecting sheep production and causes economic loss. The South African Dohne Merino sheep was bred to maximize wool and meat production and represent an important genetic resource that can be harnessed to breed for resistance to GIN as an alternative strategy to chemical control of nematodes. In this study, RNA-Seq and differential gene expression profiling was used to understand the underlying molecular mechanism associated with the infection of *H. contortus* in sheep. Total RNA was extracted from the abomasum, ileum, jejunum and duodenum tissue samples and used to generate an average of 6,691,243 paired-end reads with a length of 125 bp. Six adult Dohne Merino sheep were categorised into resistant (n = 3) and susceptible (n = 3) to *Haemonchus contortus* based on estimated breeding values derived from fecal egg count phenotypes. Differential gene expression analysis between resistant and susceptible animals resulted in 34 significantly expressed genes (DEGs) (false discovery rate ≤ 0.05, log2fold change values > ± 1). Genes such as *APLF*, *ART1*, *YIPF7*, *SIVA1* and *CDKN1A* were reported. Functional annotation of the DEGs revealed association with immune response process, responses to stimuli and other cellular processes. KEGG pathway analysis identified the Rap1 signaling pathway and PI3K-Akt signaling pathway. The differential gene expression analysis of the specific segments of the gastrointestinal tract resulted in 146 significantly expressed DEGs for abomasum, 302 for ileum, 584 for jejunum and 332 for duodenum. Functional annotation and pathway analysis demonstrated tissue-specific response mechanisms to *H. contortus* infection in sheep. Overall, the study provides information that forms the basis for understanding the genetics for resistance to GINs and has potential use in the selection of animals and breeding for resistance to infections by *H. contortus* nematodes.

Key Words: gastrointestinal nematode, RNA sequencing, South African Dohne Merino sheep, *Haemonchus contortus*, gene expression

OP135 Identification of genetic regions associated with resistance to gastrointestinal nematodes in Comisana sheep using a genome-wide association study based on EBV ranking. C. Persichilli[1], S. Biffani[2], G. Senczuk[1], M. Di Civita[1], M. K. Bitew[1], A. Bosco[3], S. Grande[4], and F. Pilla*[1], [1]*Department of Agricultural, Environmental and Food Science, University of Molise, Campobasso, CB, Italy,*

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Gastrointestinal nematodes (GINs) have significant economic, environmental, and animal welfare implications in small ruminant. This study is aimed to identify genetic regions responsible for sheep resistance to GINs. Fecal samples were collected from 642 Comisana sheep over 3 years to assess fecal egg counts (FEC) with the FLOTAC technique. Using pedigree data and logn(FEC+2) as phenotypes, estimated breeding values (EBVs) for GIN resistance were estimated by a BLUP animal model. The EBVs in the 99.95th and the 0.05th percentile were used to identify the most and the least genetically resistant individuals to GINs, later genotyped with the Illumina OvineSNP50 beadchip. Using the software PLINK a case/control GWAS was performed. A threshold for the 0.005% most significant FDR corrected *P*-values was chosen. Using the R package GALLO, QTLs associated with the significant SNPs were annotated and enriched. With the ToppGenes utility, genes associated with the SNPs have been enriched for KEGG pathways, using a threshold of 0.05 FDR. As a result, 18 significant SNPs involving 13 genes were identified on 12 chromosomes. Among these, many are involved in the physiology or pathology of the gastrointestinal tract (the UGT1A* family, KIF6, LOXL2, CALN1 and TWISTNB). Others play a role in adaptive processes and production traits (LOXL2, GPC6, MYT1 and SS18L1). Among the found QTLs, 6 are in the Health class, 5 of which are associated with traits related to FEC. Enrichment analysis of the found genes highlighted 11 significant pathways classifiable as involved in the regulation of the immune response, involved in drug metabolism and detoxification, and involved in other metabolic processes. Previous research has linked some of the discovered pathways to GIN resistance, such as ascorbate and aldarate metabolism, glucuronate pathway (uronate pathway), and metabolism of xenobiotics by cytochrome P450.

Key Words: sheep and related species, genome-wide association, disease resilience, single nucleotide polymorphism (SNP), quantitative trait locus (QTL)

OP136 Positional candidate genes involved in the response to heat stress in sheep. M. Ramon*[1], C. Diaz[2], M. Serrano[2], and M. J. Carabaño[2], [1]*CERSYRA-IRIAF, Valdepeñas, Ciudad Real, Spain,* [2]*INIA-CSIC, Madrid, Spain.*

Future climate scenarios derived from climate change (CC) point to the Mediterranean basin as one of the world areas that will be most affected by global warming. An important part of the world sheep and goat population is distributed in this region. A great effort is being put into the study of the consequences that CC will have on sheep and goat farming and into the development of mitigation and adaptation strategies. In line with this, this work aims to identify candidate genes involved in the response of sheep to heat stress. For this purpose, historical production data were used for the characterization of the individual response curve in milk, fat and protein yield along the temperature scale using random regression models and from them several thermotolerance indicators were developed. A total of 1,320 ewes with such thermotolerance indicators were genotyped with a 50K SNP chip and a GWAS analysis was carried out using the mixed-model approach in GCTA software and correcting for population structure. Genes were mapped using the ovine *Ovis aries* OAR v3.1 reference assembly within 500 Mbp windows flanking the markers with significant association. Potential genes associated with thermotolerance in sheep were interleukins (IL13, IL2, IL21, IL12RB1) and several genes of the SLC39 family, with a significant role in promoting the immune system; genes of the family of chaperones (HSF1, DNAJB1) with specific function related to heat stress events; LEP and LEPR involved in the regulation of fat metabolism, as well as PPARG, involved in the fixation, absorption and storage of fatty acids, and associated with the insulin resistance path that has been described to be affected by heat stress (HS). Genes FGF2 and VEGFA are involved in the activation of angiogenesis; the latter also in the inhibition of apoptosis an the induction of permeabilization of blood vessels. These functions largely related to HS, are also very appealing to explain mechanisms used to protect the normal functioning of cells and mechanisms of heat dissipation in response to HS. Information from this study might be used to enhance selection of HS-tolerant sheep.

Key Words: sheep and related species, genome-wide association, adaptation

OP137 ISAG Bursary Award: First look into the genetic architecture influencing liver copper concentration in Merinoland sheep. O. O. Adeniyi* and G. Lühken, *Institute of Animal Breeding and Genetics, Justus Liebig University, Giessen, Hessen, Germany.*

Economic losses due to copper (Cu) intoxication or deficiency is a problem encountered by sheep farmers. The aim of this study was to investigate the ovine genome for regions and candidate genes responsible for variability in liver Cu concentration. For this, a total of 134 liver samples were collected from slaughtered lambs of the Merinoland breed from 2 farms, and used for measurement of Cu concentration and genome-wide association study (GWAS). All samples were genotyped with the Illumina Ovine 50k SNP BeadChip, after which a total of 45,512 SNPs and 130 samples were left for analysis following quality control. For our analysis, single-locus and several multi-locus GWAS (SL-GWAS; ML-GWAS) methods were employed. Gene enrichment analysis was performed for identified candidate genes to detect gene ontology (GO) terms significantly ($P \leq 0.05$) associated with hepatic Cu levels in Merinoland sheep. The SL-GWAS and a minimum of 2 ML-GWAS identified 2 and 13 significant SNPs, respectively. The identified regions harbor some promising functional candidate genes such as *DYNC1I2*, *VPS35*, *SLC38A9* and *CHMP1A* associated with endosomal cargo sorting and trafficking, as well as lysosomal transport. Additionally, genes such as *SPG7*, *ATP5MF* and *SLC25A12* were identified as functional genes involved in mitochondrial membrane permeability which has been associated with Cu toxicity. Likewise, the genes *SLC9B1* and *SLC9B2* which are involved in luminal and intraluminal pH and multivesicular body fusion to lysosome, were observed as potential candidate genes influencing hepatic Cu levels in Merinoland sheep. These genes need to be further investigated to ascertain their involvement in liver Cu variation in Merinoland sheep in particular, and other sheep breeds in general. In addition, this study provides evidence for a polygenic inheritance of this trait and delivers promising clues for further studies to identify potential causal variants that may be associated with variation in Cu tolerance in sheep and used for practical breeding.

Key Words: sheep and related species, genome-wide association, candidate gene

OP138 DNA-based vaccine design against *Toxoplasma gondii* in ovines using rhoptry protein antigens through immunoinformatics approach. T. Madlala*[1], M. Adeleke[1], M. Okpeku[1], and S. Tshilwane[2], [1]*University of KwaZulu Natal, Durban, KwaZulu Natal, South Africa,* [2]*University of Pretoria, Onderstepoort, Pretoria, South Africa.*

Ovine toxoplasmosis is a zoonotic disease with significant impact on the welfare of livestock and on the economy of the farming industry worldwide. This disease threatens public health due to the high risk of transmission of parasite from livestock to humans through ingestion of undercooked meat containing parasite tissue cysts. To alleviate the economic burden imposed by *Toxoplasma gondii* parasite in the farming industry and public health, the development of novel vaccines against *T. gondii* that are safer has become imperative and shows great promise in controlling toxoplasmosis. Treatments currently used to control this disease often present critical throwbacks such as partial protection and short shelf-life, contributing to the parasite's resistance and inefficient elimination of the parasite tissue cysts. This study focused on ovine rhoptry proteins to predict potential antigenic epitope candidates and computationally design multiepitope vaccine effective against *Toxoplasma gondii* through immunoinformatics approach. The *in silico* technique implemented in this study successfully identified 20

T-cell and 2 (2) B-cell epitopes which were classified as conserved, antigenic, immunogenic, and non-allergen. These epitopes were joined to construct a vaccine adjuvanted with monophosphoryl lipid A and Cholera B subunit to enhance the vaccine's immunogenicity. The protein of the designed vaccine had molecular weight of 75.93 kDA, theoretical pI of 9.78 and was observed to be thermostable (instability index = 36.73) and hydrophilic (GRAVY = −0.235). it was observed as highly soluble with solubility of 0.946847. The structural validation of the refined vaccine revealed a Ramachandran plot with 96.1% residues in the most favored regions and a Z-score of −7.68. The properties observed from our proposed vaccine showed potential of eliciting robust cellular and humoral immunological response against *T. gondii*, crucial baseline information for future laboratory validation and designing a potential vaccine.

Key Words: infectious disease, immune system, vaccine, sheep, immunoinformatics

OP139 The benefit of genomic information for enhancing genetic prediction of production and reproduction traits in South African Merino sheep. C. Nel*[1,2], P. Gurman[3], A. Swan[3], J. van der Werf[4], M. Snyman[5], K. Dzama[2], W. Olivier[5], A. Scholtz[1], and S. Cloete[2], [1]*Directorate: Animal Sciences, Western Cape Department of Agriculture, Elsenburg, Western Cape, South Africa,* [2]*Department of Animal Sciences, Stellenbosch University, Stellenbosch, Western Cape, South Africa,* [3]*Animal Genetics & Breeding Unit, University of New England, Armidale, New South Wales, Australia,* [4]*School of Environmental and Rural Science, University of New England, Armidale, New South Wales, Australia,* [5]*Grootfontein Agricultural Development Institute, Department of Agriculture, Land Reform and Rural Development, Middelburg, Eastern Cape, South Africa.*

Genomic selection (GS) requires validation in South African (SA) sheep breeding. Testing of GS methods in SA currently depends on a reference population combining both commercial and research data sets, which have not been concatenated in a single analysis. This study compared the accuracy, bias and dispersion of pedigree BLUP (ABLUP) and single-step genomic BLUP (ssGBLUP) for genetic prediction. Animals in this study provided production records for weaning weight (WW), yearling weight (YW), fiber diameter (FD), clean fleece weight (CFW) and staple length (SL). For reproduction traits, the data set included 58,744 repeated records of the number of lambs born (NLB), the number of lambs weaned (NLW) and the total weight weaned (TWW). The single-step relationship matrix, H, was calculated using the PreGS90 software combining 2,811 medium density (~50k) genotypes and the pedigree of 88,600 animals. All flocks had animals genotyped ranging from 79 to 516 individuals per flock. The accuracy of ABLUP and ssGBLUP was compared according to the "LR-method" following single-trait analysis using ASREML V4.2 software. Validation candidates were assigned according to scenario I: born after a certain time point; and scenario II: born in a particular flock. In Scenario I, ssGBLUP increased the accuracy of prediction for all traits except NLB, ranging between + 8% (0.62 to 0.67) for FD and + 44% (0.36 to 0.52) for WW. This showed a promising gain in accuracy despite a modestly sized reference population. In Scenario II, overall accuracy was lower, but with greater differences between ABLUP and ssGBLUP, ranging between 17% (0.12 to 0.14) for TWW and 117% (0.18 to 0.39) for WW. There was little indication of severe bias, but some traits were prone to over dispersion and the use of genomic information did not improve these observations. These results were the first to validate the benefit of genomic information in South African Merinos. However, because production traits are highly heritable and easy to measure at an early age, GS methods are likely to rather focus on sex-limited or lowly heritable traits.

Key Words: marker data, genomic selection, ovine

OP140 Goat milk oligosaccharide composition determined by genes with a large effect. R. Gonzalez-Prendes*[1,2], H. Bovenhuis[2], L. Pellis[1], and R. P. M. A. Crooijmans[2], [1]*Ausnutria BV, Zwolle, the Netherlands,* [2]*Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands.*

Milk oligosaccharides (MOS) are complex molecules with direct impact on newborns' health. They promote the growth of beneficial bacteria in the gut, inhibit the adhesion of pathogens, stimulate brain development, and modulate the immune system. Goat milk has higher concentrations of MOS in colostrum and mature milk as compared with cow or sheep milk. As such, goat milk has been suggested as a potential natural source of MOS for infant formula. However, the genetic factors underlying MOS composition remain poorly understood. This study aims to address this knowledge gap by investigating the genetic parameters of the goat milk oligosaccharides (*g*MOS) and conducting a genome-wide association analysis (GWAS) in a population of 996 Dutch dairy goats. Blood and milk samples were collected from goats of 18 farms in the Netherlands. In milk samples 10 *g*MOS were determined by Eurofins using the ultra-high pressure liquid chromatography. Additionally, DNA extracted from the blood was genotyped using the Neogen GGP Goat 70k array. Sialylated oligosaccharides were found to be more abundant than neutral ones, with large differences in *g*MOS composition observed between goats. The estimated heritability varied from 0.22 (LNT) to 0.84 (3-FL) and the values of the genetic correlation (r_g) between the *g*MOS ranged from −0.59 to 0.76. The GWAS identified significant associations (q-value < 0.05) at whole-genome level between 7 *g*MOS and 176 SNPs, located in 16 genomic regions on 5 goat chromosomes. Most significant associations were detected on ARS15 (3-FL), ARS18 (2′-FL), and ARS23 (3′-SL). Interestingly, the most significant SNPs in 4 genomic regions were associated with more than one sialylated *g*MOS, suggesting that these MOS may be synthesized through a shared biosynthetic pathway. The analysis of positional candidate genes showed that genes related to the metabolism of MOS were significantly enriched, including genes involved in the transfer of L-fucose and sialic acid. Our findings suggest that genetic differences play a significant role in defining the composition of oligosaccharides in goat milk.

Key Words: goat milk oligosaccharide, genomic, infant formula

OP141 Rumen microbial composition in sheep supplemented with *Acacia mearnsii* tannin extract for methane reduction. I. Lawal, E. van Marle-Koster*, and A. Hassen, *University of Pretoria, Pretoria, Gauteng, South Africa.*

The emission of methane from ruminants can be reduced to varying degrees through the manipulation of the rumen microbiome by dietary interventions such as tannin. The aim was to study the rumen microbial composition in Merino sheep on a diet supplemented with crude or encapsulated *Acacia mearnsii* tannin extracts. Twenty-four sheep were divided into 4 groups that were fed a standard diet (negative control), standard diet with 75 mg/kg DM monensin (positive control, ionophore), standard diet with 20 g/kg DM *Acacia mearnsii* crude tannin extract and 29 g/kg DM of *Acacia mearnsii* tannin extract micro encapsulated in sunflower oil. Rumen samples were collected at slaughter and stored at −21°C. DNA was extracted for metagenomic analyses. Sequence data were analyzed using MG-RAST version 4.0.3 (Meyer et al., 2008). Although inclusion of crude or encapsulated *Acacia mearnsii* tannin extract has in previous studies shown an increase in fecal nitrogen excretion and a reduction in methane emission of 19.1–21.7%; in this study, the encapsulated tannin did not reduce digestibility and rumen fermentation parameters. *Bacteroidetes* (72%) and *Firmicutes* (21%) were the most prevalent phyla out of the 28 identified. Alpha and β diversity indices showed no significant differences ($P > 0.05$) among the 4 dietary treatment both at the phylum and genus level and was confirmed using Kruskal-Wallis test. Forty-one archaeal genera were identified with *Methanobrevibacter* having the highest abundance. The total bacteria and methanogens did not significantly differ between the tannin and non-tannin treatments suggesting that the tannin might have likely acted as a hydrogen sink.

Key Words: sheep and related species, microbiomics, DNA sequencing, digestive system, environment

OP142 Modulation of innate immune memory and systemic effects of Gum Arabica in goats. Y. Ahmed and M. Worku*, *North Carolina A&T State University, Greensboro, NC.*

Acacia senegal is part of the goat's natural diet. Gum Arabica (GA) is a water-soluble complex polysaccharide antimicrobial prebiotic derived from *Acacia senegal*. Antimicrobial functions of GA may involve Toll-like receptors (TLRs). This study evaluated the effect of GA on TLR transcription. Clinically healthy Boer and Spanish goats (n = 20) were randomly assigned to 2 groups of 10. Goats in the treatment group received 10 mL of GA in water daily for 6 weeks, controls received sterile water. Total RNA was isolated from blood using Trizol (Sigma-Aldrich). The RNA concentration (ng/µL) and purity was assessed using a Nanodrop Spectrophotometer (Thermo Scientific Inc.). Whole transcriptome Illumina sequencing via rRNA depletion was conducted on pooled RNA (GENEWIZ, South Plainfield, NJ). Bioinformatics analysis included mapping, differential gene expression, alternative splicing, and gene ontology analysis. Briefly, sequence reads were trimmed using Trimmomatic v.0.36 and mapped to the Capra_hircus reference genome available on Ensembl using the STAR aligner v.2.5.2b. Only unique reads that fell within exon regions were counted. Using DESeq2, a comparison of gene expression between wk 1 and wk 6 control and treated group samples was performed. The Wald test was used to generate *P*-values and log2 fold changes. Genes with a *P*-value <0.1 and absolute log2 fold change >1 were called as differentially expressed genes for each comparison. Time and GA treatment related differential gene expression was observed in goat blood. All goats expressed TLR 1–10. Only TLR10 was differentially expressed in blood from goats treated with GA (absolute log2 fold change >1). Toll-like receptor 10 (TLR10) is involved in trained memory, inhibits the induction of innate immune responses and inflammation. These studies have implications for application of GA in modulation of TLR 10 mediated innate immune memory. Further analyses are needed to validate individual and co-regulated genes, for applications in goat health.

Key Words: goat, blood, prebiotic, RNA-Seq, TLR

Plenary Session III: Functional Genomics (FAANG)

OP143 Using functional annotation and individual omics in genomic prediction. M. P. L. Calus*[1], B. C. Perez[2], J. de Vos[1], O. Madsen[1], L. Ayres[2], H. Bovenhuis[1], M. Ballester[3], M. J. Mercat[4], and M. C. A. M. Bink[2], [1]*Wageningen University & Research Animal Breeding and Genomics, Wageningen, the Netherlands,* [2]*Hendrix Genetics B.V., Research and Technology Center, Boxmeer, the Netherlands,* [3]*IRTA, Animal Breeding and Genetics Program, Caldes de Montbui, Spain,* [4]*IFIP-Institut du Porc and Alliance R&D, Le Rheu, France.*

Since the advent of genomic prediction, considerable efforts have been made to improve its accuracy by aligning the model more closely with the underlying biology of complex traits. In line with research aims to close the genotype-phenotype gap, intermediate omics have been used as additional information in genomic prediction. Broadly speaking, there are 2 approaches to do this: 1) generate functional annotation information at the species level, or 2) measure intermediate omics for individual animals. These approaches are investigated within the Euro-FAANG projects GENE-SWitCH and GEroNIMO, that received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreements no. 817998 and no. 101000236. Functional annotations may, for instance, indicate regulatory elements that affect the level of gene expression for different genomic regions. This prior information on the importance of genomic regions can be used in genomic prediction models. Measuring intermediate omics of individuals gives a better handle on individual differences between animals and may help to explain a larger proportion of the phenotypic variance. It may not only help to better predict phenotypes, but potentially can also help to predict breeding values more accurately. By applying the first approach to predict gene expression at 10 genes of 300 pigs using SNPs and methylation status of regulatory regions, we showed that using methylation status in the tissue for which gene expression was predicted tended to yield higher prediction accuracy. By using the second approach to predict phenotypes of 478 mice, we showed that individual gene expression explained considerably more phenotypic variation than SNP genotypes alone. In addition, more phenotypic variance was explained if gene expression and phenotypes were measured closer to each other in time. The use of gene expression considerably increased the accuracy of predicting phenotypes and the accuracy of predicting breeding values for 9 out of 13 traits. Additional examples of both approaches will be presented at the conference.

Key Words: genomic prediction, functional annotation, other omics

OP144 Aquaculture genetics, genomics and breeding to drive production growth, efficiency and sustainability. J. Kijas*, *CSIRO, Brisbane, Queensland, Australia.*

Global expansion of the aquaculture industry continues to accelerate, with farmed fish recently surpassing wild caught to become the largest source for human diets. The opportunity to transform this burgeoning industry sector using genetics, genomics and breeding is enormous because unlike livestock, the diverse range of species under management have been domesticated in the recent past and are comparatively unimproved. While the opportunity is significant, implementation of genetic technologies is highly variable and has proven challenging for a variety of reasons. Difficulties in the domestication process, low reproductive control due to biological factors, a paucity of suitable genomic resources or lack of the sustained investment required to reap the benefits of family-based breeding programs each contribute. This presentation provides an overview of progress and highlights research addressing specific production issues. The status of genomic tool development will be presented for selected major aquaculture species, before the challenge and opportunity imposed by reproductive biology will be illustrated as it relates to breeding program design. Finally, the presentation will make the case for the importance of data management systems, advanced phenotyping approaches and genomic selection to deliver long-term cumulative impact across the aquaculture industries.

Key Words: aquaculture, genetics, genomics, applied breeding

Animal Forensic Genetics

OP145 ISAG Bursary Award: Can DNA help trace the local trade of pangolins? Conservation genetics of white-bellied pangolins from the Dahomey Gap (West Africa). S. Zanvo*[1], C. A. M. S. Djagoun[1], F. A. Azihou[1], B. Sinsin[1], and P. Gaubert[2], [1]*Laboratory of Applied Ecology, Faculty of Agronomic Sciences, University of Abomey-Calavi, Cotonou, Benin,* [2]*Laboratoire Evolution et Diversité Biologique, Université Paul Sabatier, Toulouse, France.*

African pangolins are currently experiencing unprecedented levels of harvesting, feeding both local demands and the illegal international trade. So far, the lack of knowledge on the population genetics of African pangolins has hampered any attempts at assessing their

demographic status and tracing their trade at the local scale. We conducted a pioneer study on the genetic tracing of the African pangolin trade in the Dahomey Gap (DG). We sequenced and genotyped 189 white-bellied pangolins from 18 forests and 12 wildlife markets using one mitochondrial fragment and 20 microsatellite loci. Tree-based assignment procedure showed that the pangolin trade is endemic to the DG region, as it was strictly fed by the Dahomey Gap lineage (DGL). DGL populations were characterized by low levels of genetic diversity, an overall absence of equilibrium, important inbreeding levels, and lack of geographic structure. Genetic tracing suggested that wildlife markets from the DG sourced pangolins through the entire DGL range. Our loci provided the necessary power to distinguish among all the genotyped pangolins, tracing the dispatch of a same individual on the markets and within local communities. We developed an approach combining rarefaction analysis of private allele frequencies with cross-validation of observed data that traced 5 traded pangolins to their forest origin, c. 200–300 km away from the markets. Although the genetic toolkit that we designed from traditional markers can prove helpful to trace the illegal trade in pangolins, our tracing ability was limited by the lack of population structure within the DGL. Given the deleterious combination of genetic, demographic, and trade-related factors affecting DGL populations, the conservation status of white-bellied pangolins in the DG should be urgently re-evaluated.

Key Words: microsatellite, conservation genetics, demographic decline, trade tracing, white-bellied pangolin

OP146 ISAG Bursary Award: A new approach to the molecular differentiation of the wolf and the domestic dog in wildlife forensics. A. E. Hrebianchuk*[1] and I. S. Tsybovsky[2], [1]*State Forensic Examination Committee of the Republic of Belarus, Minsk, Republic of Belarus,* [2]*Republican Unitary Service Enterprise "BelJurZabespechenne," Minsk, Republic of Belarus.*

The molecular differentiation of individuals of the wolf (*Canis lupus lupus*) and the dog (*Canis lupus familiaris*) represents a difficult problem in the study of the *Canidae* family. In wildlife forensics, the first step toward identification of materials of animal origin is to determine the species of the animal. The current panels of STR loci employed both in basic science and forensics, which use domestic dog DNA with a confirmed species origin, have not been tested for cross-applicability with DNA from any other wild canids. We developed a test system for DNA-based differentiation of wild and domestic representatives of the *Canidae* family. This real-time PCR-based system allows one to quickly and reliably distinguish test samples of a wolf and a domestic dog. The test system is designed to detect 2 targets, the pancreatic amylase gene (*Amy2b*) and oncogene *vMYC*. The differentiating parameter between the wolf and the domestic dog is the number of copies of the pancreatic amylase gene. For wild canids, the copy number of the amylase gene is a constant value of 2, whereas in a domestic dog, the number of copies of this gene is always greater than 2. The robustness of the differentiation of the wolf and the domestic dog using this test system was confirmed in a study of Belarusian populations of the wolf (121 samples) and the domestic dog (216 samples), while verification was carried out using biological samples of the raccoon dog (179 samples), the red fox (including its black-brown morph; 383 samples) and the Arctic fox (29 samples). The developed test system can be implemented using 2 types of thermal cyclers, as well as droplet digital PCR (ddPCR), and reagents from various manufacturers. All validations were carried out in accordance with the protocol of the SWGDAM and the ISO5725 standard. Successful identification of a biological trace as originating from a wolf or a domestic dog using this test system makes it possible to subsequently employ identifying panels of STR loci, allowing statistical evaluation of the possibility that a biological trace belongs to a certain *Canis lupus* individual.

Key Words: wolf, dog, *Canis lupus*, forensics, genetic differentiation

OP147 Withdrawn

OP148 Identification of animal and plant species in foodstuffs using Target GBS assay. L. Forlani, D. M. Posik, M. C. Bruno, L. H. Olinera, M. E. Zappa, N. S. Castillo, G. Barbisan, E. E. Villegas Castagnasso, J. A. Crespi, P. Peral García, M. E. Fernandez, and G. Giovambattista*, *Instituto de Genética Veterinaria (IGEVET), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata–CONICET, La Plata, Buenos Aires, Argentina.*

DNA metabarcoding assay is increasingly used for species authentication in industrialized food. Targeted genotyping-by-sequencing (GBS) based on next-generation sequencing (NGS) technology allows the detection of multiple species in complex foodstuff matrices in a single run. This study aimed to evaluate the species of origin used in 32 commercially processed foods, replicated thrice, such as broth, salad dressing, sauce, milk, milk powder, hamburger, canned tuna, pepper, etc. In addition, positive (known DNA mixed) and negative controls were added. Ten ng of DNA form each samples were analyzed using a previously developed AgriSeq™ targeted GBS assay that include 319 multiplexed targets belonging to 2 mitochondrial (COI and CYTB) and 2 chloroplast (RBCL and MATK) genes from 177 plant and animal species used in the food industry in Argentina. Fastq files were aligned to a multi-specie reference genome to generate the SAM/BAM files for each sample. BAM files were filtered and a read count table was prepared. Finally, BAM files were visualized using IGV software and the reads with low percentage of identity were confirmed with nBLAST analyses against the NCBI public database. This analysis indicated a wide range of coverage for the highly processed samples tested, from several dozens/hundreds to few millions of reads. The obtained results were compared with the species composition declared on the product label by the manufacturer. Several declared species were identified, as well as no reported species were detected. In addition, nBLAST analyses allowed the addition of new species in the multi-specie reference genome. The present study demonstrates that AgriSeq™ is a viable solution for genomic applications involving the analysis of hundreds of multiple species in a single sequencing run.

Key Words: multispecies, genetic identification, GBS, mitochondrial DNA, chloroplast DNA

OP149 Invited Workshop Presentation: On the origin of our companion animals: A palaeogenomics perspective. L. Frantz*[1,2], [1]*Palaeogenomics Group, Department of Veterinary Sciences, Ludwig Maximilians University of Munich, Munich, Germany,* [2]*School of Biological and Behavioural Sciences, Queen Mary University of London, London, UK.*

Beginning with dogs, over 15,000 years ago, the domestication of animals has dramatically changed our evolutionary history. Although they are ubiquitous in our life today, we know surprisingly little about the geographic and temporal origin of our companion animals such as dogs and cats. Genomic information obtained from living animals provides information about their past, yet it only offers a contemporary snapshot of a long-term evolutionary process which can lead to contentious results. Over the last decade, recent methodological innovations, such as improved ancient DNA extraction methods and next-generation sequencing, have enabled the sequencing of whole ancient genomes. Palaeogenomics thus offers a unique opportunity to travel back in time and retrace the evolutionary history of our domestic species. Here I will present results from recent large-scale palaeogenomics studies on dogs and cats from our group which are starting to paint a detailed picture of the evolutionary history of our pet species.

OP150 ISAG Bursary Award: *RETREG1* variant causes canine acral mutilation syndrome (AMS) in purebred German spitz. A. Letko*[1,2], J. Plassais[1], P. Quignon[1], and C. André[1], [1]*Institut de Génétique et Développement de Rennes (IGDR), University Rennes, Rennes, France,* [2]*Institute of Genetics, University of Bern, Bern, Switzerland.*

AMS is a neurological disease documented for decades as part of inherited sensory neuropathy in various breeds with a severe impact on life quality. Causal variants in only 2 genes were identified to date, suggesting larger genetic heterogeneity in the dog population. In humans, the equivalent hereditary sensory autonomic neuropathies (HSAN) are characterized by insensitivity to pain, sometimes combined with self-mutilation. Sixteen loci have been associated with HSAN but do not explain the disease origin of all patients. Our aim was to explain the genetic etiology of an early-onset AMS in a purebred German spitz. The affected dog showed loss of pain sensation in the distal extremities, which lead to intense licking, biting, and self-mutilation of digits and paw pads. DNA was isolated from blood samples of the case, its parents, and 3 unaffected German spitzes. Whole-genome sequencing (WGS) of the case, its dam, and 2 controls was carried out using the reference genome assembly UU_Cfam_GSD_1.0 to identify single nucleotide variants and small indels private to the case. Variants were filtered based on autosomal recessive inheritance and further prioritized by predicted impact on the encoded protein and allele frequency in a cohort of 960 publicly available canine WGS. A single candidate causal variant on chromosome 4 in the *RETREG1* gene (c.656C > T, p.Pro219Leu) was discovered. Genotyping showed the variant segregated perfectly in the German spitz family and was absent in unrelated unaffected spitz dogs. This missense variant was previously recognized as deleterious in one mixed-breed dog family with similar clinical signs. Disruption of RETREG1 inhibits endoplasmic reticulum turnover and leads to neuron degeneration. Different *RETREG1* variants cause HSAN in humans. Our findings provide evidence that this variant underlies the recessive form of AMS in German spitz, and support the use of WGS-based veterinary precision medicine for early diagnosis, treatment, and prevention via a genetic test while showing the interest of dogs as natural models for rare human genetic diseases.

Key Words: nervous system, genetic disorder, genome sequencing, dog and related species, animal health

OP151 ISAG Bursary Award: Genomic and transcriptomic characterisation of hypertrophic cardiomyopathy in British Shorthair and Birman cats. T. Smedley*, L. Wilkie, V. Fuentes, D. Connolly, and A. Psifidi, *Royal Veterinary College, London, United Kingdom.*

Hypertrophic cardiomyopathy (HCM) is the most common heritable heart disease in cats and humans. It affects ~0.2% of humans and ~15% of cats. HCM is defined by primary left-ventricular myocardial hypertrophy and in cats is associated with increased risk of congestive heart failure, aortic thromboembolism and sudden cardiac death. The prevalence of feline HCM and lack of treatments to modify the disease process demonstrates the importance of studies aiming to understand the genetic architecture and underlying mechanisms of HCM susceptibility. To date, there are limited feline HCM genomic studies and only 5 HCM-associated variants have been identified. Moreover, the transcriptomic signature in myocardium for feline HCM remains largely unknown. In this study, we focused on 2 predisposed breeds, British Shorthairs and Birmans. DNA was extracted using the Qiagen DNeasy Blood and Tissue kit from 167 individuals, 111 HCM and 56 Control (>9 years old) phenotyped for HCM using echocardiography. Samples were genotyped using the Illumina Feline 63K SNP array. Genome-wide association studies (GWAS) and Fst analyses within and across breeds were performed. Additionally, total RNA-sequencing has been generated from myocardial tissue from 28 individuals (16 cases and 12 controls). RNA was extracted using the Qiagen RNeasy Minikit. RNA-Seq reads after quality control were aligned to the reference genome using STAR and differentially expressed genes were identified using DESeq2. The GWAS revealed 3 SNPs located on chromosome C1 with suggestive significance associated with HCM in Birmans. Similarly, GWAS identified several SNPs located on chromosomes B1, C2, E2 with suggestive significance in BSHs. The across breeds GWAS revealed a genome-wide significant peak of SNPs associated with HCM on chromosome D4. Some of these SNPS were located close to potentially good candidate genes linked to myocardial function. Identifying risk-increasing genetic variants and relevant gene networks and pathways involved in HCM susceptibility could lead to development of novel DNA-diagnostic tests and drug-treatment targets.

Key Words: cat and related species, biomarker, candidate gene, animal health, genome-wide association

OP152 Comparative genomics of the natural killer cell receptor genes in felids. J. Futas[1,2], A. Jelinek[1], M. Plasil[2], J. Bubenikova[2], P. Burger[3], and P. Horin*[1,2], [1]*Department of Animal Genetics, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno, Brno, Czech Republic,* [2]*Ceitec Vetuni, RG Animal Immunogenomics, University of Veterinary Sciences Brno, Brno, Czech Republic,* [3]*Research Institute of Wildlife Ecology, University of Veterinary Medicine, Vienna, Austria.*

The functional heterogeneity of Natural Killer (NK) cells is due to the differential expression of germline-encoded activating and inhibitory natural killer cell receptors (NKRs). Two complex genomic regions encode NKRs in mammals. The Leukocyte Receptor Complex (LRC) contains genes encoding killer immunoglobulin-like receptors (KIR), while the Natural Killer Complex (NKC) codes for NKRs with lectin-like structure (KLR). Both types of receptors can bind Major Histocompatibility Complex (MHC) class I molecules as ligands. Even closely related mammalian taxa may differ in their LRC/NKC genomic structure and gene contents. The objective of this work was to characterize the LRC and NKC complexes of felids. Bioinformatic analyses (tBLASTn, Splign, SignalP 6.0, TMHMM, Vista, MEGA X) were carried out on the most recent genome assemblies. Altogether, 10 assemblies of 10 species (long-read, chromosome level) and 12 additional short-read or scaffold level Felid assemblies of 12 different species were explored to provide a detailed annotation of both complexes. The genomic architecture of the LRC is highly conserved across the Felidae, containing one *KIR3DL* pseudogene and a novel Ig-like gene, along with a consistent complement of framing genes. The LILR ge-

nomic sub-region is also highly conserved among the studied species, with 7 orthologous genes in each species. All 7 genes are potentially functional in most studied Felids. The NKC is more variable in felid genomes, with one *KLRA* gene along with framing genes *KLRD*, *KLRK*, *KLRJ*, and *KLRH-like*. Variable numbers of *KLRC* and *KLRH* genes were found. Phylogenetic analyses identified presumed orthologs within the Felidae family. We also searched MHC class I sequences that could encode NKR ligands. No orthologs of non-classical class I genes were unambiguously identified. The detailed annotation of the LRC and NKC regions based on bioinformatic tools and resources is supported by our ongoing re-sequencing of selected genes (amplicons) from 32 individuals of 17 felid species including the domestic cat. This work was supported by the GA CR/FWF joint project GA CR 21–28637L/I5081-B.

Key Words: Felidae, NK cell receptor gene, LRC, NKC, MHC

OP153 Genomic resources for the domestic cat. L. Lyons*[1], G. Habacher[2], R. Malik[3], L. Coghill[4], and 99 Lives Consortium[5], [1]*Department of Veterinary Medicine & Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO,* [2]*Raddenstiles Veterinary Surgery, CVS UK Ltd., Exmouth, UK,* [3]*Centre for Veterinary Education, The University of Sydney, Sydney, NSW, Australia,* [4]*Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, MO,* [5]*99 Lives Cat Genome Consortium.*

The 99 Lives Cat Genome Sequencing Consortium develops genomic resources for the domestic cat and establishes working groups focusing on specific diseases, phenotypic traits, and genomic resources and investigations. Recent discoveries of inherited diseases in cats have included vitamin D deficiency due to a *vitamin D receptor* variant, a cat with pycnodysostosis with a variant in *cathepsin K*, and a new form of muscular dystrophy caused by a *dystrophin* loss of function variant. Each of these affect cats represent novel, singular cases as efforts to conduct Precision Medicine in veterinary health care. Active working groups include focuses on hypertrophic cardiomyopathy, amyloidosis, genomic analyses, annotation, and wildcat sub-speciation. A long-read haploid-based phased reference assembly for the domestic cat (Fca126) is available to the research community. Based on trio binning of a F_1 (Bengal) hybrid felid and because of the 6 to 8 million years of divergence between the domestic cat and Asian leopard cat parental species, this reference assembly is of telomere-to-telomere quality and is consider one of the best available for most any other mammalian species. This improved cat reference assembly is now being used to produce new genomic resources for the domestic cat as part of the 99 Lives Consortium. Using GATK best practices, the genome sequence data from approximately 666 domestic cats is currently being analyzed in comparison to the new genome assembly to develop a variant call file for the research community and to identify causal variants for diseases and phenotypic traits. All genomic data will be deposited in public data sites including the variants from the variant calling. The genome sequences and data will also be supporting groups interested in developing imputation panels and algorithms for the domestic cat. The Fca126 reference assembly has also been used to construct a new exome capture array, produced by Twist Biosciences as a commercial product and available in the spring of 2023. Combined, these resources feline genetics should support diverse research interests including disease and phenotypic studies, investigations of felid evolution, and studies on neoplastic disease.

Key Words: *Felis catus*, precision medicine

OP154 ISAG Bursary Award: *PCYT2* missense variant in Saarloos Wolfhounds with neurodegeneration. M. Christen*[1], M. K. Hytönen[2], H. Lohi[2], A. Kehl[3], V. Jagannathan[1], and T. Leeb[1], [1]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [2]*Department of Medical and Clinical Genetics, University of Helsinki, and Folkhälsan Research Center, Helsinki, Finland,* [3]*Laboklin GmbH & Co. KG, Bad Kissingen, Germany.*

We investigated hereditary progressive neurodegeneration and retinal atrophy in Saarloos Wolfhounds. Affected dogs developed heterogeneous clinical signs characterized by blindness, ataxia, epileptic seizures and aggression toward owners. The age of onset for the first signs was between 6 mo and 4.5 years. Combined linkage analysis in one family and homozygosity mapping in 7 affected dogs delineated a 14.4 Mb critical interval. The comparison of whole-genome sequence data of an affected dog to 958 control genomes revealed a private homozygous missense variant in the critical interval. It affected the *PCYT2* gene encoding the ethanolamine-phosphate cytidylyltransferase, which is important in the biosynthesis of phosphatidylethanolamine, a major phospholipid involved in normal development and function of the brain. The identified variant, XM_038546296.1:c.4A > G, is predicted to lead to a single amino acid change from isoleucine to valine at the very beginning of the encoded protein, XP_038402224.1:(p.Ile2Val). Genotypes at the variant were consistent with the monogenic autosomal recessive mode of inheritance in a complete family with 2 affected dogs. Apart from the 7 dogs used for homozygosity mapping, genotyping revealed 4 additional homozygous mutant dogs, which were of unknown phenotypes at the beginning of our investigation. A neurological phenotype was subsequently confirmed in 2 dogs. Further clinical characterization and follow-up are planned in the other 2 dogs, which were too young to show any clinical signs at the time of genotyping. *PCYT2* variants have previously been shown to cause autosomal recessive spastic paraplegia in humans. Homozygous knockout mice die during embryogenesis, suggesting that residual protein function is necessary for viability. Our data in dogs and the existing functional knowledge of *PCYT2* variants in other mammalian species suggest the identified variant as the potential candidate causative defect for the observed phenotype. Additional histopathological investigations to further characterize the phenotype are currently ongoing.

Key Words: dog and related species, diagnostics, DNA sequencing, genetic disorder, nervous system

FAANG Workshop

OP155 FAANG 2023: Community input on FAANG Task Force activities and future priorities. C. Tuggle*[1], H. Zhou[2], E. Clark[3], and E. Giuffra[4], [1]*Iowa State University, Ames, IA,* [2]*University of California–Davis, Davis, CA,* [3]*The Roslin Institute, University of Edinburgh, Edinburgh, Scotland, UK,* [4]*Paris-Saclay University, INRAE, Jouy-en-Josas, France.*

The Functional Annotation of Animal Genomes (www.faang.org) is interested in gathering inputs on the current activities and future priorities of the global FAANG community. FAANG has recently reorganized itself into a series of Task Forces (TF) including FAANGCompGen, FAANGPrediction, FAANGSingleCell, FarmGTEx, HTP-DS, and MetaFAIR. These task forces are open to all FAANG members. Since September 2022 several virtual meetings have been initiated to discuss TF-specific priorities, challenges, and future directions. The Plant and Animal Genome 2023 FAANG Workshop devoted time for roundtable (small group) discussions in gathering inputs on the first TF activities and the community suggestions for future FAANG priorities and needs. The general discussions turned on the potential expansion of current TFs, on the importance to share protocols, metadata and pipelines for the most recent assays. The role of new large-scale projects, network and infrastructures of EU, US and other FAANG-related communities, and how these initiatives can increase the benefits for the whole community, were also discussed (see PAG 2023 Workshop report at www.faang.org). This ISAG FAANG workshop will provide an opportunity for attendees to use a similar small group environment to weigh in on

the same questions posed in San Diego, as well as provide input on new emerging subjects. We will conclude with a Workshop-wide forum to allow general discussion. The feedback will be reported to the FAANG Steering Committee. The PAG and ISAG discussions will be combined and reported back to the wider FAANG community to guide the future direction of FAANG.

Key Words: Functional Annotation of Animal Genomes (FAANG), community input, task force

Avian Genetics and Genomics

OP156 ISAG Bursary Award: Invited Workshop Presentation: Chicken2K: A panel for global chicken genomic diversity and evolutionary inference. C. Ma*[1], M.-S. Peng[1,12], J. Smith[2], X. Huang[3], S. Zhang[1], X. Li[4], A. Esmailizadeh[1,5], S. C. Ommeh[6], D. W. Burt[7], A. C. Adeola[1,12], M.-S. Wang[1,12], O. Hanotte[8,9], J. Han[10,11], Y. Dong[4], Y.-P. Zhang[1,13], [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, Yunnan, China,* [2]*The Roslin Institute and R(D)SVS, University of Edinburgh, Midlothian, UK,* [3]*Guangdong Provincial Key Laboratory of Conservation and Precision Utilization of Characteristic Agricultural Resources in Mountainous Areas, School of Life Science, Jiaying University, Meizhou, Guangdong, China,* [4]*State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, Kunming, Yunnan, China,* [5]*Department of Animal Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran,* [6]*Institute for Biotechnology Research (IBR), Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya,* [7]*UQ Genomics, The University of Queensland, Brisbane, Australia,* [8]*Cells, Organisms and Molecular Genetics, School of Life Sciences, University of Nottingham, Nottingham, UK,* [9]*Livestock Genetics Program, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [10]*CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China,* [11]*Livestock Genetics Program, International Livestock Research Institute (ILRI), Nairobi, Kenya,* [12]*Sino-Africa Joint Research Center, Chinese Academy of Sciences, Kunming, Yunnan, China,* [13]*State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan University, Kunming, Yunnan, China.*

As the most widely distributed and abundant domestic animal on Earth, chickens play vital roles in human society. A growing number of studies have focused on the genetic changes involved in early domestication, local adaptation, and artificial breeding of chickens. Nevertheless, these studies to date have been limited to small numbers of populations or restricted in geographic regions. Much remains to be understood about the extent and structure of genomic diversity across diverse chicken populations as well as wild junglefowls. Herein, we developed Chicken2K, a resource panel for global chicken genomic diversity to present a global genomic landscape for domestic chickens and wild junglefowls. Chicken2K archives 39,753,026 SNPs and 5,848,802 INDELs for across 1,986 genomes, including 833 newly sequenced genomes, representing 170 global populations. Referring to the updated reference GRCg6a and its annotation, Chicken2K provided the largest whole-genome sequencing reference data set for chicken. We reconstructed the dispersal as well as breeding history of domestic chickens, mirroring the accelerated globalization via human population migration and interaction since the Middle Holocene. Then, we leveraged multi-omics approaches and multiple functional experiments to explore the evolutionary history and phenotypic diversity of chicken populations. Also, we built a database Chicken2K (http://chicken.ynau.edu.cn/index/home/index) to include miscellaneous functions and a user-friendly graphical interface for visualization of genomic diversity, genetic affinity, population structure, selective signal, and demographic history.

Key Words: chicken, admixture, breeding, dispersal, history

OP157 ISAG Bursary Award: A lncRNA gene-enriched atlas for GRCg7b chicken genome and its functional annotation across 47 tissues. F. Degalez*[1,2], M. Charles[2], S. Foissac[2], H. Zhou[3], D. Guan[3], C. Alain[1,2], L. Fang[4], C. Klopp[2], L. Lagoutte[1,2], B. Lebez[1,2], F. Lecerf[1,2], F. Pitel[2], B. Vourc'h[1,2], T. Zerjal[2], S. Lagarrigue[1,2], [1]*Institut Agro, France,* [2]*INRAE, France,* [3]*University of California–Davis, Davis, CA,* [4]*Aarhus University, Denmark.*

Considering the latest GRCg7b chicken genome assembly and differences between both associated reference genome annotations from NCBI (RefSeq) and EMBL-EBI (Ensembl), here we provide a new chicken lncRNA-enriched gene atlas by gathering RefSeq and Ensembl annotations, as well as additional FAANG and NONCODE resources. The Ensembl and Refseq chicken gene catalogs respectively grew from 17,007 and 18,010 to 24,102 PCGs and from 11,946 and 5,789 to 44,428 lncRNAs for a total of 78,323 genes. In addition to the genome annotation, we provide a functional gene annotation with a total of 1,400 RNA-seq samples representing 47 tissues. As a result, we found 64,478 (82.3%) of the 78,323 gene models considered as expressed, with an expression of TPM ≥0.1 for 35,257 (79.4%) lncRNAs and 22,468 (93.2%) PCGs or an expression of TPM ≥1 for 20,252 (45.6%) lncRNAs and 19,819 (82.2%) PCGs in at least one tissue. As expected, lncRNAs were less expressed and more tissue-specific than PCGs (39% versus 21%). Tissue-specificity was refined, indicating if a gene was specific to a single tissue or to a set of tissues which often share a common function. We also provide a set of differentially expressed genes (DEGs) between sexes and across 10 ages/development stages in 7 tissues. Moreover, we classified lncRNAs, PCGs and miRNAs according to their closest PCG and lncRNA and computed for each pair (excluding miRNAs) the co-expression across the 47 tissues. All the information and results are illustrated by concrete cases. Moreover, to facilitate the transition, we provide a gene correspondence table for galgal5, GRCg6a and GRCg7b. In summary, our work provides new gene models, especially for lncRNAs, by gathering information from Refseq, Ensembl and other databases, as well as functional gene information, thus constituting a unified and valuable resource for future genomic studies in chicken. Results are accessible at http://www.fragencode.org. Project funded by the European Union's Horizon 2020 research and innovation program under grant agreement N°101000236 and by ANR CE20 under EFFICACE program.

Key Words: genome annotation, chicken, GRCg7b, lncRNA, functional genomics

OP158 ISAG Bursary Award: Genetic diversity and relationship between Nigerian Muscovy duck populations using the mitochondria cytochrome *b* gene. O. Yusuf*[1], F. Sola-Ojo[1], and C. Adeola[2], [1]*Faculty of Agriculture, Department of Animal Production, University of Ilorin, Kwara state, Nigeria,* [2]*State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China.*

Muscovy ducks (*Cairina moschata*) are locally adapted to Nigeria climatic condition. They are known for their hardiness, resistance to disease, low feed requirement, ability to survive harsh weather condition and high prolificacy. However, the genetic information on Muscovy duck in Nigeria is limited, thus this study investigated the genetic diversity and relationship between 15 Muscovy duck populations sampled from 4 different states (Kaduna, Kwara, Niger and Oyo) in Nigeria using mitochondria DNA cytochrome *b* gene. A total of one hundred (100) Muscovy duck blood samples were collected with no cross contamination through brachial vein puncture and labeled according to the selected areas. Genomic DNA was extracted and 940 bp mtDNA cy-

tochrome *b* gene fragment was amplified and sequenced using the Big Dye terminator cycle sequencing kit. The result showed a total of 40 polymorphic sites consisting of 19 singletons variables. The 72 cytochrome *b* sequences obtained from the population were assigned into 17 distinct haplotypes and low genetic diversity is seen among populations. Phylogenetic analysis showed close clustering across all locations with the exception of BON 10, BON 20 and ADE 11. The extent of haplotype-sharing in the network indicates the absence of a definite population structure in Nigerian Muscovy duck and this was supported by the low genetic distance value between most Nigerian Muscovy duck populations being studied. These findings indicated low genetic differentiation between and within Nigerian Muscovy duck and also suggest that Nigerian Muscovy ducks are from a single domestication ancestral line with indiscriminate mating and are prone to inbreeding depression. More research evidence from genetics and archeology with wider geographic coverage of Nigeria is still required.

Key Words: Muscovy duck, cytochrome *b*, haplotype diversity, nucleotide diversity

OP159 ISAG Bursary Award: Potential of a chicken AIL population to decipher the genetic mechanisms of complex traits in the integrative omics era. X. Zhu*[1], C. Li[1], C. Luo[2], H. Zhou[3], L. Fang[4], H. Qu[2], Y. Wang[1], and X. Hu[1], [1]*State Key Laboratory for Agro-Biotechnology, China Agricultural University, Beijing, China,* [2]*State Key Laboratory of Livestock and Poultry Breeding, Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou, China,* [3]*Department of Animal Science, University of California, Davis, CA,* [4]*Center for Quantitative Genetics and Genomics (QGG), Aarhus University, Aarhus, Denmark.*

Integrative analysis of multi-omics data can elucidate valuable insights into genetic mechanisms for complex traits. Here we reported an F_{16} advanced intercross line (AIL) for QTL fine-mapping, characterized by sufficiently randomized the founder genomes, rapid linkage disequilibrium decay and abundant haplotype diversity. Utilizing 7.9 million SNPs and 75 phenotypes from 5 categories of about 1200 individuals, a total of 682 QTL were identified in 43 phenotypes. Gene-level mapping resolution was achieved at about 154 loci, of which 65 (involved 53 unique genes) were associated with growth and development phenotypes, had been identified in gene-edited mice. Next, we integrated the mQTL of the ChickenGTEx (~5000 transcriptome samples from 28 tissues) and the epigenetic annotation of the functional annotation of animal genomes (FAANG). We found that mQTL and regulatory regions of FAANG were significantly enriched in GWAS signals and narrowed down the range of candidate genes. For instance, body weight had a significant signal at the distal end of chromosome 1, in which *A gene* could be co-localized in muscle by summary mendelian randomization and transcriptome-wide association studies. The causative mutation was located in the muscle's strong enhancer region and significantly increased the promoter activity of *A gene*. In addition, we used hundreds of chicken samples from the world to illustrate the origin and transmission of causative haplotype, likely by combining standing variants from the red jungle fowl during the 1000s of years of chicken domestication, before they were rapidly accumulated in the high-weight chicken breeds during intense artificial selection. These results integrated mQTL and FAANG annotation information to determine the functional genes and causative mutations of complex traits, which have good guiding significance for in-depth analysis of the genetic structure of complex traits.

Key Words: chicken, integrated omics, GWAS, complex trait, MolQTL

OP160 The Chicken Genomic Diversity Consortium: Tracking immune diversity from ancient chickens to the present day. S. Fiddaman*[1], A. Smith[1], and L. Frantz[3,2], [1]*University of Oxford, Oxford, UK,* [2]*QMUL, London, UK,* [3]*LMU, Munich, Germany.*

The Chicken Genomic Diversity Consortium (CGDC), established 2021, represents an ongoing effort to capture a significant proportion of the extant variation in the chicken genome. Currently, we have aligned and processed ~5,000 genomes of chickens from a diverse range of geographic locations, fancy breeds, commercial lines, red junglefowl subspecies and other congeneric *Gallus* species. The aims of the consortium are numerous and varied, but specifically, our group focuses on the chicken in a historical context. We are in the process of sequencing > 200 ancient (from the last ~2000 years) chicken genomes from Europe with the primary aim of determining when, and how many times, the chicken was introduced into Europe from Asia. We are also interested in how the immune system of the chicken changed during domestication and the global expansion through exposure to novel pathogens. In this presentation, I will provide an overview of the CGDC and will outline some preliminary data on immune diversity in chickens. We found that the chicken is rich in immune diversity and that there are several-fold more variants in the Consortium data set compared with online databases. We also found that there have been selective sweeps in several Toll-like receptors, which are innate immune receptors responsible for detecting pathogen presence. Furthermore, we found evidence that some well-known chicken breeds have high-frequency pseudogenes for certain TLRs. These immune changes point to a changing relationship between the chicken and its pathogens over the course of its domestication and expansion. A comprehensive understanding of extant, extinct and interspecific immune variation could inform the breeding of more resilient chickens in the poultry industry.

Key Words: chicken, genomics, domestication, immune adaptation

OP161 Accumulated variations in the promoter regions play an important role for complex traits during duck domestication. Z.-T. Yin*[1], X.-Q. Li[1], Y.-X. Sun[1], J. Smith[2], N. Yang[1], and Z.-C. Hou[1], [1]*National Engineering Laboratory for Animal Breeding and Key Laboratory of Animal Genetics, Breeding and Reproduction, MARA; College of Animal Science and Technology, China Agricultural University, Beijing, China,* [2]*The Roslin Institute & R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, UK.*

Identifying the key factors that underlie complex traits domestication is a great challenge for evolution and biology. In addition to the protein-coding region differences caused by missense mutations, a large number of variations are located in the non-coding regions containing multiple type of gene elements. However, the genome-wide patterns and roles of gene elements during domestication and economic trait improvement are understudied. In this study, we combined large-scale genome resequencing, transcriptome, epigenetic analysis, and functional experiments to assess the role of selection on gene promoters during duck domestication. A comprehensive multi-omics map was constructed, including 12.6 million single nucleotide polymorphisms (SNPs), 3 million insertions/deletions (InDels), 74,490 structural variants (SVs), 249,326 potential regulatory elements, over 1,000 topologically associating domains (TAD), and gene expression levels of 16 tissues, from which we demonstrated the important role of gene promoter selection for complex traits during domestication. In total, 304 (42.94%) gene promoters have been specifically selected in Pekin duck among all selected genes. Joint multi-omics analysis reveals that most genes proximal to selected promoters are located in open and active chromatin, of which 267 genes (87.83%) were highly and differentially expressed in domestic trait-related tissues. Finally, we identify that the strong selection on the *ELOVL3* promoter, which is the key gene regulating high-fat content and unsaturated fatty acid in birds, has resulted in several variations which are nearly fixed in Pekin ducks, show increased expression in Pekin duck liver and confirm with in vitro mutation experiments. Overexpression of *ELOVL3* can increase lipid deposition by around 50% and also unsaturated fatty acid content by around 39%. Our results shed new light on the genetic mechanisms of domestication and modern breeding in Pekin ducks, with important implications for the future improvement of this important species.

Key Words: promoter, variation, duck, domestication

OP162 Allele-specific expression in the jejunal transcriptome profiles of two laying hen strains over the entire production pe-

riod. S. Ponsuksili*, F. Hadlich, M. A. Iqbal, H. Reyer, M. Oster, N. Trakooljul, E. Murani, and K. Wimmers, *Research Institute for Farm Animal Biology, Dummerstorf, Germany.*

The 2 laying lines (Lohmann Brown-Classic [LB], Lohmann LSL-Classic [LSL]) have approximately the same laying performance, but differ in many physiological, endocrine, metabolic and immunological traits. Obviously, they reach similar levels in the selection criterion laying performance by recruiting different functional pathways. During development, metabolic and mineral requirements change in parallel with maturation, growth and egg production. The intestine plays a critical role in the digestion and absorption of nutrients. We have previously shown that transcriptional patterns of the jejunum mucosa vary between the 2 layer strains and in response to changing metabolic and nutritional profiles at the onset of laying. Allele-specific expression (ASE) is a phenomenon involving imbalanced expression of the 2 parental alleles. This phenomenon occurs in selection processes where one allele is preferentially expressed over another having functional effects on the phenotype. Here, ASE analysis was performed in the jejunal mucosa of LB and LSL at 10, 16, 24, 30 and 60 weeks of age to detect *cis*-regulated gene expression variation associated with the functional biodiversity of the strains and adaptation to changing metabolic requirements. At the different ages, the 2 strains showed significantly different allele-specific expression in the intestinal mucosa and changes in allelic imbalance across the lifespan. Most ASE genes are involved in energy metabolism, including sirtuin signaling pathways, oxidative phosphorylation and mitochondrial dysfunction. A high number of ASE genes was found during the peak of laying, which were particularly enriched in cholesterol biosynthesis. These results suggest that both genetic architecture and biological processes that impose specific metabolic and nutritional requirements shape allelic heterogeneity. These processes are significantly influenced by breeding and management, and elucidating allele-specific gene regulation is an essential step toward deciphering the genotype-phenotype map or functional diversity between chicken populations.

Key Words: poultry, RNA-seq, allele-specific expression

OP163 Different stress response strategies of an Arctic breeding bird (*Calcarius lapponicus*) under inclement weather conditions revealed by the genome and RNA-seq analyses. Z. Wu*[1], M. M. Hindle[1], A. M. A. Reid[1], J. H. Pérez[2,3], J. S. Krause[2,4], J. C. Wingfield[2], S. L. Meddle[1], and J. Smith[1], [1]*The Roslin Institute and Royal (Dick) School of Veterinary Studies R(D)SVS, University of Edinburgh, United Kingdom,* [2]*Department of Neurobiology Physiology Behavior, University of California, Davis, CA,* [3]*Department of Biology, University of South Alabama, Mobile, AL,* [4]*Department of Biology, University of Nevada–Reno, Reno, NV.*

Developing strategies to cope with the increasing extreme global weather events requires a thorough understanding of how organisms respond to environmental perturbations. We performed RNA sequencing on samples from wild free-living male Lapland longspurs (*Calcarius lapponicus*) during their arrival on the breeding grounds and during incubation on the Arctic tundra of Alaska, USA. Samples were collected during an extremely cold arrival period in 2013 and incubation during a severe snowstorm in 2016. We performed RNA-seq analyses on liver, hypothalamus, heart, and testicular tissue to understand how this Arctic species responds to extreme weather events. We present a high-quality genome assembly and gene annotation of Lapland longspurs, identified differentially expressed genes associated with inclement weather events. We identified that *FKBP5* was significantly upregulated in hypothalamus during snowstorm, suggesting that FKBP5 is functionally important for the environmental stress response. FKBP5 is a regulator of the hypothalamic-pituitary-adrenal (HPA) axis during the stress response, and acts to modulate glucocorticoid receptor sensitivity in mammals. FKBP5 acts as a co-chaperone, negatively regulating the glucocorticoid signaling pathway providing a mechanism by which an individual can rapidly and accordingly adjust its HPA axis function in response to unpredictable environmental perturbations. Other pathways in the hypothalamus and other tissues were seen to be involved during these stressful conditions, specifically those involved in metabolism, the immune response and circadian rhythms. Such findings will contribute to the understanding of gene expression changes in multiple physiological systems to mediate stress in wild free-living birds.

Key Words: genome assembly, RNA-seq, Lapland Longspur, hypothalamic-pituitary adrenal (HPA) axis, climate change

OP164 Genome-wide association study of nucleotide and peptide contents of breast meat in Korean native chickens. M. Kim*[1], E. Cho[1], J. Munyaneza[1], A. Jang[2], H. Choo[3], and J. Lee[1], [1]*Chungnam National University, Daejeon, Korea,* [2]*Kangwon National University, Chuncheon, Gangwon-do, Korea,* [3]*National Institute of Animal Science, Rural Development Administration, Pyeongchang, Gangwon-do, Korea.*

Chicken meat flavor is one of the major factors in determining meat quality and customer acceptance. Overall meat flavor is developed during cooking, by taste-active components such as nucleotides and peptides. To identify genetic markers related to the proportion of the components, we conducted a genome-wide association study using the Korean native chicken red-brown line, which is known for its unique meat quality. We collected breast meat and blood samples from 637 birds slaughtered at 10 weeks of age. We used nuclear magnetic resonance spectroscopy to measure the amounts of nucleotides (inosine, inosine-5′-monophosphate [IMP], and hypoxanthine) and peptides (carnosine and anserine) contents. We extracted DNA from the blood and generated genotype data using the Illumina chicken 60K single nucleotide polymorphism (SNP) chip. The association test and heritability calculation were conducted using GCTA software, and the significance was corrected based on Bonferroni correction. As a result, heritability estimates of inosine and carnosine contents were the highest values at 0.50 and 0.43, respectively. The results of the association test on inosine, IMP, and hypoxanthine showed 45, 16, and 1 significant SNPs, respectively, on chromosome 5. Every significant SNP of IMP and hypoxanthine was listed in significant SNPs of inosine. The significant genomic region of nucleotides was centered on markers located from 10.21 to 19.40 Mbp. In the association test result of carnosine and anserine, 9 and 4 SNPs were captured, respectively, and a region from 29.67 Mbp to 23.05 Mbp was defined on chromosome 7. Reviewing literature, gene ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis suggested *LOC770392*, *IGF2*, and *DUSP8* as candidate genes of nucleotide contents, which are involved in nucleotide metabolism and insulin activity in muscle. *LOC771456*, *HNMT*, *NXPH2*, and *SPOPL* were identified as candidate genes of peptide contents, related to histamine metabolism, and muscle development. These results will be the basis for improving the flavor of chicken meat through genomic selection.

Key Words: chicken, genome-wide association, SNP, product quality

OP165 Contribution of STR genotyping to animal clinical cytogenetics. T. Raudsepp*, J. Kjöllerström, and R. Juras, *School of Veterinary Medicine, Texas A&M University, College Station, TX.*

A progressive tendency in animal parentage testing is to replace short tandem repeat (STR) genotyping panels with single nucleotide variation (SNV) genotyping chips. The latter are more comprehensive, have a better representation of genome variation of the species, and include variants for the detection of genetic diseases and select phenotypes. Despite the obvious advantages of SNV panels, STR genotyping remains as an efficient, fast, and reliable method for some genetic analyses, such as ancestry testing in horses because of the wealth of the available STR data for diverse breeds. Also, in the past decade, we have used on regular basis STR genotyping as a complementary approach for clinical cytogenetics in horses and occasionally in cattle to verify animal or sample identity, parentage, and genetic sex. It is also a method of choice to validate karyotyping-based XX/XY blood chimerism. In cases of X-monosomy, which is a frequent cause of sterility in female horses, STR genotyping is an efficient tool for detecting low level mosaicism for an XX cell line and determine the parental origin of the single X chromosome. Likewise, STR genotyping is a feasible tool for determining the parental origin of autosomal and sex chromosome trisomies and discriminating between centric fusions and isochromosomes. On a few occasions, where the karyotype appears normal, STR genotyping has suggested uniparental disomy which is not detectable by chromosome analysis. STR genotyping combined with mitochondrial DNA analysis is also an excellent tool for the detection of interspecific hybrids and their parental origins. In addition, comparison of STR genotyping-based ancestry with the breed information available for cytogenetics samples shows over 95% concordance, illustrating the high accuracy of STR genotyping to determine breed ancestry in horses. Because of the feasibility, speed, efficiency, low cost, and in-house availability, STR genotyping remains an important complementary method for clinical cytogenetics and will not be replaced with SNV genotyping soon.

Key Words: chromosome aberration, parental origin, ancestry

OP166 Invited Workshop Presentation: Improving parentage verification, transiting from STR to SNP and beyond from a bovine perspective. M. McClure*, *ABS-Global, Deforest, WI.*

Pedigree errors bias estimates of heritability, breeding values, estimates of genetic parameters, depress the rate of genetic progress, and can negatively influence which animal an individual chooses. To reduce the amount of pedigree errors the bovine industry initially used blood typing, then microsatellite (MS) markers, and finally to increasing sizes of SNP panels in the pursuit of a more perfect pedigree while balancing the financial and technical aspects. While the initial parentage panel of 100 SNP for multiple breeds was developed in 2002, and ISAG's bovine working group developed guidelines in 2012 the transition to SNP is still not fully complete in all breeds. Multiple strategies have been used to transition from MS to SNP: genotyping a single generation with both technologies, phase to using SNP overtime, genotype highly impactful animals with SNP, imputation of MS alleles, allow use of either technology but a preference for SNP, and others. The largest historical hinderance in transition has been cost as historically MS was much cheaper than SNP genotyping and that the higher cost was not outweighed by added benefits that can come with a large SNP panel. This presentation will provide an overview of bovine's experience as it transitioned to using SNP for parentage verification and prediction. Along with sharing many of the lessons learned along the way and how unique '1 in a million' cases were identified and handled. SNP genotypes have allowed for additional benefits to be created that bring added value to the customer and herd book—sex prediction, breeding value predictions, breed composition, traceability, genetic defect carrier status via the causative allele or associated haplotype. SNP genotyping technology has vastly transformed over the last decade and the bovine community has been at the forefront of incorporating it. From using ever larger SNP arrays to recent sequencing advancements that allow Genotyping-By-Sequencing and skim-sequencing to be commercialized products that are currently used in cattle. The advantages and disadvantages of each will be explored and shared to provide guidance to other species that wish to transition to using SNP genotyping.

OP167 Development of a robust across breed equine parentage SNP panel for ISAG approval. R. R. Bellone*[1,2], T. A. Mansour[2,3], E. Esdaile[1], B. Wallner[4], T. Raudsepp[5], B. Till[1], A. Kallenberg[1], S. Hughes[1], S. Chadaram[6], S. Shrestha[6], R. A. Grahn[1], Equine ISAG SNP Panel Consortium[9], F. Avila[1], M. McCue[7], and P. Flynn[8], [1]*Veterinary Genetics Laboratory, School of Veterinary Medicine, UC Davis, Davis, CA,* [2]*Department of Population Health and Reproduction, School of Veterinary Medicine, UC Davis, Davis, CA,* [3]*Department of Clinical Pathology, School of Medicine, Mansoura University, Mansoura, Egypt,* [4]*Institute of Animal Breeding and Genetics, Veterinary University of Vienna, Wien, Austria,* [5]*Veterinary Integrative Biosciences, School of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX,* [6]*Thermo Fisher Scientific, Austin, TX,* [7]*Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN,* [8]*Weatherbys Scientific, Kildare, Ireland,* [9]*Various Affiliations.*

Microsatellite markers (STRs) have been utilized for equine parentage testing since the 1990s to ensure the integrity of pedigrees. Historically the industry has not been motivated to transition to SNPs, however advances in technologies and use of marker assisted selection have made the transition attractive. The aim of this project was to develop an across breed SNP panel for use in equine parentage and ISAG comparison testing. To achieve this aim, 3 phases were developed: phase 1) identify a panel of 1500 SNPs from available across breed array data 2) genotype this panel in a reference sample set of 192 horses, across laboratories and platforms, to choose the most concordant SNP set and 3) evaluate this set in known trios with the goal of refining the panel to one that improves efficacy of parentage testing. Array data from 8465 horses from 50 breeds were mapped to EquCab3 and pruned for quality control and linkage disequilibrium, leaving 4878 autosomal SNPs for consideration. The top 1291 autosomal SNPs, distributed across all autosomes, were selected based on ranking by the number of breeds with MAF > 0.3. Additionally, to include markers for quality control for sex and X chromosomal aneuploidies, 39 markers from the male specific region on Y and 20 in the pseudoautosomal (PAR) region along with 150 non-PAR markers on X were included. The 1500 SNPs were submitted for AgriSeq Targeted GBS SNP panel design. Eleven SNPs failed design and were not considered further. Genomic DNA from 192 horses, representing 15 breeds, was distributed to 17 laboratories and genotyping on the Agriseq T-GBS SNP panel (n = 12) or commercially available SNP arrays (n = 7) was performed. Concordance analysis is ongoing. Preliminary analysis of 47 samples genotyped by 2 laboratories identified 5 markers that failed on the Agriseq T-GBS panel (1 SNP each on ECA3, 11, 13, X and Y). Data from the remaining markers that passed quality controls were 99.7% concordant in the samples evaluated. Genotyping trios with available STR genotypes from 16 diverse breeds is underway. The results of this work should identify a robust set of SNP markers to be considered for ISAG approval.

Key Words: equine, parentage testing, SNP

OP168 Invited Workshop Presentation: Epigenomic and microbiome signatures of early rearing conditions in aquaculture. S. Consuegra*, *Department of Biosciences, School of Biosciences, Geography, and Physics, Swansea University, Wales, UK.*

Fish farming has grown rapidly over the last few decades, but fish domestication has tended to focus almost exclusively on genetic improvements, which in highly fecund fish can exhaust genetic variation and increase vulnerability to disease, ultimately reducing fitness. Yet, non-genetic effects (such as maternal and epigenetic effects) can greatly affect fish production. For example, DNA methylation may alter gene expression without modifying DNA sequences and such changes may have phenotypic effects that can be transmitted across generations. Such an epigenetic programming is believed to have played a major role in the rapid domestication of birds and mammals, but little is known about its role in farmed fish. Epigenetic variation could act as a surrogate for immunogenetic diversity in domesticated species that have lost genetic diversity through intense selective breeding. Environmental factors (diet, temperature, pathogens, stress) and host-intrinsic factors (age, genetic background, immunological status) affect the epigenome but also host microbiome, particularly at early developmental stages. In turn, microbiome composition and function affect host nutrition and health. Yet, the potential application of epigenetic programming and microbiome engineering in aquaculture settings, where environmental conditions can be easily controlled, is still largely unexplored. Our group is investigating how contrasting fish species adapt to crowding and environmental stress, and how such response may differ between wild and farmed fish in relation disease. For this, we use commercial and model species to examine the role of genetic and non-genetic (maternal, epigenetic, microbiome) factors in the response to environmental stress, inbreeding and pathogen transmission, all common crowded farm conditions, particularly in view of predicted increases in water temperature.

OP169 ISAG Bursary Award: A high-density genetic linkage map and QTL mapping for growth traits in South African abalone (*Haliotis midae*). T. Tshilate*[1], E. Ishengoma[2], and C. Rhode[1], [1]*Department of Genetics, Stellenbosch University, Stellenbosch, South Africa,* [2]*Mkwawa University College of Education, University of Dar es Salaam, Iringa, Tanzania.*

A high-density genetic linkage map is important for QTL fine mapping, comparative genome analysis, identification of candidate genes and marker-assisted selection for economic traits in aquaculture species. The South African abalone (*Haliotis midae*) is one of the most important aquaculture species in South Africa. However, limited genetic and genomic resources have been developed for the genetic improvement of economically important traits for the species. Therefore, a 2b-RAD (2b-restriction site-associated DNA) sequencing technique was used to sequence 68 *H. midae* specimens at around 5 years of age and obtain 7173 single nucleotide polymorphism (SNP) markers. A high-density linkage map was constructed using 2266 SNP markers spanning 1646.01 cM in length and an average marker interval of 0.73 cM to 18 linkage groups (LGs). Using the genetic linkage map, 13 QTLs for growth-related traits were detected on 3 linkage groups (1, 7 and 13) with an average shell of 70.17 mm, average shell length of 99.31 mm and average total body weight of 174.92 g. The phenotypic variance explained (PVE) ranges from 13.6 to 25.1%, with LOD scores ranging from 4.17 to 9.72. Finally, some important candidate genes such as *egf*-1, *megf10*, *megf6*, *tnx*, *sevp1*, *kcp*, *notch1* and *scube2* which may regulate growth in *H. midae* were identified. This genetic map may provide a basis for genome assembly and comparative genomics studies, and the QTLs derived candidate genes and genetic markers are useful genomic resources for future marker-assisted selection (MAS) for growth-related traits in the South African abalone.

Key Words: growth trait, *Haliotis midae*, linkage map, quantitative trait loci

OP170 A technology for producing all-female progenies of the Flathead grey mullet by selecting sex-reversed males. L. David*[1], G. Hirsch[1], I. Oz[1], D. Agiv[1], E. Marcos-Hadad[1], A. Bennet-Perlberg[2], A. Naor[2], and B. Ginzbourg[3], [1]*The Hebrew University of Jerusalem, Rehovot, Israel,* [2]*Israel Ministry of Agriculture and Rural Development, Dor, Israel,* [3]*Dagon Fish Hatchery, Kibbutz Maagan-Michael, Israel.*

Flathead gray mullet (*Mugil cephalus*) is a cosmopolitan marine food-fish, mainly fished in seas, but also increasingly farmed on-land. Aquaculture relies on capturing wild fry in estuaries and acclimating them to grow out in ponds. Capturing wild adults and fry puts pressure on natural populations. Natural populations yield irregular fry supply, hampering the development of mullet aquaculture. Mullet is a desirable aquaculture species, in which females grow faster than males. Only recently, the life cycle of mullet in captivity was closed allowing to produce fry in hatcheries. Breeding for desirable traits starts when hatchery production is in place. We developed a technology to genetically select brood fish producing all-female progeny. Sex was oftentimes evolutionarily shaped as a trait with only 2 phenotypes (female and male) and a fixed phenotypic segregation ratio of 1:1. Fish are a prime group to study sex determination as different species evolved different sex-determination mechanisms. Mullet has a genetic mechanism influenced by hormonal manipulations. The technology involves a hormonal treatment producing sex-reversed mature males (milt producing males with a female sex genotype), which are then crossed to normal females for producing all-female progenies. While control groups had a 1:1 sex-ratio, in 3 treatment groups an excess of 63%, 74% and 84% males were identified, indicating that some males there were sex-reversed. Control groups were screened using ddRAD-seq to identify several thousands of SNP markers, from which 280 were significantly associated with sex and their mapping identified a single genomic position, suggesting a monogenic sex-determination system. Tightly linked markers had over 80% accuracy in determining the genetic sex of fish and allowed identifying sex-reversed males. Then, mature sex-reversed males were crossed with normal females to produce, for the first time, an all-females progeny group. Incorporating this technology into hatchery production will advance further development of mullet aquaculture and profitability of this industry.

Key Words: fish, genome-enabled breeding, DNA sequencing, candidate gene, aquaculture

OP171 Atlantic salmon miRNAs associated with smoltification and sea-water adaptation. R. Andreassen*[1], A. Shwe[1], S. Ramberg[1], A. Krasnov[2], and T. Østbye[2], [1]*Oslo Metropolitan University, Oslo, Norway,* [2]*Nofima (Norwegian Institute of Food, Fisheries and Aquaculture Research), Ås, Norway.*

Smoltification is a developmental process transforming parr into saltwater-adapted smolt. It is induced by artificial photoperiod in Atlantic salmon (AS) production. It is highly energy demanding and associated with suppressed immune function. About 15% of farmed AS transferred to sea die, and mortality rate is high during the first months post seawater transfer (SWT). Suboptimal smoltification and disease are considered contributory factors to death shortly after SWT. miRNAs are small ncRNAs regulating gene expression at the post-transcriptional level. However, their involvement in regulation of smoltification and SW adaptation is less well studied, and here we aimed to gain a better understanding of miRNA's role in regulation of this developmental transition. Head kidney (n = 48), liver (n = 42) and gill (n = 42) samples from 6 time points: parr (T1), early and late light treatment, smoltified, one week post SWT, one month post SWT were small-RNA sequenced. DESeq2 was applied for differential expression analysis comparing each of the time points with T1. Heatmap2 (R-package gplots) was used for hierarchical clustering to group differentially expressed miRNAs (DE-miRNAs) with similar dynamic changes. Samples from

each group (n = 6) were analyzed on the 44k Salgeno-2 microarray to identify differentially expressed genes (DEGs) that were used for in silico target gene predictions and enrichment analysis by use of the MicroSalmon GitHub repository and PANTHER Overrepresentation test (ORT). DE-miRNAs were revealed in head-kidney (n = 54), liver (n = 62) and gill (18). They were clustered into groups by increasing or decreasing expression patterns, commonly showing largest changes at smolt or post SWT stages. The ORT showed smoltification associated biological processes as enriched, e.g regulation of hormone levels, stress response, and ion transport (head-kidney), lipid homeostasis, energy metabolism, circadian rhythm and growth (liver), immune system networks, extracellular matrix and lipid metabolism (gill). Collectively, this indicate that the DE-miRNAs are important post-transcriptional regulators of smoltification and SW adaptation.

Key Words: miRNA, Atlantic salmon, smoltification, small RNA sequencing

OP172 ISAG Bursary Award: Construction of a high-density genetic linkage map using 2b-RAD sequencing in dusky kob (*Argurosomus japonicus*). T. Jackson and C. Rhode*, *Stellenbosch University, Stellenbosch, South Africa.*

A high-density genetic linkage map is a useful resource in genomic research, providing a means for quantitative trait loci (QTL) mapping, gene mapping, and comparative genomic analysis. Information which can be incorporated into current selective breeding strategies, leading to the increased success of finfish aquaculture. To provide the fundamental basis for future genomics research, this study constructed the first high-density genetic linkage map for dusky kob (*Argyrosomus japonicus*) using 2b-restriction site-associated DNA (2b-RAD) sequencing for genome-wide single nucleotide polymorphism (SNP) genotyping. This approach was performed using 3 full-sib F1 families, where a total of 21,515 SNPs were discovered and genotyped. Of which, 4,482 high-quality SNPs were assigned to 24 linkage groups (LGs), which agreed with the haploid chromosome number. The map spanned a length of 1,784 cM with an average marker space of 0.54 cM and a genome coverage rate of 96.3%. These results are consistent with the high-quality maps produced for the closely related species, *Argyrosomus regius* and *Larimichthys crocea*, apart from the male vs female recombination rates which have yet to be reported for these species. The recombination rate for this species was determined to be higher in females than males (an average female-to-male ratio of 1.2:1), coinciding with the previous research conducted on other teleost species. Overall, this study was able to produce a high-quality linkage map which will serve as an important tool and resource for detecting and fine mapping of QTLs, which will contribute considerably to the development and improvement of ongoing marker assisted selection (MAS) initiatives in the species.

Key Words: linkage map, 2b-RAD sequencing, single nucleotide polymorphism, aquaculture

OP173 Population genetics of two critically endangered rhino rays from the Southwest Indian Ocean region. M. Groeneveld*[1], J. Klein[1], R. Bennett[2], M. Bond[3], D. Ebert[4,5], K. Gledhill[6], S. Jaquemet[7], J. Kiszka[3], A. Macdonald[8], B. Mann[9], J. Nevill[10], A. Price[1], M. van Staden[1], B. Wueringer[11,12], A. Bester-van der Merwe[1], [1]*Department of Genetics, Stellenbosch University, Stellenbosch, South Africa,* [2]*Wildlife Conservation Society,* [3]*Institute of Environment, Department of Biological Sciences, Florida International University, Miami, FL,* [4]*Pacific Shark Research Center, Moss Landing Marine Laboratories, Moss Landing, CA,* [5]*South African Institute for Aquatic Biodiversity, Grahamstown, South Africa,* [6]*Fish Ecology Lab, University of Technology Sydney, Broadway, Sydney, Australia,* [7]*UMR Entropie, Université de La Réunion, La Réunion, France,* [8]*School of Life Sciences, University of KwaZulu-Natal, Westville, South Africa,* [9]*Oceanographic Research Institute, Durban, South Africa,* [10]*Environment Seychelles, Mahé, Seychelles,* [11]*Sharks And Rays Australia, Bungalow, Queensland, Australia,* [12]*Department of Biological Sciences, Faculty of Science and Engineering, Macquarie University, Macquarie Park, New South Wales, Australia.*

Wedgefishes (Rhinidae) are threatened by unsustainable trade globally and in the Southwest Indian Ocean (SWIO) due to their high-value fins. The whitespotted wedgefish *Rhynchobatus djiddensis* and the bottlenose wedgefish *Rhynchobatus australiae* are classified as Critically Endangered on the International Union for Conservation of Nature's Red List of Threatened Species, yet a lack of species-specific knowledge and taxonomic uncertainty still exists within this genus. Delineating populations and understanding the genetic connectivity of endangered and exploited species are important for their conservation management. Species identity of samples (n = 189) collected across the SWIO was confirmed based on the cytochrome oxidase c subunit 1 (*COI*) and nicotinamide adenine dehydrogenase subunit 2 (*ND2*) gene regions, where a ~98% sequence similarity match was considered reliable. The genetic diversity and population structure within and between species and sampling locations were investigated using a dual marker approach: (a) 2 concatenated mitochondrial gene regions, *COI* and the control region (n = 120), and (b) 9 nuclear microsatellite markers (n = 151). The overall genetic diversity was low, with an indication that different evolutionary forces are at play on a mitochondrial versus nuclear level. Analyses based on both marker types (haplotypes, *F*-statistics, multivariate and clustering analyses, with statistical significance defined at a 0.05 level) indicated clear differentiation of species. For *R. djiddensis*, the sampling populations from South Africa and Mozambique were generally homogeneous with no intraspecific genetic structure. For *R. australiae*, significant differentiation was found between the majority of sampling populations especially Australia, Reunion Island and Seychelles, whereas those from Madagascar and Tanzania were genetically the most similar. This information provides insights into the distribution range and population structure of the whitespotted wedgefish species complex that can support the sustainable management of wedgefishes.

Key Words: conservation genetics, genetic identification, population structure, genetic marker, fishery

OP174 Genetic variation in disease resistance traits in hybrid striped bass. J. Abernathy*[1], M. Lange[1], B. Farmer[2], M. McEntire[2], and S. Rawles[2], [1]*United States Department of Agriculture, Agricultural Research Service, Auburn, AL,* [2]*United States Department of Agriculture, Agricultural Research Service, Stuttgart, AR.*

White bass (*Morone chrysops*) and striped bass (*Morone saxatilis*) are parental species of hybrid striped bass (*M. chrysops* × *M. saxatilis*), a fish of increasing commercial importance throughout the US. One chief constraint to the expansion of hybrid striped bass production arises from the use of wild-catch parents in breeding programs. This is costly, unsustainable and leads to uncontrolled variation in the offspring. Our goal is to advance progress in the genetic improvement of hybrids by building additional white bass resources to facilitate selective breeding for agriculturally important traits. White bass were gathered from Arkansas, Texas and Alabama along with available domesticated strains and used to establish a breeding population for familywise evaluations of growth and nutrient utilization on alternative, sustainable diets. In addition to growth and nutritional traits, we are also interested in understanding disease resistance traits and characterizing genetic variation in this trait among moronid fish. Toward this goal, we have established baseline disease susceptibility for several pathogens among white bass, striped bass and their hybrids using our in-house disease challenge system. We are examining the genetic basis of disease resistance using both gene expression data during active infections as well as through genetic mapping of backcross populations. From our studies, we identified differentially expressed genes among species at multiple early time points post infection with 3 of the most important bacterial pathogens in moronid aquaculture including *Flavobacterium*, *Aeromonas*, and *Streptococcus* species. Mapping of important gene pathways also revealed unique host-pathogen signature differences among the parental white

striped bass and their hybrid. Quantitative trait loci (QTL) data is being investigated.

Key Words: morone, disease, moronid, striped bass, white bass

OP175 Multi-functional genomic analyses identify causal gene and variants modulating viral nervous necrosis resistance in European seabass. R. Mukiibi*[1], L. Peruzza[2], C. Penaloza[3], M. Babbucci[2], R. Franch[2], M. Freguglia[5], S. Laureau[5], G. Dalla Rovere[2], D. Bertotto[2], S. Ferraresso[2], C. Tsigenopoulos[4], R. D. Houston[3], L. Bargelloni[2], and D. Robledo[1], [1]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, United Kingdom,* [2]*Department of Comparative Biomedicine and Food Science, University of Padova, Padova, Italy,* [3]*Benchmark Genetics, Edinburgh Technopole, Edinburgh, United Kingdom,* [4]*Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (H.C.M.R.) Crete, Gournes Pediados Heraklion, Crete, Greece,* [5]*Valle Cà Zuliani Società Agricola s.r.l., Conselice (RA), Italy, Rovigo, Italy.*

Viral nervous necrosis (VNN) is a major infectious disease threatening the European seabass aquaculture industry. VNN causes high economic losses emanating from high mortality rates and slow growth of infected fish. Selective breeding has the potential to increase the disease resistance of aquaculture stocks, reducing the impact of disease. The most efficient selection strategy depends on the genomic architecture of the trait, which is currently unknown for resistance to VNN. In the current study we employed whole-genome resequencing and transcriptomic tools to define the genomic architecture of VNN resistance in European seabass using a VNN challenged population of 1400 juvenile fish. Similar to previous studies, our results demonstrated that VNN resistance is moderately heritable ($h^2 = 0.45$). Additionally, GWAS results revealed a major QTL on LG12, which hosted over 90% (37,357 of 48,787) of the significant (FDR >0.05) variants. Variants in this QTL region explained up to 37% of the genetic variance of resistance to the disease. Interestingly the most significant variants were located within the *IFI27L2A* gene. Furthermore, our analyses showed remarkable association between *IFI27L2A* gene expression and VNN resistance in the brain and head kidney. Expression quantitative trait loci analyses further revealed that GWAS significant variants also had significant impact on the expression of *IFI27L2A* in both brain and head kidney. *IFI27L2A* is an interferon inducible gene demonstrated to have antiviral properties in other species. Together, our results provide a more refined insight into the potential causal genetic background of resistance to VNN that could further be utilized for enhancing genomic selection or genome editing to produce more resistant fish.

Key Words: European seabass, viral nervous necrosis, genomics, QTL, eQTL

OP176 Utilizing of genetic evaluation system using genomic information of the Korean flatfish population. D. Lee*[1], J. Kang[1], Y. Chung[1], S. Lee[1], Y. Kim[2], J. Park[3,1], D. Lee[3], J. Kim[3], H. Yang[3], J. Lee[3], and S. Lee[1], [1]*Chungnam National University, Yuseong-gu, Daejeon, Republic of Korea,* [2]*Quantomic Research & Solution, Yuseong-gu, Daejeon, Republic of Korea,* [3]*Fish Genetics and Breeding Research Center, Geoje, Republic of Korea.*

The olive flounder (*Paralichthys olivaceus*) is one of the most popular fish species in Southeast Asia and around the world. However, a breeding program design such as genomic prediction for this species have been limited compared with other livestock breeds. Genetic analysis using genomic information has been lacking in breeding-related research in fish industry of Korea. National Institute of Fisheries Science was started a breeding program since 1990s, total 54,159 animal were measured breeding objective traits, body weight (BW), total length (TL), and condition factor (CF) at 11, 18, and 22 mo. Pedigree information was recorded in total 8 generation. Genomic data was generated from at generation 8 in this breeding population. In this study, the genomic analysis was conducted using a total of 3,223 individuals, all of which underwent custermized Affymetrix array. After quality control, a total of 67,467 SNPs were used for analysis. In addition, pedigree and genomic-based analyses were compared for accuracy and efficiency using pedigree information from 3 or more generations. Using the BLUP method with single traits, each trait's genetic parameter and breeding values were estimated for pedigree-based BLUP (PBLUP) and genomic-based BLUP (GBLUP) methods. The estimated breeding values (EBV) accuracy was compared using the prediction error variance and the correlation with the actual phenotype. The estimated heritabilities by PBLUP method were 0.425, 0.468, and 0.384 for the traits, respectively, while those estimated by GBLUP method were 0.491, 0.505, and 0.594 for the traits. The heritability showed a similar trend when compared with research using pedigree information and showed 0.03–0.21 higher when using genomic information. The theoretical accuracy difference between PBLUP and GBLUP methods was 0.15 to 0.16, depending on the trait, but in actual accuracy (correlation), the difference ranged from 0.1 to 0.3. These results indicate that introducing genetic improvement using genomic information in the olive flounder population could effectively enhance genetic improvement.

Key Words: genomic selection, GBLUP, EBV, heritability, olive flounder

OP177 Whole-genome sequencing data provide a landscape picture of genetic variability in sea cucumber species. F. Bertolini*[1], A. Ribani[1], V. Taurisano[1], A. Rakaj[2], A. Fianchini[2], F. Capoccioni[3], D. Pulcini[3], S. Bovo[1], and L. Fontanesi[1], [1]*Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Bologna, Italy,* [2]*Department of Biology, University of Rome Tor Vergata, Rome, Italy,* [3]*Centro di Ricerca "Zootecnia e Acquacoltura," Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Monterotondo (Rome), Italy.*

Holothuria is a genus of marine invertebrates commonly called sea cucumbers, that belongs to the phylum Echinodermata. *Holothuria* species are deposit feeders, ingesting sediment with oral tentacles, extracting and digesting the organic matter with associated microorganisms, and voiding the sand through the anus. In this way they bioturbate significant areas of the seabed, playing a central role in the benthic habitats where these species live. The interest in *Holothuria* spp. for aquaculture began in Asia and soon reached the European market, making these species promising candidates to establish novel aquaculture production systems worldwide. The genus counts more than 160 species, many of which are uncharacterised at the genome level. Here, we provide a comparative genome analysis between 2 Mediterranean sea cucumber species, *Holoturia polii* (HP) and *Holoturia tubulosa* (HT) utilizing the reference genomes of both *Holothuria glaberrima* (HG) and *Holothuria scabra* (HS), the only 2 species of this genus for which a reference genome is available thus far. The HG and HS reference genomes are composed of 89,105 and 4,345 scaffolds, respectively. Whole-genome sequencing data sets of 4 HP and 3 HT were produced, filtered and then mapped to the 2 reference genomes with standard options using BWA tool. The genome coverage of the sequencing data obtained for HT was 59.58% and 20.24% against the HG and HS genomes respectively, whereas the genome coverage obtained for HP was 55.62% and 19.16% against the same 2 genomes respectively. Variant calling was performed utilizing the HG-based alignment, retrieved more than 25 million high-quality SNPs. These SNPs were used for the study of population differentiation via the F_{ST} statistics; HP and HT that was 0.34 presented a F_{ST} index equal to 0.34. This information provided a first comparative analysis of genome information between HT and HP species and underlined the need to further expand genomic research in holothurian species. This information will be used to detect genomic regions of high genetic divergence that might differentiate HT and HP species.

Key Words: aquaculture species, holothuria, SNP; F_{ST}

OP178 The history and future of African cattle diversity and adaptation: The known and the possible. O. Hanotte*[1,2], [1]*ILRI, Ethiopia,* [2]*The University of Nottingham, Nottingham, United Kingdom.*

We will review here our current knowledge about the origin, migration, dispersion, crossbreeding and adaptation of indigenous African cattle presenting today's available genomics and archeological information. It is now well established that the first cattle to reach the African continent were of the taurine type. Subsequently, the continent saw the arrival of humped cattle (indicine). It was followed by centuries of crossbreeding and hybridizations which shaped today's African cattle genomics landscape. At the roots of these events were trading networks (within the continent and between Africa and Asia), natural selection (climatic, vector-borne diseases) and ancient human dispersion events. Here, the role and importance of African civilisations and societies remain underrated despite the socio-cultural importance of cattle pastoralism across the continent. European colonisation saw the arrival of exotic improved germplasm adding a new layer of cattle diversity. In today's urgent need to improve cattle production sustainably, exotic × indigenous crossbreds have an immediate impact on productivity. Market-assisted selection and gene-editing offer new opportunities for the enhanced utilization of the unique diversity of African cattle.

Key Words: cattle, history, diversity, productivity, Africa

OP179 Whole-genome diversity of dromedary camels from the entire geographic distribution range. G. Senczuk*[1], S. Bruno[2], M. Di Civita[1], V. Landi[3], S. Brooks[4], F. Almathen[5,6], B. Faye[7], S. B. S. Gaouar[8], M. Piro[9], K. S. Kim[10], H. Dadi[11], C. Iglesias Pastrana[12], H. Al-Haddad[13], M. Al-Abri[14], C. Persichilli[1], F. Pilla[1], P. Burger[15], and E. Ciani[2], [1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, 86100 Campobasso, Italy,* [2]*Department of Biosciences, Biotechnologies and Environment, University of Bari "Aldo Moro", 70126 Bari, Italy,* [3]*Department of Veterinary Medicine, University of Bari "Aldo Moro", Valenzano, 70010 Bari, Italy,* [4]*Department of Animal Sciences, University of Florida, Gainesville, FL 32610, USA,* [5]*Department of Public Health, College of Veterinary Medicine, King Faisal University, Al-Ahsa 31982, Saudi Arabia, Camel Research Center, King Faisal University, Al-Ahsa 31982, Saudi Arabia, CIRAD-ES, UMR SELMET, 34398 Montpellier, France, Department of Biology, Abou Bakr Belkaid University of Tlemcen, Tlemcen 13000, Algeria, Department of Medicine, Surgery and Reproduction, Institut Agronomique et Vétérinaire Hassan II, Rabat BP 6202, Morocco,* [10] *Department of Animal Sciences, Chungbuk National University, Chungbuk 28644, Korea,* [11]*Ethiopian Biotechnology Institute (EBTi), Addis Ababa, Etiopia,* [12]*Department of Genetics, Faculty of Veterinary Sciences, University of Córdoba, Córdoba, Spain,* [13]*Department of Biological Sciences, Kuwait University, Kuwait City, Kuwait,* [14] *Department of Animal and Veterinary Sciences, Sultan Qaboos University, Muscat, Oman,* [15]*Research Institute of Wildlife Ecology, Vetmeduni, Vienna, Austria.*

During human history, dromedaries have played a central role in many essential aspects including commercial transportation and for human sustenance in terms of meat, milk and leather. Still now, dromedaries represent an essential social and cultural element in many Arabian and Asiatic countries. This was possible mainly due to dromedary strong adaptation to extreme environmental condition in marginal agro-natural zones. Under the frame of the 2019 Illumina® Agricultural Greater Good initiative, the whole-genome sequencing of 336 dromedaries from the entire geographic distribution range has been carried out. Other 22 WGS samples were included from public databases. All samples were mapped against the reference genome (CamDro3) and a variant calling analysis was performed using the Illumina® Dragen germline platform. A total of 505,662 SNPs were retained after filtering for minor allele frequency and missing call rate. The overall variability was explored by using genetic diversity indices (H_o, pairwise F_{ST} and F_{ROH}), while the population genetic relationships were assessed by the use phylogeographic inference tools (PCA, ADMIXTURE and Neighbor-net). The overall H_o values were higher than those observed for African cattle and sheep breeds, while all F_{ROH} values were positive with the highest values observed in Australia and Kenya populations. Both PCA and ADMIXTURE analyses revealed a weak genetic structure, however a phylogeographical pattern was observed. At the best K value (K = 5), a first separation involved samples from Ethiopia and Kenya following a subsequent split between African and Asian populations. A genomic cline from East to West was also evident, suggesting a possible dominant role of ancient caravan routes along de Sahara Desert in molding the observed genetic pattern. The topology inferred by the Neighbor-net graph basically confirms the genetic repartition into 5 main groups. Interestingly, the proportion of ancestral components together with the Neighbor-net basal position, would seem to confirm the Southern Arabian Peninsula as an important cradle of domestication.

Key Words: old world camelid, evolutionary genomics, population genomics, biodiversity

OP180 ISAG Bursary Award: Genome-wide scan for selection signatures in South African indigenous goat ecotypes. A. M. Magoro*[1,2], A. Zwane[2], K. Hadebe[3], and B. Mtileni[2], [1]*Tshwane University of Technology, Pretoria, South Africa,* [2]*Agricultural Research Council-Animal Production, Pretoria, South Africa,* [3]*Agricultural Research Council-Biotechnology Platform, Pretoria, South Africa.*

Whole-genome scan of signatures of selection contributes to the identification of genomic regions that are functionally important for agricultural production. Indigenous goats have adapted to different environments for survival, breeding and production. Their genomes are likely to have acquired unique alleles for various traits of economic importance. The objective of this study was to investigate signatures of selection in indigenous goat ecotypes from 4 provinces of South Africa. Non-descript goat ecotypes representing populations from the Free State (n = 18), Gauteng (n = 24), Limpopo (n = 28), North West (n = 30) provinces and a conservation flock from the Agricultural Research Council (ARC)-Animal Production (n = 25) were genotyped using Illumina Goat SNP65K BeadChip. Quality control was performed using PLINK v1.9. A total of 43 726 autosomal SNPs remained for downstream analyses. Pairwise F_{ST} of the populations was observed to be lower in non-descript populations (FS, GP, LP and NW) and higher in the ARC population. The R package rehh was used to detect selection signatures implementing 2 complementary approaches: Integrated Haplotype Score (iHS) and cross-population-extended haplotype homozygosity (XP-EHH). The most significant regions were identified at a threshold of -log (iHS) > 4 ($P < 0.0001$). A total of 161 top selection signature regions were identified across all the populations with some genes relating to adaptation (*PRKCB*), reproduction process (*BMPR2*, *HOOK1*, *ROR2*), feed efficiency (*DACH1*), response to stress (*HSMCE1*, *ALS2*, *GTF2I*, *ROR2*) and skeletal system development (*ROR2*, *BMPR2*, *HOOK1*) based on the iHS analysis. The XP-EHH analysis identified 215 regions, with several genes relating to cell development (*ABCA12, Figure 4, CASP8, IL4R*) and response to growth factors (*BMPER*, *VWC2L*). The genes identified in this study are highly significant to the biological function and traits of economic importance in goats. The results indicate that various selection pressures have influenced the genome of indigenous goats in South Africa and provide new knowledge important for breeding and conservation of these indigenous goat ecotypes.

Key Words: adaptation, genomic region, non-descript goat

OP181 ISAG Bursary Award: Differential proteomics revealed the impact of heat stress on milk whey proteins in indigenous Deoni (*Bos indicus*) and Holstein Friesian (*Bos taurus*) crossbred cows. E. Rana*[1,4], K. P. Ramesha[1], N. Azharuddin[1], M. A. Najar[2], M. K. Sinha[1], S. Jeyakumar[1], L. Gopalakrishnan[2,3], P. Nag[1], S. Mall[1], M.

Ashokan[1], M. Dasgupta[1], A. Kumaresan[1], D. N. Das[1], and T. S. K. Prasad[2], [1]*Southern Regional Station, ICAR- National Dairy Research Institute, Bangalore, India,* [2]*Center for Systems Biology and Molecular Medicine, Yenepoya Research Centre, Yenepoya (Deemed to be University), Mangalore, India,* [3]*Institute of Bioinformatics, International Technology Park, Bangalore, India,* [4]*Livestock Development Department, Government of Chhattisgarh, Chhattisgarh, India.*

Heat stress is a significant financial threat to animal husbandry. Though, indigenous (*Bos indicus*) cattle can survive and perform better under heat stress conditions as compared with exotic (*Bos taurus*) breeds or their crossbreds. Still, the mechanisms at a molecular level associated with heat tolerance among these cattle are ill-understood to date. Proteins are the phenotypic product of genes that explain the effect of direct environment in a better way. In the present study, high-throughput milk whey proteomics was performed to identify the subtle changes occurring at protein level compared between normal (Temperature Humidity Index, THI = 66.6) and heat stress (THI = 82.2) conditions in Deoni and Holstein Friesian crossbred cows. A total of 412 proteins were identified in milk whey samples by LC-MS/MS technique coupled with bioinformatics analysis. Differential milk whey proteomics revealed that 27 and 53 proteins were upregulated (fold > 1.5), whereas, 10 and 8 proteins were downregulated (fold < 0.6) during heat stress as compared with normal condition in indigenous and crossbred cows, respectively. It was observed that the upregulated proteins were mainly related to defense response, metabolic process and response to external stimuli. The Gene Ontology analysis showed 38.09 and 48.15 percent of the enriched biological processes were related to the defense mechanism in indigenous and crossbred cows, respectively. The study was validated by ELISA method which revealed that the expression of haptoglobin, an acute phase protein, was highly significant during heat stress condition, thus, could act as a potential biomarker associated with thermo-tolerance of the animal. Collectively, it is concluded that a definite difference exists in the milk whey protein profile of dairy cows and the information on their relative abundance during heat stress condition would act as an aid to select and propagate thermo-tolerable dairy cows. This study would serve as a foundation for further proteomics studies on milk.

Key Words: cattle and related species, proteomics, mass spectrometry, gene ontology, biomarker

OP182 ISAG Bursary Award: Whole genome sequencing of Landim pigs of Mozambique reveals a close relationship with Angola native pigs and suggests selection for immune response. F. Teixeira*[1,2], P. Sá[1], D. Santos[1], C. Garrine[3], R. Zimba[4], L. Souza[3], H. Chiaia[2], A. Leitão[1], J. M. Cordeiro[2], L. T. Gama[1], and A. J. Amaral[1,5], [1]*Centre for Interdisciplinary Research in Animal Health and Associate Laboratory for Animal and Veterinary Sciences, Faculty of Veterinary Medicine, University of Lisbon, Alto da Ajuda, Lisbon, Portugal,* [2]*Faculty of Veterinary Medicine, University José Eduardo dos Santos, Huambo, Angola,* [3]*Faculty of Veterinary Medicine, University Eduardo Mondlane, Maputo, Mozambique,* [4]*Escola Superior de Desenvolvimento Rural de Vilankulo, University Eduardo Mondlane, Mozambique,* [5]*Escola de Ciências e Tecnologia Universidade de Évora, Évora, Portugal.*

Landim pigs, a population of native pigs from Mozambique are currently threatened by the recent introduction of European exotic breeds. Like most African pig populations, Landim pigs have never been characterized at the genome level and are well adapted to harsh conditions which include adaptation to endemic diseases. In this study, we provide a comprehensive genetic characterization of Landim pigs using whole-genome sequencing (WGS). We generated genomes from Landim pigs (n = 6) and compared these genomes to local pigs from Angola and European and Asian domestic pigs and wild boars currently in the public domain (n = 78). Analyses of population structure showed that Angola local and Landim pigs are closely related, and both are more closely related to European than Asian breeds. Preliminary results suggest that Landim pigs display a duplication in Chr4 that has been reported only in Chinese domestic pigs, overlapping the *TBX19* gene that is associated with development and growth. The functional analysis of missense SNPs in Landim pigs shows that these occur in genes related to immune system response. The integrated haplotype score (iHS) analysis revealed candidate regions under selection overlapping genes also related to immune response. This study represents the first assessment of the genetic background of native pigs from Mozambique and opens the path to understanding the dynamic and interlinked history of African pig populations. The present study further reports unique genetic attributes related to an immune response that should be further explored.

Key Words: pigs and related species, genome sequencing, population structure, adaptation, conservation

OP183 Structural variant calling using ONT long-read whole genome sequencing of indigenous Zulu sheep. N. Nxumalo*[1], A. Molotsi[1], C. Rhode[1], and N. Kunene[2], [1]*Stellenbosch University, Stellenbosch, Matieland, South Africa,* [2]*University of Zululand, Empangeni, Kwadlangezwa, South Africa.*

Livestock genetic variation has been based on genetic markers such as single nucleotide polymorphisms, indels (<50 bp) and short tandem repeats. Even though structural variants (SV) have been reported as a significant constituent underpinning livestock phenotypic variation, their exploration has been minimal. Structural variants calling requires long-read sequencing reads that covers the whole length of the variant. The aim of this study was to explore Zulu sheep breed genetic divergence through SV calling using long-read sequencing. Blood samples were collected from 2 purebred Zulu sheep kept at the University of Zululand, high molecular weight DNA was extracted and used for whole genome sequencing. Sequencing was done using Oxford Nanopore technology at Central Analytical Facility at Stellenbosch University. After raw reads quality control, competent reads were mapped to ARS-UI_Ramb_v2.0 sheep reference genome (96.08 mapping percentage) using minimap2/2.1. Structural variants were called using sniffles/2.0.7 and included duplication (12), inversions (33), breakend (166), deletions (9541) and insertions (15986). Variant Effect Predictor (VEP) was then used to predict their possible genomic effect. There were 62014 identified SV's that overlapped genes and 19749 transcripts. Some identified SVs may significantly affect Zulu sheep breed phenotypic uniqueness based on their functional genomic location and may underpin consequences on intergenic variants, intron variants (HDAC4, SCLY, UBE2F, MLPH, AGAP1) and feature elongation (ZMYM4, AGO3, SH3D21, MANEAL, UTP11) thus altering gene regulation mechanisms, splicing site recognition and extended transcriptions and/ translations. Further investigation on the identified SVs using genome wide association studies may assist to understand genomic bases of Zulu sheep phenotypic unique characteristics.

Key Words: genetic heterogeneity, structural variation, whole genome sequencing, Zulu sheep

OP184 Studying cattle structural variation and pangenome using whole genome sequencing. G. Liu*, *Animal Genomics and Improvement Laboratory, Henry A. Wallace Beltsville Agricultural Research Center, Agricultural Research Service, USDA, Beltsville, MD.*

A cattle pangenome representation was created based on the genome sequences of 898 cattle representing 57 breeds. The pangenome identified 83 Mb of sequence not found in the cattle reference genome, representing 3.1% novel sequence compared with the 2.71-Gb reference. A catalog of structural variants developed from this cattle population identified 3.3 million deletions, 0.12 million inversions, and 0.18 million duplications. Estimates of breed ancestry and hybridization between cattle breeds using insertion/deletions as markers were similar to those produced by single nucleotide polymorphism–based analysis. Hundreds of deletions were observed to have stratification based on subspecies and breed. For example, an insertion of a Bov-tA1 repeat element was identified in the first intron of the *APPL2* gene and correlated with cattle breed geographic distribution. This insertion falls within a segment overlapping predicted enhancer and promoter regions of the gene, and could affect important traits such as immune response, olfactory functions, cell

proliferation, and glucose metabolism in muscle. The results indicate that pangenomes are a valuable resource for studying diversity and evolutionary history, and help to delineate how domestication, trait-based breeding, and adaptive introgression have shaped the cattle genome.

Key Words: cattle, structural variation, pangenome, whole genome sequencing

OP185 Poultry genomics within the Centre for Tropical Livestock Genetics and Health. J. Smith*[1], A. Gheyas[1], A. Trujillo[1,2], A. Kebede[3], G. Gebru[4,5], N. Seboka[5,6], M. Rachman[2], T. Dessie[7], and O. Hanotte[2,7], [1]*Centre for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, University of Edinburgh, Edinburgh, UK,* [2]*University of Nottingham, Nottingham, UK,* [3]*Amhara Regional Agricultural Research Institute, Bahir Dar, Ethiopia,* [4]*Tigray Agricultural Research Institute, Mekelle, Tigray, Ethiopia,* [5]*Addis Ababa University, Addis Ababa, Ethiopia,* [6]*Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia,* [7]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia.*

The Centre for Tropical Livestock Genetics and Health (CTLGH; https://www.ctlgh.org/) is a collaboration between The Roslin Institute (RI; UK), Scotland's Rural College (SRUC; UK) and the International Livestock Research Institute (ILRI; Kenya/Ethiopia). The ethos behind the center is tackling global challenges through genetic improvements in tropical livestock. Working as part of CTLGH we use genomics to identify ways to improve productivity in indigenous chickens. Our main interest is in understanding the tolerance of these birds, which are resilient to environmental stresses such as temperature, altitude and food/water scarcity. Our main aim is to understand the genes controlling these resilience traits so that favorable characteristics can be used in breeding programmes to produce birds which are not only environmentally adaptable, but are also healthier and more productive. This would benefit livelihoods of both smallholder farmers and larger farming enterprises across low-to-middle income countries (LMICs). As our global climate continues to change it is important that we are able to produce livestock that can withstand that change. We have used whole genome sequencing of hundreds of birds from different areas across Ethiopia and Nigeria to identify candidate genes for environmental adaptation (e.g., heat stress). Ecological niche modeling has also allowed for definition of ecotypes and creation of environmental suitability maps. Investigation of the MHC in these birds has also identified many novel haplotypes, with implication for disease resistance. As we now enter a new phase of research within CTLGH, we look to investigate new populations of birds across different African countries and use different genomic approaches (transcriptomics, epigenetics) to gain deeper insight into our candidate genes and regions. Our data are also included in efforts by The Chicken Diversity Consortium, bringing together sequence data from thousands of chickens representing ancient, commercial, research, exotic and indigenous breeds from around the world, to enable pan-genomic analysis of the chicken genome.

Key Words: chicken, genetics, environmental adaptation, genomics

OP186 Tracking the adaptive history of African cattle using low-coverage genomes. S. I. Ng'ang'a*[1,2], J. A. Ward[3], G. V. Smith[4], S. Rossiter[2], C. Faulkes[2], D. G. Bradley[5], O. Hanotte[6,7], D. E. MacHugh[8], and L. A. F. Frantz[1,2], [1]*Palaeogenomics Group, Department of Veterinary Sciences, Ludwig Maximilian University, Munich, Germany,* [2]*School of Biological and Chemical Sciences, Queen Mary University of London, London, United Kingdom,* [3]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Dublin, Ireland,* [4]*SilverStreet Capital, London, United Kingdom,* [5]*Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland,* [6]*International Livestock Research Institute, Addis Ababa, Ethiopia,* [7]*School of Life Sciences, University of Nottingham, Nottingham, United Kingdom,* [8]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland.*

Africa is home to more than 150 distinct breeds of domestic cattle, with rich phenotypic diversity reflecting adaptation to a wide range of agro-ecological conditions. The genetic basis of cattle adaptations to the myriad of African ecosystems, however, is poorly understood, and many breeds are endangered, due to uncontrolled crossbreeding and replacement by non-native breeds. Low-coverage sequencing (LCS) can improve our knowledge of genomic diversity in African cattle and pave the way for cost-effective genome-enabled breeding programs. The LCS approach requires high-coverage genome sequence data to generate a reference panel that is then used to impute low-coverage genomes. Importantly, for accurate imputation, the LCS reference panel should include sufficient genomic representation of the cattle breeds that are to be imputed to a whole-genome scale. Previous studies have shown that this is difficult to achieve in African cattle due to a lack of existing genomic information. To address this issue, we developed an imputation pipeline based on a large reference panel combining 150 newly generated high-coverage genomes from multiple African cattle populations, together with publicly available genome data, which comprises over 3,200 cattle genomes from 133 cattle populations. Through imputation of down-sampled high-coverage genomes from various cattle populations, we show that our imputation pipeline provides genotype imputation accuracies > 99% for common variants (minor allele frequency [MAF] ≥ 5%; compared with 75% in previous studies) and between 92% and 98% for rare variants (0.5% ≤ MAF < 1%; compared with 30% in previous studies) in 0.5 × coverage of African cattle genomes. The accuracy of our LCS imputation pipeline, however, was lower for Asian cattle of primarily *B. indicus* ancestry, reflecting the need to sequence more cattle from Asian countries. We then used this reference panel to impute > 1,000 low-coverage African cattle genomes. Population genomic analyses from this data set will provide a new perspective on how natural selection and human-mediated breeding has shaped the genetic composition of African cattle.

Key Words: cattle and related species, genome-enabled breeding, imputation, adaptation

OP187 Virginia Tech research education programs: Models for increasing STEM participation in middle- and low-income countries. E. Smith*, *Virginia Tech, Blacksburg, VA.*

Middle- and low-income countries have the same challenges faced by the United States of America and other high-income countries: engaging, recruiting, and training students from low-income backgrounds in STEM and related fields. We describe training programs, and their statistics, and suggest they can be used to train the next generation of aspiring animal geneticists from developing countries. Our training programs are based on the hypothesis that a cohort approach to graduate education have better outcomes than the professor-driven tradition of the land grant system. A total of 227 trainees have participated in these research education programs. The undergrad institutions of origin have been national and The US Territories and Puerto Rico. The training plan has included innovative approaches such as an Ombudsperson for graduate education, retrospectives by scientists as "scientific journeys" on YouTube, peer and near-peer mentoring, and a committed engagement with alumni for their lived experiences and to continue impacting their careers. Additional required courses include "Effective Grant Writing" and Scientific Writing. The courses help us to not only provide additional training in "soft skills," but to stay in regular contact with trainees in the early months of the PhD. Together, these efforts have resulted in 91 PhDs, 3 MDs, 30 MS and 1 DPharm. Trainees are pursuing diverse careers including 13 in tenure-track academic positions, one of whom was recently tenured. Our recent rate continues to be above 75%. Beyond research skills, our trainees acquire networking, mentoring, and communication (written and oral) skills that have contributed to their successes. Our creativity as scientists, I will argue, has made these successes more likely in a department not generally considered biomedical or behavioral. Our future efforts with these research training programs will include the use of "lived experiences" by our distinguished alumni as a training tool.

Key Words: research education, STEM, developing country, average student

OP188 Withdrawn

OP189 Identification the genetic resistance genes and biosynthesis pathways to gastrointestinal nematodes infection in goat using RNA-sequencing. A. A. Bhuiyan[1], A. Bhuyan*[2], A. S. Afsana[3], S. Zhao[4], and X. Du[4], [1]*Bangladesh Agricultural Research Council, Dhaka, Dhaka, Bangladesh,* [2]*National Institute of Biotechnology, Savar, Dhaka, Bangladesh,* [3]*Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh,* [4]*Huazhong Agricultural University, Wuhan, Hubei, China,* [5]*Huazhong Agricultural University, Wuhan, Hubei, China.*

Gastrointestinal nematodes (GINs) are one of the most economically important parasites of small ruminants and a major animal health concern in many regions of the world. However, the molecular mechanisms of the host response to GIN infections in goat are still little known. In this study, 2 genetically distinct goat populations, one relatively resistant and the other susceptible to GIN infections, were identified in *Yichang* goat and then 4 individuals in each group were chosen to compare mRNA expression profiles using RNA-seq. Field experiment showed lower worm burden, delayed and reduced egg production in the relatively resistant group than the susceptible group. The analysis of RNA-Seq showed that 2369 genes, 1407 of which were upregulated and 962 downregulated, were significantly ($P < 0.001$) differentially expressed between these 2 groups. Functional annotation of the 298 genes more highly expressed in the resistant group yielded a total of 46 significant ($P < 0.05$) functional annotation clusters including 31 genes (9 in innate immunity, 13 in adaptive immunity, and 9 in innate immune response) related to immune biosynthetic process as well as transforming growth factor (TGF)-β, mitogen-activated protein kinase (MAPK), and cell adhesion molecules (CAMs) pathways. Our findings provide insights that are immediately relevant for the improvement of host resistance to GIN infections and which will make it possible to know the mechanisms underlying the resistance of goats to GIN infections.

Key Words: transcriptome, genetic resistant, gastrointestinal nematode, RNA-Seq, goat

OP190 A continent-wide genomic resource for African buffalo (*Syncerus caffer*). L. Morrison*[1,2], [1]*Roslin Institute, University of Edinburgh, Edinburgh, UK,* [2]*Centre for Tropical Livestock Genetics and Health, University of Edinburgh, Edinburgh, UK.*

The African buffalo (*Syncerus caffer*) is a wild bovid with a historical distribution across much of sub-Saharan Africa. Genomic analysis enables insights into the evolutionary history of the species, and potentially the key selective pressures shaping the current populations, including an assessment of population level differentiation, population fragmentation, and population genetic structure. In this study we generated the highest quality de novo genome assembly (2.65 Gb, with a scaffold N50 of 69.17 Mb) of African buffalo to date, and sequenced a further 195 genomes from populations representing the distribution of all subspecies. Principal component and admixture analyses provided surprisingly little support for the currently described 4 subspecies, but indicated 2 main lineages, located in Western/Central and Eastern/Southern Africa, respectively, with secondary differentiation between Eastern and Southern African populations. Estimating Effective Migration Surfaces analysis suggested that geographical barriers across the continent have played a significant role in shaping gene flow and the population structure. Estimated effective population sizes (N_e) indicated a substantial drop in N_e occurring in all populations 5–10,000 years ago, coinciding with the rapid increase in human populations on the continent. Finally, signatures of selection were enriched for key genes associated with the immune response, suggesting infectious disease exert a substantial selective pressure in shaping the genetics of African buffalo. The data is suggestive of protozoan parasites (the African buffalo is the primary host for the tick-borne *Theileria parva,* an important pathogen of cattle) perhaps exerting particularly strong selection. These findings have important implications for understanding bovid evolution, buffalo conservation and population management, and the pathways involved in bovid tolerance to pathogens.

Key Words: African buffalo, reference genome, population genomics, conservation genomics

OP191 A time-resolved multi-omics atlas of transcriptional regulation in response to high-altitude hypoxia across the whole-body tissues. Z. Yan*[1] and M. Li[1], [1]*China Agricultural University, Beijing, China,* Hypoxia serve as the most representative environmental stressors in high-altitude area and profoundly challenge life survive. Previous studies identified a suit of traits favoring local adaptation from indigenous high-altitude population, and indicated the polygenicity of hypoxia adaptation, suggesting that downstream of transcription and participate more directly in crucial cellular activities. Here, we conduct environmental shift experiment (e.g., plain and plateau) and generate time-resolved (i.e., 0 d, 6 d, 13 d, 20 d and 8 mo) phenotypic and RNA-Seq, single-cell RNA-Seq, ATAC-Seq data from 19 diverse tissues. Our phenotypic changes reveal that short-term hypoxia acclimatization is a 2-stage process. We systematically depict multi-tissue temporal dynamic of transcriptome and interplay network under hypoxia. We illustrate that genomic variants associated with high-altitude evolution play a crucial role in cerebellum and exhibit distinct expression pattern. We identify TAD-constrained *cis*-regulatory elements (CREs) and found hypoxia suppress the transcriptional activity. Moreover, we capture

phenotypic (e.g., oxygen saturation) and transcriptional (e.g., *UCP3*, *CAT*, *SIK1*) evidences indicate that antenatal hypoxia may increase hypoxia tolerance for offspring. Take together, our study provides a comprehensive view of hypoxia acclimatization and new insight for human disease research.

Key Words: hypoxia acclimatization, multi-omics, dynamic regulatory, sheep

OP192 Copy number variation mapping and copy number variation contribution to genetic variance of complex traits in dairy cattle. G. Ladeira[1], P. Pinedo[2], J. Santos[1], W. Thatcher[1], and F. Rezende*[1], [1]*University of Florida, Gainesville, FL,* [2]*Colorado State University, Fort Collins, CO.*

Copy number variation (CNV) are structural genomic variants that can play active role in gene dosage and expression. Hence, CNV can provide substantial insights into the genetic architecture of complex traits and account for some of the additive genetic variance which cannot be accounted for by SNPs. First, CNVs were mapped from 3,601 Holsteins genotyped with the Illumina BovineHD BeadChip. The Log R ratio and B Allele Frequency of 720,732 autosomal markers were used for CNV calling. After quality control, 3,952 non-redundant CNV events were identified spanning the autosomal genome in 2,422 Holsteins. At the population level, individual CNVs were compiled into 943 CNV regions (CNVR). Of those, 927 CNVRs overlapped 12,258 QTLs, and 698 CNVRs overlapped 1,762 genes, most of them (85.81%) classified as protein-coding genes. CNV information was then included into a SNP-based model to assess its contribution in estimating genetic parameters for health traits in 2,900 cows. The variance components were estimated by fitting either only a SNP-derived genomic relationship matrix (SNP_GMR) or both SNP_GMR and CNV_GMR matrices in threshold models implemented in a Bayesian framework using THRGIBBS1F90. The additive genetic variances captured by the pure SNP_GMR model and heritability estimates were, respectively, 0.18 ± 0.09 and 0.14 ± 0.06 for mastitis and 0.16 ± 0.06 and 0.13 ± 0.04 for metritis. When SNP_GMR and CNV_GMR were considered jointly, CNVs accounted for additional genetic variances (0.02 ± 0.02 for mastitis, and 0.03 ± 0.02 for metritis), resulting in greater heritability estimates for mastitis (0.17 ± 0.09) and metritis (0.16 ± 0.06). Therefore, the SNPs and CNVs in the present study were not redundant forms of genomic data, and CNVs accounted for part of the heritability which was not account for by SNPs alone as genetic markers (so called, "missing heritability"). These findings will provide opportunities for a better understanding of the genetic architecture of complex traits and may contribute to the development of more accurate genomic selection methods in Holstein dairy cattle.

Key Words: copy number variation, variance component, health trait

OP193 Analysis of differential isoform usage in production relevant tissues across pre- and post-natal development in sheep. S. A. Woolley[1], J. G. D. Prendergast[1], M. Salavati[1,2], and E. L. Clark*[1], [1]*The Roslin Institute, Edinburgh, Midlothian, UK,* [2]*SRUC, Edinburgh, Midlothian, UK.*

Understanding transcription during early development in sheep can help to inform breeding programmes by identifying the genomic drivers of healthy growth in production relevant tissues. This study aims to identify genes that are differentially expressed and exhibit differential isoform usage across pre- and post-natal developmental stages. RNA-sequencing was generated for whole embryo, placenta, liver and skeletal muscle bicep tissue from 6 different developmental stages from 48 Texel × Scottish Blackface sheep in total. Gene expression levels were estimated across tissues and developmental stages using Kallisto and compared using DESeq2. Tissue- and developmental stage-specific differences in gene expression were observed, especially between d 100 of gestation and one week of age when genes related to muscle growth and development were upregulated (e.g., *MYH3*, *GDF5* and *COL9A2*) in skeletal bicep muscle. Analysis of allele-specific expression also revealed imbalances in expression from either parent in gene families related to growth. In addition, long read Iso-Seq data for 12 of the 48 Texel × Scottish Blackface sheep was generated to investigate differential isoform usage through development. Using the Iso-Seq data we identified 16 isoforms per gene on average across 4 tissue types and 6 time points. The transcription start sites for these transcripts were validated using CAGE-Sequencing data from the same set of samples. The isoform models predominantly originated form 1–2 TSS sites already annotated in the reference gene models for the sheep reference genome ARS-UI_Ramb_v2.0 (NCBI v108). Analysis of the Iso-Seq, RNA-Seq and CAGE data, using the FLAIR analysis pipeline, revealed dominant isoforms across developmental stages, along with functionally important transcript isoforms linked to growth traits in sheep. Integration of these results with GWAS data will assist the identification of expressed genomic variation associated with growth traits in sheep. This information can then be used to inform genomics enabled breeding programmes and provide breed-specific annotation information for sheep.

Key Words: sheep, transcriptome, muscle, growth, Iso-Seq

OP194 Functional mapping of alternative polyadenylation in cattle. Z. Jiang[1], H. Wang[1], X. Zhou[1], J. J. Michal[1], S. A. Carrion[1], S. Zhang[1], Y. Zhang[1], M. J. Stotts[1], S. He[1], Y. Zhang[1], X. Zhang[1], X. Han[1], W. Wang[1], L. Qu[1], R. Li[1], M. Maquivar[1], M. Du[1], L. K. Fox[1], M. L. Bernhardt[2], Y. Wang[3], J. Velez[4], B. Hans[4], B. M. Murdoch[5], C. Gill[6], H. Jiang[7], H. Zhou[8], J. E. Koltes[9], J. Reecy[9], M. Rijnkels[10], P. J. Ross[8], S. McKay[11], T. P. L. Smith[12], W. Liu[13], K. Ren[14], L. Low[14], J. Yang[15], and S. P. Miller[16], [1]*Department of Animal Sciences and Center for Reproductive Biology, Washington State University, Pullman, WA,* [2]*Animal Production Core, Center for Reproductive Biology, Washington State University, Pullman, WA,* [3]*Department of Mathematics and Statistics, Washington State University, Pullman, WA,* [4]*Aurora Organic Farms, Platteville, CO,* [5]*Department of Animal and Veterinary Science, University of Idaho, Moscow, ID,* [6]*Department of Animal Science, Texas A&M University, College Station, TX,* [7]*Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA,* [8]*Department of Animal Science, University of California Davis, Davis CA,* [9]*Department of Animal Science, Iowa State University, Ames, IA,* [10]*Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX,* [11]*Department of Animal and Veterinary Science, University of Vermont, Burlington, VT,* [12]*Roman L. Hruska U.S. Meat Animal Research Center, USDA-ARS-PA-MARC, Clay Center, NE,* [13]*Department of Animal Science, The Pennsylvania State University, University Park, PA,* [14]*School of Animal and Veterinary Science, University of Adelaide, Adelaide, SA, Australia,* [15]*Department of Human Nutrition, Food and Animal Sciences, University of Hawaii at Manoa, Honolulu, HI,* [16]*Animal Genetics and Breeding Unit, University of New England, Armidale NSW, Australia.*

Abundant use of alternative transcripts makes it possible for a finite genome to coordinate an infinite phenome in an organism. The objective of this study was to profile alternative polyadenylation (APA) sites in cattle and thus facilitate functional annotation of the bovine genome. Here we report development of a whole transcriptome termini site sequencing (WTTS-seq) method and collection of 203,491 APA sites in cattle using 5 types of cells and 10 fetal and 43 adult tissues/organs. Our WTTS-seq method involves total RNA fragmentation, polyA+ RNA enrichment, first-strand cDNA synthesis by reverse transcription and second-strand cDNA synthesis by PCR. There are at least 6 advantages of WTTS-seq over RNA-seq (RNA sequencing). Data analysis revealed that gene biotypes had significant effects on use of APA sites per gene ($c^2 = 3{,}317.6$, $P < 0.00001$). For example, 81% of protein coding genes, 50% of lncRNA (long non-coding RNAs), 33% of pseudogenes and 9% of small RNA (such as miRNAs, tRNAs, snRNAs, snoRNAs, rRNAs and guide rRNAs) utilized more than one poly(A) site per gene. The APA usage frequency per gene does have functional relevance. For example, genes involved in mating behavior have one APA site per gene only, while fertilization-related genes use up to 3 APA sites per gene. However, oocyte-related functions require genes to execute 4 and more APA sites per gene. We observed that APA events accurately sense the severity of endometritis in dairy cows. The DE-APA sites in endometrial epithelial cells from healthy cows were dominantly enriched for

pathways related to cilium organization. In contrast, the enriched pathways associated with DE-APA sites of cells from unhealthy cows were heavily involved in inflammation and immune responses. Overall, our results provide evidence that alternative transcripts, rather than individual genes are the minimal units that drive, regulate and realize genome functions. This research was supported by the USDA/NIFA grants under Award Numbers 2016–67015–24470/2020–67015–31733/2022–51300–38058 to ZJ and 2018–67015–27500 to PR and HZ.

Key Words: cattle, alternative polyadenylation, genome function

OP195 Insights into the genetic variation, gene-flow and demographic history of African cattle breeds. M. Malima[1,2], K. Nxumalo[1], A. Tijjani[3,4], M. Makgahlela*[1], F. Joubert[2], and A. Zwane[1], [1]*Department of Animal Breeding and Genetics, Agricultural Research Council-Animal Production Irene, Pretoria, South Africa,* [2]*Centre for Bioinformatics and Computational Biology, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa,* [3]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [4]*The Centre for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, The University of Edinburgh, Midlothian, UK.*

The genomes of many livestock species, including African cattle, have been shaped by domestication and selection pressures. Africa is home to various cattle breeds that are adapted to different environments and used for different purposes. However, little is known about their genomic relationships, history, and gene flow. This study aimed to examine these factors among South, East, and West African cattle breeds using whole-genome sequencing data and bioinformatics techniques. The study analyzed a total of 32 whole genome sequences, including data from Ankole, Kenana, and N'Dama, as well as sequenced data of Nguni, Afrikaner, Bonsmara, and Holstein. The data were analyzed using various methods, including phylogenetic trees, admixture and principal component analyses, D-statistics, Treemix and the Pairwise Sequentially Markovian Coalescent model. The genomic relationships analysis revealed genetic exchange between African breeds and differentiated Bos taurus, Bos indicus, and Bos taurus × Bos Indicus ancestries. The admixture/gene-flow analysis also showed evidence of several genetic exchange events between breeds from different geographical locations followed by unveiling of possible periods where African cattle populations experienced declines and expansions. These findings coincide with events such as domestication and migrations, shedding light on possible migration patterns and co-migration of cattle and humans. Overall, this study highlights the unique genetic diversity and complex genetic changes that African indigenous cattle have undergone, resulting in adaptive traits and unique features important for conservation and breeding programs.

Key Words: indigenous cattle, genomic diversity, population genomics, geneflow, demographic history

OP196 ISAG Bursary Award: Size and composition of haplotype reference panels impact the accuracy of imputation from low-pass sequencing in cattle. A. Lloret-Villas*, H. Pausch, and A. Leonard, *ETH Zürich, Universitätstrasse 2, 8092, Zürich, Switzerland.*

Millions of cattle are genotyped every year for the purpose of genomic prediction. Low-coverage whole-genome sequencing (lcWGS) followed by genotype imputation is a cheap alternative to routine microarray-based genotyping. We assessed the impact of haplotype reference panel composition and sequencing coverage on the accuracy of lcWGS imputation in a target population consisting of cattle from the Brown Swiss (BSW) breed. We showed that GLIMPSE can accurately impute sequence variant genotypes into cattle genomes sequenced at low coverages. For instance, a same-breed haplotype panel consisting of 75 sequenced samples enabled us to genotype more than 13 million sequence variants in animals sequenced at 0.5-fold sequencing coverage with F1 scores greater than 0.9. Overall, same-breed haplotype reference panels with n = 150 sequenced samples outperformed multi-breed panels for sequencing coverages lower than 1-fold, including low allele frequencies. In absence of an adequately sized breed-specific panel (e.g., when less than 30 animals with sequence data are available), F1 scores of 0.9 could also be accomplished either by increasing the sequencing coverage of the target samples or by enlarging the reference panel with distantly related samples from other breeds. Nevertheless, since suboptimal haplotype reference panels lack variants private to the target breed, the resulting imputed lcWGS data are depleted for this type of variation.

Key Words: cattle, lcWGS, imputation, genotyping, variant calling

OP197 ISAG Bursary Award: Pangenomes of haplotype-resolved assemblies enable population-scale genotyping of cattle structural variation for eQTL mapping. A. Leonard*, X. Mapel, and H. Pausch, *ETH Zurich, Zurich, Switzerland.*

Genome-wide association studies relate sequence variation to phenotype variation, and so completeness of the marker panel impacts the power to reveal trait-associated loci. Conventional short read sequencing approaches mainly capture single nucleotide polymorphisms and small insertions/deletions but largely neglect structural variation (SV). Long reads, particularly when assembled into haplotype-resolved genomes, produce a much more complete catalog of variation for any type of polymorphism. While generating sufficient samples for a statistically significant association study is still cost prohibitive, imputation of variation discovered from a representative set of haplotypes into large mapping cohorts is feasible. Here we build a Braunvieh cattle pangenome using 16 assemblies generated from PacBio HiFi reads and then use PanGenie to impute pangenome variation into a much larger short read data set of 307 Braunvieh samples. This approach enabled us to genotype 15 million variants in the 307 samples, including approximately 50k SVs of which nearly 10k exceeded 1000 bp in size. This comprehensive set of variation was then tested for association with gene expression in 117 deeply sequenced testis total RNA samples. We identified 3947 genes that were strongly affected by structural variants (approximately 35% of all significant genes). A structural variant was the most strongly associated variant for 45 genes. We were also able to collect PacBio HiFi reads at moderate coverage on 24 of the 117 eQTL samples. We find that the 16 haplotypes in the pangenome do not reach saturation for all SVs present in the Braunvieh population but capture nearly 70% of the SVs discovered directly from the unrelated 48 haplotypes. Efforts like the Bovine Pangenome Consortium or Cattle Long Read Consortium will eventually provide sufficient resources to assess all types of variation, but for the intermediate future, we demonstrate that SV genotyping through a cattle pangenome can reveal eQTL that are missed or incorrectly associated when using only short read variants.

Key Words: cattle, genome assembly, pangenome, structural variant, eQTL

Plenary Session IV: Genomics for SA Livestock and Wildlife

OP198 The African BioGenome Project: An African initiative to conserve and document Africa's biodiversity. A. W. Muigai*[1,2], J. Kuja[3], N. Mapholi[4], T. Ebenezer[5], and A. Djikeng[6], [1]*Jomo Kenyatta University of Agriculture and Technology, Kiambu, Kenya,* [2]*National Defence University-Kenya, Nakuru, Kenya,* [3]*University of Copenhagen, Denmark,* [4]*University of South Africa, Pretoria, South Africa,* [5]*5European Bioinformatics Institute (EMBL), Hinxton, United Kingdom,* [6]*International Livestock Research Institute, Nairobi, Kenya.*

Global challenges of climate change, massive degradation of the environment, and corresponding biodiversity losses have impacted Africa. To mitigate this and document African biodiversity, the African BioGenome Project was instituted to galvanize African scientists, those on the continent and across the globe, to come together and characterize African plants, animals, and microbes. The goal is to initiate and coordinate local sequencing of 100,000 genomes indigenous to Africa with a focus on their significance to African communities. The initiative will contribute to capacity building through the AfricaBP Open Institute program that focuses on the regional bioinformatics workshops that bring together stakeholders with a vested interest in the conservation and safeguarding of the biodiversity endemic to the African continent. Through this approach, we expect to get genomic trainers of trainees who would then contribute to genomic research bridging the gap between genomics and conservation approaches on the content. A coordinated partnership with the stakeholders will also ensure the effective establishment of genomic infrastructure to facilitate the sequencing of the genomes on the continent. This will ensure the long-term sustainability of the project. Most of the African endemic species not only contribute to cultural heritage, but they also serve as sources of food, medicine, and shelter to other species. To date, 9 genomes have been sequenced on the continent with many more envisaged for sequencing in the next year. During this conference, an update on AfricaBPs activities will be provided with the aim of sharing lessons learned and future prospects of the project.

OP199 Genetic biodiversity in southern Africa: Implications for wildlife conservation. P. Bloomer*[1], A. van Wyk[1], A. Klopper[1], D. de Jager[2], and I.-R. Russo[3,1], [1]*Molecular Ecology and Evolution Programme, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa,* [2]*Section of Molecular Ecology and Evolution, Globe Institute, University of Copenhagen, Copenhagen, Denmark,* [3]*School of Biosciences, Cardiff University, Cardiff, UK.*

Genetic diversity, and its distribution within species, is fundamental to evolutionary processes and is an important factor in ecosystem stability. However, this aspect of biodiversity is often underrepresented in national and international policy dealing with the conservation and management of ecosystems and species. We present a conceptual framework for using genetic diversity metrics across different time scales as a proxy for evolutionary and ecological processes. Due to anthropogenic and natural impacts many formerly widespread species now exist only as managed populations on private land and in isolated formally protected areas, especially in South Africa. Natural ecological processes, such as migration (and consequent gene flow), and evolutionary processes, such as local adaptation and reinforcement of differentiation, currently occur on a limited scale. Instead managers need to mimic natural migration through translocations and the re-establishment of dispersal corridors between isolated areas. Due to changes in the distribution patterns of populations, subspecies and species, local adaptation is broken down due to outcrossing and outbreeding, or due to other changes in selection regimens. Varied management strategies across different conservation areas and on private lands further exacerbate the situation. We are using studies of genetic diversity, based on markers that provide resolution across different time scales, to infer the evolutionary and ecological processes that have operated across populations before recent anthropogenic influences. These insights can be used by managers to identify the most feasible options for maintaining the short- and long-term benefits of these processes that ultimately contribute to species persistence.

Key Words: wild species, population genomics, biodiversity, conservation, management

J.E.D.I Symposium

OP200 **Decolonizing science: A primer on centering justice, equity, diversity, and inclusion within animal genetics and genomics.** S. Paez*[1,2], [1]*Rockefeller University, New York, New York, United States,* [2]*New York University, New York, New York, United States.*

Fulfilling the scientific potential of animal genetics and genomics is not possible without addressing the web of cultural, political, ethical, legal and socioeconomic issues contributing to the unsustainable development of biodiversity, including agriculture. These technical, policy, and institutional issues are complicated by an entangled history of structural racism and systemic oppression in the broad scientific enterprise that has excluded and marginalized many communities from participating in and benefitting from the anthropocentric and ecocentric outcomes of these fields. Additionally, uneven biodiversity distribution, differing biodiversity valuations, and disproportionate dissemination of funding, technological and educational resources add further complexities in translating these outcomes, such as in reducing the impact of the sixth mass extinction, diffusing biomedical benefits across human society, and creating new bioeconomies, biotechnologies, and biomanufacturing initiatives for climate resiliency, food protection, and enhanced biosecurity. Justice, equity, diversity, and inclusion, as a set of social justice principles, promote a transparent and intentional approach to reinstitute access and build capacity for people by integrating a plurality of epistemologies at every level of planning, decision-making, and policy development and implementation. A conceptual framework integrating the relationships between these principles with the data life-cycle and interested parties will provide an approach toward defining problems and brainstorming solutions for scientific excellence, beginning with awareness, then transitioning to amelioration, and ultimately transformation. In the same way that every species counts and is worthy, so too does engagement of all researchers and other interested parties, across intercultures and intersections, for representing human diversity, for doing good research, and for moving beyond absolutes toward more just, equitable and inclusive future in animal genetics and genomics.

P1 Gene orthology detection for long noncoding RNA (lncRNA). F. Degalez*[1,2], C. Allain[1,2], L. Lagoutte[1,2], and S. Lagarrigue[1,2], [1]*Institut Agro, France,* [2]*INRAE, France.*

Since their discovery a decade ago, long non-coding RNAs (lncRNAs) have emerged as a significant part of the regulatory elements in genomes. However, the role of most of them remains to be clarified. Exploring the lncRNA conservation between species is an approach for improving their annotation by inferring function in one species from another one more studied, as was previously done for protein coding genes (PCGs). However, unlike PCGs, lncRNA sequences are not well conserved across species, only by patches of a few nucleotides. Therefore, no lncRNA orthologs are reported in reference databases such as Ensembl BioMart, regardless of species, this in contrast to PCGs. A few specialized databases reported lncRNAs orthologs, such as SyntDB or NONCODEv6, which target specific species and use whole-genome alignment approaches based on syntenic regions identified with liftOver and reduced gap penalties. In this context, we have developed a workflow combining 3 methods which can be used for any species of interest: the 1st method uses a "PCG-lncRNA-PCG" triplet with 2 orthologous PCGs on either side of the lncRNA as an "anchor." The 2nd method is based on "lncRNA-PCG" pairs with just one orthologous PCG and the lncRNA and PCG transcripts in both species must be in the same genomic orientation. Finally, the last method considers the alignment of lncRNAs but by small patches, using the "Mercator-Pecan" multiple genome alignment method. Applied on 8 species covering a broad phylogenetic scale from mammals to chicken, these 3 methods are complementary regardless of the species pair. Considering the 18,805 human lncRNAs (e!106), around 9,000 are identified with one orthologous in chicken, of which about 3,000 and 1000 orthologous lncRNA are detected by 2 and the 3 methods respectively. Several analyses based on the conservation of the co-expression network between species have been performed to improve the lncRNA annotation. The first results show interesting cases of network conservation between human and chicken. Project funded by the French ANR CE20 under 'EFFICACE' program.

Key Words: lncRNA, orthology, functional annotation, chicken

P2 Withdrawn

P3 Withdrawn

P4 Withdrawn

P5 DNA methylation alteration patterns in repeat elements are similar during subclinical mastitis caused by *Staphylococcus chromogenes* and *Staphylococcus aureus*. M. Wang[1,2], N. Bissonnette[1], M. Laterrière[3], D. Gagné[3], and E. M. Ibeagha-Awemu*[1], [1]*Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Québec, Canada,* [2]*Département des Sciences Animales, Université Laval, Québec, Québec, Canada,* [3]*3Quebec Research and Development Centre, Agriculture and Agri-Food Canada, Québec, Québec, Canada.*

DNA methylation alterations in repeat elements (REs) may lead to abnormal RE activity which may influence genome stability and gene function during infections. Therefore, this study profiled the DNA methylation patterns of REs of milk somatic cells from dairy cows with *Staphylococcus chromogenes* (SC, n = 4), *Staphylococcusaureus* (SA, n = 16) subclinical mastitis and healthy cows (HC, n = 10) using whole genome DNA methylation sequencing and bioinformatics analyses. Abundant differentially methylated cytosines (DMCs, 641197) occurred in REs (reDMCs) between SC and HC, accounting for 30% of total DMCs. Similarly, 33.8% (964144) of total DMCs identified between SA and HC occurred in REs. Three quarters of SC (72.8%) and SA (79.8%) reDMCs were hypermethylated in SC and SA groups compared with HC group. The majority of reDMCs were found in retrotransposons, including SINE, LINE and LTR. Interestingly, 339772 reDMCs were common to SA and SC, accounting for 53% of SC-reDMCs and 35.2% of SA-reDMCs. More than 99% of common reDMCs showed methylation changes in the same direction, and ~75% and 25% were hyper- and hypo-methylated in SA and SC groups, respectively, suggesting similar roles in host responses to SC and SA mastitis. The common reDMCs were concentrated in SINEs (136733), LINEs (120813) and LTRs (48806). BovB, Bov-tA2, BOV-A2, Bov-tA1, Bov-tA3, SL2a, L2b, MIR and MIRb were the REs harboring the most common reDMCs. Moreover, 107499 common reDMCs were found within 10314 genes. These genes showed significant involvement in pathways related to mammary gland homeostasis and health, such as *Leukocyte transendothelial migration*, *T cell receptor signaling pathway*, *Th17 cell differentiation*, *Chemokine signaling pathway*, *Leishmaniasis* etc. suggesting possible regulatory roles of DNA methylation changes in REs during subclinical mastitis. In summary, this study revealed abundant and common DNA methylation changes in REs related to SC and SA subclinical mastitis, which suggests their possible involvement in similar mechanisms in the regulation of mammary gland health.

Key Words: differentially methylated cytosine, LINE, SINE, LTR, mastitis

P6 Genome-wide acetylation modification of H3K27ac in bovine rumen cell following butyrate exposure. X. Kang[1,2], C. Li[2], R. L. Baldwin[1], G. Liu[1], and C. Li*[1], [1]*ARS, USDA, Beltsville, MD,* [2]*Ningxia University, Yinchuan, Ningxia, China.*

Butyrate contributes epigenetically to cellular function and rumen development in ruminant animals, which might be achieved by its genetic or epigenetic regulation of gene expression. To explore the role of butyrate on bovine rumen epithelial function and development, this study characterized genome-wide H3K27ac modification changes and super-enhancer profiles in rumen epithelial primary cell (REPC) induced with butyrate by ChIP-seq and analyzed its effects on the genes expression and functional pathways by integrating RNA-seq data. The results showed that the genome-wide acetylation modification (H3K27ac) was observed in the REPC with 94,675 and 48,688 peaks in the butyrate treatment and control group, respectively. Totally, 9,750 and 5,020 genes with increased modification (H3K27ac-gain) and decreased modification (H3K27ac-loss) were detected in the treatment group. The super-enhancers associated genes in the butyrate-induction group were involved in the AMPK signaling pathway, MAPK signaling pathway, and ECM-receptor interaction. Finally, the upregulated genes (PLCG1, CLEC3B, IGSF23, OTOP3, ADTRP) with H3K27ac gain modification by butyrate were involved in cholesterol metabolism, lysosome, cell adhesion molecules, and PI3K-Akt signaling pathway. Butyrate treatment has the role of genome-wide H3K27ac acetylation on bovine REPC and affects the changes in gene expression. The effect of butyrate on gene expression correlates with the acetylation of the H3K27ac level. Identifying genome-wide acetylation modifications and expressed genes of butyrate in bovine REPC cells will expand the understanding of the biological role of butyrate and its acetylation.

Key Words: bovine, epigenetics, histone acetylation, transcription, gene regulation

P7 Withdrawn

P8 ISAG Bursary Award: Relationship between spleen and uterus gene expression and DNA methylation according to developmental stages of pigs. B. Ahn*[1], M. Kang[1], M. Choi[1,2], L. Rund[3], L. Shook[3], and C. Park[1], [1]*Department of Stem Cell and Regenerative Biotechnology, Konkuk University, Seoul, Korea,* [2]*Living Systems*

Institute, University of Exeter, Exeter, United Kingdom, [3]Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL.

Understanding the patters of gene expression changes during development is critical to decipher development mechanisms. To understand the changes of gene expression and associated epigenetic regulation on immune and female reproductive tissues in pigs, we collected spleens and uteri from 3 90-d-old fetuses (E90), 3 28-d postnatal pigs (P28), and 4 6-mo-old pigs (P180) and conducted RNA-seq and reduced representation bisulfite sequencing (RRBS). A total of 29,677 transcribed genes were detected and differentially expressed genes (DEGs, q-value <0.01 and fold change >1.5) between different developmental stages were determined. In spleens, 3.71% and 3.08% of the genes were up- and downregulated, respectively, in P28 than E90. In P180, 3.85% of the genes were downregulated compared with P28, while only 1.17% were upregulated. In uteri, 0.77% of the genes showed increased expression and 0.71% were decreased in P28 than E90. In P180 uteri, 1.84% of the genes were downregulated and 0.58% were upregulated comparing to P28. Gene enrichment analysis showed that the DEGs showing a gradual decrease in expression during spleen development were highly enriched in genes associated with mitotic sister chromatid segregation and DNA metabolism, indicating a decrease in cell proliferation according to the maturation of the immune system. In contrast, no consistent patterns of gene enrichment were observed among DEGs across different developmental stages in uteri. The analysis of DNA methylation showed that the spleen showed ~4% higher level of CpG methylation than that of the uterus regardless of different developmental stages. In addition, DNA methylation increases with age, and thus P28 showed ~10% higher level of DNA methylation than E90. Further analyses on differentially methylated regions (DMRs) associated with gene expression changes are in progress. Our study contribute to understanding the relationship between gene regulation and DNA methylation during porcine organ development.

Key Words: pig, organ development, RRBS, DNA methylation, differentially methylated region

P9 Extending Ensembl regulatory annotation to farmed animals. G. R. Ilsley*, G. A. Merino, P. R. Branco Lins, M. Perry, D. Urbina-Gomez, and P. Harrison, *European Molecular Biology Laboratory, European Bioinformatics Institute, Hinxton, Cambridge, UK.*

Ensembl (https://www.ensembl.org/) is a widely used genome browser that has assisted the scientific community in interpreting the genome for more than 20 years. Ensembl's regulatory annotation identifies regions in the genome that might regulate and control the expression of nearby genes. These regulatory features can be used to filter and identify regions of the genome where non-coding variation or targeted mutations could have important consequences for gene expression and hence phenotype. Ensembl has well established regulatory builds for human and mouse, which grew out of work on the ENCODE and BLUEPRINT projects. Now for the first time, we are extending this analysis beyond human and mouse. In collaboration with the GENE-SWitCH and AQUA-FAANG consortia, Ensembl is making regulatory annotation available for farmed animals including Pig, Chicken, Atlantic salmon and Turbot. EMBL's European Bioinformatics Institute also develops the FAANG Data Portal (https://data.faang.org/), which together with its rich and standardised metadata enables a wide range of experimental data sets for farmed animals to be discovered. Ensembl Regulation's computational workflow relies on this standardised metadata to retrieve and process primary data sets to identify open chromatin (ATAC-seq) and histone marks (ChIP-seq). These are then combined to produce genomic-level regulatory annotation. The analysis process will be described, along with examples of the resulting annotation and how it might be interpreted to guide further studies. Work is ongoing to add additional capabilities and further farmed animal species, and we are seeking feedback and collaborations with the community. Ensembl is primarily funded by the Wellcome Trust (WT222155/Z/20/Z), and the GENE-SWitCH and AQUA-FAANG projects have received funding from the European Union's Horizon 2020 Research and Innovation Programme under the grant agreement n°s 817998 and 817923 respectively.

Key Words: functional genomics, genome annotation, ATAC-seq, pigs and related species, fish

P10 Beyond the genome: Establishing molecular phenotypes to accelerate adaptation to a changing environment. A. Caulton*[1], R. Brauning[1], K. M. McRae[1], K. G. Dodds[1], C. Couldrey[2], P. L. Johnson[1], and S. M. Clarke[1], [1]*AgResearch, Invermay Agricultural Centre, Mosgiel, Otago, New Zealand,* [2]*Livestock Improvement Corporation, Hamilton, New Zealand.*

Breeding livestock that are resilient to environmental stresses is of critical importance considering the changing global climate. Genetic adaptation occurs through the selection of advantageous genetic variants over time, however adapting to environmental challenges often requires rapid biological responses that occur through changes in gene expression. Epigenetic modifications, including DNA methylation, alter gene expression without changing the DNA sequence, allowing for immediate and reversible modulation of physiological responses to environmental perturbation. The "Beyond the Genome" research program aims to exploit this phenomenon through the development and application of emerging DNA methylation profiling assays and high-throughput techniques to provide transformative industry-applicable tools. These will be used to select animals that are resilient against biotic/abiotic stresses that are increasing in prevalence with the changing global climate. A range of biological resources (including disease challenges and multigenerational families in ruminant species, sheep, cattle, deer) have been established to investigate the role of the methylome in adaptation to stress and to assess transgenerational inheritance of methylation patterns. In parallel, a focus on methylome profiling tools, tailored to enable core research through to industry application, have been investigated and will be presented. These include a mammalian methylation array that has been utilized to develop epigenetic clocks for livestock species, restriction enzyme DNA sequencing methods, with and without the use of deamination of cytosines, and comparisons to whole genome methylome profiles with examples from both bisulphite sequencing and nanopore sequencing. The application of these tools to profile methylome changes in response to disease stress in sheep with also be presented. Establishing the methylome as a molecular phenotype to accelerate adaptation to a changing environment will facilitate the breeding of animals that are fit for the future.

Key Words: epigenomics, sheep and related species, animal breeding, adaptation, methyl-seq

P11 RNA methylation as a mechanistic link between epigenotype and phenotype. S. Xie[1], B. Murdoch[1], and S. McKay*[2,3], [1]*University of Idaho, Moscow, ID,* [2]*University of Vermont, Burlington, VT,* [3]*University of Missouri, Columbia, MO.*

Determining the extent of epigenetic effects upon phenotypic variation involves characterization of both the epigenome and the epitranscriptome. Both DNA 5-methylcytosine (5mC) and RNA N6-methyladenosine (m6A) are common epigenetic modifications that play a role in transcription and post-transcriptional regulation, respectively. Recent evidence suggests the existence of a potential mechanism of coordinated transcriptional (5mC) and post-transcriptional (m6A) regulation in various biological processes, further emphasizing the need for epitranscriptomic annotation. Therefore, with the aim of elucidating the effects of DNA and RNA methylation on gene expression, we have performed Whole Genome Bisulfite Sequencing (WGBS), Methylated RNA Immunoprecipitation Sequencing (MeRIP-Seq) and RNA-Seq in the caruncle, spleen, and mammary gland from each of 4 cattle and 4 sheep. The results indicate that the density (5mC/C) of DNA 5mC were similar in 3 tissues (caruncle, spleen, and mammary gland) of sheep (2.04%, 2.62% and 2.53%) and cattle (2.52%, 2.77% and 2.61%), with spleen having the highest 5mC density in both species. RNA 6mA modifications identified from MeRIP data in the same 3 tissues yielded a total of 19,931, 26,463 and 11,018 peaks were identified in sheep, and

20,123, 19,467 and 17,774 peaks were identified in cattle. The average length of identified peaks are 2,063–7,213 bp and 8,270–9,036 bp in sheep and cattle, respectively. Moreover, the DNA and RNA modifications of long non-coding RNAs were simultaneously resolved in the relationship with gene expression for sheep and cattle. Subsequent analysis includes genome/transcriptome-wide associations between 5mC and m6A modifications as well as expression levels. While epigenetic annotation of modifications like DNA methylation and histone modifications has been accomplished through functional annotation of animal genomes initiatives, further epigenomic annotation is necessary to fully realize the effect of both DNA and RNA methylation on phenotypic variation. This work supports the annotation of animal epigenomes and epitranscriptomes while exploring a potential mechanistic link between epigenotype and regulation of gene expression.

Animal Forensic Genetics

P12 Withdrawn

P13 Withdrawn

P14 Withdrawn

P15 DNA barcoding in South Africa: Progress, challenges and future plans. M. Mwale*, M. T. Sethusa, and J. R. Baxter, *Foundational Biodiversity Science, South African National Biodiversity Institute (SANBI), National Zoological Gardens, Pretoria, South Africa.*

The decline and loss of biodiversity is a global concern due to ongoing habitat transformation and unsustainable levels of resource exploitation with the increasing human population. These declines have also been exacerbated by climate change, biological invasions and illegal trade. In South Africa, almost half of all ecosystems have been categorized as threatened including 12% of all animals assessed (A large number have never been assessed due to limited information on their taxonomy and unverified extent of occurrence). Current biodiversity assessments may therefore be incomplete and limiting with under-estimated species richness for several taxa. The advent of the DNA barcoding approach for species identification, including the creation of reference libraries has however contributed significantly to species verification and biodiversity assessments globally. DNA barcoding is now a valuable tool for forensics applications through identifying illegally traded species and their process products. This paper therefore aimed to conduct a DNA barcoding gap analysis aimed at highlighting the status and progress of barcoding of selected key South African animals. Barcode sequence gaps for South Africa records downloaded from the

Barcode of Life Data Systems (BOLD) and NCBI GenBank were analyzed and verified using published national species checklists. Data analyses revealed that although BOLD has the most comprehensive database of verified animal records using the mitochondrial cytochrome c oxidase gene, only 30,000 out of an estimated 65,000–100,000 South African records have species names. This represents only about 7,000 species (less than 10%) of the estimated known indigenous taxa. There is also limited information on the taxonomic and geographic coverage of species including priority groups, with the reliability and accuracy of most of the available references being unknown for use in forensic applications. It is therefore still necessary to coordinate and define barcoding targets to ensure that the critical baseline foundational biodiversity information of South African biodiversity is available based on national research priorities to support decision-making.

Key Words: DNA barcoding, forensics, genetic identification, mitochondrial DNA, sequence variation

P16 Identification of animal and plant species in foodstuffs using target GBS assay. L. Forlani, D. M. Posik, M. C. Bruno, L. H. Olinera, M. E. Zappa, N. S. Castillo, G. Barbisan, E. E. Villegas Castagnasso, J. A. Crespi, P. Peral García, M. E. Fernandez, and G. Giovambattista*, *Instituto de Genética Veterinaria (IGEVET), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata—CONICET, La Plata, Buenos Aires, Argentina.*

DNA metabarcoding assay is increasingly used for species authentication in industrialized food. Targeted Genotyping-by-Sequencing (GBS) based on next-generation sequencing (NGS) technology allows the detection of multiple species in complex foodstuff matrices in a single run. This study aimed to evaluate the species of origin used in 32 commercially processed foods, replicated thrice, such as broth, salad dressing, sauce, milk, milk powder, hamburger, canned tuna, pepper, etc. In addition, positive (known DNA mixed) and negative controls were added. A total of 10 ng of DNA from each sample was analyzed using a previously developed AgriSeq™ targeted GBS assay that include 319 multiplexed targets belonging to 2 mitochondrial (COI and CYTB) and 2 chloroplast (RBCL and MATK) genes from 177 plant and animal species used in the food industry in Argentina. Fastq files were aligned to a multi-specie reference genome to generate the SAM/BAM files for each sample. BAM files were filtered and a read count table was prepared. Finally, BAM files were visualized using IGV software and the reads with low percentage of identity were confirmed with nBLAST analyses against the NCBI public database. This analysis indicated a wide range of coverage for the highly processed samples tested, from several dozens/hundreds to few millions of reads. The obtained results were compared with the species composition declared on the product label by the manufacturer. Several declared species were identified, as well as no reported species were detected. In addition, nBLAST analyses allowed the addition of new species in the multi-specie reference genome. The present study demonstrates that AgriSeq™ is a viable solution for genomic applications involving the analysis of hundreds of multiple species in a single sequencing run.

Key Words: multispecies, genetic identification, GBS, mitochondrial DNA, chloroplast DNA

P17 Withdrawn

P18 ISAG Bursary Award: A new approach to the molecular differentiation of the wolf and the domestic dog in wildlife forensics. A. E. Hrebianchuk*[1] and I. S. Tsybovsky[2], [1]*State Forensic Examination Committee of the Republic of Belarus, Minsk, Republic of Belarus,* [2]*Republican Unitary Service Enterprise «BelJurZabespechenne», Minsk, Republic of Belarus.*

The molecular differentiation of individuals of the wolf (*Canis lupus lupus*) and the dog (*Canis lupus familiaris*) represents a difficult problem in the study of the *Canidae* family. In wildlife forensics, the first step toward identification of materials of animal origin is to determine the species of the animal. The current panels of STR loci employed both in basic science and forensics, which use domestic dog DNA with a confirmed species origin, have not been tested for cross-applicability with DNA from any other wild canids. We developed a test system for DNA-based differentiation of wild and domestic representatives of the *Canidae* family. This real-time PCR-based system allows one to quickly and reliably distinguish test samples of a wolf and a domestic dog. The test system is designed to detect 2 targets, the pancreatic amylase gene (*Amy2b*) and oncogene *vMYC*. The differentiating parameter between the wolf and the domestic dog is the number of copies of the pancreatic amylase gene. For wild canids, the copy number of the amylase gene is a constant value of 2, whereas in a domestic dog, the number of copies of this gene is always greater than 2. The robustness of the differentiation of the wolf and the domestic dog using this test system was confirmed in a study of Belarusian populations of the wolf (121 samples) and the domestic dog (216 samples), while verification was carried out using biological samples of the raccoon dog (179 samples), the red fox (including its black-brown morph; 383 samples) and the Arctic fox (29 samples). The developed test system can be implemented using 2 types of thermal cyclers, as well as droplet digital PCR (ddPCR), and reagents from various manufacturers. All validations were carried out in accordance with the protocol of the SWGDAM and the ISO5725 standard. Successful identification of a biological trace as originating from a wolf or a domestic dog using this test system makes it possible to subsequently employ identifying panels of STR loci, allowing statistical evaluation of the possibility that a biological trace belongs to a certain *Canis lupus* individual.

Key Words: wolf, dog, *Canis lupus*, forensics, genetic differentiation

P19 ISAG Bursary Award: *Can DNA help trace the local trade of pangolins? Conservation genetics of white-bellied pangolins from the Dahomey Gap (West Africa).* S. Zanvo*[1], C. A. M. S. Djagoun[1], F. A. Azihou[1], B. Sinsin[1], and P. Gaubert[2], [1]*Laboratory of Applied Ecology, University of Abomey-Calavi, Faculty of Agronomic Sciences, University of Abomey-Calavi, Cotonou, Benin,* [2]*Laboratoire Evolution et Diversité Biologique, Université Paul Sabatier, Toulouse, France.*

African pangolins are currently experiencing unprecedented levels of harvesting, feeding both local demands and the illegal interna-

tional trade. So far, the lack of knowledge on the population genetics of African pangolins has hampered any attempts at assessing their demographic status and tracing their trade at the local scale. We conducted a pioneer study on the genetic tracing of the African pangolin trade in the Dahomey Gap (DG). We sequenced and genotyped 189 white-bellied pangolins from 18 forests and 12 wildlife markets using one mitochondrial fragment and 20 microsatellite loci. Tree-based assignment procedure showed that the pangolin trade is endemic to the DG region, as it was strictly fed by the Dahomey Gap lineage (DGL). DGL populations were characterized by low levels of genetic diversity, an overall absence of equilibrium, important inbreeding levels, and lack of geographic structure. Genetic tracing suggested that wildlife markets from the DG sourced pangolins through the entire DGL range. Our loci provided the necessary power to distinguish among all the genotyped pangolins, tracing the dispatch of a same individual on the markets and within local communities. We developed an approach combining rarefaction analysis of private allele frequencies with cross-validation of observed data that traced 5 traded pangolins to their forest origin, c. 200–300 km away from the markets. Although the genetic toolkit that we designed from traditional markers can prove helpful to trace the illegal trade in pangolins, our tracing ability was limited by the lack of population structure within the DGL. Given the deleterious combination of genetic, demographic, and trade-related factors affecting DGL populations, the conservation status of white-bellied pangolins in the DG should be urgently re-evaluated.

Key Words: microsatellite, conservation genetics, demographic decline, trade tracing, white-bellied pangolin

Applied Genetics of Companion Animals

P20 Circulating exosomes and microRNAs as biomarkers for canine idiopathic epilepsy. M. García-Gracia[1], S. Usón[1], L. Moreno-Martínez1,2, J. Moral[3], D. Sanz-Rubio[4], A. Hernaiz[1], R. Osta1,2, P. Zaragoza1,2, B. Rosado[3], S. García-Belenguer[3], and I. Martín-Burriel*1,2, [1]*Laboratorio de Genética Bioquímica (LAGENBIO), Facultad de Veterinaria, Universidad de Zaragoza, Instituto de Investigación Sanitaria de Aragón, Zaragoza, Spain,* [2]*Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain,* [3]*Departamento de Patología Animal, Facultad de Veterinaria, Universidad de Zaragoza, Zaragoza, Spain,* [4]*Translational Research Unit, Instituto de Investigación Sanitaria de Aragón (IISAragón), Hospital Universitario Miguel Servet, Zaragoza, Spain.*

Idiopathic epilepsy (IE) is defined as a chronic neurological disorder manifested by repeated epileptic seizures and not associated with intracranial structural injury or metabolic or toxic problems. The cause of this disease remains unknown but this is the most prevalent form both in people and in dogs (48–70% of epileptic dogs). Individuals refractory to pharmacological treatment represent 30–40% of patients in both human and veterinary medicine. The cause of this resistance to anti-seizure medication remains unknown and more research is needed to unravel the responsible molecular mechanisms. In this work we analyzed microRNA profiles in plasma and circulating exosomes obtained from dogs with IE sensitive (SIE) and refractory (RIE) to treatment. Extracellular vesicles (EV) were isolated from plasma and their morphology and size were analyzed by transmission electron microscopy and nanoparticle tracking. Moreover, the presence of exosomal markers were confirmed by dotblot. Canine EV obtained from plasma displayed strong immunoreactivity for CD81, ALIX, ICAM and TSG101 markers. Moreover, a set of 8 microRNAs altered in human epilepsy were analyzed by RT-qPCR in plasma and plasma derived exosomes obtained from control, SIE and RIE dogs. Each group included males and females from different breeds, age and sexual status (intact or neutered). MicroRNA levels were normalized against the most stable analyzed microRNA (miR-93) and the endogenous miRNA control miR-16. In both cases, miR-142 displayed a significant higher level in SIE dogs, whereas the levels of this microRNA in RIE remained unchanged. Despite the heterogeneity of the groups, a mixed model indicated that group significantly influences the levels of this microRNA ($P = 0.0045$) explaining 46% of the variability. Small-RNA sequencing studies are warranted to investigate the true depth of miRNA alterations in naturally occurring canine IE, especially in refractory animals, which would deep in the knowledge of drug resistance mechanisms and could be a source of prognostic biomarkers.

Key Words: dog, non-coding RNA, nervous system, animal health

P21 Candidate gene analysis of primary ciliary dyskinesia in the English cocker spaniel. R. T. Cheng[1], L. Hambrook[2], and C. M. Wade*[1], [1]*The University of Sydney, Camperdown, NSW, Australia,* [2]*Advanced Vet Care, Kensington, VIC, Australia.*

The goal of the current study is to identify the mutation(s) causing a familial respiratory disorder in the English cocker spaniel dog breed. Primary ciliary dyskinesia (PCD) is an inherited disorder that affects the dog's ability to properly clear mucus from the airways, leading to symptoms including recurrent rhino-sinusitus, bronchitis, bronchiectasis, and bronchopneumonia. Our team has conducted whole genome sequencing, alignment, and variant calling of 7 individuals (3 affected and 4 unaffected) from a family with 3 littermates with chronic rhinitis/bronchitis since d 3 of life and a more distantly related case. The results of high-speed video-microscopy on one exemplar affected puppy were strongly suggestive of ciliary dysfunction with diagnostic criteria being met for PCD. Transmission electron microscopy findings supported the presence of an ultrastructural defect affecting the dynein arms in all 4 affected pups. Whole genome sequence analysis has revealed that the affected animals have wild-type/normal alleles for all previously described canine gene variants impacting this disorder as reported by On-line Mendelian Inheritance in Animals (OMIA). These include the N-acetyltransferase 10 (NAT10) (OMIA 000573–9615) missense mutation at CFA18:34,074,175.g.A > C described in the Schnauzer, the in-frame deletion in non-metastatic cells 5, protein expressed in (nucleoside-diphosphate kinase) (NME5) (OMIA 002206–9615) at CFA11:25,839,016del described in the Alaskan Malamute breed, the coiled-coil domain containing 39 (CCDC39) (OMIA 001540–9615) missense mutation CFA34:14,126,119G > A described in the Old English Sheepdog breed; and the Serine/threonine kinase 36 (STK36) (OMIA 002623–9615) splice-site mutation at CFA37:25,167,072G > A described in the Australian Shepherd. All variant positions are reported relative to UU-GSD1 reference genome. Later analyses will consider candidate genes obtained from Online Mendelian Inheritance in Man (OMIM) for phenotypic series (OMIM PS 244400) and de-novo genome-wide analysis.

Key Words: dogs and related species, functional genomics, animal health

P22 Obligatory testing in dogs: Input from breeders and organizations. E. Beckers*, N. Buys, and S. Janssens, *Center for Animal Breeding and Genetics, KU Leuven, Leuven, Belgium.*

Cats and dogs are burdened with many genetic diseases and several breeds have low genetic diversity. Breeding Healthy Pets was started in association with the Flemish government (Belgium) to create a sustainable breeding policy for all cat and dog breeds in Flanders. The latter aims to reduce the frequency of disease-causing variants while maintaining genetic diversity. One objective is to make several genetic tests obligatory. A list of relevant genetic diseases was compiled based on available scientific literature for 21 dog breeds (15 with low genetic diversity, 5 brachycephalic breeds and the most popular breed in Bel-

gium). While studies exist on many genetic diseases, literature on allele frequencies and disease prevalences in some breeds can be scarce. Moreover, frequencies can change over time and geographic location. With this in mind, a survey was sent to dog breeders and breed organizations, inquiring about the occurrence of genetic disorders within the Flemish population of their dog breed. Forty replies contained minimal useful information: at least one breed and genetic disease was mentioned. The majority of the respondents were dog breeders (n = 30) and/or board members of a breed club (n = 18). Others were dog show judges (n = 7), board members for another kind of organization (n = 5), or future one-time hobby breeders (n = 2). Of the board members, 16 were authorized to represent their organization and were asked to answer according to their organization's official point of view. Information was gathered on 29 breeds, 13 of which fall under the 21 dog breeds selected for this study. Most of the answers were in line with what was found in the literature. Genetic conditions with little or no available prevalence information were added to the list of relevant genetic diseases when these emerged from the survey, like hemivertebra in the pug. The feedback, therefore, proved useful for fine-tuning the list. Other countries compiling a sustainable breeding policy could likewise benefit from the input of breeders and organizations. A final list will be confirmed after input from veterinarians with several clinical specializations.

Key Words: animal breeding, population genomics, animal health

Avian Genetics and Genomics

P24 ISAG Bursary Award: Combined effect of microbially-derived caecal SCFA and host genetics on feed efficiency in broiler chickens. Z. He*[1,2], R. Liu[1], Q. Wang[1], J. Zheng[1], J. Ding[1], J. Wen[1], A. Fahey[2], and G. Zhao[1], [1]*Institution of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*School of Agriculture and Food Science, University College Dublin, Dublin, Ireland.*

Improving feed efficiency is the most important goal for modern animal production. The regulatory mechanisms of controlling feed efficiency traits are extremely complex and include the functions related to host genetics and gut microbiota. Short-chain fatty acids (SCFAs), as significant metabolites of microbiota, could be used to refine the combined effect of host genetics and gut microbiota. However, the association of SCFAs with the gut microbiota and host genetics for regulating feed efficiency is far from understood. In this study, we examined host genome sequence, microbial data and SCFA concentration of cecal chyme in 300 broilers. The SNP-based heritability analysis found that the SCFA concentrations had moderate to high heritability (h^2 = 0.183~0.401). Genome-wide association studies (GWAS) showed that 4 out of 7 SCFAs had significant associations with genome variants. One locus (gga4: 29414391–29417189) was significantly associated with propionate, locating near or inside the genes *MAML3, SETD7 and MGST2*, and had a modest effect on feed efficiency traits and the microbiota. The genetic effect of the top SNP explained 8.43% phenotype of propionate. Individuals with genotype AA had significantly different propionate concentrations (0.074 vs. 0.131 ug/mg), feed efficiency (FCR: 1.658 vs. 1.685), and relative abundance of 14 taxa compared with those with the GG genotype. *Christensenellaceae* and *Christensenellaceae_R-7_group* were identified being associated with feed efficiency, propionate concentration and top SNP genotypes, and they were found to be lipid metabolism-related. Individuals with a higher cecal abundance of these taxa showed better feed efficiency and lower concentrations of cecal SCFAs. Our study concluded that cecal taxa *Christensenellaceae* and *Christensenellaceae_R-7_group* were identified as representative taxa contributing to the combined effect of host genetics and SCFAs on chicken feed efficiency. These findings proved strong evidence of the combined effect of host genetics and gut microbial SCFAs in regulating feed efficiency traits.

Key Words: feed efficiency, caecal microbiota, genetic variation, SCFA, broiler

P25 Production performance of four lines of Japanese Quail reared under tropical climatic conditions of Tamil Nadu, India. K. Vishal Arunrao[1], D. Kannan[1], R. Amutha[1], A. K. Thiruvenkadan[2], A. Yakubu[3], and S. O. Peters*[4], [1]*Department of Poultry Science, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India, Namakkal, Tamil Nadu, India,* [2]*Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Salem, Tamil Nadu, India, Salem, Tamil Nadu, India,* [3]*Centre for Sustainable Agriculture and Rural Development, Department of Animal Science, Faculty of Agriculture, Nasarawa State University, Keffi, Shabu-Lafia Campus, Nigeria, Nigeria,* [4]*Department of Animal Sciences, Berry College, Rome, GA.*

This study was conducted on the growth and other production performance of 4 different lines (L1, L2, L3 and L4) of Japanese quail (*Cortunix japanoica*) maintained under tropical climatic conditions of Tamil Nadu, India. The data on various production and reproduction parameters were collected and analyzed. The results revealed a highly significant difference ($P < 0.01$) in body weight from hatch to 5th week of age, where the highest value was recorded in L3 (235.31 g) and the lowest in L4 (203.62 g). The cumulative 5th week feed conversion ratio showed non-significant difference ($P > 0.05$) at first and second week, and highly significant difference ($P < 0.01$) from third to fifth week of age. The age ($P < 0.05$) at 50 per cent egg production was 60.2 (L4), 61.4 (L2), 65.1 (L3) and 66.0 (L1) days. The highest bodyweight (g) ($P < 0.01$) during the laying period (at 15 weeks of age) was observed in L2 (327.08) followed by L3 (326.54), L1 (309.24) and L4 (288.69) lines, respectively. The mean egg weight (g) of different lines showed non-significant difference ($P > 0.05$) at all weeks, except at 11th week of age ($P < 0.01$). The mean feed consumption (g)/bird/day differed significantly ($P < 0.01$) from 6th to 16th week of age, except at 6th and 8th week of age, where it was non-significant ($P > 0.05$). The overall feed efficiency/dozen of eggs (from 6th to 16th weeks) ($P > 0.05$) ranged from 1.33 (L1) to 1.98 (L3). The livability from 6 to 16 week of age was 100 per cent in all the lines. To boost Japanese quail production in the tropics, L3 and L4 may be selected for body weight and egg production, respectively.

Key Words: Japanese quail, breeding line, production trait, livability, tropics

P26 Withdrawn

P27 Occurrence and genetic diversity of *Haemoproteus* and *Leucocytozoon* parasites in selected captive birds in South Africa. R. Gaorekwe*[1,2], V. Phetla[2], D. Malatji[2], and M. Chaisi[1,3], [1]*South African National Biodiversity Institute, Pretoria, South Africa,* [2]*University of South Africa, Florida, Roodepoort, South Africa,* [3]*University of Pretoria, Onderstepoort, South Africa.*

Avian malaria parasites are transmitted by blood-sucking insects, infecting a wide range of bird spp. globally. Infection can result in severe disease and sometimes mortality. This study aimed to determine the occurrence and genetic diversity of avian hemosporidian parasites in 15 species of captive birds from a conservation facility in South Africa. One hundred and 83 (183) blood samples from different species of flamingos, vultures, owls, ibis and parrots were analyzed for the presence of hemosporidia by a nested PCR assay. Infections were characterized by sequencing of the *Cytochrome b* gene from positive samples. Sixty-four (35%) samples were positive for infection by hemosporidian parasites. Infections by *Leucocytozoon* spp. were more prevalent (31.7%) than those of *Plasmodium* and/or *Hemoproteus* spp. (14.2%) and the observed difference was statistically significant ($P < 0.0001$). A total of 8. 7% of the samples had mixed infections. The generated sequences from the South African birds were 96 – 100% similar to those of *Leucocytozoon* spp. (lineages CHRKLA02, CIAE02, BUVIR02) and *Hemoproteus* spp. (lineage TYTAL6), isolated from birds in different parts of the world. Two novel lineages were identified from the Cape Eagle-Owl (*Bubo capensis*) and Hadeda Ibis (*Bostrychia hagedash*) respectively and designated as BUBCAP01 and BOSHAG02. *Plasmodium* spp. lineages were not identified in this study. This study indicates that infection rates in captive birds at the national zoological garden are high, consequently, posing a health concern to conservation efforts of these birds. Regular monitoring of infections of these pathogens and insect control is recommended to avoid disease outbreaks.

Key Words: avian haemposporidia, *Haemoproteus* spp., *Leucocytozoon* spp., prevalence, diversity

P28 ISAG Bursary Award: Characterization of chicken strains in Isin local government based on phenotypic parameters, blood polymorphism, and 18s mitochondria genes. P. A. Owolabi*, F. E. Sola-Ojo, R. Y. Eseyin, A. G. Aremu, F. T. Sa'ad, E. O. Omidiji, A. O. Adeyanju, A. T. Fakayode, N. T. Fadairo, S. O. Oni, A. A. Odumade, K. A. Ganiyu, A. O. Muhammad-Nasir, S. D. Aniyi, and S. D. Lawal, *University of Ilorin, Ilorin, Kwara, Nigeria.*

Ninety adults' chickens were sampled from Isin Local Government Area of Kwara State Nigeria for determination of genetic diversity within the chicken population using phenotypic traits, blood protein polymorphism and 18S mitochondria genes. Body parameter were measured on live birds, blood was collected for cellulose acetate electrophoresis and DNA extraction for further analysis, data obtained were analyze using SPSS 2017, Pop gene 32 and Clustal Omega accordingly. Phenotypic correlation results show a significant positive correlation between the body weight and morphometric body parameters in the chickens studied. 55.55% of the population were HbAA, 22.22% were of HbAB, HbBB type each. The transferrin was distributed at the rate of 66.66%, 0%,33.33% between TrfAA; TrfAB and TrfBB. The Carbonyl anhydrase were 50%, 0% and 50% for FF, FS and SS, while 33.33%, 55.56% and 11.11% of the population were of AA, AB and BB Albumin, respectively. There was a close relationship between the sequence obtained for Yoruba Ecotype Female and Exotic Female in the phylogenetic tree as they are found in the same monophyletic clade. This study showed that all the body parameters measured in the chickens contributed significantly to their overall body weight, it also showed that population of the chickens sampled have not been initially selected and they are in Hardy Weinberg equilibrium. Lastly, the use of 18S mitochondria genes shows that most of the sampled birds clustered within a clade, thus representing a major haplogroup. This study indicates that there is room for improvement of Nigeria local chickens, thus breeders should concentrate their effort in selection and improvement of Nigeria local chickens for production of more animal protein for the teeming population.

Key Words: chicken, electrophoresis, phylogenetic, polymorphism, sequence.

P29 ISAG Bursary Award: Estimation of genetic diversity and population structure of Korean domestic chickens by comparison with SYNBREED data. E. Cho*, M. Kim, and J. Lee, *Chungnam National University, Daejeon, Republic of Korea.*

Genetic diversity depends on the rates of allele loss and fixation, and it reflects the balance of emergent genetic variants within populations. It is a key aspect of disease prevention and trait enhancement research for the sustainable livestock industry. Korean domestic chickens (KDCs) are a significant genetic resource in Korea in terms of economics and conservation. KDCs are generally classified into native and commercial breeds. We conducted genetic diversity study using high-density single nucleotide polymorphism (SNP) genotype data targeting several KDC populations. For comparison with the KDC populations, global chicken data consisting of African, American, Asian, European, and commercial breeds collected from the SYNBREED project in Germany were also analyzed. The data set consisted of a total of 200 breeds and 4,224 individuals, however, to decrease data bias, 4 individuals from each breed were randomly selected. A total of 465,518 SNPs derived from 600K chicken SNP array were subjected to this study. Principal component analysis (PCA) was performed for the entire population. The results revealed that the KDC populations clustered together with parts of the European and commercial populations. The genetic distances (GD) among the chicken populations were calculated using Reynold's equation, and GD values were then used to generate a phylogenetic tree. The GD analysis indicated that the native KDC breeds were clearly distinguished from other global breeds, and the commercial KDC breeds were confirmed to be similar to groups of the same origin. Furthermore, the native KDC populations were identified to be closely related to some European populations, including Hungarian Yellow, Rhode Island Red, and New Hampshire. The result of this study suggests that genetic diversity, genetic fixation, and high population uniformity occur in the KDC populations. As well, this study can provide basic genetic information that could speed up breed improvement by selecting various characteristics of native chickens.

Key Words: Korean native chicken, genetic diversity, population structure, SNP

P30 Genomic analysis of long-tailed chicken (Onagadori) offers insight into the evolution of avian molting. C. Ma[1,2], M.-S. Wang[1,2], F.-J. Wang[1,2], K. Kinoshita[3,4], Z.-F. Cai[1,5], K. Srikulnath[6], J.-L. Han[7,8], L. Zeng[1], F. Wu[1,2], H.-J. Wei[3,4], Y.-P. Zhang[1,5], and M.-S. Peng*[1,2], [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, Yunnan, China,* [2]*Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan, China,* [3]*State Key Laboratory for Conservation and Utilization of Bio-resources in Yunnan, Yunnan Agricultural University, Kunming, Yunnan, China,* [4]*Key Laboratory of Animal Gene Editing and Animal Cloning in Yunnan Province, Kunming, Yunnan, China,* [5]*State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan University, Kunming, Yunnan, China,* [6]*Animal Genomics and Bioresource Research Unit (AGB Research Unit), Faculty of Science, Kasetsart University, Bangkok, Thailand,* [7]*CAAS-ILRI Joint Laboratory on Livestock and Forage*

Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China, [8]Livestock Genetics Program, International Livestock Research Institute (ILRI), Nairobi, Kenya.

Regular feather molting has occurred over hundreds of millions of years during the life history of Aves along with foraging, breeding, migration and overwintering. However, little is known about the genetic basis of molting evolution and development. Here, we focus on a unique phenotype in birds, the Japanese long-tailed chicken (Onagadori), which has fast and continuous growth of non-molting long tail feathers. Comparative population genomic analyses revealed that the genes involved in growth, rhythm, and hormone-related pathways were under strong selection in Onagadori. Fast growth and non-molting loci as the *FGF14* and *TRHDE* genes, respectively were characterized. The selective rhythm gene *ENOX1* was proposed to play a key role in the regulation of feather cycle. Integrated networks consisting of multiple pathways with selective genes were depicted to illustrate the formation of long tail feathers. Results indicated that Onagadori originated from domestic chickens in Northern China around 2,300 years ago. Our study shed novel insights into the growth and molting of feathers, and provided a novel case for how artificial selective breeding can answer classical evolutionary biology questions.

Key Words: feather, molting, chicken, selection, genome

P31 Metabolomic approach to investigate the effect of β-alanine and L-histidine supplementation on carnosine synthesis in slow-growing Korat chicken jejunum tissue. K. Promkhun*[1], C. Suwanvichanee[1], K. Thumanu[2], W. Molee[1], S. Kubota[1], P. Uimari[3], and A. Molee[1], [1]*School of Animal Technology and Innovation, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand,* [2]*Synchrotron Light Research Institute (Public Organization), Nakhon Ratchasima, Thailand,* [3]*Department of Agricultural Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Helsinki, Finland.*

The slow-growing Korat chicken (KR) crossbreed was developed to provide an alternative breed for smallholder farmers in Thailand. A previous study generated KR breast meat containing high carnosine concentration by dietary supplementation with the constituent amino acids of carnosine. The aim of this study was to investigate the effect of enriched carnosine synthesis, obtained by the β-alanine (BA) and L-histidine (L-His) supplement on diet, on changes in metabolomic profiles in jejunum tissue. Four hundred 21-d-old female KR were divided into 4 experimental groups: basal diet (A), a basal diet supplemented with 1.0% BA (B), 0.5% L-His (C), and 1.0% BA combined with 0.5% L-His (D). On 70 d, 10 randomly-selected chickens from each treatment were slaughtered, and metabolic profiles using 500 MHz ^{1}H nuclear magnetic resonance (NMR) spectroscopy in jejunum were analyzed. Results showed that significant changes in the concentrations of 28 metabolites were identified. Partial least squares discriminant analysis was applied to identify the distinguishing metabolites. Based on a variable of importance > 1 and $P < 0.05$. These changed metabolites included β-alanine, choline, myo-inositol, creatine, lactate, aspartate, tyrosine, isoleucine, valine, and taurine. The detected metabolites involved in 34 potential metabolic pathways were predicted by metabolite content using MetaboAnalyst 5.0 software. The change of the abovementioned metabolite concentrations in chicken jejunum was related to potential metabolic pathways. This study may identify pathways associated with the transportation and synthesis of BA and L-His such as Taurine and hypotaurine metabolism was related to the transport of β-alanine in jejunum and Pantothenate and CoA biosynthesis was associated with carnosine synthesis. Therefore, these results contribute to a better understanding of metabolic changes in jejunum tissue as a pathway of precursor for carnosine synthesis and reveal the biomarker for facilitating the development of nutrient selection programs in slow-growing chickens.

Key Words: slow-growing Korat chicken, β-alanine, L-histidine, jejunum, ^{1}H-NMR

P32 ISAG Bursary Award: Genetic diversity in Nigeria laughing dove population using the mitochondria cytochrome C oxidase gene. I. A. Abubakar*[1], F. E. Sola-Ojo[1], C. A. Adeola[2], and M. O. Adesina[3], [1]*University of Ilorin, Ilorin, Kwara, Nigeria,* [2]*Chinese Academy of Sciences, Kunming, China,* [3]*Kwara State University, Malete, Kwara State, Nigeria.*

The laughing dove (*Streptopelia senegalensis*) is a small pigeon with long-tailed typically 25 cm (9.8) in length that is a resident breeder in Africa, the Middle East, the South Asia, and Western Australia and it has established itself in the wild. Laughing dove belongs to bird family *Columbidae* and found in dry scrub and semi-desert habitats where pairs can often be seen feed on seeds, grasses, other vegetable matter and small ground insects, they are used as source of animal protein by farmers in Nigeria. This study was designed to provide basic knowledge of genetic diversity and relationship between this germane poultry species for future selection and improvement, by analyzing the genetic diversity and relationship between their populations sampled from 3 different Agroecological locations in Nigeria using Cytochrome c Oxidase I (Mt-COI) gene. The results show a total of46 haplotype in Nigerian *Streptopelia species* based on the 618-base pair (bp) of COI. It also shows that Haplotype 2 had the highest number (13), followed by Haplotype 5 which was 8 in number and Haplotype 12 with 5. The ML tree of 46–618bp of *Streptopelia species* used in this study shows a monophyletic relationship within the Nigeria population studied from different location, thus an indication of a close niche relationship which might be a result of continuous in breeding within the population. High haplotype diversity and low nucleotide diversity obtained in this study shows that there is room for population expansion and selection of laughing dove for breeding and genetic improvement is possible.

Key Words: genetic diversity, haplotype, nucleotide, laughing dove.

P33 ISAG Bursary Award: Transcriptome analysis of pre-hierarchical follicles highlights dominance as the major mode of gene expression that underpins heterosis for egg number and clutch size in crossbred laying hens. A. M. Isa*[1,2], Y. Sun[1], and J. Chen[1], [1]*Key Laboratory of Animal (Poultry) Genetics Breeding and Reproduction, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Department of Animal Science, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria.*

Heterosis is the major benefit of crossbreeding and has been exploited in the breeding of laying hens for over a century. This genetic phenomenon has been linked to various modes of non-additive gene action. However, the molecular mechanism of heterosis for egg number and clutch size in laying hens has not been fully elucidated. To fill this research gap, we sequenced the transcriptome of the pre-hierarchical follicles in White Leghorn and Rhode Island Red hens, as well as their reciprocal crossbreds that demonstrated heterosis for egg number (8–38%) and clutch size (13–56%). We identified dominance as the principal mode of gene expression in the crossbred hens. Important pathways enriched by genes with higher expression in the crossbreds compared with purebred(s) were cell adhesion molecules, tyrosine and purine metabolism. In contrast, ECM-receptor interaction, focal adhesion, phagosome, PPAR signaling, and ferroptosis were enriched in genes with low expression in the crossbred compared with either parental purebred(s). Hub genes identified by protein interaction network include apolipoprotein B (*APOB*), transferrin, acyl-CoA synthetase medium-chain family member (APOBEC) 3, APOBEC1 complementation factor, and cathepsin S. *APOB* was the only gene with under-dominance expression common to the 2 reciprocal crossbred lines, and has been linked to oxidative stress. In conclusion, high proportion of conserved gene expression between the 2 purebred lines suggests little divergence. Genes with dominance expression play crucial roles in follicle growth and atresia via improved follicle competence and increased oxidative stress, respectively. These 2 phenomena could underpin heterosis for egg number and clutch size in crossbred laying hens.

Key Words: heterosis, transcriptome, dominance, egg number, hen

P34 Comparative proteomics reveal the chicken sperm freezability. Y. Li*, Y. Zong, Y. Sun, J. Yuan, H. Ma, and J. Chen, *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

The sperm freezability during cryopreservation varies widely in chicken breeds and individuals. This study aimed to investigate this mechanism at protein levels. Five Beijing-you chickens with higher freezability and 5 chickens with lower freezability were selected. The sperm proteins were extracted and quantified using the 4D label-free quantitative proteomic analysis strategy in combined with parallel reaction monitoring (PRM). A total of 2,309 proteins were identified. Among them, 42 proteins were differentially expressed (DEPs). The top-ranked GO BP terms were metabolic process, localization, biological regulation, regulation of biological process, and response to stimulus. And the DEPs were mainly associated with binding, catalytic activity, molecular function regulator, and transporter activity. The DEPs were annotated to be localized in the organelle, membrane, and protein-containing complex. According to the KEGG analysis, pathways involved in the maintaining of freezability were lysosome, amino sugar and nucleotide sugar metabolism. In addition, PRM of 6 proteins including VDAC1, ATP5S, TOM1L2, G6PC3, LNS2 domain-containing protein, and PSAP further validated label-free quantification. ATP5S is involved in the regulation of mitochondrial membrane ATP synthase, which is necessary for ATP synthase H^+ conduction by blocking proton leakage through another proton exit pathway and promoting energy-driven catalytic ATP synthesis. The higher ATP5S in the high freezability and the observation that the ATP content in spermatozoa decreased after cryopreservation, indicating that the energy metabolism may be disturbed in frozen-thawed sperm. In conclusion, the difference in sperm proteome composition might be one of the reasons leading to different freezability of sperm. The DEPs identified in this study could potentially serve as potential biomarkers for evaluating the post-thaw quality of chicken sperm. This study was provided by National Key R&D Program of China (No. 2021YFD1200300) and National Natural Science Foundation of China and The Egyptian Academy of Scientific Research and Technology (No.31961143028).

Key Words: chicken sperm, conservation, freezability, proteomics

P35 Effects of single-nucleotide polymorphisms in histamine n-methyl transferase (hnmt) gene on anserine and carnosine contents in Korean native chickens. J. Munyaneza*[1], M. Kim[1], E. Cho[1], A. Jang[2], H. Choo[3], and J. Lee[1], [1]*Chungnam National University, Daejeon, Republic of Korea,* [2]*Kangwon National University, Chuncheon, Republic of Korea,* [3]*National Institute of Animal Science, Pyeongchang, Republic of Korea.*

The meat of Korean native chicken is very well known and preferred by consumers due to its good taste and flavor resulting from the higher content of taste-active compounds such as anserine and carnosine. A previous GWAS reported the HNMT Gene as the potential candidate gene to influence the content of anserine and carnosine in Korean native chickens-red-brown line (KNC- R line), thus affecting the meat flavor. The HNMT gene is mapped on chromosome 7 and has 7 exons and 6 introns, and is involved in the metabolism of histidine, which is then used in the formation of carnosine and anserine. However, the polymorphisms of HNMT gene and their effects on anserine and carnosine in chickens remain unclear. Therefore, the objectives of this study were to identify the SNPs in HNMT gene and investigate associations between identified SNPs and the content of anserine and carnosine in KNC-R chickens. This study used 10 weeks old KNC-R line, and a sample of 277 (males, n = 123; females n = 154) for genotyping of HNMT gene. Four missense and 4 synonymous SNPs were identified in KNC-R line based on the NGS data analysis, and one synonymous SNP (rs29009298C > T) in HNMT gene was used for genotyping by PCR-RFLP. Two-way ANOVA of the R program was used to analyze the association between HNMT genotypes and anserine as well as carnosine contents. The results indicated the significant association ($P < 0.05$) for the SNP with anserine and carnosine contents in KNC-R chickens. This study also, observed the significant effect of sex on anserine content in KNC-R line. Our findings suggested that SNPs in HNMT gene might be used as genetic markers in selection and production of chickens with flavored meat.

Key Words: HNMT gene, Korean native chicken, meat flavor, NGS, SNP

P36 Withdrawn

P37 Assessment of incubation eggs quality of the local ducks crosses population. M. Saginbayeva*[1], R. Sharipov[2], A. Shamshidin[3], and G. Temirbekova[4], [1]*S. Seifullin Kazakh AgroTechnical Universit, Astana, Akmola Region, Kazakhstan,* [2]*Union of Poultry Breeders of Kazakhstan, Astana, Akmola Region, Kazakhstan,* [3]*Zhangir Khan University, Uralsk, Kazakhstan,* [4]*North-Kazakhstan Research Institute of Agriculture, Astana, Kazakhstan.*

The use of the gene pool of domestic bird breeds in breeding work is of particular importance, due to their high adaptation properties and their adaptability to local feed and climatic conditions. The production of duck meat in Kazakhstan was previously based on the use of 4–5 lines and populations of the Peking breed, where the Medeo cross lines (M-1 paternal and M2 maternal) were most widely used. Many domestic lines, such as the "Bishkulskaya colored" and "Kyzylzharsky" crosses, represent rich genetic material and can be used to create new crosses. In this regard, our study investigated the incubation parameters of the maternal stock of local ducks in the Northern region of Kazakhstan. The research was conducted at the "Bishkulskaya Poultry Farm" LLP of the North Kazakhstan. The study focused on populations of ducks "Kyzylzharsky" (KZ) and "Bishkulskaya Colored" (BC) crosses. During the study, the selection of incubation eggs was conducted from the breeding nests. The "Kyzylzharsky" (K12) duck cross is represented by paternal (K1) and maternal (K2) lines of the Pekin breed. The paternal line "K1" was created based on the gene pool of ducks from the M-1 line of the

"Medeo" cross. The maternal line K2 was developed from the M-2 line of the "Medeo" cross. The K2 line ducks are well-matched with the K1 paternal line in terms of their main economically valuable traits. As a result, a high fertilization rate of eggs was established, amounting to 87.1% for the "BC" cross, and 86.4% for the "KZ" cross. The cross "KZ" (K12) showed good results in terms of hatchability and had a high survival rate. The average egg weight of the "KZ" cross was 6.3% higher than the "BC" cross, and the protein index by 14.6%, the yolk index by 22.5%, the albumen height by 6.5%, and the Haugh units by 6.1% higher, the thickness of the eggshell was 11.1% thicker. Thus, it is necessary to annually evaluate the lines for compatibility at the poultry farm, and to assess the resulting offspring in terms of hatchability, body weight, preservation, and meat yield per laying duck of the maternal parent form, for further targeted breeding work.

Key Words: cross, duck, selection, reproduction, fertility

P38 Genome-wide association analysis (GWAS) and accuracy of genomic selection on growth traits in two duck lines using imputed genotypes. O. Matika*[1], E. Tarsani[1], S. Desire[1], K. McIntosh[1], A. Kranis[1], A. Rae[2], and K. Watson[1,3], [1]*The Roslin Institute and Royal (Dick) School of Veterinary Studies University of Edinburgh, Edinburgh, Midlothian, United Kingdom,* [2]*Cherry Valley Farms (UK) Ltd., Grimsby, United Kingdom,* [3]*Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Edinburgh, Midlothian, United Kingdom.*

The Pekin duck is well described for both its fast growth and meat qualities. Traditional breeding programmes rely on laborious and costly pedigree gathering usually through trap nests. The change to open pen-mating system with many drakes and hens in a single pen is closer to production environments and allows free mate choice. In this system, low density SNP array made it possible to reconstruct parentage. Subsequently, using imputation to the 60K Duck SNP array, we estimated variance components, conducted genome-wide association study (GWAS) and genomic prediction for growth traits in 2 commercial duck lines and compared these to pedigree-based estimates. The GWAS results were similar to those obtained using 60K Duck SNP array genotypes, albeit with more power since we used more animals in the imputation study (3K records for 2 lines vs 13K records per line). The estimates of heritability were consistently lower for genomic matrix than when using pedigree information (G-Matrix vs A-Matrix). We also observed that the use of markers improved prediction accuracies and reduced biases across all traits analyzed. The results, in one line, of the comparison between accuracy prediction estimates for A-Matrix vs G-Matrices in several growth traits such as normal start weight, finish weight, feed intake, breast depth, gait score, average daily gain, feed conversion ratio and residual feed intake were respectively 0.38 vs 0.70, 0.35 vs 0.68, 0.35 vs 0.63, 0.26 vs 0.50, 0.24 vs 0.58, 0.40 vs 0.65, 0.36 vs 0.67, 0.36 vs 0.67, 0.43 vs 0.68 obtained when predicting 6th generation phenotype from previous generation data. Similar improvements were observed in the other line considered. There was less bias estimated using the markers as opposed to pedigree-based selection. These major increases in accuracies will have higher impact on low heritable traits like gait. In combination with the use of a low cost parentage panel being imputed to 60k SNP chip, genomic selection will be able to increase genetic improvements, reduce mating costs (labor and pens) and improve welfare of the ducks.

Key Words: imputation, duck, genomic selection, growth traits

P40 Genome-wide circular RNAs signatures involved in sexual maturation and its heterosis in chicken. Y. Wang, J. Yuan, Y. Sun, Y. Li, and J. Chen*, *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing China.*

Sexual maturation heterosis has been widely used in animal crossbreeding. However, the underlying mechanism have long remained elusive. In the current study, the reciprocal crossing between White Leghorns and Beijing You chickens were performed to measure the sexual maturation related traits, and the ovary circRNAs of purebreds and crossbreds were profiled to illustrate molecular mechanism of sexual maturation heterosis. Pubic space and oviduct length exhibited positive heterosis and age at first egg showed negative heterosis in crossbreds. We identified 3,025 known circRNAs and 624 putative circRNAs, which were mainly derived from the coding exons. Among these, 141 and 178 circRNAs were specific expressed in WY and YW, respectively. The high proportion of non-additive circRNAs in the 2 crossbreds (54.38% and 64.63%) indicated non-additivity was the major expression pattern. GO and KEGG analysis showed that the host genes of non-additive circRNAs were associated with TGF-β signaling pathway, oocyte development, ATPase activator activity, oocyte meiosis, progesterone-mediated oocyte maturation and GnRH signaling pathway. Weighted gene co-expression network analysis identified that 4 modules were significantly correlated with oviduct length and pubic space. The nonadditive circRNAs harbored in the 4 modules were enriched in MAPK signaling pathway and Wnt signaling pathway. Furthermore, ceRNA network analysis characterized non-additive circRNA *chr6:31878072–31892396* and *chr17:9977659–10017457* could interact with *gga-miR-1612* and *gga-miR-12235–5p* to regulate *FGF7*, *COL8A1*, and *FHL2*, which were essential for ovary development, indicating that the non-additive circRNAs involved in sexual maturation heterosis through regulating genes related to the metabolic and developmental process. The findings provide a deeper understanding of the molecular mechanism underlying sexual maturation heterosis from a novel perspective.

Key Words: poultry and related species, animal breeding, crossbreeding, egg production

P41 Profiling the diversity of the village chicken faecal microbiota using Amplicon and Shotgun metagenomic sequencing data. M. E. Nene*[1,2], N. W. Kunene[1], R. Pierneef[3], and K. Hadebe[2], [1]*University of Zululand, Mpangeni, KwaZulu Natal, South Africa,* [2]*Agricultural Research Council—Biotechnology Platform, Pretoria, Gauteng, South Africa,* [3]*University of Pretoria, Pretoria, Gauteng, South Africa.*

According to metabolism studies, the gut microbiome's functional role can be cataloged and annotated to reveal a complex interaction between the microbiome and the immunological and metabolic systems of the host. We hypothesize that the gut microbial community is crucial to an animal's ability to flourish in the environment. The aim of this study was to investigate microbiome diversity within and between communities using Amplicon and Shotgun metagenomic sequencing of chicken fecal samples from Limpopo and KwaZulu-Natal provinces. The objectives of this study were to study the composition and diversity of the fecal microbiome in Chicken from Villages between KwaZulu-Natal (KZN) and Limpopo (LIMP) provinces, districts and villages, and attributing its metabolic function to the microbiome between the 2 areas. A total of 85 village chicken fecal samples from LIMP and KZN were collected, of which 45 were used for Amplicon sequencing and 40 for Shotgun sequences. Reads were analyzed in R4.2.2. Shotgun sequencing data were analyzed in KBase. Through published research on the chicken gut microbiome, metabolic functional analysis of both the Amplicon and Shotgun Metagenomic data was done. *Firmicutes*, *Proteobacteria*, and *Bacteriodota* were highly abundant in KZN and LIMP, according to Amplicon sequencing data. According to Shotgun sequencing data, *Pseudomonas*, *Brevundimonas*, and *Paenibacillus* bacteria are abundant in Limpopo. The KwaZulu-Natal Shotgun sequencing data revealed high levels of *Escherichia*, *Lactobacillus*, and *Enterococcus.* The sequencing data from the provinces of KZN and LIMP show that *Bacteriodetes*, *Firmicutes*, and *Proteobacteria* are the most frequent microbial phyla in the chicken Gastrointestinal Tract (GIT). *Pseudomonas*, *Brevundimonas*, and *Paenibacillus* are abundant in the provinces of Limpopo, whereas *Escherichia*, *Lactobacillus*, and *Enterococcus* are prevalent in the province of KwaZulu Natal. Metabolic functional analysis revealed microbial population associated with health, immunity and overall productivity.

Key Words: Amplicon, Shotgun, agroecological zone, faecal microbiome

P42 Identification of potential candidate genes for plumage color in Korean native duck based on whole-genome sequencing. E. Cho[1], M. Kim[1], H. Choo[2], and J. Lee*[1], [1]*Chungnam National University, Daejeon, Republic of Korea,* [2]*National Institute of Animal Science, Rural Development Administration, Pyeongchang, Gangwon-do, Republic of Korea.*

Duck is one of the best sources of protein and, compared with other meats, duck meat is known to be rich in essential fatty acids that help prevent diseases such as arteriosclerosis or hypertension. The supply of most broiler ducks in Korea relies on imports of the Pekin duck breed for more than 90%. Korean native ducks (KNDs) originated as hybrids between mallard ducks and Pekin ducks, and have white and dark brown plumage phenotypes. Among these, the white populations are highly preferred for broiler ducks because there is no fine hair left on the meat surface after slaughter. Therefore, this study aims to search for candidate genes that affect plumage color to provide a genetic basis for the linebreeding of white KNDs. We generated whole-genome sequence data of 4 animals, 2 each of white and colored KNDs, through next-generation sequencing. After the quality control and calibration steps, the data were aligned to the *Anas platyrhynchos* reference genome following the GATK best practice pipeline, and variant filtration and annotation step were conducted by SnpEff software. As a result of previous literature research on candidate genes for plumage color in ducks, it was reported that *MITF* and *DCT* genes affect plumage color through an association study on Asian duck breeds including KNDs. To identify candidate causal genes other than these 2 genes, the additional analysis should be performed using the previously obtained variant files of 4 KNDs. It is necessary to search for the common differential variants of the 2 groups with different phenotypes and to perform functional gene analysis such as enriched gene ontology (GO) and Kyoto encyclopedia of genes and genome (KEGG). The results of this additional analysis could be used as a meaningful basic study for improving and using white KNDs in the Korean poultry industry.

Key Words: Korean native duck, whole-genome sequence, plumage color, candidate gene

P43 Using selection population revealed the mechanism of intramuscular fat formation in chicken. Y. Wang, L. Liu, H. Cui, and J. Wen*, *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

[Introduction] The purpose of this study was to investigate the formation mechanism of intramuscular fat (IMF) in chicken. [Materials and Methods] A total of 516 JXY chickens were selected, including IMF selection line (S) and control line (C), to determine the content of IMF, TG, PLIP, TCHO and fatty acid (FA) in breast muscle. Then, identifying the key genes affecting IMF/TG deposition by GWAS and using isotope tracer and scRNA-seq technology to determine cell types of FA de novo synthesis. [Results] (1) The content of PLIP and TG in IMF were significantly higher than that in TCHO . Only TG content in the S line was significantly higher than that in the C line ($P < 0.05$). TG plays a decisive role in IMF deposition. (2) Under the 2 modes of FA de novo synthesis and extracellular uptake, the proportion of long-chain FA such as C14:0 and C16:0 increased, and TG was synthesized by esterification, which promoted the deposition of IMF. (3) *SLC16A7* was a key candidate gene for TG, which may affect TG deposition by regulating the FA de novo synthesis. The top 1% SNPs with IMF trait were annotated, like *FASN*. FA de novo synthesis occurs in muscle and is directly involved in the deposition of IMF. (4) Malonyl-CoA content in muscle was about 40 ng/g, and myocytes was significantly higher than that adipocytes ($P < 0.01$). The proportion of FA de novo in myocytes was about 40%. FA de novo synthesis occurred in muscle, and myocytes had stronger ability than adipocytes. (5) scRNA-seq showed that muscle contained satellite cells, adipocytes and other types. The mRNA level of *FASN* was high in satellite cells, myoblasts and myocytes, which were the main cell type of FA de novo synthesis. (6) Overexpression of the *SLC16A7* was followed by a significant increase in malonyl-CoA and TG content. SLC16A7 regulates FA de novo synthesis and ultimately promotes IMF deposition. In conclusion, FA de novo synthesis occurs in chicken muscle tissue, and myocytes are the main cell type of FA de novo synthesis. Both FA de novo synthesis and extracellular uptake jointly promote IMF deposition.

Key Words: intramuscular fat, triglyceride, FA de novo synthesis, myocyte

P44 Assessment of the usefulness of an additional set of six pigeon microsatellite markers for parentage testing and genetic diversity. A. Masior*, A. Szumiec, A. Radko, and K. Ropka-Molik, *Department of Animal Molecular Biology, National Research Institute of Animal Production, Krakowska, Balice, Poland.*

Pigeons have been used as a means of communication for centuries. They are famous for their amazing homing abilities, speed, and skills to train in various types of competitions. This study aimed to examine the usefulness of an additional panel of 6 microsatellite markers: CliμD32, PG2, PG3, PG5, PG6, and PG7 in parentage testing of pigeons and to determine genetic diversity. This was a continuation of an earlier preliminary study (Radko et al., 2016). 475 randomly selected homing pigeon DNA samples were used. Amplifications were performed using multiplex PCR while capillary electrophoresis was performed on a 3130xl Genetic Analyzer. The samples were genotyped in GeneMapper 4.0., and the obtained DNA profiles were subjected to bioinformatics analysis in the CERVUS, GenAlEx, and STRUCTURE tools. DNA profiles were obtained for all tested pigeons. The average PIC value for the tested markers was 0.582, however, in PG5 and PG6 markers a value <0.5 was observed, which indicates that these markers are weakly polymorphic. For all 6 markers combined exclusion probability for one candidate parent and the combined exclusion probability for a candidate parent pair was 84.31% and 99.69% respectively. The highest values of Ne, I, Ho, and He were characterized by the PG2 marker, even though not the most polymorphic marker in terms of alleles found. In turn, CliμD32 had the most different alleles and the lowest inbreeding coefficient. The conducted molecular analysis shows that the tested set of 6 microsatellite markers is not an indication for use as a separate panel for individual identification and parentage testing. However, it can successfully serve as an additional panel in situations where the pedigree analyses may be questionable after performing analyses using the core panel recommended by ISAG. In addition, it was also observed that the population structure of racing pigeons kept in Poland does not indicate a loss of genetic diversity, but population monitoring is strongly recommended.

Key Words: pigeon, parentage testing, genetic variability

P45 Landscape genomic approach to estimate the environmental suitability of village-based indigenous chickens in South African major production regions. R. R. Mogano*[1,2], T. J. Mpofu[1], B. J. Mtileni[1], K. Madlala[2], and T. Chokoe[3], [1]*Tshwane University Technology, Tshwane University Technology, Pretoria, Gauteng, South Africa,* [2]*Agricultural Research Council, Biotechnology Platform, Pretoria, Gauteng, South Africa,* [3]*Department of Agriculture, Land Reform and Rural Development, Pretoria, Gauteng, South Africa.*

Village-based indigenous chickens are an important source of livelihood. Presently, they are under threat due to changes in the market demand for breeds and production potential, emerging arising diseases, and predators. Due to the nature of the extensive management; they are not buffered by the production system. The current study was conducted to investigate the environmental suitability of indigenous chickens and estimate the risk status of 356 village-based indigenous chickens from rural areas of the Capricorn (n = 88), Sekhukhune (n = 144) from Limpopo province, Harry Gwala (n = 27), and uMzinyathi (n = 27) Districts from KwaZulu-Natal. Reference breeds from the conservation flock of the Agricultural Research Council were Ovambo (n = 10), Venda (n = 20), Potchefstroom Koekoek (n = 20), and Naked Neck (n = 20). These animals were genotyped using Illumina chicken iSelect SNP60k Bead chip and analysis were done on PLINK, *SNeP*, as well as R packages *adegenet*, *GGally, Raster,rdgal, ENMeval* and *vegan*. The *Ne* was higher than 50 in generation 31, with over 50 animals for each

population. Bioclimatic variables Bio6, Bio8, Bio11, Bio18, elevation, solar-radiation of the 6th and 7th months of the year, and soil cation exchange capacity, soil pH, soil clay content, and soil organic carbon content were not correlated (<0.5) and used for downstream analysis. A total of 43 SNP were significantly associated with one or more variables using the Redundancy Analysis. The ENMeval identified Hinge (H) feature with regularization multiplier = 1 as the best parameter, this had the lowest delta AICc value. The results indicate that various environmental variables have varying effects on a chicken's ability to adapt to local environments. The findings of this study will contribute to the understanding the of the effects of environmental selection and ecotype suitability. In turn, it can inform current and future ecotype management and conservation efforts for these chickens.

Key Words: population structure, adaptation, landscape genomics

P46 ISAG Bursary Award: Complex genetic architecture of the chicken genome. An example of *Growth1* QTL region. J.-H. Ou*, T. Rönneburg, and C.-J. Rubin, *Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.*

Chicken body weight is an important trait in both commercial and research, and it is known to be controlled by multiple effects. To comprehend more of the genetic effect, the Virginia Chicken Lines were introduced, including 2 bidirectional selected lines, high-weight (HWS) and low-weight (LWS) lines, and an advanced intercross line (AIL) produced from the 41st generation of HWS and LWS lines. Several research works on parts of this data have succeeded, such as finding 13 suggestive QTLs linked to body weight, a GxG radiation network, and selected sweeps. This study focuses on the known QTL region, *Growth1*, to determine if the complex genetic architecture is due to genetic variance heterogeneity and/or linkage disequilibrium between functional alleles. Taking advantage of extending the AIL population to the F18 generation, we have a higher resolution for association studies. Genome-wide association study and variance-heterogeneity GWAS were performed to select SNP markers that carried either mean or variance effect in this region. Haplotype-based analysis was conducted to include information about LD from multiple markers. The natural and orthogonal interaction (NOIA) model was used to estimate the main and interaction effects among selected markers. The results show that epistasis causes a complex genetic architecture with minor haplotype effects. Haplotype analysis shows a similar result as GWAS while providing a higher statistical significance to candidate regions. Pairwise interaction among selected SNPs gave statistical support for epistasis. Annotation information was then added to provide biological insights.

Key Words: poultry and related species, computational biology, genome-wide association, statistical genetics, complex trait

P47 Identification of runs of homozygosity in a commercial laying hen population. M. Neuditschko*[1], B. Makanjuola[3], C. Baes[2,3], and M. Toscano[2], [1]*Agroscope, Posieux, Fribourg, Switzerland,* [2]*University of Bern, Bern, Bern, Switzerland,* [3]*University of Guelph, Guelph, Ontario, Canada.*

Runs of homozygosity (ROH) are continuous homozygous segments that arise through the transmission of haplotypes that are identical by descent. The length and distribution of ROH segments provide insights into the genetic diversity of populations and can be associated with selection signatures. Here, we analyzed a total of 61'485 birds genotyped for 59'481 genome-wide SNPs. ROH were derived with a sliding windows approach and the following settings: minimum SNP density was set to one SNP every 50 kb, with a maximum gap length of 1 Mb. For each ROH, one missing genotype was permitted. The genomic based-inbreeding coefficients (F_{ROH}) of the birds were calculated by dividing the total length of ROH segments (S_{ROH}) by the length of the autosomal genome (L_{AUTO}), which was set to 931 Mb. Model-based clustering successfully separated the birds into sire and dam line and simultaneously highlighted 2 admixed birds (>10%) within the dam line. The bird with the highest admixture proportion (29%) was also the least inbred bird (F_{ROH} = 3.6%), while the other one showed a moderate reduced of 14.1%. After excluding the 2 admixed birds the average F_{ROH} within the sire and dam line was 29.4% and 46.7%, respectively. We observed low negative correlations between the admixture proportions and F_{ROH} within the sire (r = −0.05) and dam line (r = −0.31). Therefore, it seems that the observed admixture proportions, with the only exception of the highly admixed bird, are associated with internal substructures and does not indicate cross-breeding between the lines, which should result in a high negative correlation between F_{ROH} and admixture proportions. To explore the difference between 2 breeding lines in more depth, we will conduct a high-resolution population structure analysis, including the identification of key contributors, and examine ROH and heterozygosity-rich regions (HRR) islands within the respective line.

Key Words: runs of homozygosity, genomic inbreeding, admixture, key contributor, high-resolution population structure

P48 Genome wide association study to investigate shank skin colour of indigenous village chickens from Limpopo and KwaZulu-Natal. M. G. Segakoeng* and K. Hadebe, *Agricultural Research Council—Biotechnology Platform, Onderstepoort, Pretoria, South Africa.*

Indigenous chickens present an invaluable animal genetic pool for conservation with the looming climate change and for improvement for food security. While their phenotypic definitions vary by breed, natural adaptation and selection response; their cultural relevance also play a major role in shaping the phenotypes we see today. Some consumers have a preference for black shank skin for cultural purposes. The aim of this study was to investigate the genomic regions associated with the shank skin color in South African indigenous chickens from regions of Limpopo and KwaZulu-Natal provinces. Shank skin color is influenced by pigmentation genes environmental factors thus it acts as both a production and an adaptation trait. Illumina 60K genotyping data of 115 village-based non-descript chickens from 4 District Municipalities from Limpopo (Elias Motsoaledi; n = 35), Mole Mole = 38; Fetakgomo = 37) and KwaZulu-Natal in (Mzinyathi n = 5) were selected based on shank color of either white (n = 20), yellow (n = 45), pink (n = 20), black (n = 30). Our approach combined genome-wide association studies (GWAS) and scans for signatures of selection at the regional level. PC2 and PC6 showed a single major cluster with subpopulations from observed from Mole Mole district. No SNPs were found to be significantly associated with the shank color at *P*-value = $-\log_{10}(0.05)$. This may be due to small sample size. The indigenous chickens exhibited an admixed ancestry, due to uncontrolled mating as well the continuous introduction of exotic cocks into their flocks for improvement. Additional genotypes, inclusion of reference populations and other production and adaptation traits is necessary to confirm breed composition and increase the differentiation power GWAS. The results of this study can be used to support community level breeding and conservation strategies in South Africa.

Key Words: indigenous chicken, shank skin colour, GWAS

P49 The TuBaVi project: An example of biodiversity management in Italian local chicken breeds. D. Soglia[1], F. Perini[2], N. Stoppani[1], A. Schiavone[1], and E. Lasagna*[3], [1]*Department of Veterinary Sciences, University of Turin, Grugliasco, Italy,* [2]*Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Legnaro, Italy,* [3]*Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy.*

Biodiversity loss in local chicken breeds is a growing concern due to the increasing popularity of commercial chicken lines. Efforts to preserve and promote local chicken breeds can help to maintain genetic diversity. The aim of the work is to preserve the genetic variability of local poultry breeds using mating plans based on individual genetic variability indices estimated by molecular markers. Under the TUBAVI project, the breeding animals from 17 breeds were characterized by 26 microsatellites. For each animal the heterozygosity index (H) and the parentage index (P) have been calculated. The H index is the number of heterozygous loci/number of loci analyzed. This index ranges from 1 (maximum variability, all loci are polymorphic) to 0 (no locus is poly-

morphic). Index P is the proportion shared alleles between breeding animals. This index also ranges between 0 (no common alleles) and 1 (all common alleles). The choice of animals with high H index allows to optimize the conservation of the genetic variability, while the choice of breeding animals with low P allows to contain the inbreeding. To maximize the selection results, the 2 indices have been combined into a conservation index (C) calculated as difference between H and P. The index ranges from 1 (H = 1 and $P = 0$) to −1 (H = 0 and $P = 1$). Individual females are selected as breeding animals on the basis of the highest H index and the males on the basis of highest C index. As preliminary trial, these indices have been applied to Bionda Piemontese breed over 3 generations. The results showed an increase in the average observed heterozygosity (from 0.65 to 0.69) and an increase by 6% in the average parentage (P). The allelic variability of the breed has been preserved. The application of the new conservation index (C) in small populations could be a valid tool to contain inbreeding. The monitoring of both heterozygosity and parentage in the offspring will allow to evaluate the results of the mating plans carried out. Funding: PSRN project: "Protection of biodiversity of Italian poultry breeds" - TuBAvI-2 −2021–2024. Sub-measure 10.2., project n 0011078.

Key Words: biodiversity, local breeds, mating plans, molecular markers, STR

P50 Some genetic factors controlling water intake in meat-type chickens. S. E. Aggrey*[1], A. F. A. Ghareeb[1], M. C. Milfort[1], A. L. Fuller[1], M. I. El-Sabry[2], F. K. R. Stino[2], and R. Rekaya[1], [1]*University of Georgia, Athens, GA,* [2]*Cairo University, Giza, Egypt.*

Water is an essential nutrient for poultry but data on individual water intake in meat-type chicken is scant. Understanding the genetic basis of water intake is essential for better management and breeding for sustainability. Individual water and feed intake, and growth data were collected on 520 commercial broilers aged 14 to 42 d. Water intake was evaluated by water conversion ratio (WCR) and residual water intake (RWI). Based on the distribution of RWI, the bottom 5 birds (LRWI) and the top 5 birds (HRWI) for RWI were selected for mRNA expression differences. The average broiler consumed about 7.8 L (±1L) of water from 14 to 42 d of age. The mRNA expression of arginine vasopressin (AVP) antidiuretic hormone, calcium sensing receptor (CasR), sodium channel epithelial 1 subunit α (SCNN1A) and SCNN1D in the hypothalamus was upregulated in the LRWI group compared with the HRWI group. Similarly, kidney aquaporins (AQP) 2, and 3 were upregulated in the LRWI group compared with the HRWI group. The upregulation of AVP and AQP gene mRNA expressions seem to indicate that the LRWI birds were more efficient in water reabsorption in the kidney compared with their HRWI counterparts. Increased water reabsorption will reduce the amount of water consumed to attain hydration. The water reabsorption potential was reflected in the excreta moisture levels as the LRWI birds had significantly lower excreta moisture than the HRWI birds. The kidney potassium inwardly rectifying channel subfamily J (KCNJ1), potassium calcium-activated channel subfamily M (KCNMA1), potassium calcium-activated potassium voltage-gated channel subfamily A (KCNA3), SCNN1A and SCNN1B were upregulated in the HRWI compared with the LRWI birds. It is plausible that, the HRWI birds reabsorbed more sodium, potassium and calcium compared with their LRWI counterparts. We therefore hypothesize that excreta moisture and sodium levels require further studies and could be considered as potential proxy traits for water intake.

Key Words: water intake, broiler, kidney, sodium, excreta moisture

P51 lncRNA analysis in response to diet changes in broiler chickens. F. Degalez[1], L. Lagoutte[1], C. Allain[2], and S. Lagarrigue*[2], [1]*INRAE, Saint-Gilles, France,* [2]*Institut Agro, Rennes, France.*

We have recently generated a new lncRNA gene-enriched atlas for GRCg7b chicken genome gathering gene models from different databases, in particular NCBI-RefSeq and EMBL-EBI Ensembl databases. This chicken gene atlas is composed of 24,102 protein coding genes (PCG) and 44,428 long non-coding RNAs (lncRNAs) (see abstract Degalez et al., ISAG 2023). We used this new atlas to revisit a transcriptomic study published in 2018 showing the main role of liver in the chicken adaptive response to a switch in dietary energy source through the transcriptional regulation of lipogenesis; the switch consisting in a starch substitution in the diet (CTR diet) with fat and fiber (HF diet) was analyzed in the context of alternative diets to cope with the unstable cost of cereals which represents a large part of production charges for meat-type chicken. The aim of this study was to better understand the molecular mechanisms of this adaptation to changes in dietary energy sources, considering lncRNAs, since the latter have emerged a decade ago as a significant part of the regulatory elements in genomes. PolyA+ RNA from the liver of 47 chickens (half per diet) was sequenced resulting on average in 83 M stranded and paired-end reads per sample that aligned on GRCg7b assembly. 15442 genes were expressed including 2119 lncRNAs and 12903 PCGs (normalized expression ≥0.1). The differential analysis using edgeR package shows 363 differential expressed genes (DEG) for PCGs ($adj.pVal < 0.05$, $|\log2(FC)| \geq 1$), with 133 and 230 over and under expressed in the HF diet compared with the control diet. A Kegg pathway enrichment revealed as expected that the under expressed PCGs in HF diet were associated with fatty acid biosynthetic process. Interestingly, we found 111 DEG for lncRNA ($adj.pVal < 0.05$, $|\log2(FC)| \geq 1$), with 51 and 60 over and under expressed in the HF diet compared with the standard diet. Several analyses based on the PCG-lncRNA genomic colocalization combined to co-expression network are currently performed to better annotate the role of these differential expressed lncRNA in response to dietary energy source changes.

Key Words: lncRNA, chicken, RNA-seq, adaptation, diet

P52 One Health Poultry Hub: A multidisciplinary project that aims to increase poultry sustainability in Southeast Asia. A. Hinsu[1], M. Hay[1], P. Koringa[2], M. A. Hoque[3], H. T. T. Pham[4], A. Conan[5], G. Fournie[1], D. Blake[1], F. Tomley[1], and A. Psifidi*[1], [1]*Royal Veterinary College, United Kingdom,* [2]*Anand Agricultural University, Anand, India,* [3]*Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh,* [4]*CIRAD, Hanoi, Vietnam,* [5]*City University of Hong Kong, Hong Kong SAR, China.*

Increased human demand for dietary protein is driving intensification of chicken production, and amplifying the risk posed by pathogens including zoonoses. The One Health Poultry Hub, an interdisciplinary program, focuses on increasing sustainability of poultry farming and marketing while reducing zoonotic disease (*Campylobacter*, *E. coli*, avian influenza virus) and antimicrobial resistance (AMR). The hub applies a multidisciplinary approach integrating biological and social science studies in south and southeast Asian countries namely India, Vietnam, Bangladesh, and Sri Lanka. Around 3300 chickens including commercial broilers, hybrid and indigenous chickens were sampled from partner sites to study pathogen prevalence, microbiota structure, AMR gene catalogs and chicken genomic makeup. Specifically, blood samples on FTA cards for genomic work, intestinal tissues for RNA-seq, cecal content to explore microbiota and AMR genes, muscle and feathers to quantify antimicrobial residues and swabs for culturing microbes were analyzed. Detailed metadata were collected in the form of questionnaires and ethnographic observations to complement the social science, epidemiological, microbiome and genetic analyses. Preliminary analyses revealed the presence of *Campylobacter*, *E. coli* and AIV in 24%, 65%, and 7% of analyzed samples, respectively. AMR gene analyses showed a high level of aminoglycoside and tetracycline resistance genes. So far, 500 chickens have been genotyped using a skim sequencing approach. Using the genomic relationship matrix, principal component analysis revealed that the commercial and indigenous chickens clustered separately, and multiple substructures were observed within both commercial and indigenous birds. Fst analyses-based population differentiation revealed several genomic regions that differed between broilers and indigenous birds, many with known associations with morphological features including cell morphogenesis and organ development. Ongoing work includes microbiome and genome wide

association studies to investigate pathogen colonisation and microbiota structure.

Key Words: poultry and related species, One Health, genome-wide association

P53 Withdrawn

P54 The Chicken Genomic Diversity Consortium: Tracking immune diversity from ancient chickens to the present day. S. Fiddaman*[1], A. Smith[1], and L. Frantz[3,2], [1]*University of Oxford, Oxford, UK,* [2]*QMUL, London, UK,* [3]*LMU, Munich, Germany.*

The Chicken Genomic Diversity Consortium (CGDC), established 2021, represents an ongoing effort to capture a significant proportion of the extant variation in the chicken genome. Currently, we have aligned and processed ~5,000 genomes of chickens from a diverse range of geographic locations, fancy breeds, commercial lines, red junglefowl subspecies and other congeneric *Gallus* species. The aims of the consortium are numerous and varied, but specifically, our group focuses on the chicken in a historical context. We are in the process of sequencing > 200 ancient (from the last ~2000 years) chicken genomes from Europe with the primary aim of determining when, and how many times, the chicken was introduced into Europe from Asia. We are also interested in how the immune system of the chicken changed during domestication and the global expansion through exposure to novel pathogens. In this presentation, I will provide an overview of the CGDC and will outline some preliminary data on immune diversity in chickens. We found that the chicken is rich in immune diversity and that there are several-fold more variants in the Consortium data set compared with online databases. We also found that there have been selective sweeps in several Toll-like receptors, which are innate immune receptors responsible for detecting pathogen presence. Furthermore, we found evidence that some well-known chicken breeds have high-frequency pseudogenes for certain TLRs. These immune changes point to a changing relationship between the chicken and its pathogens over the course of its domestication and expansion. A comprehensive understanding of extant, extinct and interspecific immune variation could inform the breeding of more resilient chickens in the poultry industry.

Key Words: chicken, genomics, domestication, immune adaptation

P55 Genome-wide association study of nucleotide and peptide contents of breast meat in Korean native chickens. M. Kim*[1], E. Cho[1], J. Munyaneza[1], A. Jang[2], H. Choo[3], and J. Lee[1], [1]*Chungnam National University, Daejeon, Korea,* [2]*Kangwon National University, Chuncheon, Gangwon-do, Korea,* [3]*National Institute of Animal Science, Rural Development Administration, Pyeongchang, Gangwon-do, Korea.*

Chicken meat flavor is one of the major factors in determining meat quality and customer acceptance. Overall meat flavor is developed during cooking, by taste-active components such as nucleotides and peptides. To identify genetic markers related to the proportion of the components, we conducted a genome-wide association study using the Korean native chicken red-brown line, which is known for its unique meat quality. We collected breast meat and blood samples from 637 birds slaughtered at 10 weeks of age. We used nuclear magnetic resonance spectroscopy to measure the amounts of nucleotides (inosine, inosine-5′-monophosphate (IMP), and hypoxanthine) and peptides (carnosine and anserine) contents. We extracted DNA from the blood and generated genotype data using the Illumina chicken 60K single nucleotide polymorphism (SNP) chip. The association test and heritability calculation were conducted using GCTA software, and the significance was corrected based on Bonferroni correction. As a result, heritability estimates of inosine and carnosine contents were the highest values at 0.50 and 0.43, respectively. The results of the association test on inosine, IMP, and hypoxanthine showed 45, 16, and 1 significant SNPs, respectively, on chromosome 5. Every significant SNP of IMP and hypoxanthine was listed in significant SNPs of inosine. The significant genomic region of nucleotides was centered on markers located from 10.21 Mbp to 19.40 Mbp. In the association test result of carnosine and anserine, 9 and 4 SNPs were captured, respectively, and a region from 29.67 Mbp to 23.05 Mbp was defined on chromosome 7. Reviewing literature, gene ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis suggested *LOC770392*, *IGF2*, and *DUSP8* as candidate genes of nucleotide contents, which are involved in nucleotide metabolism and insulin activity in muscle. *LOC771456*, *HNMT*, *NXPH2*, and *SPOPL* were identified as candidate genes of peptide contents, related to histamine metabolism, and muscle development. These results will be the basis for improving the flavor of chicken meat through genomic selection.

Key Words: chicken, genome-wide association, SNP, product quality

P56 ISAG Bursary Award: Invited Workshop Presentation: Chicken2K: A panel for global chicken genomic diversity and evolutionary inference. C. Ma*[1], M.-S. Peng[1,12], J. Smith[2], X. Huang[3], S. Zhang[1], X. Li[4], A. Esmailizadeh[1,5], S. C. Ommeh[6], D. W. Burt[7], A. C. Adeola[1,12], M.-S. Wang[1,12], O. Hanotte[8,9], J. Han[10,11], Y. Dong[4], and Y.-P. Zhang[1,13], [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, Yunnan, China,* [2]*The Roslin Institute and R(D)SVS, University of Edinburgh, Midlothian, UK.,* [3]*Guangdong Provincial Key Laboratory of Conservation and Precision Utilization of Characteristic Agricultural Resources in Mountainous Areas, School of Life Science, Jiaying University, Meizhou, Guangdong, China,* [4]*State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, Kunming, Yunnan, China,* [5]*Department of Animal Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran,* [6]*Institute for Biotechnology Research (IBR), Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya,* [7]*UQ Genomics, The University of Queensland, Brisbane, Australia,* [8]*Cells, Organisms and Molecular Genetics, School of Life Sciences, University of Nottingham, Nottingham, UK,* [9]*Livestock*

Genetics Program, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, [10]CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China, [11]Livestock Genetics Program, International Livestock Research Institute (ILRI), Nairobi, Kenya, [12]Sino-Africa Joint Research Center, Chinese Academy of Sciences, Kunming, Yunnan, China, [13]State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan University, Kunming, Yunnan, China.

As the most widely distributed and abundant domestic animal on Earth, chickens play vital roles in human society. A growing number of studies have focused on the genetic changes involved in early domestication, local adaptation, and artificial breeding of chickens. Nevertheless, these studies to date have been limited to small numbers of populations or restricted in geographic regions. Much remains to be understood about the extent and structure of genomic diversity across diverse chicken populations as well as wild junglefowls. Herein, we developed Chicken2K, a resource panel for global chicken genomic diversity to present a global genomic landscape for domestic chickens and wild junglefowls. Chicken2K archives 39,753,026 SNPs and 5,848,802 INDELs for across 1,986 genomes, including 833 newly sequenced genomes, representing 170 global populations. Referring to the updated reference GRCg6a and its annotation, Chicken2K provided the largest whole-genome sequencing reference data set for chicken. We reconstructed the dispersal as well as breeding history of domestic chickens, mirroring the accelerated globalization via human population migration and interaction since the Middle Holocene. Then, we leveraged multi-omics approaches and multiple functional experiments to explore the evolutionary history and phenotypic diversity of chicken populations. Also, we built a database Chicken2K (http://chicken.ynau.edu.cn/index/home/index) to include miscellaneous functions and a user-friendly graphical interface for visualization of genomic diversity, genetic affinity, population structure, selective signal, and demographic history.

Key Words: chicken, admixture, breeding, dispersal, history

P57 Withdrawn

P58 Allele-specific expression in the jejunal transcriptome profiles of two laying hen strains over the entire production period. S. Ponsuksili*, F. Hadlich, M. A. Iqbal, H. Reyer, M. Oster, N. Trakooljul, E. Murani, and K. Wimmers, *Research Institute for Farm Animal Biology, Dummerstorf, Germany.*

The 2 laying lines (Lohmann Brown-Classic (LB), Lohmann LSL-Classic (LSL)) have approximately the same laying performance, but differ in many physiological, endocrine, metabolic and immunological traits. Obviously, they reach similar levels in the selection criterion laying performance by recruiting different functional pathways. During development, metabolic and mineral requirements change in parallel with maturation, growth and egg production. The intestine plays a critical role in the digestion and absorption of nutrients. We have previously shown that transcriptional patterns of the jejunum mucosa vary between the 2 layer strains and in response to changing metabolic and nutritional profiles at the onset of laying. Allele-specific expression (ASE) is a phenomenon involving imbalanced expression of the 2 parental alleles. This phenomenon occurs in selection processes where one allele is preferentially expressed over another having functional effects on the phenotype. Here, ASE analysis was performed in the jejunal mucosa of LB and LSL at 10, 16, 24, 30 and 60 weeks of age to detect cis-regulated gene expression variation associated with the functional biodiversity of the strains and adaptation to changing metabolic requirements. At the different ages, the 2 strains showed significantly different allele-specific expression in the intestinal mucosa and changes in allelic imbalance across the lifespan. Most ASE genes are involved in energy metabolism, including sirtuin signaling pathways, oxidative phosphorylation and mitochondrial dysfunction. A high number of ASE genes was found during the peak of laying, which were particularly enriched in cholesterol biosynthesis. These results suggest that both genetic architecture and biological processes that impose specific metabolic and nutritional requirements shape allelic heterogeneity. These processes are significantly influenced by breeding and management, and elucidating allele-specific gene regulation is an essential step toward deciphering the genotype-phenotype map or functional diversity between chicken populations.

Key Words: poultry, RNA-seq, allele-specific expression

P59 Accumulated variations in the promoter regions play an important role for complex traits during duck domestication. Z.-T. Yin*[1], X.-Q. Li[1], Y.-X. Sun[1], J. Smith[2], N. Yang[1], and Z.-C. Hou[1], [1]*National Engineering Laboratory for Animal Breeding and Key Laboratory of Animal Genetics, Breeding and Reproduction, MARA; College of Animal Science and Technology, China Agricultural University, Beijing, China,* [2]*The Roslin Institute & R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, UK.*

Identifying the key factors that underlie complex traits domestication is a great challenge for evolution and biology. In addition to the protein-coding region differences caused by missense mutations, a large number of variations are located in the non-coding regions containing multiple type of gene elements. However, the genome-wide patterns and roles of gene elements during domestication and economic trait improvement are understudied. In this study, we combined large-scale genome resequencing, transcriptome, epigenetic analysis, and functional experiments to assess the role of selection on gene promoters during duck domestication. A comprehensive multi-omics map was constructed, including 12.6 million single nucleotide polymorphisms (SNPs), 3 million insertions/deletions (InDels), 74,490 structural variants (SVs), 249,326 potential regulatory elements, over 1,000 topologically associating domains (TAD), and gene expression levels of 16 tissues, from

which we demonstrated the important role of gene promoter selection for complex traits during domestication. In total, 304 (42.94%) gene promoters have been specifically selected in Pekin duck among all selected genes. Joint multi-omics analysis reveals that most genes proximal to selected promoters are located in open and active chromatin, of which 267 genes (87.83%) were highly and differentially expressed in domestic trait-related tissues. Finally, we identify that the strong selection on the *ELOVL3* promoter, which is the key gene regulating high-fat content and unsaturated fatty acid in birds, has resulted in several variations which are nearly fixed in Pekin ducks, show increased expression in Pekin duck liver and confirm with in vitro mutation experiments. Overexpression of *ELOVL3* can increase lipid deposition by around 50% and also unsaturated fatty acid content by around 39%. Our results shed new light on the genetic mechanisms of domestication and modern breeding in Pekin ducks, with important implications for the future improvement of this important species.

Key Words: promoter, variation, duck, domestication.

P60 ISAG Bursary Award: Genetic diversity and relationship between Nigerian Muscovy duck populations using the mitochondria cytochrome b gene. O. Yusuf*[1], F. Sola-Ojo[1], and C. Adeola[2], [1]*Faculty of Agriculture, Department of Animal Production, University of Ilorin, Kwara state, Nigeria,* [2]*State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China.*

Muscovy ducks (*Cairina moschata*) are locally adapted to Nigeria climatic condition. They are known for their hardiness, resistance to disease, low feed requirement, ability to survive harsh weather condition and high prolificacy. However, the genetic information on Muscovy duck in Nigeria is limited, thus this study investigated the genetic diversity and relationship between 15 Muscovy duck populations sampled from 4 different states (Kaduna, Kwara, Niger and Oyo) in Nigeria using mitochondria DNA Cytochrome *b* gene. A total of one hundred (100) Muscovy duck blood samples were collected with no cross contamination through brachial vein puncture and labeled according to the selected areas. Genomic DNA was extracted and 940bp mtDNA Cytochrome *b* gene fragment was amplified and sequenced using the Big Dye terminator cycle sequencing kit. The result showed a total of 40 polymorphic sites consisting of 19 singletons variables. The 72 Cytochrome *b* sequences obtained from the population were assigned into 17 distinct haplotypes and low genetic diversity is seen among populations. Phylogenetic analysis showed close clustering across all locations with the exception of BON 10, BON 20 and ADE 11. The extent of haplotype-sharing in the network indicates the absence of a definite population structure in Nigerian Muscovy duck and this was supported by the low genetic distance value between most Nigerian Muscovy duck populations being studied. These findings indicated low genetic differentiation between and within Nigerian Muscovy duck and also suggest that Nigerian Muscovy ducks are from a single domestication ancestral line with indiscriminate mating and are prone to inbreeding depression. More research evidence from genetics and archeology with wider geographic coverage of Nigeria is still required.

Key Words: Muscovy duck, cytochrome *b*, haplotype diversity, nucleotide diversity

P61 Different stress response strategies of an arctic breeding bird (*Calcarius lapponicus*) under inclement weather conditions revealed by the genome and RNA-seq analyses. Z. Wu*[1], M. M. Hindle[1], A. M. A. Reid[1], J. H. Pérez[2,3], J. S. Krause[2,4], J. C. Wingfield[2], S. L. Meddle[1], and J. Smith[1], [1]*The Roslin Institute and Royal (Dick) School of Veterinary Studies R(D)SVS, University of Edinburgh, United Kingdom,* [2]*Department of Neurobiology Physiology Behavior, University of California, Davis, CA,* [3]*Department of Biology, University of South Alabama, Mobile, AL,* [4]*Department of Biology, University of Nevada Reno, Reno, NV.*

Developing strategies to cope with the increasing extreme global weather events requires a thorough understanding of how organisms respond to environmental perturbations. We performed RNA sequencing on samples from wild free-living male Lapland longspurs (*Calcarius lapponicus*) during their arrival on the breeding grounds and during incubation on the Arctic tundra of Alaska, USA. Samples were collected during an extremely cold arrival period in 2013 and incubation during a severe snowstorm in 2016. We performed RNA-seq analyses on liver, hypothalamus, heart, and testicular tissue to understand how this Arctic species responds to extreme weather events. We present a high-quality genome assembly and gene annotation of Lapland longspurs, identified differentially expressed genes associated with inclement weather events. We identified that *FKBP5* was significantly upregulated in hypothalamus during snowstorm, suggesting that FKBP5 is functionally important for the environmental stress response. FKBP5 is a regulator of the Hypothalamic-Pituitary-Adrenal (HPA) axis during the stress-response, and acts to modulate glucocorticoid receptor sensitivity in mammals. FKBP5 acts as a co-chaperone, negatively regulating the glucocorticoid signaling pathway providing a mechanism by which an individual can rapidly and accordingly adjust its HPA axis function in response to unpredictable environmental perturbations. Other pathways in the hypothalamus and other tissues were seen to be involved during these stressful conditions, specifically those involved in metabolism, the immune response and circadian rhythms. Such findings will contribute to the understanding of gene expression changes in multiple physiological systems to mediate stress in wild free-living birds.

Key Words: genome assembly, RNA-seq, Lapland longspur, hypothalamic-pituitary adrenal (HPA) axis, climate change

P62 ISAG Bursary Award: Potential of a chicken AIL population to decipher the genetic mechanisms of complex traits in the integrative omics era. X. Zhu*[1], C. Li[1], C. Luo[2], H. Zhou[3], L. Fang[4], H. Qu[2], Y. Wang[1], and X. Hu[1], [1]*State Key Laboratory for Agro-Biotechnology, China Agricultural University, Beijing, China,* [2]*State Key Laboratory of Livestock and Poultry Breeding, Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou, China,* [3]*Department of Animal Science, University of California, Davis, CA,* [4]*Center for Quantitative Genetics and Genomics (QGG), Aarhus University, Aarhus, Denmark.*

Integrative analysis of multi-omics data can elucidate valuable insights into genetic mechanisms for complex traits. Here we reported an F_{16} advanced intercross line (AIL) for QTL fine-mapping, characterized by sufficiently randomized the founder genomes, rapid linkage disequilibrium decay and abundant haplotype diversity. Utilizing 7.9 million SNPs and 75 phenotypes from 5 categories of about 1200 individuals, a total of 682 QTL were identified in 43 phenotypes. Gene-level mapping resolution was achieved at about 154 loci, of which 65 (involved 53 unique genes) were associated with growth and development phenotypes, had been identified in gene-edited mice. Next, we integrated the mQTL of the ChickenGTEx (~5000 transcriptome samples from 28 tissues) and the epigenetic annotation of the functional annotation of animal genomes (FAANG). We found that mQTL and regulatory regions of FAANG were significantly enriched in GWAS signals and narrowed down the range of candidate genes. For instance, body weight had a significant signal at the distal end of chromosome 1, in which *A gene* could be co-localized in muscle by summary mendelian randomization and transcriptome-wide association studies. The causative mutation was located in the muscle's strong enhancer region and significantly increased the promoter activity of *A gene*. In addition, we used hundreds of chicken samples from the world to illustrate the origin and transmission of causative haplotype, likely by combining standing variants from the red jungle fowl during the 1000s of years of chicken domestication, before they were rapidly accumulated in the high-weight chicken breeds during intense artificial selection. These results integrated mQTL and FAANG annotation information to determine the functional genes and causative mutations of complex traits, which have good guiding significance for in-depth analysis of the genetic structure of complex traits.

Key Words: chicken, integrated omics, GWAS, complex traits, MolQTL

P63 ISAG Bursary Award: A lncRNA gene-enriched atlas for GRCg7b chicken genome and its functional annotation across 47 tissues. F. Degalez*[1,2], M. Charles[2], S. Foissac[2], H. Zhou[3], D. Guan[3], C. Alain[1,2], L. Fang[4], C. Klopp[2], L. Lagoutte[1,2], B. Lebez[1,2], F. Lecerf[1,2], F. Pitel[2], B. Vourc'h[1,2], T. Zerjal[2], and S. Lagarrigue[1,2], [1]*Institut Agro, France,* [2]*INRAE, France,* [3]*University of California Davis, Davis, CA,* [4]*Aarhus University, Denmark.*

Considering the latest GRCg7b chicken genome assembly and differences between both associated reference genome annotations from NCBI (RefSeq) and EMBL-EBI (Ensembl), here we provide a new chicken lncRNA-enriched gene atlas by gathering RefSeq and Ensembl annotations, as well as additional FAANG and NONCODE resources. The Ensembl and Refseq chicken gene catalogs respectively grew from 17,007 and 18,010 to 24,102 PCGs and from 11,946 and 5,789 to 44,428 lncRNAs for a total of 78,323 genes. In addition to the genome annotation, we provide a functional gene annotation with a total of 1,400 RNA-seq samples representing 47 tissues. As a result, we found 64,478 (82.3%) of the 78,323 gene models considered as expressed, with an expression of TPM ≥0.1 for 35,257 (79.4%) lncRNAs and 22,468 (93.2%) PCGs or an expression of TPM ≥1 for 20,252 (45.6%) lncRNAs and 19,819 (82.2%) PCGs in at least one tissue. As expected, lncRNAs were less expressed and more tissue-specific than PCGs (39% versus 21%). Tissue-specificity was refined, indicating if a gene was specific to a single tissue or to a set of tissues which often share a common function. We also provide a set of differentially expressed genes (DEGs) between sexes and across 10 ages/development stages in 7 tissues. Moreover, we classified lncRNAs, PCGs and miRNAs according to their closest PCG and lncRNA and computed for each pair (excluding miRNAs) the co-expression across the 47 tissues. All the information and results are illustrated by concrete cases. Moreover, to facilitate the transition, we provide a gene correspondence table for galgal5, GRCg6a and GRCg7b. In summary, our work provides new gene models, especially for lncRNAs, by gathering information from Refseq, Ensembl and other databases, as well as functional gene information, thus constituting a unified and valuable resource for future genomic studies in chicken. Results are accessible at *http://www.fragencode.org*. Project funded by the European Union's Horizon 2020 research and innovation program under grant agreement N°101000236 and by ANR CE20 under EFFICACE program.

Key Words: genome annotation, chicken, GRCg7b, lncRNA, functional genomics

P64 High-throughput detection of single nucleotide polymorphisms with flexible content panels. S. Camiolo[1], J. Yeakley[1], E. Clark[2], B. Seligmann[1], and J. McComb*[1], [1]*BioSpyder Technologies Inc., Carlsbad, CA,* [2]*Zoetis Inc., Kalamazoo, MI.*

Detection of single nuclear polymorphisms (SNPs) is a powerful tool for genetic selection and maximization of the breeding potential of farm animals. It can also be used to estimate disease susceptibility or for pathogen detection. Most approaches to SNP calling, however, have significant limitations. Microarrays can measure many SNPs simultaneously but come with fixed content that cannot be customized or easily expanded without distorting original performance. Due to high costs of creating and qualifying each production lot, microarrays are usually available only for a subset of species, and often suffer from significant levels of lot-to-lot variability. qPCR detection is work intensive and severely limited in the number of samples and gene targets that can be evaluated simultaneously. Direct sequencing is expensive and produces data that is difficult to interpret correctly. TempO-SNP is a novel targeted assay capable of inexpensive high-throughput and high-plexity detection of SNPs from any species. It relies on direct hybridization of 2 adjacent barcoded oligomers to the target DNA, which are ligated into the reporter probe only if the correct SNP base is present. The content of such SNP panels is flexible as new probes can be added to the mixture easily and without affecting prior content. The assay does not require specialized instrumentation and the TempO-SeqR software pipeline makes SNP calling and report creation straightforward and painless. In partnership with Zoetis, we demonstrate TempO-SNP detection of hundreds of targets from hundreds of samples simultaneously, across multiple species. We also show that the assay can measure SNPs from crude tissue lysates or without need for DNA extraction, as well as from hair and blood. TempO-SNP shows excellent call and accuracy rates in a side-by-side comparison of data from the same samples produced by Zoetis' current microarray approaches. Additionally, TempO-SNP can be combined with existing commercial TempO-Seq technology to obtain RNA expression data from the same tissue lysates. Robust samples like dried blood spots on paper can also be used for both RNA and DNA readouts.

Key Words: SNP, genotyping, genetics, parentage, RNA

Cattle Molecular Markers and Parentage Testing

P66 Genetic diversity and population structure of Zambian indigenous cattle. E. Musimuko*[1,3], K. S. Nalumamba[1], V. C. Zulu[1], K. I. Odubote[2], and W. Muleya[1], [1]*University of Zambia, School of Veterinary Medicine, Lusaka Zambia,* [2]*University of Zambia, School of Agricultural Sciences, Lusaka Zambia,* [3]*Ministry of Fisheries and Livestock, Department of Livestock Research and Development, Lusaka Zambia.*

In Zambian, smallholder famers' livelihoods depend on well adapted indigenous cattle for several functions. Globally studies have been conducted to evaluate cattle genetic diversity in Africa, unlike in Zambia. This study was conducted to determine genetic diversity and population structure of Zambian indigenous cattle using 32 specific microsatellite markers that included AGLA 232, BL 1043, BM 1818, BM 1824, BM203, BM 2113, BM 305, BM 5004, BMS 2047, BMS 2742, BMS 510, BMS 650, CSSM 016, CSSM 022, CSSM 036, CSSM 042, ETH 3, ETH 10, ETH 225, INRA 005, INRA 23, MGTG 4, RM 067, RME 40, SPS 115, TGLA 057, TGLA 122, TGLA 126, TGLA 227, TGLA 263, TGLA 293 and TGLA 53. Results analyzed by ARLEQUIN 3.1 version, demonstrated that all marker loci contributed to cattle breed differentiation. However, CSSM66 (27%), ETH225 (22.6%), and TGLA122 (13.9%) and the least powerful being RM067 (0.7%), BM1824 (1.0%), and ETH10 (1.1%) contributed mostly and further revealed significant deviations from Hardy-Weinberg equilibrium (HWE) proportions (V_D 8.672 vis v_e5.5908) using Lian linkage analysis. The Angoni and Barotse breeds exhibited excess average observed heterozygosity (1.0% and 0.9%) while Tonga breed exhibited deficit observed heterozygosity of 3.6%. Global heterozygosity (Fit) was 4.2%, significantly different ($P < 0.001$). These cattle were slightly differentiated (Fst 3.2%), significantly different ($P < 0.001$). In addition, high gene flow (11.3%) was evident between populations of cattle. Evidence delivered from AMOVA exhibited a higher level of genetic diversity within populations (97.7%) than between population (2.3%). These results provide evidence of existing divergent gene pool and genetic admixtures between and within cattle population that is available for breed selection and conservation.

Key Words: genetic marker, breed diversity, admixture, microsatellite, heterozygosity

P67 Effects of training population sizes in detecting genomic markers for low heritability traits in beef cattle. J. K. Macharia*, J. H. Lee, and S. H. Lee, *Chungnam National University, Division of Animal and Dairy Sciences, Republic of Korea.*

This study aimed to assess the effect of training population size in detecting genomic markers associated with low heritability traits in beef cattle. Genome-wide association study (GWAS) is a valuable tool

for mapping genetic variants that are associated with complex traits in cattle. The statistical power of GWAS in the detection of significant markers is influenced by several factors including the heritability of the trait and the training population size. In this study, we used simulated data to assess the effects of training population sizes in mapping markers associated with traits of low heritability in beef cattle. Phenotypes and 50K SNP markers for 3 traits of heritability 0.05, 0.1, and 0.3 were simulated using QMSim software. Three training population sizes of 4500, 9000, and 13500 animals respectively were created for each trait, and the association between phenotypes and markers was calculated using a mixed-effect linear model in the GCTA software. No significant markers were detected for the trait with the heritability of 0.05 for all 3 population sizes tested. For the trait with a heritability of 0.1, 4 significant markers were detected using the 13,500 population size while no significant markers were detected for the other 2 populations. For the trait with a heritability of 0.3, 6 significant markers were detected using the 4,500 population size, 9 markers using the 9000 population size, and 11 markers using the 13500 population size. The results from this study suggest that when designing genome-wide association studies, large training population sizes are required to detect genomic markers for low heritability traits in beef cattle.

Key Words: genome-wide association studies, heritability, markers, QMSim, training population size

P68 Association of copy number variants with coat colour in Nguni cattle investigated using BovineHD SNP and Bionano optical mapping data. N. M. Dlamini*[1,2], E. F. Dzomba[2], M. Magawana[3], S. Ngcamu[3], and F. C. Muchadeyi[1], [1]*Agricultural Research Council-Biotechnology Platform, Onderstepoort, Pretoria, South Africa,* [2]*University of KwaZulu-Natal, Scottsville, Pietermaritzburg, South Africa,* [3]*KwaZulu-Natal (KZN) Department of Agriculture & Rural Development, Pietermaritzburg, South Africa.*

Copy number variants (CNVs), are some of the most prevalent types of structural changes in mammalian genomes. In this study we characterized CNVs in South African Nguni cattle that were phenotyped for base coat color, forehead stripe and color-sidedness using Illumina's BovineHD genotype data and Bionano optical mapping. In the first analysis, Bovine HD genotype data was used to call CNVs on the autosomes of 133 cattle obtained from 2 Nguni conservation cattle populations. CNVs were called across 26, 91, and 16 animals in PCA based clusters 1, 2, and 3, respectively. A total of 1 742, 8 524, and 1 225 CNV events were detected in genetic clusters 1, 2 and 3, respectively. For Cluster 1, the CNVs were merged into 981 CNV regions (CNVRs), consisting of 632 losses (64.42%), 203 gains (20.69%) and 146 mixed states (14.88%). Cluster 2 had a total of 1837 CNVRs that consisted of 855 losses (46.54%), 396 gains (21.56%) and 586 mixed states (31.90%) while cluster 3 had 614 CNVRs which consisted of 293 losses (47.72%), 175 gains (28.50%) and 146 mixed states (23.78%). Altogether, the CNVRs (3 432) covered 270.48 Mb of the cattle genome corresponding to 10.87% of the ARS-UCD1.2/*bosTau*9 genome assembly. The observed CNVRs were either fully within or partially overlapping 5 434 genes. About 106 of the reported genes were associated with coat color/pigmentation and included *PRKCA, CALML5, MAPK1, WNT5B, POMC, WNT3A, PLCB4*. In addition, we performed a CNV analysis in Nguni cattle (n = 8) using Bionano optical mapping data and identified a total of 26 314 CNVs across all autosomes. A total of 35 569 candidate genes overlapped with the CNVRs including well-known coat color genes of *KIT*, *KITLG*, *ASIP, TYR, TYRP1, WNT3, MAPK1* and *MC1R*. These genes were enriched in pathways such as MAPK signaling, cAMP signaling and Melanogenesis that are all linked with coat color traits. This study provides insights into the coat color patterns observed in Nguni cattle and demonstrates the utility of Bionano optical mapping technology in coat color genetics studies.

Key Words: CNV, Coat colour gene, Nguni cattle, BovineHD SNP data, Bionano optical mapping

P69 Genomic assessment of inbreeding and identification of markers associated with carcass weight gain in Portuguese Preta cattle using a medium-density SNP-chip. M. C. Feliciano[1,2], A. J. Amaral*[3,4], F. Teixeira[3,5], F. Ferreira[6], E. Bettencourt[1], and L. T. Gama[3], [1]*Instituto Mediterrâneo para Agricultura Ambiente e Desenvolvimento, University of Évora, Polo da Mitra, Évora, Portugal,* [2]*University Lusófona, Campo Grande, Lisboa, Portugal,* [3]*Centre for Interdisciplinary Research in Animal Health and Associate Laboratory for Animal and Veterinary Sciences, Faculty of Veterinary Medicine, University of Lisbon, Lisboa, Portugal,* [4]*Escola de Ciências e Tecnologia University of Évora, Largo dos Colegiais, Évora, Portugal,* [5]*Faculty of Veterinary Medicine, University José Eduardo dos Santos, Huambo, Angola,* [6]*Associação de Criadores de Bovinos da Raça Preta, Samora Correia, Portugal.*

Preta is a Portuguese local cattle breed raised in the Ribatejo and Alentejo regions. With the aim of enhancing the results of the breed management plan, that has been in place for nearly 20 years, genotyping using the GGP Bovine 100K SNP array has been performed since 2020. A total of 317 animals with available information for carcass weight were selected for this study. Data on carcass weight per day of age were first analyzed with a BLUP-Animal Model, using phenotypic records for carcass weight daily gain in 4194 animals, collected since 2002, and the full relationship matrix (n = 48,728). The estimated breeding values were deregressed for genome-wide association analyses. Genotype data were filtered using PLINK. Markers with minor allele frequency < 0.01, missing genotypes < 0.10, not in Hardy-Weinberg equilibrium < 0.001, and located in sexual chromosomes were removed. Inbreeding (FROH) and association analysis were performed using PLINK. After quality filtering, 83,689 SNPs remained. Results show that the inbreeding level is low (FROH = 0.046) and long ROH segments (>16Kb) per individual are quite rare, showing that inbreeding has occurred only in distant generations. We have identified markers located in BTA6 and in BTA26 which displayed significant association ($P < 10^{-5}$) with carcass weight gain. These overlapped LCORL and RNLS genes, respectively. The identified markers also overlap previously described QTLs for body height and carcass weight, which suggests that these novel variants are relevant for the future improvement of this trait in Preta cattle breed.

Key Words: local breed, GWAS, ROH, adaptation, conservation

P70 Low-density genotype panels performance for parentage verification in South African beef cattle breeds. Y. Sanarana*[1,2], D. Berry[1,3], A. Maiwashe[2], C. Banga[2,4], and E. Van Marle-Köster[1], [1]*University of Pretoria, Hatfield, Pretoria, Gauteng, South Africa,* [2]*Agricultural Research Council, Irene, Pretoria, Gauteng, South Africa,* [3]*Teagasc, Fermoy, County Cork, Ireland,* [4]*Botswana University of Agriculture and Natural Resources, Gaborone, Botswana.*

The available ISAG SNP marker panel has some limitations for parentage verification in local breeds. In this study, the information content and efficiency of 2 (multi-breed and breed population-specific) low-density genotype panels were tested in South African Bonsmara (BON) and Drakensberger (DRB) cattle breeds. SNPs for the multi-breed and population-specific panels were selected across and within the 2 breeds. The number of SNPs chosen per chromosome for the development of these panels was directly proportional to the chromosome length, which was measured from the first to the last SNP's genomic position. The block method dividing each chromosome into equal-length blocks was used to predetermine the number of SNPs required per chromosome. All SNPs were ranked based on the minor allele frequency (MAF) and a SNP with the highest MAF, good clustering quality and high call rate per block was selected. To minimize linkage disequilibrium (LD) among selected SNPs, the selected SNPs had to be at least 1 Mb apart. A total of 200 informative SNPs was compiled for each low-density genotype parentage panel. The panels were tested per breed of already validated 45 BON and 74 DRB sire-offspring pairs. Parentage exclusion was considered whenever the genotype of

the sire was discordant with that of the offspring for more than once per SNP. The multi-breed panel performed comparatively lower than the population-specific panel. Five and 2 discordant SNPs were observed in the BON and DRB, respectively when the multi-breed panel was tested. The multi-breed panel had MAF values of 0.38 and 0.40 while the breed-specific panel had 0.49 and 0.48 MAF values in the BON and DRB, respectively. The multi-breed panel falsely excluded 3 (6.6%) sire–offspring relationships of the BON whereas population-specific panels were free from false-negatives. These results suggest that breed-specific panels are better than a set of markers selected across breeds.

Key Words: pedigree, Sanga cattle, opposing homozygous, parentage testing

P71 Genetic diagnosis of sex chromosome aberrations in cattle based on parentage test by microsatellite DNA, X- and Y-linked markers. L. Borreguero*[1], M. R. Maya[2], A. Trigo[2], I. Bonet[2], and J. A. Bouzada[1], [1]*Laboratorio Central de Veterinaria, Algete, Madrid, Spain,* [2]*Tecnologias y Servicios Agrarios S.A., Madrid, Spain.*

Chromosomal abnormalities may result in a substantial loss of animal production or infertility, especially in the case of sex chromosome alterations, which are not often phenotypically visible for breeders. Through the routine DNA genotyping of animals, it is possible to identify profiles that are indicative of chromosome abnormalities by including additional DNA markers in usual panels for pedigree and parentage verification. Abnormal profiles of genetic markers located on sex chromosomes can help identify animals with chromosomal defects. Markers panel used for cattle DNA testing by Laboratorio Central de Veterinaria of Algete (Madrid) consisting of 20 autosomal microsatellite markers (BM1818, BM1824, BM2113, CSRM60, ETH10, ETH152, ETH225, ETH3, ILTST006, INRA023, MGTG7, SPS115, TGLA122, TGLA126, TGLA227, TGL48, TGLA53, TGLA57), 3 microsatellite markers linked to sex chromosomes (BM6017, BM4604 and BYM) and the Amelogenin marker, a gene with distinct X and Y alleles, has proved been very useful for genealogical control and detection of chromosomal abnormalities, presenting a 99,99999% of exclusion probability. A new panel with additional sex-linked markers (BM6017, BM4604, HEL14, BMC6021, IDVGA82, TGLA325, BM861 and BYM) was implemented 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 cattle kept for breeding.

Key Words: cattle, genetic identification, genotyping, sex determination

Companion Animal Genetics and Genomics

P72 Independent *COL17A1* variants in cats with junctional epidermolysis bullosa (JEB). S. Kiener[1,2], H. Troyer[3], D. Ruvolo[3], A. Rostaher[4], P. Grest[5], S. Soto[2,6], E. A. Mauldin[7], C. Yang[7], V. Jagannathan[1,2], and T. Leeb*[1,2], [1]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [2]*Dermfocus, University of Bern, Bern, Switzerland,* [3]*Oradell Animal Hospital, Paramus, NJ,* [4]*Clinic for Small Animal Internal Medicine, Vetsuisse Faculty University of Zurich, Zurich, Switzerland,* [5]*Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland,* [6]*Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [7]*University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA.*

Epidermolysis bullosa (EB) is a heterogenous group of inherited skin diseases characterized by detachment of the epidermis from the dermis resulting in blister formation. Currently, variants in 16 different genes have been shown to cause different forms of EB in human patients. We investigated 2 unrelated domestic shorthair cats with clinical and histopathological features of EB. PCR free libraries from genomic DNA of both cats were prepared and their genomes were sequenced at ~20x coverage on a NovaSeq 6000 instrument. We identified private variants in each of these cats individually by comparing the data to the genomes of 80 genetically diverse other cats. The analyses revealed independent homozygous variants in the *COL17A1* gene encoding the collagen XVII α 1 chain in both affected cats. Case 1 had a splice site variant, XM_006938156.5:c.3019+1delG and case 2 had a splice region variant, XM_006938156.5:c.769+5G > A. Experimental confirmation of the suspected splice defect at the transcript level in case 2 is currently under way. Loss of function variants in *COL17A1* in humans and mice cause an autosomal recessive form of junctional epidermolysis bullosa (JEB). Collagen type XVII links basal keratinocytes of the epidermis to the basal membrane and is an essential component of healthy skin. The functional knowledge on *COL17A1* together with comparative data from humans and mice support the pathogenicity of the detected feline *COL17A1* variants. The fact that 2 cats with comparable phenotypes carried independent *COL17A1* variants proves their causality. The genetic findings confirmed and refined the clinical and histopathological diagnosis. To the best of our knowledge, we report the first pathogenic *COL17A1* variants in domestic animals.

Key Words: cats and related species, biochemical genetics, genome sequencing, candidate gene, animal health

P73 Withdrawn

P74 Design and validation of high density SNP array for Indian dog populations. R. Kolandanoor Nachiappan*, R. Arora, S. Ahlawat, U. Sharma, M. Raheja, M. Maggon, A. K. Mishra, and R. K. Vijh, *ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana, India.*

India, has 15.31 million dog populations with diverse phenotype and utility viz.Caravan Hound, Combai, Chippiparai, Rajapalayam, Rampur Hound, Mudhol Hound and Gaddi. Accessibility of whole genome based SNP analysis has facilitated the characterization of animal

populations at the genome level; further applied for detection of selection signatures, fine mapping of quantitative trait loci etc. Commercially available canine SNP chips may not provide enough polymorphism in Indian dogs due to ascertainment bias, as they have been designed using exotic breeds. Hence, it was necessary to develop a high density SNP array for genomic characterization of Indian dogs. DNA samples from 4 morphologically and geographically diverse dog populations, namely, Chippiparai, Mudhol Hound, Rajapalayam (registered dog breeds) and Gaddi were used for paired-end (150bp) sequencing through Illumina HiSeq with 10X coverage. Clean reads with a Phred score of 30 (Q30) or above were used for further analysis. Standard pipeline as per Axiom Array technology was used to design the HD SNP array. More than 23 million raw SNPs were identified after preliminary screening. After MAF, indels and 21bp gap filtering, about 11 million SNPs with unique flanking regions were submitted for propriety screening and quality controls based on recommendations provided by Affymetrix. More than 6 lakh SNP markers were tiled on the Indian canine array. Array was validated by genotyping 192 samples covering more than 5 dog breeds/populations of India. A total of 186 samples passed the SNP QC thresholds. Average call rate for QC passing samples was 99.609 and 92.415% markers were best recommended. The high call rate (99%) of SNPs on the designed chip suggests the suitability of its use for Indian dog populations and availability of sufficient/high genetic diversity in Indian dogs. This high density SNP array may be used in future for population genetics, parentage verification, identification of breed/selection signatures, development of trait specific biomarkers for sniffing, hunting, and guarding abilities and genome wide data mining.

Key Words: Indian dog, canine HD SNP array, genetic diversity, parentage testing

P75 Genetic parameters and genome wide association studies for feed efficiency-related traits in F2 Nguni × Angus cattle. L. Nesengani*[1], N. Nemukondeni[1], N. Mkize[2], B. Dube[2], T. Masebe[1], and N. Mapholi[1], [1]*University of South Africa, Florida, South Africa,* [2]*Agricultural Research Council, Irene, South Africa.*

Feed efficiency (FE) traits are of economic importance in cattle production. Genetic improvement of FE traits has greater potential to reduce production cost and energy consumption in the livestock industry. The objectives of this study were to estimate genetic parameters of FE traits (Average Daily gain = ADG, Average Daily Feed Intake = ADFI and Feed Conversion Ratio = FCR) and to identify SNP associated with FE traits in F2 Nguni × Angus cattle. Feed intake and weight gain were measured weekly for 10 weeks at the Agricultural Research Council-Irene, South Africa. Genetic parameters were estimated using an animal model on the ASReml software package. A genome-wide association study was performed for FE traits in 216 F2 Nguni × Angus cattle. DNA was isolated from hair samples and genotyped using the Illumina BovineSNP150 assay. Quality control (QC) for the genotype data was performed using Plink software (call rate >90%, minor allele frequency >0.01), after which 128257 SNPs were retained for further analysis. The genome wide association analysis (GWAS) was performed with GEMMA software. Summarizing and visualization of the results from GEMMA were done using R packages. Heritability estimates for FE traits were 0.43, 0.44 and 0.53 for ADG, ADFI and FCR respectively. A total of 7 significant SNPs from chromosome 7, 16, 21 and 24 associated with 2 FE related traits (FCR and ADFI) were identified. Our results provide more insights into the genetic mechanisms of FE related traits in cattle. With these findings, we have further provided important genetic tools for use in developing genetic programs to advance genetic progress of FE.

Key Words: genetic parameters, F2 Nguni × Angus cattle, genome-wide association study, feed efficiency trait

P76 Are the rules of World Union of German Shepherd Clubs (WUSV) enough for the maintenance of genetic diversity of the breed in Brazil? F. M. de Andrade*[1], I. A. Scabello[1], A. V. L. Pereira[2], A. G. Sedrez[3], and J. A. Cobuci[1], [1]*Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil,* [2]*Universidade Federal da Bahia, Salvador, BA, Brazil,* [3]*Universidade Federal de Pelotas, Pelotas, RS, Brazil.*

German Shepherd is one of the most popular dog breeds in the world, and its breeding is regulated mainly by World Union of German Shepherd Clubs (WUSV), based in Germany. The aim of this work was to analyze the population structure of this breed bred in Brazil, to evaluate if limitations on inbreeding imposed by WUSV are being enough to maintain the genetic diversity. A pedigree database of 91,386 dogs was provided by CBPA (Brazilian Club of German Shepherd, which is the Brazilian WUSV club), and these data were analyzed using PopRep, CFC and PEDIG software. The average inbreeding coefficient was 0.83%, with only 106 dogs with F higher 25%. The rate of change in F per generation was of 0.0036, and the change in coancestry (C) was of 0.0013. A significant loss of original founders genetic was detected, once the relation f/fe was 17.8. However, the fraction fe/fa of 0.99 did not indicate the existence of bottlenecks, even if only 147 dogs were responsible by 50% of current genetic diversity. Effective population size was 332 ($Ne_C = 1/2\Delta C$), and its division by Ne_F ($Ne_F = 1/2\Delta F$), resulted in 1.99, indicating the existence of populational subdivision. This stratification was influenced by hair length phenotype, since dogs with long and short hair doesn't breed each other, through WUSV rules. When only short hair dogs were evaluated, the relation Ne_C/Ne_F was smaller (1.44). The total loss of genetic diversity, calculated through $1 – (1/2 \times fg)$ was of 0,4%, and the loss because unequal breeding of animals, calculate through $1 – (1/2 \times fe)$, was of 0,2%. Taking altogether, these results show that the breed bred in Brazil has a good genetic diversity, in spite of the reproduction of relatively few dogs, which probably is due to inbreeding restrictions imposed by WUSV. Still, to perpetuate this effect, it would be important to regulate the maximum use of popular sires, and implement the use of coancestry evaluation, to detect rare blood lines dogs and encourage the reproduction of these dogs.

Key Words: dogs and related species, animal breeding, population structure, breed diversity, management

P77 AgriseqPI 1.0: Reporting utility for SNP based parentage determination with targeted genotyping by sequencing panels. S. Chadaram*[1], A. Burrell[1], K. R. Gujjula[1], C. Carrasco[1], S. Daly[3], S. Udumudi[2], N. Anjuri[2], V. H. Kema[2], and A. Udumudi[2], [1]*Thermo Fisher Scientific, Austin, TX,* [2]*ATS GeneTech Pvt, Ltd., Hyderabad, Telangana, India,* [3]*Thermo Fisher Scientific, Lissieu, Lyon, France.*

The AgriSeqPI is a bulk report generation plugin primarily developed for SNP based parentage and identity analysis using the AgriSeq Targeted Genotyping by Sequencing (T-GBS) parentage panels for canine, feline, bovine, equine, run using the Torrent Suite Software (TSS). The reporting plugin is based on the ISAG rules and guidelines for parentage determination using SNP markers. End users can generate individual reports for each sample on a sequencing run using AgriSeqPI. AgriSeqPI can be run manually or automatically (via plan run) after a sequencing run is completed on Ion Torrent. The plugin retrieves the genotype data from Torrent Variant Caller (TVC) and AgriSum Toolkit and outputs a comprehensive report for each sample for all parentage markers in each panel. The report includes sample, reference, panel (test) details, and a summary of the results. AgriSeqPI allows users to export the reports in bulk mode per customer for ease of processing. Supported output formats include JSON and PDF. The report can be fully customized allowing users to add or /remove the report section and even facilitating custom logos on the final report. AgriSeqPI enables veterinarians, and veterinary service labs to enhance their product offerings related to the AgriSeq parentage panel by automating the report creation process. The plugin will support the users to adhere to the ISAG parentage determination guidelines. AgriSeqPI is publicly available and can be downloaded via Thermo Fisher Cloud and installed on TSS at no additional cost. For Research Use Only. Not for use in diagnostic procedures.

Key Words: multispecies, genotyping, bioinformatics, breed/population identification, parentage

P78 ISAG Bursary Award: *RETREG1* variant causes canine acral mutilation syndrome (AMS) in purebred German spitz. A. Letko*[1,2], J. Plassais[1], P. Quignon[1], and C. André[1], [1]*Institut de Génétique et Développement de Rennes (IGDR), University Rennes, Rennes, France,* [2]*Institute of Genetics, University of Bern, Bern, Switzerland.*

AMS is a neurological disease documented for decades as part of inherited sensory neuropathy in various breeds with a severe impact on life quality. Causal variants in only 2 genes were identified to date, suggesting larger genetic heterogeneity in the dog population. In humans, the equivalent hereditary sensory autonomic neuropathies (HSAN) are characterized by insensitivity to pain, sometimes combined with self-mutilation. Sixteen loci have been associated with HSAN but do not explain the disease origin of all patients. Our aim was to explain the genetic etiology of an early-onset AMS in a purebred German spitz. The affected dog showed loss of pain sensation in the distal extremities, which lead to intense licking, biting, and self-mutilation of digits and paw pads. DNA was isolated from blood samples of the case, its parents, and 3 unaffected German spitzes. Whole-genome sequencing (WGS) of the case, its dam, and 2 controls was carried out using the reference genome assembly UU_Cfam_GSD_1.0 to identify single nucleotide variants and small indels private to the case. Variants were filtered based on autosomal recessive inheritance and further prioritized by predicted impact on the encoded protein and allele frequency in a cohort of 960 publicly available canine WGS. A single candidate causal variant on chromosome 4 in the *RETREG1* gene (c.656C > T, p.Pro219Leu) was discovered. Genotyping showed the variant segregated perfectly in the German spitz family and was absent in unrelated unaffected spitz dogs. This missense variant was previously recognized as deleterious in one mixed-breed dog family with similar clinical signs. Disruption of RETREG1 inhibits endoplasmic reticulum turnover and leads to neuron degeneration. Different *RETREG1* variants cause HSAN in humans. Our findings provide evidence that this variant underlies the recessive form of AMS in German spitz, and support the use of WGS-based veterinary precision medicine for early diagnosis, treatment, and prevention via a genetic test while showing the interest of dogs as natural models for rare human genetic diseases.

Key Words: nervous system, genetic disorder, genome sequencing, dogs and related species, animal health

P79 Comparative genomics of the natural killer cell receptor genes in felids. J. Futas[1,2], A. Jelinek[1], M. Plasil[2], J. Bubenikova[2], P. Burger[3], and P. Horin*[1,2], [1]*Department of Animal Genetics, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno, Brno, Czech Republic,* [2]*Ceitec Vetuni, RG Animal Immunogenomics, University of Veterinary Sciences Brno, Brno, Czech Republic,* [3]*Research Institute of Wildlife Ecology, University of Veterinary Medicine, Vienna, Austria.*

The functional heterogeneity of Natural Killer (NK) cells is due to the differential expression of germline-encoded activating and inhibitory natural killer cell receptors (NKRs). Two complex genomic regions encode NKRs in mammals. The Leukocyte Receptor Complex (LRC) contains genes encoding killer immunoglobulin-like receptors (KIR), while the Natural Killer Complex (NKC) codes for NKRs with lectin-like structure (KLR). Both types of receptors can bind Major Histocompatibility Complex (MHC) class I molecules as ligands. Even closely related mammalian taxa may differ in their LRC/NKC genomic structure and gene contents. The objective of this work was to characterize the LRC and NKC complexes of felids. Bioinformatic analyses (tBLASTn, Splign, SignalP 6.0, TMHMM, Vista, MEGA X) were carried out on the most recent genome assemblies. Altogether, 10 assemblies of 10 species (long-read, chromosome level) and 12 additional short-read or scaffold level Felid assemblies of 12 different species were explored to provide a detailed annotation of both complexes. The genomic architecture of the LRC is highly conserved across the Felidae, containing one *KIR3DL* pseudogene and a novel Ig-like gene, along with a consistent complement of framing genes. The LILR genomic sub-region is also highly conserved among the studied species, with 7 orthologous genes in each species. All 7 genes are potentially functional in most studied Felids. The NKC is more variable in felid genomes, with one *KLRA* gene along with framing genes *KLRD*, *KLRK*, *KLRJ*, and *KLRH-like*. Variable numbers of *KLRC* and *KLRH* genes were found. Phylogenetic analyses identified presumed orthologs within the Felidae family. We also searched MHC class I sequences that could encode NKR ligands. No orthologs of non-classical class I genes were unambiguously identified. The detailed annotation of the LRC and NKC regions based on bioinformatic tools and resources is supported by our ongoing re-sequencing of selected genes (amplicons) from 32 individuals of 17 felid species including the domestic cat. This work was supported by the GA CR/FWF joint project GA CR 21–28637L/I5081-B.

Key Words: felidae, NK cell receptor genes, LRC, NKC, MHC

P80 ISAG Bursary Award: *PCYT2* missense variant in Saarloos Wolfhounds with neurodegeneration. M. Christen*[1], M. K. Hytönen[2], H. Lohi[2], A. Kehl[3], V. Jagannathan[1], and T. Leeb[1], [1]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [2]*Department of Medical and Clinical Genetics, University of Helsinki, and Folkhälsan Research Center, Helsinki, Finland,* [3]*Laboklin GmbH & Co. KG, Steubenstraße 4, Bad Kissingen, Germany.*

We investigated hereditary progressive neurodegeneration and retinal atrophy in Saarloos Wolfhounds. Affected dogs developed heterogeneous clinical signs characterized by blindness, ataxia, epileptic seizures and aggression toward owners. The age of onset for the first signs was between 6 mo and 4.5 years. Combined linkage analysis in one family and homozygosity mapping in 7 affected dogs delineated a 14.4 Mb critical interval. The comparison of whole genome sequence data of an affected dog to 958 control genomes revealed a private homozygous missense variant in the critical interval. It affected the *PCYT2* gene encoding the ethanolamine-phosphate cytidylyltransferase, which is important in the biosynthesis of phosphatidylethanolamine, a major phospholipid involved in normal development and function of the brain. The identified variant, XM_038546296.1:c.4A>G, is predicted to lead to a single amino acid change from isoleucine to valine at the very beginning of the encoded protein, XP_038402224.1:(p.Ile2Val). Genotypes at the variant were consistent with the monogenic autosomal recessive mode of inheritance in a complete family with 2 affected dogs. Apart from the 7 dogs used for homozygosity mapping, genotyping revealed 4 additional homozygous mutant dogs, which were of unknown phenotypes at the beginning of our investigation. A neurological phenotype was subsequently confirmed in 2 dogs. Further clinical characterization and follow-up are planned in the other 2 dogs, which were too young to show any clinical signs at the time of genotyping. *PCYT2* variants have previously been shown to cause autosomal recessive spastic paraplegia in humans. Homozygous knockout mice die during embryogenesis, suggesting that residual protein function is necessary for viability. Our data in dogs and the existing functional knowledge of *PCYT2* variants in other mammalian species suggest the identified variant as the potential candidate causative defect for the observed phenotype. Additional histopathological investigations to further characterize the phenotype are currently ongoing.

Key Words: dogs and related species, diagnostics, DNA sequencing, genetic disorder, nervous system

P81 Genomic resources for the domestic cat. L. Lyons*[1], G. Habacher[2], R. Malik[3], L. Coghill[4], and 99 Lives Cat Genome Sequencing Consortium[5], [1]*Department of Veterinary Medicine & Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO,* [2]*Raddenstiles Veterinary Surgery, CVS UK Ltd., Exmouth, UK,* [3]*Centre for Veterinary Education, The University of Sydney, Sydney, NSW, Australia,* [4]*Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, MO,* [5]*99 Lives Cat Genome Sequencing Consortium.*

The 99 Lives Cat Genome Sequencing Consortium develops genomic resources for the domestic cat and establishes working groups focusing on specific diseases, phenotypic traits, and genomic resources

and investigations. Recent discoveries of inherited diseases in cats have included vitamin D deficiency due to a *vitamin D receptor* variant, a cat with pycnodysostosis with a variant in *cathepsin K*, and a new form of muscular dystrophy caused by a *dystrophin* loss of function variant. Each of these affect cats represent novel, singular cases as efforts to conduct Precision Medicine in veterinary health care. Active working groups include focuses on hypertrophic cardiomyopathy, amyloidosis, genomic analyses, annotation, and wildcat sub-speciation. A long-read haploid-based phased reference assembly for the domestic cat (Fca126) is available to the research community. Based on trio binning of a F1 (Bengal) hybrid felid and because of the 6 to 8 million years of divergence between the domestic cat and Asian leopard cat parental species, this reference assembly is of telomere-to-telomere quality and is consider one of the best available for most any other mammalian species. This improved cat reference assembly is now being used to produce new genomic resources for the domestic cat as part of the 99 Lives Consortium. Using GATK best practices, the genome sequence data from approximately 666 domestic cats is currently being analyzed in comparison to the new genome assembly to develop a variant call file for the research community and to identify causal variants for diseases and phenotypic traits. All genomic data will be deposited in public data sites including the variants from the variant calling. The genome sequences and data will also be supporting groups interested in developing imputation panels and algorithms for the domestic cat. The Fca126 reference assembly has also been used to construct a new exome capture array, produced by Twist Biosciences as a commercial product and available in the spring of 2023. Combined, these resources feline genetics should support diverse research interests including disease and phenotypic studies, investigations of felid evolution, and studies on neoplastic disease.

Key Words: *Felis catus*, precision medicine

P82 ISAG Bursary Award: Genomic and transcriptomic characterisation of hypertrophic cardiomyopathy in British Shorthair and Birman cats. T. Smedley*, L. Wilkie, V. Fuentes, D. Connolly, and A. Psifidi, *Royal Veterinary College, London, United Kingdom.*

Hypertrophic cardiomyopathy (HCM) is the most common heritable heart disease in cats and humans. It affects ~0.2% of humans and ~15% of cats. HCM is defined by primary left-ventricular myocardial hypertrophy and in cats is associated with increased risk of congestive heart failure, aortic thromboembolism and sudden cardiac death. The prevalence of feline HCM and lack of treatments to modify the disease process demonstrates the importance of studies aiming to understand the genetic architecture and underlying mechanisms of HCM susceptibility. To date, there are limited feline HCM genomic studies and only 5 HCM-associated variants have been identified. Moreover, the transcriptomic signature in myocardium for feline HCM remains largely unknown. In this study, we focused on 2 predisposed breeds, British Shorthairs and Birmans. DNA was extracted using the Qiagen DNeasy Blood and Tissue kit from 167 individuals, 111 HCM and 56 Control (>9 years old) phenotyped for HCM using echocardiography. Samples were genotyped using the Illumina Feline 63K SNP array. Genome-wide association studies (GWAS) and Fst analyses within and across breeds were performed. Additionally, total RNA-sequencing has been generated from myocardial tissue from 28 individuals (16 cases and 12 controls). RNA was extracted using the Qiagen RNeasy Minikit. RNA-Seq reads after quality control were aligned to the reference genome using STAR and differentially expressed genes were identified using DESeq2. The GWAS revealed 3 SNPs located on chromosome C1 with suggestive significance associated with HCM in Birmans. Similarly, GWAS identified several SNPs located on chromosomes B1, C2, E2 with suggestive significance in BSHs. The across breeds GWAS revealed a genome-wide significant peak of SNPs associated with HCM on chromosome D4. Some of these SNPS were located close to potentially good candidate genes linked to myocardial function. Identifying risk-increasing genetic variants and relevant gene networks and pathways involved in HCM susceptibility could lead to development of novel DNA- diagnostic tests and drug-treatment targets.

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

Comparative and Functional Genomics

P83 Long-term effect of dietary antioxidants supplementation to pregnant sows on early ovarian functionality in gilt progeny. Y. Núñez*[1], G. Gómez[2], H. Laviano[3], F. García[1], M. Muñoz[1], J. García Casco[1], R. Benítez[1], F. Sánchez-Esquiliche[4], A. González-Bulnes[5], A. Rey[3], C. López-Bote[3], and C. Ovilo[1], [1]*Instituto Nacional Investigacion y Tecnologia Agraria y Alimentaria—Consejo Superior de Investigaciones Cientificas, Madrid, Spain,* [2]*Instituto Regional de Investigacion y Desarrollo Agroalimentario y Forestal, Oropesa, Toledo, Spain,* [3]*Facultad de Veterinaria, Universidad Complutense, Madrid, Spain,* [4]*Sanchez Romero Carvajal, Jabugo, Huelva, Spain,* [5]*Facultad de Medicina Veterinaria, Universidad Cardenal Herrera—CEU, Valencia, Spain.*

Early ovarian functionality and onset of puberty are important for gilt selection and has been related to piglet vitality and health at birth and early stages of growth. The supplementation of the diet of pregnant sows with antioxidants improves the function of the placenta, colostrum and milk composition and piglet health and weight. The objective of this work was to study the ovarian transcriptome of pre-pubertal gilts whose mothers were supplemented with different antioxidant agents during late gestation and lactation. Thirty pregnant Iberian sows were supplemented with antioxidants from gestation d 85th until weaning. Three experimental groups were made: control (C) (30 mg VE/kg); supplemented with vitamin E (VE) (100 mg VE/kg); supplemented with hydroxytyrosol (HT) (30 mg VE+1.5 mg HT/kg). When piglets were 110 ± 5 d old, 6 females from each experimental group (n = 18) were surgically castrated and ovarian tissue was obtained for RNaseq. Transcriptome analysis was carried out with HISAT, HTSeq-Count and DESeq2 software. Differentially expressed genes (DEG) were considered when $q < 0.1$ and Fold-change (FC) $\geq |1.2|$. We obtained scarce DEG (C *vs* HT, 6 DEGs and C *vs* VE, 5 DEGs) with one gene, *CDC42EP5*, upregulated in both antioxidant groups. With a exploratory focus, a functional analysis was done using a filtering with $P < 0.01$ and FC $\geq |1.2|$. Functional analysis of C *vs* HT comparison (152 genes) showed inhibition of functions related to nervous system development and function, organismal and tissue development in HT group. Functional analysis in C *vs* VE comparison (139 genes) showed activation in VE group of functions related to organismal development and lipid metabolism and inhibition of functions as perinatal death, hypoplasia or organismal death. Transcriptome differences between C and antioxidant groups (HT or VE) were small but results allow us to hypothesize that the treatment given to pregnant mothers influences the ovarian transcriptome of their daughters, with VE showing more positive effects on ovarian function and homeostasis than HT

Key Words: pig, transcriptome, ovary, antioxidants

P84 ISAG Bursary Award: Functional variants associated with male fertility in reproductive tissues of Brown Swiss bulls. X. Mapel*, N. Kadri, Q. He, A. Leonard, A. Lloret-Villas, and H. Pausch, *ETH Zürich, Zürich, Switzerland.*

Male fertility is a crucial component of the beef and dairy industries, yet the impact of genotypic variation on gene expression in reproductive tissues has barely been explored. Here we employ whole genome and total RNA sequencing to characterize variants that influence gene

expression in 3 male reproductive tissues. We sampled testis, epididymis, and vas deferens tissue from 117, 103, and 84 Brown Swiss (BS) bulls, respectively, and sought (i) to identify variants that effect gene expression and splicing in each tissue, (ii) explore similarities across tissues, and (iii) determine if these variants are associated with male fertility. We detected 21,847 expressed and 15,493 spliced genes across the 3 tissues and used over 21,000,000 sequence variants to conduct *cis* e/sQTL mapping. Testis had the most e/sQTL, with 15,642 eQTL in 11,164 genes (eGenes) and 11,450 sQTL in 7,000 genes (sGenes). We observed 4,768 eQTL (4,347 eGenes) and 3,165 sQTL (2,662 sGenes) in epididymis, and 4,211 eQTL (3,889 eGenes) and 1,920 sQTL (1,718 sGenes) in vas deferens. Gene expression, splicing, and e/sQTL effects were similar between epididymis and vas deferens. Testis had a unique functional profile; it contained over 1,000 tissue-specific expressed genes and 8,291 tissue-specific eQTL effects. We used PrediXcan to integrate expression and splicing phenotypes through transcriptome wide association studies (TWAS) with fertility phenotypes from 3,736 BS bulls and semen phenotypes from 902 BS bulls. We conducted genome wide association studies (GWAS) with these phenotypes and colocalized significant peaks with e/sQTL. TWAS revealed 14 genes associated with at least one fertility phenotype ($P < 1.0 \times 10^{-6}$), 5 of which had an e/sQTL that was colocalized with a GWAS peak (PP4 > 0.8). Our results validated previously described male fertility QTL (*WDR19*) and identified new possible causal genes (*KCTD19*). In conclusion, our results indicate that characterizing expression and splicing variation in male reproductive tissues may provide mechanistic insight into differential insemination success in bulls.

Key Words: functional genomics, system genetics (eQTL), cattle and related species, fertility

P85 Identification of consensus homozygous regions and their associations with growth and feed efficiency traits in American mink. P. Davoudi*[1], D. Ngoc Do[1], S. Colombo[1], B. Rathgeber[1], M. Sargolzaei[2,3], G. Plastow[4], Z. Wang[4], and Y. Miar[1], [1]*Department of Animal Science and Aquaculture, Dalhousie University, Truro, NS, Canada,* [2]*Department of Pathobiology, University of Guelph, Guelph, ON, Canada,* [3]*Select Sires Inc., Plain City, OH,* [4]*Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

The recent chromosome-based genome assembly followed by the newly developed 70K single nucleotide polymorphism (SNP) array in American mink (*Neogale vison*) facilitate the identification of genetic variants underlying complex traits in this species. The objective of this study was to evaluate the association between consensus runs of homozygosity (ROH) with growth and feed efficiency traits in American mink. A subsample of several mink populations (n = 2,986) were genotyped with the Affymetrix Mink 70K SNP array. After quality control, 49,268 SNPs remained for the association studies. The identified ROH were concatenated into consensus regions and the association studies were carried out with linear mixed models considering a genomic relationship matrix for 11 growth and feed efficiency traits. Statistical analyses used ASReml-R version 4. In total 298,313 ROH were identified across all individuals, with an average length and coverage of 4.16 Mb and 414.8 Mb, respectively. We detected 196 consensus ROH regions by merging ROH segments, these were used to perform the genome-wide ROH-phenotype association analysis. Thirteen regions were significantly ($P < 0.01$) associated with the traits studied. Several candidate genes within the significant regions are known to be related to growth and body size development, such as *MEF2A*, *ADAMTS17*, *PPP2R2B*, *POU3F2*, and *TYRO3*. In addition, we found 10 consensus ROH regions with frequencies over 80%. These regions contain 12 annotated genes, some of which were related to immune systems processes such as *DTX3L*, *PARP9*, *PARP14*, *CD86*, and *HCLS1*. This is the first study to explore the associations between homozygous regions with growth and feed efficiency traits in American mink. The findings of this study display the effects of homozygosity in the mink genome on growth and feed efficiency traits, that can be utilized in designing a sustainable breeding program for mink.

Key Words: homozygosity, genome-wide association, feed efficiency, candidate gene, animal breeding

P86 Single-cell RNA sequencing reveals thoracolumbar vertebra heterogeneity and rib-genesis in pigs. J. Li*[1], L. Wang[2], D. Yu[3], H. Xie[4], and Y. Zhang[5], [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, Yunnan, China,* [2]*Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [3]*State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing China,* [4]*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, Yunnan, China,* [5]*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, Yunnan, China.*

Development of thoracolumbar vertebra (TLV) and rib primordium (RP) is a common evolutionary feature across vertebrates, although whole-organism analysis of the expression dynamics of TLV- and RP-related genes has been lacking. Here, we investigated the single-cell transcriptome landscape of thoracic vertebra (TV), lumbar vertebra (LV), and RP cells from a pig embryo at 27 d post-fertilization (dpf) and identified 6 cell types with distinct gene expression signatures. In-depth dissection of the gene expression dynamics and RNA velocity revealed a coupled process of osteogenesis and angiogenesis during TLV and RP development. Further analysis of cell type-specific and strand-specific expression uncovered the extremely high level ofHOXA10 3′-UTR sequence specific to osteoblasts of LV cells, which may function as anti-HOXA10-antisense by counteracting the HOXA10-antisense effect to determine TLV transition. Thus, this work provides a valuable resource for understanding embryonic osteogenesis and angiogenesis underlying vertebrate TLV and RP development at the cell type-specific resolution, which serves as a comprehensive view on the transcriptional profile of animal embryo development.

Key Words: scRNA-seq, thoracolumbar vertebra transition, rib-genesis, osteogenesis, angiogenesis

P87 Online Mendelian Inheritance in Animals—Introducing OMIA variant IDs and the Vertebrate Breed Ontology. I. Tammen*[1], M. Mather[1], S. Toro[2], K. R. Mullen[2], H. M. Rondo[2], N. Vasilevsky[2], N. Matentzoglu[3], M. Haendel[2], Z.-L. Hu[4], C. A. Park[4], G. Leroy[5], and F. W. Nicholas[1], [1]*The University of Sydney, Sydney, NSW, Australia,* [2]*University of Colorado Anschutz Medical Campus, Aurora, CO,* [3]*Semanticly, Athens, Greece,* [4]*Department of Animal Science, Ames, IA,* [5]*Food and Agricultural Organization of the United Nations, Rome, Italy.*

Online Mendelian Inheritance in Animals (OMIA: https://omia.org/home/) is a freely available, curated knowledgebase which provides users with up-to-date information on inherited disorders and traits in animals. Entries are annotated with information on genetics, clinical and pathological signs, and links to related databases. OMIA is modeled on and reciprocally hyperlinked to Online Mendelian Inheritance in Man. It also provides links to PubMed and Gene records at NCBI and Ensembl. Recent links have been created to the Mondo Disease Ontology and the newly developed Vertebrate Breed Ontology (VBO). The need for standardised nomenclature for variants has been widely discussed to ensure greater transparency in relation to genetic testing. OMIA provides downloadable tables with more than 1400 likely causal variants presented using standardized nomenclature following Human Genome Variation Society (HGVS) recommendations. Numerical OMIA variant IDs have recently been added to all OMIA variant tables. An OMIA

variant ID provides a unique, unchanging ID for each likely causal variant annotated in OMIA, including those variants for which European Variation Archive IDs are lacking or HGVS nomenclature is complex or lengthy. Recognizing the need to replace OMIA's home-grown breed list with a computable, comprehensive list of standardised breed names for species of interest, we instigated the creation of the VBO (https://github.com/monarch-initiative/vertebrate-breed-ontology). VBO is based on the breed list of FAO's Domestic Animal Diversity Information System (DAD-IS) and has been updated (especially for cat and dog breeds) with information from other international organizations, communities, and experts. Breed information in OMIA phene-species entries and in variant tables has been mapped to the corresponding VBO term. Our vision for the future is that in addition to summarizing information about inherited conditions in animals, OMIA becomes a global repository for standardised information on likely causal variants for diseases to allow transparent delivery of genetic testing

Key Words: multispecies, databases, breed standardisation, polymorphism, monogenic trait

P88 Is a combination of biomarkers a good strategy to assign animals to stress categories when studying differences in transcriptomic profiles? C. Diaz*[1], J. Rosa[1], R. Peiro[1], C. Meneses[1], J. de la Fuente[2], C. Gonzalez-Verdejo[1], M. Ramon[3], and M. Carabaño[1], [1]*INIA-CSIC, Madrid, Spain,* [2]*UCM, Madrid, Spain,* [3]*CERSYRA-IRIAF, Valdepeñas, Spain.*

Animals along their production lifespan are affected by several stressors that compromise their welfare as well as their performances. Stress is a complex phenomenon and the use of a combination of biomarkers to characterize the stress level is often recommended. The objective of this study was to evaluate the impact of the level of physiological stress characterized by a combination of 7 biomarkers, in the detection of DE genes in muscles (*Psoas major* (PM) and *Flexor digitorum* (FD), liver (LI) and adrenal gland (AG) at slaughter. Seven biomarkers Albumin, Creatine phosphokinase (CPK), Cortisol, Globulin, Glucose, Lactate and LDH were measured in plasma of 80 Avileña-Negra Ibérica (ANI) male calves.ANI is a breed with a large variability in temperament which is expected to result in substantial stress response differences. Principal Components were applied on the correlation matrix between biomarkers and the first component was used to identify the 10 most (HS)/least (LS) stressed calves. Sequencing of stranded mRNA was performed to obtain 100bp long paired-end reads. Bovine Bos taurus ARS-UCD1.2 reference ensemble was used. Number of genes DE between HS and LS groups in LI, AG, PM and FD were 11, 5, 19, and 77 DE genes, respectively. In contrast with the small number of genes DE when comparing stress levels, the number of genes DE between muscles were 9374 (regardless of stress level). The number of DE genes between PM and FD varied with the level of stress, being larger in the HS group (7647 genes) than in the LS (5474 genes). Common DE genes to both stress levels were 4800, while 2847 were unique to the HS group and 674 to the LS. From this study we conclude that the use of combined biomarkers to identify stress level of animals may not be a sensible strategy when the aim is to detect genes DE under different stress levels and tissues. This strategy does not seem to provide enough power to capture differences in transcriptomic profiles but somehow it has an impact when searching for DE genes between muscles.

Key Words: functional genomics, animal welfare, cattle

P89 Withdrawn

P90 Withdrawn

P91 Phenotypic and genotypic identification of hard ticks (Acari: Ixodidae) species infesting cattle in South Africa. T. Makwarela*[1], N. Mapholi[1], T. Masebe[1], L. Nesengani[1], R. Smith[1], N. Nyangiwe[2], and D. Appolinaire[3], [1]*University of South Africa, Florida, Gauteng, South Africa,* [2]*Döhne Agricultural Development Institute,*

Stutterheim, Eastern Cape, South Africa, [3]University of Edinburgh, Edinburgh, UK.

The diversity and taxonomy of hard cattle tick species were assessed from farms covering the tropical wet and dry zone and the tropical semi-arid zone of South Africa. A total of 3218 ticks were collected from 21 farms (n = 9 Limpopo (LP), n = 1 Gauteng (GP), n = 1 Mpumalanga (MP), n = 4 KwaZulu Natal (KZN), n = 2 Free State (FS) and n = 4 Eastern Cape (EC) from 5 provinces of South Africa. A combination of tick morphology analysis and 16S rRNA gene sequences identified 12 tick species, namely *A. hebraeum, Haemaphysalis silacea, H. rufipes, H. truncatum, R. decoloratus, R. evertsi, R. exophthalmos, R. glabroscutatus, R. microplus, R. appendiculatus, R. sanguineus, and Ixodes pilosus.* This study showed how regional tick prevalence varies depending on climatic zones. different regions with similar climatic zones showed similar tick prevalence and similar species occurrence. The predominant species was *A. hebraeum* in Limpopo, KwaZulu Natal and Eastern Cape, and the infestation of *Haemaphysalis silacea,Ixodes pilosus, Rhipicephalus simus* was restricted to the Eastern Cape. The overall prevalence of tick infestation per province were as follows: LP (25%), MP (3%), GP (4%), KZN (46%), Free State (2%) and EC (20%). The identified species clustered into discrete clades, demonstrating the utility of rDNA genes for tick species identification using Maximum Likelihood. The phylogenetic analysis revealed a monophyletic group of genera *Amblyomma*, *Hyalomma*, *Haemaphysalis*, *Ixodes*, and *Rhipicephalus*. When analyzing intra- and interspecies K3P distances, most samples revealed that intraspecific distances ranged between 0 and 0.05. According to PCA analysis, all other genera clustered separately from one another except for genera *Haemaphysalis* and *Ixodes*, which clustered closely together. Additionally, high tick prevalence on livestock farms may be correlated with several risk factors, including grazing, lack of acaricidal treatment, traditional rural animal husbandry practices, and the scarcity of rural poultry.

Key Words: cattle, 16s rDNA, sequence, morphology, hard tick

P92 Phylogenetic analysis of bacterial tick-borne pathogen species found in South Africa. N. Mametja* and T. Masebe, *University of South Africa, Florida, Johannesburg, South Africa.*

Numerous tick-borne pathogens, including *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Ricketssia* spp., and *Theileria* spp., have been documented as significant pathogens of domestic ruminants, including cattle, goats, and sheep, and are transmitted by different types of ticks. Several AAA proteins have been documented to have a central role in reshaping cellular membranes among other functions. Methodology: This study aimed to use bioinformatics tools to analyze the AAA proteins protein from ticks-borne bacteria found prevalently in South Africa. 41 initial amino acid sequences of AAA domain were retrieved from NCBI database and only 22 qualified in this study and were analyzed for homology and phylogenetic relationships using MEGA11. A phylogenetic tree representing evolutionary relationships among the bacterial tick-borne species found in South Africa was drawn. Results: Results showed that the amino acid sequence lengths of AAA gene among the selected tick born bacterial pathogens differ, around 87–781 residues. *Borreliella burgdorferi* and *Coxiella burnetii* group together as an outlier. *Anaplasma marginale* group more closely together with *Rickettsia africae* than with *Borreliella burgdorferi* and *Coxiella burnetii*. The equality of evolutionary rate between sequences A (*WP_040933642.1 AAA family ATPase Coxiella burnetii*) and B (*WP_231014803.1 AAA family ATPase, Borreliella burgdorferi)* were used as an outgroup in Tajima's relative rate test to estimate the evolutionary molecular clock. Thus, confirming the outcome observed on the tree itself. Discussion: Implicitly, though the AAA gene sequences in the selected ticks differ in lengths physiochemical properties, GC content and motif. The result of this study revealed that *Anaplasma marginale* sequences are closely related to that of *Rickettsia africae* but distant to *Borreliella burgdorferi* and *Coxiella burnetii*. Therefore, results affirmed the hypothesis that the AAA protein of the bacterial tick-borne pathogens are somewhat related and share a common ancestor.

Key Words: tick borne, South Africa, AAA, phylogenetic, bioinformatics

P93 Explore of major adipogenic regulation factors and genes for pork belly parameters using the AWM-PCIT network analysis. J.-M. Kim*, *Department of Animal Science and Technology, Chung-Ang University, Gyeonggi-do, Anseong-si, Republic of Korea.*

In Korea and many of Asian countries, pork belly, which is called *Samgyeopsal* in Korean, is the most favorable parts in pork. However, pig-breeding has largely focused on improving *longissimus thoracis*, representing the lean-meat production capacity, and the quality of pork belly has not improved because of the absence of established standards. Previous studies have suggested a standard for the establishment of such standards and relevant genetic parameters, but no key gene has been identified yet. Here, we predicted key genes, including transcription factors, related to pork belly formation. We analyzed 543 Yorkshire pigs and their SNP chip data and identified 3,238 genes and 135,025 edges related to pork belly traits by using the association weight matrix. Based on these results, in silico validation was performed. The predicted 9 transcription factors, including the key trio, were enriched in adipogenesis-related signaling pathways. The transcription factors and corresponding genetic network identified in this study may be useful in improving pork belly parameters.

Key Words: pork belly, GWAS, association weight matrix

P94 Genetic resources and biodiversity biobanks: A win-win situation. K. Labuschagne*, *South African National Biodiversity Institute, Pretoria, Gauteng, South Africa.*

Permit nightmares. Nagoya considerations. Access and Benefit Sharing. Import. Export. All terrifying words to the modern-day researcher trying to conduct genetic-level research. However, this is where well managed collections of genetic resources are able to assist. Biodiversity Biobanks in South Africa have been around for 20+ years and include wildlife tissues and DNA extracts (2), fungal cultures (2), yeast cultures (1), bacterial cultures (3), indigenous plant extracts (1), herpetology tissues for DNA studies (1), aquatic biodiversity tissues and DNA extracts (1), and various agrobiodiversity biobanks including a seed bank holding local crops and crop wild relatives, and an indigenous sheep and goat breed biobank. In total, over 500 000 samples are held in these biodiversity collections. Recently, the Biodiversity Biobanks South Africa project funded by the National Department of Science & Innovation was launched, and this is to ensure the longevity of these resources. These core facilities include collections from government departments and universities among many others. The South African National Biodiversity Institute (SANBI) Biobank is just one of these collections dedicated to the long-term curation of wildlife biomaterials. South Africa is considered one of the megadiversity countries, with high levels of species richness and endemism. With more species being placed on the International Union for Conservation of Nature (IUCN) Red List, the importance of collecting and storing biomaterials from this natural heritage is critical. The SANBI Biobank currently curates approximately 200 000+ samples from more than 85 000 individual animals, representing over 1 000 species. This collection ensures access for research and technological applications, and the platform supports project and data management and coordination including permit applications, optimal sample storage temperatures, sampling protocols, as well as becoming a BSL2 extraction facility to assist with animal disease restrictions, and setting up the need for linking digital sequence information (DSI) to samples accessed from the collection.

Key Words: genetic, resources, biobank, collection, biodiversity

P95 Global phylogeography and population genomics of the commercially exploited smoothhound shark, *Mustelus mustelus*. J. C. Winn*[1], A. E. Bester-Van der Merwe[1], and S. N. Maduna[2], [1]*Stellenbosch University, Stellenbosch, Western Cape, South Africa,* [2]*Norwegian Institute of Bioeconomy Research, Svanhovd Research Station, Svanvik, Norway.*

Accelerated global warming and over-exploitation of marine species threaten to eradicate the unique genetic archives of modern Chondrichthyans (sharks, rays, and chimeras), an evolutionarily robust lineage with a 400-million-year genealogy. Understanding the impact of rapidly changing global conditions on elasmobranchs (sharks and rays) and devising conservation responses relies on the identification of unique genetic populations adapted to specific environmental niches. This research focuses on delineating the global phylogeography of the common smoothhound shark, *Mustelus mustelus,* using whole mitogenome data from 10 sampling populations collected across the species' distribution range. The same samples have been used to generate reduced-representation sequencing (RRS) (RADSeq) data to reveal associations between single nucleotide polymorphism (SNP) alleles and environmental variables hypothesized to drive selection while controlling for the confounding effects of neutral genetic structure. It is predicted that species inhabiting the rapidly warming, yet diverse, biogeographic regions of South Africa and the Mediterranean Sea are likely locally adapted to their environment which can lead to differences in genetic variation among populations even in the face of high gene flow. The eventual goal is to better characterize the genomic mechanisms of environmental adaptation and provide a system for predicting responses to new selective challenges in this species and exploited houndsharks. We have submitted a manuscript to the Journal of Genomics which characterizes 5 newly assembled houndshark mitochondrial genomes and remaps the Carcharhiniform phylogeny using a rigorous, statistically guided pipeline to uncover the root of the discordance between phylogenies for this order.

Key Words: comparative mitogenomics, convergent evolution, multi-species coalescence, positive selection, environmental adaptation

P96 ISAG Bursary Award: Ribosome profiling reveals stage-specific translational regulation during muscle differentiation. A. Goldkamp*[1], L. Okamoto[2], K. Thornton[2], and D. Hagen[1], [1]*Oklahoma State University, Stillwater, OK,* [2]*Utah State University, Logan, UT.*

Myogenesis is an essential process, in which skeletal muscle fibers are formed. In most mammalian species, this process largely occurs prenatally as the number of muscle fibers is fixed close to birth. However, the differentiation of myogenic cells is crucial to support hypertrophy of skeletal muscle, in which the size of muscle fibers is increased postnatally resulting in enhanced muscle volume and mass. C2C12 cell lines are a widely-used model for studying muscle myogenesis. Transcriptional changes during myogenesis have been well defined and differentially expressed transcripts throughout muscle development have been identified, yet transcriptome profiling often overlooks post-transcriptional events contributing to a phenotype. Thus, advancements in sequencing technologies have recently permitted the study of the translatome, which refers to the entire population of mRNA associated with ribosomes for protein synthesis and can be investigated through ribosome profiling. As skeletal muscle ultimately becomes meat in livestock animals, a deeper understanding of the regulatory factors involved in skeletal muscle growth is needed. In this study, we performed genome-wide ribosome profiling coupled with RNA sequencing in C2C12 cells in a temporal manner (0 h, 30 min, 1 h and 4 h) post-induction of differentiation. Through ribosome profiling, we have characterized key transcripts regulated at transcriptional and/or translational levels throughout myogenesis. In addition, we have identified codons contributing to stalling events across each development stage, which may be governed by alterations in tRNA expression. Overall, this work offers a snapshot of all active ribosomes at each time point of muscle development and can aid in our understanding of gene regulation in one of the most important economic traits in livestock, skeletal muscle.

Key Words: growth, muscle, myogenesis, differentiation

P97 ISAG Bursary Award: Adipose gene expression profiles of four cattle breeds highlight selective pressures and tissue functions. D. Ruvinskiy*[1], K. Pokharel[1], A. Amaral[2], M. Weldenegodguad[1], M. Honkatukia[1,3], H. Lindberg[1], J. Peippo[1,3], P. Soppela[4], P. Uimari[5], C. Ginja[6], and J. Kantanen[1], [1]*Natural Resources Institute Finland (Luke), Jokioinen, Finland,* [2]*CIISA—Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal,* [3]*Nordic Genetic Resources Center, Ås, Norway,* [4]*Arctic Centre, University of Lapland, Rovaniemi, Finland,* [5]*Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland,* [6]*BIOPOLIS-CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal.*

Less functional genomic characterization of cattle breeds has been applied in studies focusing on population genomics, adaptation genomics and cattle genetic resources. Adipose tissues are crucial in regulating metabolism and energy balance with their ability to re-structure based on external changes. We have sequenced RNA samples extracted from metacarpal, perirenal, tailhead and prescapular adipose tissues of 81 individuals of Yakutian cattle (Sakha Republic), Northern Finncattle (Finland), Mirandesa (Portugal), and the commercial Holstein breed, and compared differentially expressed genes (DEGs) between tissues, breeds, and sexes. In total, 20 714 genes were expressed in our data, and we found the highest number of tissue-specific expressed genes in the metacarpal adipose tissue (672). The gene with the highest mean abundance in the metacarpal adipose tissue was *HOXD13* associated with adipogenesis. Moreover, the Principal Component Analysis of normalized expression profiles showed a separation of the metacarpal adipose tissue from the others. In breed comparisons, some upregulated genes in in Yakutian cattle are associated with energy metabolism and response to cold temperatures (*NR4A3, TEKT3*, and *FGGY)*. In the Mirandesa cattle breed, the upregulated genes are related to immune response and inflammation (*AVPR2, CCN1*, and *IL6*), while in the Northern Finncattle the upregulated genes appear to be involved in various physiological processes, including energy metabolism (*IGFBP2*). In the sex-based comparisons, *TPRG1* was upregulatedin 3 tissues in Yakutian cattle females, suggesting adaptation related to feed efficiency. Most DEGs were found between Yakutian cattle vs Holstein and several DEGs associated with immunity were upregulated in Yakutian cattle indicating potential differences in disease resistance and immunity between the 2 breeds. This study shows the vast difference in gene expression profiles in adipose tissues between breeds from different environments, most likely highlighting selective pressure and the potential significance of metacarpal adipose tissue in regulatory functions.

Key Words: cattle and related species, adaptation

P98 Withdrawn

P99 Chromosome conformation comparison in Piedmontese × Gaur F1 fetal muscle tissue. M. R. Stegemiller*[1], K. L. Kuhn[2], T. P. Smith[2], B. D. Rosen[3], and B. M. Murdoch[1], [1]*Department of Animal, Veterinary, and Food Sciences, University of Idaho Moscow, ID,* [2]*USDA, ARS, US Meat Animal Research Center, Clay Center, NE,* [3]*USDA, ARS, Animal Genomics and Improvement Laboratory, Beltsville, MD.*

The 3-dimensional (3D) genome organization within the nucleus is vital for correct DNA interactions. The hierarchal 3D organization of the genome is comprised of chromosome territories, compartments, topologically associated domains (TADs), and loops. There are 2 chromosome compartments, compartment A represents open genomic regions and compartment B are closed or inactive regions of the genome. Chromatin conformation has been shown to be tissue specific and previous studies demonstrated as high as 80% conserved conformation in species including chicken, goats, and pigs, although little 3D organization research has been completed in cattle and very little information about haplotype-specific organization has been produced in any species. The present study uses a fetal F1 cross (*Bos taurus* × *Bos gaurus*) harvested at 120 d gestation with muscle tissue subjected a variation of Hi-C called Micro-C, which uses a micrococcal nuclease to identify nucleosome positioning. The interspecies nature of the cross supports improved resolution of haplotype specific variation within a single tissue type. Compartments and TADs at 50kb resolution were identified independently for each parental haplotype by mapping sequence reads separately to "complete" telomere-to-telomere genome assemblies of each haplotype generated using trio binning approach with parental data to separate haplotypes. Comparison of 3D organization between the species shows 41 locations organized in opposite compartments in the 2 haplotypes, for example on chromosome 4 between 45 and 47.5Mb the cattle haplotype is in the B compartment while the corresponding region of the gaur haplotype is in the A. There were 1613 TADs identified in the Piedmontese but only 1556 in the Gaur haplotype, and differences in TAD conformation in the chromosome 4 region of compartment switch were observed. Defining compartment determination and 3D conformation during fetal development can show parts of the genome that are interacting, and which segments of DNA are active and identification of regions that diverge within the Bovidae family could indicate regions of biological significance between the species.

Key Words: Micro-C, 3D chromosome conformation

P100 Exploring tissue-specificity in the regulatory landscape of bovine genome. G. Costa Monteiro Moreira*[1], C. Yuan[1], S. Dupont[1], L. Tang[1], Y. Lee[1], D. Becker[2], M. Salavati[3], R. Clark[4], E. Clark[3], G. Plastow[5], C. Kühn[2,6], C. Charlier[1], and BovReg consortium[7], [1]*Unit of Animal Genomics, GIGA Institute, University of Liège, Liège, Belgium,* [2]*Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany,* [3]*The Roslin Institute, University of Edinburgh, Edinburgh, UK,* [4]*Genetics Core, Edinburgh Clinical Research Facility, The University of Edinburgh, Edinburgh, UK,* [5]*Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada,* [6]*Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [7]*https://www.bovreg.eu/project/consortium/.*

Transcriptomic (mRNA-, totalRNA- and small-RNA-Seq), ATAC- and ChIP-Seq assays were compiled in a catalog of 129 tissue samples collected from 6 individuals of both sexes, different ages, kept in different environments and from 3 divergent dairy and beef cattle breeds/crosses: Holstein, Kinsella composite and Charolais × Holstein F2 crossbred. From the de novo transcriptome assembly, 43,117 gene models including ≥ 15k potentially novel transcripts were assembled; BovReg expanded the catalog of bovine non-coding RNAs by including non-polyadenylated transcripts (totalRNA assay). Long-read mRNA sequencing (ONT-Seq) is being performed to support predicted isoforms. 1,265 (638 known and 627 novel) miRNAs were detected and, for ≥ 90%, potential primary transcripts (pri-miRNA) were identified. Interestingly, ~39.71% of the novel miRNAs overlapped with repeats with a strong enrichment for an ancient DNA transposon (Mariner). On average, 105,245 (ATAC), 28,187 (H3K4me3), 152,646 (H3K4me1), 127,855 (H3K27me3), 77,967 (H3K27ac) and 71,868 (CTCF) peaks (q-value ≥ 0.05) per sample were annotated. Investigating open chromatin regions in the same tissue across the different ages/environment/breeds, differentially accessible regions were identified. By applying nonnegative matrix factorization on regulatory elements *x* tissue samples, we detected tissue and/or organ-system (muscle, digestive system, etc.) specific components. Using WGS (>30*X*) from the 6 individuals, ~8.5k CNV and ~400 polymorphic long-terminal repeat transposons were annotated; we highlighted candidates potentially affecting gene expression based on their co-localization with regulatory elements. Tissue-specific unannotated genes, miRNAs/pri-miRNA and regulatory elements were detected contributing to the understanding of vital body functions in bovine. The results presented herein represent a substantial improvement on the regulatory landscape annotation in bovine. The BovReg project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 815668.

Key Words: cattle and related species, functional annotation of animal genomes (FAANG), functional assay, regulatory element

P101 A multi-omic approach to understanding genetic and phenotypic variation in mass reared black soldier flies (*Hermetia illucens*). C. Rhode*, K. Hull, and M. Greenwood, *Stellenbosch University, Stellenbosch, Western Cape, South Africa.*

The need for renewable, sustainable and environmentally friendly animal protein production has been intensified by a growing human population, adverse effects of climate change and diminishing natural resources. Black Soldier Fly (BSF) farming has been proposed as an alternative livestock production system that may meet the challenges for future food security, with low resource requirements, higher feed conversion ratios and similar nutritional value as conventional animal protein. BSF has also proven highly advantageous due to the dual potential of the larvae to act as a bioremedial agent, converting organic waste into useable biomass, creating a circular agricultural production system. Despite the industrial scale of BSF mass rearing, little is known about the drivers of genetic and phenotypic variation under these production conditions. This study, therefore used a muti-omic approach to assess the interplay between organismal genetics, functional genomics,

the microbiome and feed-substrate on phenotypic development in BSF larvae. The population genomic assessment revealed that genetic drift is the major evolutionary force shaping genomic diversity, even in the presence of direct artificial selection for production traits. Additionally, few loci were significantly associated with these production traits, further illustrating the influence of stochastic evolutionary processes during the mass rearing period. The effects of selection on gene expression were also weak and differential transcriptomic profiles highlighted functional trade-offs between growth metabolism and immune function. Metagenomic analysis found significant associations been bacterial taxa and protein-fat ratios in BSF, and that both feed-substrate and the interaction between feed- and host genetics played a significant role in the composition of larval gut microbiomes. The findings highlight the multidimensional and complex nature of BSF production and its impact on phenotypic development, with applications for future genetic management and improvement strategies for enhanced production.

Key Words: functional genomics, GWAS, insect farming, microbiome, population genomics

P102 ISAG Bursary Award: DNA methylation dynamics regulating embryonic development in pig. J. de Vos*[1], M. Derks[1], H. Acloque[2], S. Djebali[3], S. Foissac[4], C. Guyomar[4], C. Kurylo[4], E. Giuffra[2], M. Groenen[1], and O. Madsen[1], [1]*Animal Breeding and Genomics, Wageningen University, Wageningen, the Netherland,* [2]*Paris-Saclay University, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [3]*IRSD, Université de Toulouse, INSERM, INRA, ENVT, UPS, Toulouse, France,* [4]*GenPhySE, Université de Toulouse, INRAE, ENVT, Toulouse, France.*

A main aim of the Horizon 2020 GENE-SWitCH project (grant agreement n° 817998) is to generate exhaustive functional genomic annotations for several key tissues in both pigs and chickens across development. DNA methylation is an epigenetic modification which plays a crucial role in mammalian development, however there is still limited understanding of the overarching dynamics in the developing fetus. In this research we used both whole genome- and reduced representation bisulphite sequencing (WGBS and RRBS) data to evaluate the methylome dynamics during development at 30 d post fertilization (dpf), 70dpf, and new born (NB) of 7 tissues (liver, kidney, brain, muscle, skin, small intestine and lung) in the pig. Developmental transitions were investigated using a 2-fold approach: 1) Dividing the methylome into unmethylated regions (UMR), indicative of promoters, and lowly methylated regions (LMR) indicating enhancers, and 2) performing differential methylation analyses. The number of UMRs across developmental stages within the various tissues ranged from 10,822 to 14,680 and from 35,073 to 75,477 for LMRs. The defined methylation states (UMRs, LMRs and fully methylated regions) were used to define the dynamic changes of the methylome and cis-regulatory elements during embryological development. The most notable finding was the shift of methylation states, from hypo to hyper methylation, in liver 70dpf to NB. Lastly, differentially methylated regions were integrated and combined with differentially expressed genes from the same samples. The combined differentially methylated and expressed genes were involved in biological pathways related with general growth, e.g., regulation of developmental processes, during initial stages of development (30dpf to 70dpf), and with tissue specific functions during maturation transition (70dpf to NB). This trend was observed for most tissues, except in liver and brain which showed tissue specific functions during early developmental stages like e.g., neurogenesis in brain.

Key Words: functional annotation of animal genomes (FAANG), integrative genomics, development, DNA methylation, epigenomics

P103 Genomic and functional characterization of frequently used bovine cell lines. D. Becker*[1], G. C. M. Moreira[2], C. Mörke[1], M. Charles[3], F. Hadlich[1], C. Lopez-Roques[10], M. Schmicke[4], V. Blanchet[5], H. Taniguchi[6], E. Clark[7], C. Pfarrer[8], J. Vanselow[1], C. Charlier[2], D. Rocha[3], and C. Kuehn[1,11], [1]*Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [2]*Unit of Animal Genomics, GIGA, Liege, Belgium,* [3]*INRAE, Jouy-en-Josas, France,* [4]*Veterinary Endocrinology and Laboratory Diagnostics, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany,* [5]*Unité de Génétique Moléculaire Animale (UGMA), University of Limoges, Limoges, France,* [6]*Institute of Genetics & Animal Biotechnology, Polish Academy of Sciences, Magdalenka, Poland,* [7]*The Roslin Institute, Edinburgh, UK,* [8]*Institute of Anatomy, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany,* [9]*Agricultural and Environmental Faculty, University Rostock, Rostock, Germany,* [10]*INRAE, US 1426, GeT-PlaGe, Genotoul, Castanet-Tolosan, France,* [11]*Agricultural and Environmental Faculty, University Rostock, Rostock, Germany.*

There is a strong demand for fully characterized cell lines from farmed animal species e.g., for functional genome, physiology or veterinary medicine. Particularly for validating potentially regulatory variants in non-coding regions of the genome, thoroughly described cell lines are essential for target tissues. However, while cell lines are heavily used as in vitro surrogates for in vivo experiments, most of them lack a comprehensive functional annotation of active genomic regions as well as a catalog of genetic variants. In our project, 4 cell lines frequently used in bovine research were characterized at functional and structural level: EBL (embryonic lung cell line), F3 (generated from bovine trophoblast cells), MAC-T (mammary gland epithelial cell line) and MDBK (kidney cell line). All cell lines were monitored for whole transcriptome (mRNA, totalRNA, miRNA) and for their regulatory genomic landscape by epigenomic profiling of open chromatin (ATAC-seq) and histone modifications (chromatin marks H3K4me3, H3K4me1, H3K27me3, H3K27ac) and CCCTC- binding factor (CTCF) binding sites via ChIP-seq. Regarding the whole transcriptome level, we further explored the effect of different passages and differences between diverse sources of the cell lines. At genomic level, the cell lines were subjected to whole genome short-read sequencing at high coverage (40–81 X). We found on average 6.54 million single nucleotide polymorphisms, and 1.15 million small insertions/deletions were discovered. Comparing the transcriptomic profile of the cell lines to their tissue counterparts revealed profound differences e.g., in genes related to energy metabolisms in the MDBK cells or protein synthesis in MAC-T cells. Furthermore, we observed a substantial variation of the transcriptome associated with passage number and source of cell clones. An increased awareness of particular cell line-specific limitations is recommended when interpreting in vitro cell line experiments. This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 815668.

Key Words: cattle, cell line, transcriptome, epigenome, genetic variant

P104 ISAG Bursary Award: Single cell atlas of developing ovine tail tissue reveals multi cellular origins contributing to fat deposition. J. Han*[1,2], [1]*Institute of Animal Science, Chinese Academy of Agriculture Science, Beijing, China,* [2]*School of Agriculture and Food Science, University College Dublin, Dublin, Ireland.*

Fat-tail sheep exhibit a unique trait whereby substantial adipose tissue accumulates in the tail, a phenotype that is advantageous in many agroecological environments. However, the genetic factors underpinning this phenotype are still not clear. Previous studies indicated that developing tail tissue is an ideal biomaterial to study adipogenesis and fat metabolism. Therefore, we collected embryonic tail tissues at 9 time points covering the stages before and after fat deposition to perform single cell transcriptome sequencing and single cell assay for transposase-accessible chromatin sequencing. Based on the expression of canonical markers, all cells obtained in 2 assays were assigned as 23 cell types, including progenitor cells with 10 subpopulations (progenitor and stem cell, PSC; connective tissue progenitor, CTP; myogenic progenitor), precursors (e.g., preadipocyte and preosteoblast), terminally differentiated cells (e.g., adipocyte, osteoblast, vascular smooth muscle cell (VSMC)) and several other cell types, suggesting high heterogeneity within tail tissue. Furthermore, we evaluated the specificity of markers identified by differently expressed genes analysis, which were divided into 3 categories, strong marker (A+), general marker (A) and

highly expressed gene (A-), and the A+ markers were mainly identified in terminally differentiated cells, such as *TMEM120A*, *FASN* and *CYB5A* for adipocytes. We constructed the differentiation trajectories for all lineages, including adipogenic, myogenic, osteogenic, chondrogenic lineage and VSMC generation, importantly, cellular origins for preadipocyte have been identified, including one subset of PSC, one subset CTP and VSMC. Multi origins of adipocyte would be one vital reason that results in quick and massive fat deposition within ovine fat tail tissue. In our following work, we will focus on the cellular interaction across cell to reveal the influence of microenvironment on adipogenesis, and integrating the results of 2 assays to construct gene regulation networks governing fat deposition.

Key Words: single cell sequencing, adipogenesis, developmental biology, differentiation trajectory

P105 A multi-tissue porcine single-cell immune atlas: Resources for comparative and systems immunology. C. Tuggle*[1,2], L. Daharsh[1], M. Kapoor[1,2], P. BK[2], S. Sivasankaran[3], K. Byrne[3], J. Herrera-Uribe[1], and C. Loving[3], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Bioinformatics and Computation Biology, Iowa State University, Ames, IA,* [3]*USDA-Agriculture Research Service, National Animal Disease Center, Food Safety and Enteric Pathogens Research Unit, Ames, IA.*

A single cell-level understanding of the porcine immune system will provide tools for improving both disease resistance and use of the pig in biomedical modeling. We performed scRNA-seq on bone marrow, lymph node, spleen, and thymus from healthy adult pigs. After quality control to remove duplicate cells and cells with high mitochondrial content, 50,559 cells and 18,673 genes were used for downstream analysis. Each tissue was individually mapped using nonlinear dimensional reduction, and distinct clusters were found and analyzed using the following computational tools: IKAP, ROGUE, pair-wise differential gene expression testing, and random forest models. Using a combination of model defined and canonical immune cell markers, we were able to annotate each cluster as part of a diverse set of immune cell types identified. We validated these annotations using publicly available human scRNA-seq data sets specific for each corresponding porcine immune tissue. We used our published porcine PBMC scRNA-seq data set to predict cells that may be tissue-resident or match circulating cells, and integrated all of the immune tissue data to compare all cell types across the 4 tissues, to identify shared and tissue specific cell types. Finally, we created an on-line visualization tool for users to explore expression of individual genes and the annotation of clusters in each tissue as well as combined. We are currently using these data and SCENIC software to create a first-generation regulatory network across all tissues. These studies of immune tissues will be an important resource for improved annotation of porcine immune genes and cell types, including providing information for development of new reagents and as a tool for systems approaches in porcine immunity. Further, these data can inform human translational biomedical research using pigs as a biomedical model.

Key Words: scRNAseq, porcine, immune tissue, atlas

P106 Withdrawn

Comparative MHC Genetics: Populations and Polymorphism

P107 Successful reduction of proviral load by a novel bovine leukemia virus vaccine targeting cattle carrying susceptible bovine leukocyte antigen (BoLA)-*DRB3* allele. Y. Aida*[1,2], S.-N. Takeshima[2,3], L. Bai[2,4], J. Kim[2], Y. Matsumoto[2], R. Matsuura[1,2], and J. Kohara[5], [1]*Laboratory of Global Infectious Diseases Control Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan,* [2]*Viral Infectious Diseases Unit, RIKEN, Saitama, Japan,* [3]*Department of Food and Nutrition, Jumonji University, Saitama, Japan,* [4]*Graduate School of Science and Engineering, Iwate University, Iwate, Japan,* [5]*Animal Health Group, Animal Research Center, Hokkaido Research Organization, Hokkaido, Japan.*

Bovine leukemia virus (BLV), the etiological agent of the enzootic bovine leucosis, spread worldwide. Previously study demonstrated that the cattle with bovine major histocompatibility complex (*BoLA)-DRB3*016:01/*016:01* genotype tended to develop lymphoma and high BLV proviral load. One of problems to develop BLV vaccine is unstable effect of the vaccine due to the individual difference for disease susceptibility. We here tried to develop the BLV vaccine for susceptible cattle with *BoLA-DRB3*016:01/*016:01* genotype which are difficult to create vaccines and evaluated its vaccine effect in cattle. First, we determined the Th1 epitope in BLV against susceptible cattle using a total 118 peptides corresponding to GAG p15, p24 and p12, and ENV gp51 and gp30. Next, we optimized our determined Th1 epitopes by in silico peptide modeling to improve low affinity binding between peptide and susceptible BoLA DR molecule. To 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 subcutaneous and intradermal vaccination of p12-4R1/gp51R1 peptides-conjugated CO_3Ap. Finally, to demonstrate effect of this vaccine in cattle, we produced 6 susceptible cattle by fertilized ovum transplantation technique. All of 6 animals were infected by BLV and intradermally immunized 3 times with either vaccine or PBS as a control at 2 weeks post infection. The proviral load, lymphocyte count, and antibody titer were measured for 119–161 d. Among 6 BLV-infected susceptibility cattle, vaccinated cattle (n =

3) significantly decreased the level of proviral load, lymphocyte count, and CD5^{+}B cell count, compared with PBS control cattle (n = 3). This vaccine successfully suppresses proviral load in susceptible cattle.

Key Words: *BoLA-DRB3*, bovine leukemia virus, susceptible, vaccine

P108 MHC haplotype diversity in the main equine breeds of the Iberian Peninsula. M. García-Martínez[1], A. Cequier[1,2], E. Bernad[1], B. Serrano[1], A. Romero[1,2], F. Vázquez[1,2], A. Vitoria[1,2], S. Fuente[1,2], C. Cons[1], C. Rodellar*[1], and L. Barrachina[1,2], [1]*Laboratorio de Genética Bioquímica LAGENBIO-Instituto Agroalimentario de Aragón-IA2 (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.*

Major histocompatibility complex (MHC) genes are related with immune functions and can reflect genetic diversity, but furthermore, their study can have applications for cell therapy. The immunogenicity of mesenchymal stem cells (MSC) is key for safe and effective allogenic treatments and can vary upon the degree of MHC matching between donor and patient. Thus, establishing the most common equine MHC haplotypes provides critical information for biobanks aiming at providing allogenic cells for therapy in horses. The goal of this study was to enlarge our current knowledge on equine MHC diversity, focusing on Purebred Spanish (PRE), Purebred Arabian (PRá), Hispano-Arabian (Há), and Lusitano (PSL), as the most common breeds in the Iberian Peninsula. Using a validated panel of 10 microsatellite markers, MHC haplotypes were determined in related and/or homozygous animals, and haplotype frequencies were calculated in unrelated animals. In PRE horses (n = 110 unrelated animals) the most common haplotype was HapPRE10 (20.45%), followed by HapPRE11 (9.54%) and HapPRE13 (7.72%). Haplotype frequencies in PRá, Há, PSL have not been calculated due to the limited number of unrelated horses currently available. Nevertheless, preliminary results showed that the most common haplotypes were HapPRA02 in PRá, HapPRA09 in Há, and HapPRE06 in PSL. Provided the preference for homozygotes as MSC donors, we found 12 homozygous animals in the whole PRE population analyzed (n = 268): 7 for HapPRE10, 2 for HapPRE11. From the total PRá (n = 34), Há (n = 40), and PSL (n = 16) horses studied, only 2 PRá homozygotes were found (COR42 and HapPRA02). Finally, allelic frequencies showed 6–15 alleles with no marked predominance except for region UMNJH-38, where around 60% of animals of all breeds shared the allele 156. Moreover, one new allele (208) in ABGe9030 region was found in 5 horses (4 PRE, 1PSL) shared in all of them HapPRE36 haplotype. Although the sample needs to be enlarged, this study provides relevant information for MHC diversity in equine breeds in the Iberian Peninsula, which can have applications in genetic diversity and cell-based therapies in horses.

Key Words: major histocompatibility complex (MHC), horses and related species, haplotype, microsatellite, immunology

Domestic Animal Sequencing and Annotation

P109 ISAG Bursary Award: Comparative genomics reveals common diversity and signature of selection in Saudi Arabian indigenous chickens. A. Assiri*[1,2], [1]*University Of Nottingham, Nottingham, United Kingdom,* [2]*King Faisal University, Al-Hufuf, Saudi Arabia.*

Indigenous chickens possess unique adaptations to harsh conditions such as high temperatures and cold challenges. In the Arabian Peninsula, local chickens are mostly found outdoors, being reared by sheep and camel herders for secondary production purposes. These birds show high resilience to extreme temperatures (hot, cold), typical to the desert environment of the country. Here, we aimed to investigate the genome diversity and to identify candidate genome regions and candidate genes that show strong evidence of positive selection for thermotolerance in 15 indigenous chicken populations. A total of 156 chickens grouped in 15 populations were investigated: 5 local Saudi chicken populations and 10 other populations that are known to adapt to the harsh environment were mapped to the chicken reference genome *Gallus gallus* (GRCg6a). Data from SNPs were generated following the GATK best practices protocol restricted to bi-allelic sites. All populations were inferred with principal component analysis and the estimation of the proportion of admixture ancestry. To detect genomic signatures to cold and hot adaptation, we performed *FST* and *Hp* analysis. The results of genetic structure analysis reveal the clustering of all populations with their geographic region of origin. In all populations, 24,906,132 SNPs were detected. The average nucleotide diversity for the 15 populations is about 0.0021. About 38% of the bi-allelic SNPs are novel (dbSNP release 107(2022). Among all the studied populations, the highest level of linkage disequilibrium (~0.30) is observed in Chantecler and Fayoumi, at marker pairs distance of 1 kb, while the lowest is recorded in both Omani and Black populations (~0.10). PC1 clearly separates the Ethiopian populations from the Chinese and Arabian Peninsula populations, while PC2 separates Arabian Peninsula populations from the Chantecler, as well as the Ethiopian population from Dulug and Chantecler. A total of 95 candidate regions and genes were detected by ZHp analysis in both Black and Brown, the majority in common. Also, 37 candidate regions were detected by ZFst of Black vs. Brown.

Key Words: Saudi local chicken, signature of selection, adaptation

P110 AgriSeqSV: A solution to genotype structural variants on AgriSeq™. K. R. Gujjula[1], A. Burrell[1], S. Daly[2], M. Lelievre[2], and S. Chadaram*[1], [1]*Thermo Fisher Scientific, Austin, TX,* [2]*Thermo Fisher Scientific, Lissieu, Lyon, France.*

AgriSeq targeted GBS is being used as a high-throughput and cost-effective genotyping solution in various applications from animal and plant breeding studies to parentage testing and genetic screening. One of the many features of this technology is supporting different type of markers including single nucleotide polymorphisms (SNPs), multiple nucleotide polymorphisms (MNPs), insertions and deletions (InDels), long insertions and deletions (LongIndels) and other structural variants (SVs; e.g., inversions, duplications). Structural variants are variants of size larger than 50 bp, and its primer design is slightly different than regular SNP design, having 2 amplicons per marker (one for wild-type and another for mutant). Because the current variant caller is incapable of making calls for such a marker, we developed an analysis method to genotype long indels and other structural variants: AgriSeqSV. The robustness of this technology has been demonstrated across 96 samples using 13 canine long indel markers (insertion, deletion, and inversion). Overall, 97% marker call rate across samples and 100% concordance were observed (when compared with truth data). It has also been shown that primer design and downstream analysis were not impacted by the indel size. High concordance across multiple samples with varying indel size indicates the reproducibility and flexibility of the method. Enhanced AgriSeq targeted GBS offers customers an end-to-end solution for genotyping diverse marker types simultaneously using the analysis workflow. AgriSeqSV is available as a plugin on Torrent Suite Software (TSS). For research use only. Not for use in diagnostic procedures.

Key Words: multispecies, genotyping, complex trait, bioinformatics tools, genetic improvement

P111 Growth and development of Kazakh white-head breed bulls of different genotypes depending on the type of temperaments. R. Uskenov*, S. Bostanova, and B. Akkair, *Saken Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan.*

Temperament is an important trait in beef production systems that has practical and economic implications. Temperament has been reported to affect almost every aspect of animal husbandry, including growth, reproduction, and immunity. The objective of the research is to study the growth and development of bulls of Kazakh white-head breed of different genotypes, depending on the type of temperament. For the experiment, purebred bulls of Kazakh white-head breed were selected in the amount of 117 heads of 7–8 mo of age. At the time of completion of the test, the bulls were about 11–12 mo old. During the test period, the bulls were in the same conditions of feeding and keeping. The average live weight of bulls with temperament score 4 was 319.2 ± 5.0 kg, which is 1.5% less than bulls with temperament score 3 (324 ± 3.1 kg). The average live weight of bulls with temperament type 2 was 316 ± 2.8 kg, which is 1.1% less than their peers with temperament 4. In excitable type 301 ± 2.2, which is 5.7% less compared with bulls with a rating of temperament 3. The average daily gain of bulls with temperaments 3 and 4 was 1,404 ± 30 g and 1,155 ± 30 g, respectively. It should be noted that the calmer bulls belonging to the 3rd and 4th groups come from the same line, the Veteran genotype. The average daily gain of the most temperamental bulls with a score of 1 was lower compared with the rest of the bulls. Their average daily gain was 682 ± 10 g, which is 51.43% less compared with bulls with a temperament score of 4. The results of the data obtained show that there is a relationship between the live weight of bulls and types of temperament, and a positive correlation (R = 0.98) was found between the average daily gain and the temperaments of bulls. An analysis of the data on the lines showed that the heritability of the temperament types of bulls, although low, is positive and requires further detailed study.

Key Words: animal breeding, behavior, breed diversity, growth and development

P112 Identification of characteristic aroma substances and their metabolic precursors in chickens. Y. Wang, Y. Jin, X. Liu, H. Cui, and J. Wen*, *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

The characteristic of aroma is one of the most important indexes to measure the quality of chicken meat. In this study, GC-O-MS and OAV methods were used to conduct a combined comparative analysis of volatile aroma substances from 4 main animal species, chickens of 3 different breeds and 4 different days of age to determine the characteristic aroma substances of chicken. And key metabolic markers of important aroma substances were screened by multi-omics analysis. The results showed that (1) hexanal, nonanal, octanal, heptanal, 1-octen-3-ol, (E)-2-nonenal, (E,E)-2,4-decadienal, and dimethyl tetrasulfide were the key common aroma substances in meat. (2) Hexanal and 1-octen-3-ol were the main content aroma substances of Chinese native chicken high-quality meat. (3) Nonanal, octanal, and dimethyl tetrasulfide were present in the 3 breeds, so it can be seen that they were the basic characteristic aroma compounds of chicken. (4) Hexanal, 1-octen-3-ol, (E)-2-nonenal, heptanal, and (E,E)-2,4-decadienal were the only characteristic aroma compounds in Chinese native chicken breeds. (5) A multi-omics analysis revealed a significant correlation between 1-octen-3-ol and mevalonate, and the regulation of mevalonate content by genes such as *HSP90AA1* and *PTPN9*, leading to an increase in 1-octen-3-ol. (6) Proline inhibits hexanal formation and can eliminate this inhibition by modulating pyrimidine metabolic pathways through the *DGUOK*, *ENTPD1*, *NME4*, and *RRM2* genes. (7) Arachidonic acid was identified as a metabolic marker of 5 kinds of olefine aldehyde, and L-glutamine was identified as a metabolic marker of hexanal, nonanal, octanal, heptanal, and 1-octen-3-ol. In conclusion, the characteristic aroma substances of chicken are not unique, and their metabolic precursors identified by metabolome are not unique. These results provide a theoretical basis for improving chicken quality through genetic breeding and nutrition regulation.

Key Words: characteristic aroma substances, metabolic precursors, GC-O-MS, OAV, multi-omics

P113 Withdrawn

P114 ISAG Bursary Award: An organism-wide ATAC-Seq peak catalogue for the bovine and its use to identify regulatory variants. C. Yuan*[1], L. Tang[1], T. Lopdell[2], C. Oget-Ebrad[1], G. Costa Monteiro Moreira[1], J. L. Gualdron[1], Z. Cheng[3], M. Salavati[3], D. C. Wathes[3], M. A. Crowe[4], W. Coppieters[1], C. Charlier[1], T. Druet[1], M. Georges[1], H. Takeda[1], [1]*GIGA Institute, University of Liège, Liège, Belgium,* [2]*Livestock Improvement Corporation, Hamilton, New Zealand,* [3]*Royal Veterinary College, Herts, UK,* [4]*School of Veterinary Medicine, University College Dublin, Dublin, Ireland.*

We herein report the generation of an organism-wide catalog of 976,813 *cis*-acting regulatory elements detected by ATAC-Seq. We regroup these regulatory elements in 15 tissue-specific and one tissue-shared components by nonnegative matrix factorization. Correlation between the genome-wide density of peaks and transcription start sites, between peak accessibility and expression of neighboring genes, and enrichment in transcription factor binding motifs supports their regulatory potential. Using a previously established catalog of 12,736,643 variants, we show that the proportion of single nucleotide polymorphisms mapping to ATAC-Seq peaks is higher than expected and that this is due to an ~1.3-fold higher mutation rate within than outside peaks. Their site frequency spectrum indicates that variants in ATAC-Seq peaks are subject to purifying selection. We generate eQTL data sets for liver and blood and show that variants that drive eQTL fall into liver and blood-specific ATAC-Seq peaks more often than ex-

pected by chance. We combine ATAC-Seq and eQTL data to estimate that the proportion of regulatory variants mapping to ATAC-Seq peaks is approximately 1 in 3, and that the proportion of variants mapping to ATAC-Seq peaks that are regulatory is approximately 1 in 25. We discuss the implication of these findings on the utility of ATAC-Seq information to improve the accuracy of genomic selection.

Key Words: cattle and related species, epigenomics, ATAC-seq, regulatory element, genomic selection

P115 Withdrawn

P116 ISAG Bursary Award: Identification and comparison of plant-derived miRNAs based on massive public data. H. Liu*[1], P. Xu[1], Y. Liao[1], C. Li[1], J. Dou[1], Y. Wang[1], Z. Tang[1], J. Xu[1], D. Yin[1], S. Zhu[1], L. Yin[1,2], M. Yu[1], S. Zhao[1,2], X. Liu[1,2], Y. Fu[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei, China,* [2]*Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, Hubei, China.*

Numerous studies have reported that plant miRNAs could be absorbed into the circulatory system and organs of animals, and these studies have broadened our view of cross-kingdom communication. However, limited by the scale of data, the relationship between plant-derived miRNAs and animal diets, and the distribution of plant-derived miRNAs in various tissues are lacking in-depth exploration. In this study, we collected 5,834 miRNA sequencing data involving 46 tissues from 6 common animals including pig, cow, sheep, chicken, mouse, and monkey. After bioinformatics analysis, we identified a total of 5,430 plant-derived miRNAs in 6 species, which is distinctly higher than 7 in carnivores (crocodiles, seals) and 3 in yeast. It suggests that these miRNAs are indeed related to the diet of animals. To investigate the potential dietary sources of these miRNAs, we mapped them to miRBase and found most of them were from crops or grasses, such as *Zea mays*, *Glycine max*, and *Medicago truncatula*. However, we also found that miRNAs, which have higher expression level in each species, mainly from the miR166, miR167, miR168, miR159, miR396, and miR482 families but not affected by the dietary source of animals. It indicates that the absorption of the dietary miRNAs in the recipient may be affected by their stability, and most of the plant miRNAs are difficult to be absorbed. Our study also demonstrates that plant-derived miRNAs widely exist in various tissues, with an average detection rate of 38.06%, among which the detection rates in the oral cavity, body fluids (blood, milk), and excreta (urine and feces) is higher than 50%, which may be related to their absorption and transportation pathways. In summary, our study confirmed that plant-derived miRNAs are derived from diet based on large-scale sequencing data, and their expression level in various tissues is highly associated with their absorption and transport pathways. It will advance the understanding of plant miRNAs regulation across kingdoms and provides new theoretical insights into the effects of diet on animal breeding.

Key Words: miRNA, plant-derived miRNAs, tissue expression profile, cross-kingdom regulation

P117 Overview of Ruminant T2T Consortium. B. M. Murdoch*[1], S. D. McKay[2], B. D. Rosen[3], and T. P. L. Smith[4], [1]*University of Idaho, Moscow, ID,* [2]*University of Missouri, Columbia, MO,* [3]*USDA, Agricultural Research Service, USDA, Animal Genomics and Improvement Laboratory, Beltsville Agricultural Research Center, Beltsville, MD,* [4]*USDA, Agricultural Research Service, USDA, Genetics and Animal Breeding, Clay Center, NE.*

The first draft human genome assembly was released over 20 years ago, but a gapless telomere to telomere (T2T) "complete" assembly was elusive until last year. The highly repetitive nature of the pericentromeric, subtelomeric and duplicated gene families such as the rRNA arrays made them impossible to assemble until advances in long read sequencing technologies, coupled with new bioinformatic tools, resolved these structures. We recently proposed application of these new resources, tools and knowledge in support of a "Ruminant T2T Consortium" with the goal of generating complete genomes for the ruminant evolutionary lineage. The ruminant suborder is represented by 6 families and 66 living genera, found in geographically dispersed areas, adapted to a wide variety of environments, and subjected to both natural and artificial selection. Our hypothesis is that generating T2T assemblies of ruminant species with a resolution from closely related (e.g., capable of interbreeding, even if only generating sterile offspring) to higher evolutionary distance (up to the estimated 25 million years ago last common ancestor) will inform our understanding of the underpinnings of ruminant evolution, shed light on the genomic consequences of domestication, and enhance our knowledge of the functional roles of heterochromatin and other repeat regions of the genome. A ruminant T2T workshop, held in February at the USDA Meat and Animal Research Center in Nebraska, featured presentations from the founders of the human T2T effort and leaders of the vertebrate genomes and earth biogenome projects to provide details of their experience and lend assistance in designing and optimizing the project. The consortium developed working groups to conduct orthogonal data production and analyses. The working groups include Chromosome Evolution, 3D Genome Architecture, Comparative Methylome, Assemblers and Curation, Annotation, Variant Discovery and population sequencing, Immunome analyses, Cytogenetic. This update is intended to share the outcomes of the ruminant T2T workshop and provide an opportunity for interested members of the international genomes community to participate.

Key Words: Ruminant T2T, genome assembly, pangenomes

P118 Discovering the missing structural variation in the bovine genome. A. Chamberlain*[1,2], T. Nguyen[1], J. Wang[1], and I. Macleod[1,2], [1]*Agriculture Victoria, Bundoora, Victoria, Australia,* [2]*La Trobe University, Bundoora, Victoria, Australia.*

Structural variations (SV) often show large and sometimes deleterious effects on phenotypes and remain largely unexplored in livestock. The Bovine Long Read Consortium (BovineLRC) aims to use long read sequencing technologies to sequence cattle at population scale to characterize the structural variation of the bovine genome for downstream

applications. As a pilot study 41 animals from 2 breeds were sequenced. In an analysis of 2 parent-offspring trios we show that between 10× to 20× coverage resulted in some reduction in the SV discovery rate versus higher read depth, but this may be an acceptable compromise for population scale studies to spread sequencing costs over a larger number of animals. However, if the purpose is to discover a deleterious Mendelian mutation among a small group of known affected or carrier animals, the results here suggest that at least 30× would be preferable. The SNP and INDEL called from various read depths were compared with calls from short read sequences. At read depths from 5 to 60× recall and precision of SNP was considerably higher than for INDEL. At ≥10× coverage, SNP recall was 0.86 and reached 0.99 at 60×. The precision for both SNP and particularly INDEL suggested that the long-read variant calls include a relatively high, but likely overestimated proportion of false positives. Using sequences from all animals a total of 76,572 SVs were detected across all samples, one-third of which were segregating in only one breed. Insertions and deletions tended to be smaller and duplications larger. Insertions and deletions more often segregated across both breeds, while inversions were more often breed specific. Few duplications were detected but they tended to be slightly more likely to be breed specific. The results highlight that it would be beneficial to have a data set with large numbers of animals and breeds to understand the structural variation that exists and explore the impact of SVs on traits of interest.

Key Words: bovine, structural variation, long read sequencing

Equine Genetics and Thoroughbred Parentage Testing

P119 Characterization of genetic variants of equine cathelicidin. T. Ishige*, M. Kikuchi, H. Kakoi, K.-I. Hirota, A. Ohnuma, T. Tozaki, and S.-I. Nagata, *Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan.*

Cathelicidins (CATHs) are host defense peptides that are conserved across numerous vertebrate species and play an important role in innate immunity. The activity of host defense peptides can be affected by amino acid substitution (AAS) and copy number polymorphism (CNP). Three equine CATHs (namely, eCATH, eCATH2, and eCATH3) have been identified and their antimicrobial activities have been reported. Several AASs of those eCATHs have been deposited in Ensemble database. However, the presence of AASs and CNPs in eCATHs has not been investigated in different equine breeds and needs to be studied. Therefore, we first investigated AASs and CNPs of eCATHs using whole-genome sequencing data of 101 thoroughbred racehorses by mapping sequence-reads to the horse reference genome. We then sequenced the eCATHs and estimated the AASs in 150 horses, including 6 Japanese native horse populations (namely, Hokkaido, Kiso, Taishu, Tokara, Miyako, and Yonaguni). The net charge was calculated by the peptide property calculator (http://www.innovagen.com). Finally, we analyzed CNPs for Japanese native horse populations using qPCR. Among the 101 thoroughbred racehorses, no AAS was found in eCATHs. In the Japanese native horses, no AAS was found in eCATH and eCATH3. However, 3 AASs were observed in the eCATH2 of Hokkaido and Kiso populations. Of these, 2 of 3 AASs (Arg130Pro and His131Arg) were deposited and a novel AAS (Leu141Gly) was found in both populations. Because 2 homozygous genotypes were shown in the Kiso population, at least 2 AAS types can be identified: the reference type (eCATH2a: 130Arg, 131His, and 141Leu) and the alternative type (eCATH2b: 130Pro, 131Arg, and 141Gly). The frequencies of eCATH2b in Hokkaido and Kiso were 1.7% and 21.7%, respectively. The net charge of eCATH2a (3.1) was higher than that of eCTAH2b (3.0). Thus, it was suggested that the antimicrobial activity of eCATH2b may be less than that of eCATH2a. Further analysis of CNPs in eCATHs is ongoing. These results can aid in further understanding the innate immunity in horses.

Key Words: horses and related species, polymorphism, innate immunity, copy number variation (CNV)

P120 Overlapping allelic ranges in equine STR panel for parentage verification—Technical notes. A. Bieniek* and A. Piestrzynska-Kajtoch, *National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

AHT4 and HTG4 are loci in the core equine STR panel recommended by ISAG. A kit provided by ThermoFisher Scientific for horse parentage testing includes those 2 loci and both are labeled with the same fluorescent dye (6-FAM). AHT4 and HTG4 are adjacent loci in this kit; size range according to the technical manual are 116–138 and 140–166 respectively. We detected issues with those 2 loci in Polish coldblood horse samples. We found the atypical and shifted peak of AHT4 in the typical range for HTG4. As a result, HTG4 homozygotes looked like heterozygotes or third peak was present in HTG4 heterozygotes, and the sample looked as if there were 3 alleles in this loci. To verify the profiles of the putative parents, blood and hair follicles were tested. Analysis of the DNA profiles of the offspring and the alleged parent indicated the correctness of the inherited variant. To check for potential new allelic variant, samples were amplified in monoplex PCR for both STR alleles using primer pairs recommended by ISAG, labeled with the same fluorescence dye as in multiplex PCR. Samples were also sequenced by Sanger sequencing to check the repeats of the STR motifs. We confirmed multiplex reaction results by monoplex PCR and sequencing. Both loci are autosomal di-nucleotide STRs, and analyses indicate the presence of new allelic variant in AHT4 loci on the left of range pointed in the technical manual. To date, new variant of AHT4 was detected only in Polish coldblood horses. The breed dominates the Polish horse population (about 50% share in the Polish population). Therefore, it can be expected that the variant may appear in other coldblood horses in the future. It also shows the need of redesigning the STR panel (e.g., by labeling some of primers with additional dye) and indicates the potential benefits of developing SNP panels for parentage verification in horse.

Key Words: parentage verification, horse, coldblood horse, STR

P121 ISAG Bursary Award: Investigating the effect of chromosome 20 on lordosis in Saddlebred horses. N. Yousefi-Mashouf*, K. Graves, T. Kalbfleisch, and E. Bailey, *University of Kentucky, Lexington, KY.*

Juvenile Onset Lordosis (JOL) or swayback, is a common hereditary conformation defect in Saddlebred horses where the back curvature drops within the first 2 years of life. The phenotype is quantified using a Measurement of Back Contour (MBC), where horses of MBC >7.0 cm are considered high-MBC swayback. Genome-wide association studies identified a recessive haplotype on chromosome 20 associated with high-MBC. Whole-genome sequencing was conducted on 11 horses (5 high-MBC, 6 low-MBC) to compare for the target region of chr20:41000000–44000000. No variants were found that fit the hypothesis of a single, recessive factor. This led to a new hypothesis, that high-MBC is caused by multiple genetic factors with a major effect from chr20. This study aimed to evaluate all the genomic variation in the target region of chr20:41M–44M to identify variants that might alter gene function contributing to high-MBC. A total of 9,691 variant loci were detected that make 21,463 transcript variations. Of these, 599 made coding sequence variations, including 315 synonymous, 250 missense, 14 frameshift, 9 in-frame deletion, 7 in-frame insertion, and 2 splice-donor and 2 start-loss variants. The distribution among affected and unaffected horses was compared along with computer predictions for impact of the variants on gene function. Potentially deleterious variants were found for 4 candidate genes: *MDFI*, *C6orf132*, *PTK7*, and *NCR2*. The strongest candidate based on predicted function was a frameshift deletion

of 7bp in the exon 1 of the *MDFI* gene, at 20:41873061–41873068. *MDFI*-knockout mice show defects in the formation of thoracic vertebrae and ribs, which restrains fusion of the spinous processes. This is consistent with necropsy reports on juvenile lordotic horses, where the spinous processes of the vertebral bones are underdeveloped. Further RNA expression studies are suggested to compare the expression of the *MDFI* gene in the affected spinous process of the vertebral bones with healthy bone tissues. Also, an additional genome-wide association study to control for the chr20 factor between affected and unaffected animals could reveal further loci affecting JOL.

Key Words: swayback, fine-mapping, whole-genome sequencing

P122 Withdrawn

P123 Contribution of STR genotyping to animal clinical cytogenetics. T. Raudsepp*, J. Kjöllerström, and R. Juras, *School of Veterinary Medicine, Texas A&M University, College Station, TX.*

A progressive tendency in animal parentage testing is to replace short tandem repeat (STR) genotyping panels with single nucleotide variation (SNV) genotyping chips. The latter are more comprehensive, have a better representation of genome variation of the species, and include variants for the detection of genetic diseases and select phenotypes. Despite the obvious advantages of SNV panels, STR genotyping remains as an efficient, fast, and reliable method for some genetic analyses, such as ancestry testing in horses because of the wealth of the available STR data for diverse breeds. Also, in the past decade, we have used on regular basis STR genotyping as a complementary approach for clinical cytogenetics in horses and occasionally in cattle to verify animal or sample identity, parentage, and genetic sex. It is also a method of choice to validate karyotyping-based XX/XY blood chimerism. In cases of X-monosomy, which is a frequent cause of sterility in female horses, STR genotyping is an efficient tool for detecting low level mosaicism for an XX cell line and determine the parental origin of the single X chromosome. Likewise, STR genotyping is a feasible tool for determining the parental origin of autosomal and sex chromosome trisomies and discriminating between centric fusions and isochromosomes. On a few occasions, where the karyotype appears normal, STR genotyping has suggested uniparental disomy which is not detectable by chromosome analysis. STR genotyping combined with mitochondrial DNA analysis is also an excellent tool for the detection of interspecific hybrids and their parental origins. In addition, comparison of STR genotyping-based ancestry with the breed information available for cytogenetics samples shows over 95% concordance, illustrating the high accuracy of STR genotyping to determine breed ancestry in horses. Because of the feasibility, speed, efficiency, low cost, and in-house availability, STR genotyping remains an important complementary method for clinical cytogenetics and will not be replaced with SNV genotyping soon.

Key Words: chromosome aberrations, parental origin, ancestry

P124 Withdrawn

P125 ISAG Bursary Award: Introgressive hybridization levels of tilapiines species in Lake Victoria basin, Kenya, inferred from microsatellite and mitochondrial DNA genotyping based on next-generation sequencing. G. Kwikiriza*[1,2], T. Vijayan[1], P. D. Tibihika[3], M. Curto[1,4], G. Winkler[5], J. K. Nattabi[2], J. Kariuki[6], and H. Meimberg[1], [1]*Institute for Integrative Nature Conservation Research, University of Natural Resources and Life Sciences Vienna (BOKU), Vienna, Austria,* [2]*Makerere University Kampala, Kampala, Uganda,* [3]*National Fisheries Resources Research Institute, Aquaculture Research and Development Center, Kampala, Uganda,* [4]*MARE-Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Lisbon, Portugal,* [5]*Institute of Hydrobiology and Water Management, University of Natural Resources and Life Sciences Vienna (BOKU), Vienna, Austria,* [6]*Department of Biochemistry, University of Nairobi, Nairobi, Kenya.*

Despite their high abundance and species richness, tilapiines have been compromised by various factors especially overfishing, climate change, and uncontrolled fish translocations. Fish translocations have negatively impacted native tilapiine populations through competition, predation, hybridization, and introgression compromising their genetic integrity. The hybridization levels of different tilapiines in the Lake Victoria basin remains an understudied aspect relatively. The study utilized nuclear microsatellite and mitochondrial DNA (mtDNA) genetic markers to investigate hybridization signals and compare the genetic diversity of different tilapiines in Lake Victoria, Kenya, using next-generation sequencing. Low levels of hybridization from *Oreochromis niloticus* into other *Oreochromis* species were detected by Bayesian clustering analysis and principal coordinate analysis (PCoA). The results contribute to the need for conservation measures of these fish species.

Key Words: tilapiines, next-generation sequencing, hybridization, admixture, conservation

P126 ISAG Bursary Award: Phylogenetic status and origin of monogenean gill parasites of *Synodontis* spp. (Actinopterygii, Siluroidei) from Cameroon: Influence of the ichthyological province. J. A. Mbondo*[1], D. N. D. Bahanak[1], E. D. Bayiha[2], and C. F. Bilong Bilong[2], [1]*Institute of Agricultural Research for Development, Yaounde, Centre, Cameroon,* [2]*University of Yaounde I, Yaounde, Centre, Cameroon.*

Members of the catfish family, Mochokidae, are among the most important teleost species suitable for aquaculture. Fish parasite diseases remain a constraint for successful fish farming as they usually affect the marketability of produced fish and raise economic losses in aquaculture industry. Thus, fishery development programs also depend on the intensification of parasitological researches so that improved fish yield can be achieved from healthy fish stocks. The present work provides additional molecular data on *Synodontella* spp. (Monogenea) and reveals their phylogenetic status and explores the origin of *Synodontella* species. Molecular analyzes were based on 28S rDNA. *Synodontella* spp. form a monophyletic group with 3 clusters. The existence of 2 or more lineages within the genus *Synodontella* suggested by the previous morphological studies is here molecularly confirmed and suggest that it can be due to the ecological influence or phylogenetical relationship of parasites and/or hosts. *Synodontella* appears to be closer to *Schilbetrema* than other datylogyrid species taken in account and could originate from West Africa.

Key Words: phylogenetic, catfish, parasites, 28 S rDNA, fishery development

P127 Comparative gene expression and regulation of the response of head kidney immune-related cells of turbot (*Scophthalmus maximus*) to common virus (Poly I:C) and bacteria (*Vibrio*) triggers after in vitro and in vivo challenges. O. Aramburu[1], B. G. Pardo[1], P. R. Villamayor[1], J. Lamas[1], P. S. Dewari[2], D. Perojil[2], D. J. Macqueen[2], C. Bouza[1], and P. Martínez*[1], [1]*Universidade de Santiago de Compostela, Lugo, Spain,* [2]*University of Edinburgh, Midlothian, United Kingdom.*

Infectious diseases affecting turbot aquaculture cover a broad spectrum of pathogens, from viruses and bacteria to different parasites. Here, we present a genomic atlas of regulatory elements and differential gene expression of immune-stimulated turbot aimed at understanding general and pathogen-specific immune response, as well as for a comparative analysis with other fish aquaculture species pertaining to different taxonomic groups. Following the same experimental protocol as in other 5 species of the AQUAFAANG project, we carried out in vivo and in vitro immune challenges in live specimens and head kidney primary cell cultures of turbot, respectively, using viral mimic (Poly I:C) and bacterial (inactive *Vibrio*) stimuli. Both live fish head kidneys and cell cultures were processed after 24hpi, to be studied using RNA-, ATAC-, and ChIP-seq, for transcriptomic and regulomic characterization of the turbot immune response. Upregulated differentially expressed genes were enriched on pathogen-specific pathways during viral (JAK-STAT) and bacterial (glycosaminoglycan catabolism) infections, as well as on the IFN-gamma pathway and general gene expression activation; meanwhile, downregulated genes were enriched on energetic metabolism and cell cycle GO terms. The atlas of regulatory elements was constructed using highly reproducible peaks of open chromatin regions and binding sites for histone marks associated with active promoters (H3K4me3), enhancers (H3K27ac), and inactive regions (H3K27me3) using the IDR software, annotated with HOMER and later used to generate chromatin structure models (ChromHMM) and lists of differential binding peaksets (DiffBind). Our results provide the first picture of the turbot genomic resources being generated in the AQUAFAANG project, providing brand new information on regulation of gene response n during immuno-stimulation useful for more efficient farm breeding programs.

Key Words: flatfish, resistance to pathologies, regulomics, immune response, head kidney

P128 ISAG Bursary Award: Metagenomics analysis of salt-fermented hilsa (*Tenualosa ilisha*) at different processing stages. H. Muhammad Shahdat*[1] and S. Islam Sarkar[2], [1]*National Institute of Biotechnology, Savar, Dhaka, Bangladesh,* [2]*Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.*

We investigated the microbial communities of salted hilsa fish (*Tenualosa ilisha*) using a sequence-based assessment of the 16S rRNA gene (V3-V4 region) at different stages, namely initial salted fish (after 1–2 d of salting), interim salted fish (after 4–5 d of salting), and ripe salted product (final product after 15 d of salting). The eDNA was collected and then sequenced on the Illumina (NovaSeq 6000) paired-end sequencing. Using reads splicing, an average of 82,231 tags per sample was measured, and an average of 79,945 valid data was obtained. A total of 3,248 OTUs (operational taxa units) were found with 97% identity. In the annotation results, a total of 1,228 (37.81%) OTUs were annotated at the genus level using the Silva138 database. Of the 48 phyla that have been identified, the predominant bacteria were *Firmicutes*, *Proteobacteria*, and *Bacteroidota*. In the initial stage, *Proteobacteria* were higher, but the *Firmicutes* replaced both *Proteobacteria* and *Bacteroidota* in the final salted product. The dominant bacterial genera were *Enterobacter*, *Shewanella*, *Myroides*, and *Kurthia* among 636 identified genera in the initial stage. *Cohnella* and *Enterobacter* were higher in the interim salted product, but *Cohnella* and *Bacillus* were higher in the final product. Pathogenic zoonosis *B. anthracis* was dominant in the final salted product, possibly due to salt contamination or unsanitary conditions. The bacterial richness, diversity and evenness were higher in the final product of salted hilsa according to chao1, ACE, Shannon, and Simpson indices ($P < 0.05$). There were significant differences among groups according to AMOVA analysis. According to PCoA and UPG-

MA, there was a cluster of initial and interim salted products that made a different group with the bacterial diversity of the final salted product. Significant differences were found in the functional gene profile category between initial and final salted products. These findings shed new light on the in-depth characterization of hilsa microbiota and enhance the current understanding of salting impact on the bacterial assemblage of hilsa microbiota.

Key Words: aquatic animal, hilsa fish, salt-fermentation, metagenomics

P129 Digital phenotyping of omega-3 fatty acid content in Atlantic salmon (*Salmo salar*) using Raman spectroscopy. G. F. Difford*[1,2], J. Park[1,2], S. S. Horn[2], B. Ruyter[2], B. Hillestad[3], A. Sonesson[2], and N. K. Afseth[2], [1]*Norwegian University of Life Sciences (NMBU), Ås, Norway,* [2]*Norwegian Insitute of Food, Fisheries and Aquaculture (NOFIMA), Tromsø, Norway,* [3]*Benchmark Genetics AS, Bergen, Norway.*

Atlantic salmon are world-renowned in sushi and seafood dishes as a source of omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, in the last decade, the Norwegian salmon industry has shifted from marine ingredients high in EPA and DHA to greener more sustainable feed ingredients which are deficient in EPA and DHA. The resultant drop in EPA and DHA has spurred interest in breeding for Atlantic salmon better able to retain EPA and DHA in their muscle. The EPA and DHA content in Atlantic salmon fillets has been found to be significantly heritable. The biggest bottleneck to breeding for improved EPA and DHA content is the costs associated with liquid chromatography needed to measure EPA and DHA content and thus limits phenotype acquisition. Raman spectroscopy is a rapid and non-invasive method that measures the light scattered by chemical bonds such as those found in the EPA and DHA. The objective of this study was to evaluate the use of EPA and DHA content predicted from Raman spectra as rapid and non-destructive alternative phenotypes in commercial breeding. We recorded the EPA and DHA content in 668 genotyped (57 K SNP chip) adult Atlantic salmon from the breeding nucleus of Benchmark genetics and concurrently used Raman spectroscopy on their fillets. Remarkably, using as few as 50 fish and partial least squares regression, it was possible to predict EPA and DHA content from Raman spectra in the fillet. The predicted EPA and DHA content was as heritable as the "true" EPA and DHA content and encouragingly the genetic correlations between predicted and true phenotypes was close to unity (genetic correlations >0.90). Raman spectroscopy has the potential to break the bottleneck in EPA and DHA phenotyping and holds promise for future selective breeding to improve EPA and DHA content in Atlantic salmon.

Key Words: fish, animal breeding, quantitative genetics, fat/lipid, aquaculture

P130 Withdrawn

P131 ISAG Bursary Award: A high-density genetic linkage map and QTL mapping for growth traits in South African abalone (*Haliotis midae*). T. Tshilate*[1], E. Ishengoma[2], and C. Rhode[1], [1]*Department of Genetics, Stellenbosch University, Stellenbosch, South Africa,* [2]*Mkwawa University College of Education, University of Dar es Salaam, Iringa, Tanzania.*

A high-density genetic linkage map is important for QTL fine mapping, comparative genome analysis, identification of candidate genes and marker-assisted selection for economic traits in aquaculture species. The South African abalone (*Haliotis midae*) is one of the most important aquaculture species in South Africa. However, limited genetic and genomic resources have been developed for the genetic improvement of economically important traits for the species. Therefore, a 2b-RAD (2b-restriction site-associated DNA) sequencing technique was used to sequence 68 *H. midae* specimens at around 5 years of age and obtain 7,173 single nucleotide polymorphism (SNP) markers. A high-density linkage map was constructed using 2,266 SNP markers spanning 1,646.01 cM in length and an average marker interval of 0.73 cM to 18 linkage groups (LGs). Using the genetic linkage map, 13 QTLs for growth-related traits were detected on 3 linkage groups (1, 7 and 13) with an average shell of 70.17 mm, average shell length of 99.31 mm and average total body weight of 174.92 g. The phenotypic variance explained (PVE) ranges from 13.6 to 25.1%, with LOD scores ranging from 4.17 to 9.72. Finally, some important candidate genes such as *egf-1*, *megf10*, *megf6*, *tnx*, *sevp1*, *kcp*, *notch1* and *scube2* which may regulate growth in *H. midae* were identified. This genetic map may provide a basis for genome assembly and comparative genomics studies, and the QTLs derived candidate genes and genetic markers are useful genomic resources for future marker-assisted selection (MAS) for growth-related traits in the South African abalone.

Key Words: growth traits, *Haliotis midae*, linkage map, quantitative trait loci

P132 Utilizing of genetic evaluation system using genomic information of the Korean flatfish population. D. Lee*[1], J. Kang[1], Y. Chung[1], S. Lee[1], Y. Kim[2], J. Park3,1, D. Lee[3], J. Kim[3], H. Yang[3], J. Lee[3], and S. Lee[1], [1]*Chungnam National University, Yuseong-gu, Daejeon, Republic of Korea,* [2]*Quantomic research & solution, Yuseong-gu, Daejeon, Republic of Korea,* [3]*Fish Genetics and Breeding Research Center, Geoje, Republic of Korea.*

The olive flounder (*Paralichthys olivaceus*) is one of the most popular fish species in Southeast Asia and around the world. However, a breeding program design such as genomic prediction for this species have been limited compared with other livestock breeds. Genetic analysis using genomic information has been lacking in breeding-related research in fish industry of Korea. The National Institute of Fisheries Science was started a breeding program in the 1990s, total 54,159 animal were measured breeding objective traits, body weight (BW), total length (TL), and condition factor (CF) at 11, 18, and 22 mo. Pedigree

information was recorded in total 8 generations. Genomic data were generated from at generation 8 in this breeding population. In this study, the genomic analysis was conducted using a total of 3,223 individuals, all of which underwent customized Affymetrix array. After quality control, a total of 67,467 SNPs were used for analysis. In addition, pedigree and genomic-based analyses were compared for accuracy and efficiency using pedigree information from 3 or more generations. Using the BLUP method with single traits, each trait's genetic parameter and breeding values were estimated for pedigree-based BLUP (PBLUP) and genomic-based BLUP (GBLUP) methods. The estimated breeding values (EBV) accuracy was compared using the prediction error variance and the correlation with the actual phenotype. The estimated heritabilities by PBLUP method were 0.425, 0.468, and 0.384 for the traits, respectively, while those estimated by GBLUP method were 0.491, 0.505, and 0.594 for the traits. The heritability showed a similar trend when compared with research using pedigree information and showed 0.03–0.21 higher when using genomic information. The theoretical accuracy difference between PBLUP and GBLUP methods was 0.15 to 0.16, depending on the trait, but in actual accuracy (correlation), the difference ranged from 0.1 to 0.3. These results indicate that introducing genetic improvement using genomic information in the olive flounder population could effectively enhance genetic improvement.

Key Words: genomic selection, GBLUP, EBV, heritability, olive flounder

P133 Multi-functional genomic analyses identify causal gene and variants modulating viral nervous necrosis resistance in European seabass. R. Mukiibi*[1], L. Peruzza[2], C. Penaloza[3], M. Babbucci[2], R. Franch[2], M. Freguglia[4], S. Laureau[4], G. Dalla Rovere[2], D. Bertotto[2], S. Ferraresso[2], C. Tsigenopoulos[5], R. D. Houston[3], L. Bargelloni[2], and D. Robledo[1], [1]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom,* [2]*Department of Comparative Biomedicine and Food Science, University of Padova, Padova, Italy,* [3]*Benchmark Genetics, Edinburgh, United Kingdom,* [4]*Valle Cà Zuliani Società Agricola s.r.l., Conselice (RA), Italy, Rovigo, Italy,* [5]*Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR) Crete, Heraklion, Greece.*

Viral nervous necrosis (VNN) is a major infectious disease threatening the European seabass aquaculture industry. VNN causes high economic losses emanating from high mortality rates and slow growth of infected fish. Selective breeding has the potential to increase the disease resistance of aquaculture stocks, reducing the impact of disease. The most efficient selection strategy depends on the genomic architecture of the trait, which is currently unknown for resistance to VNN. In the current study we employed whole-genome resequencing and transcriptomic tools to define the genomic architecture of VNN resistance in European seabass using a VNN challenged population of 1400 juvenile fish. Similar to previous studies, our results demonstrated that VNN resistance is moderately heritable ($h^2 = 0.45$). Additionally, GWAS results revealed a major QTL on LG12, which hosted over 90% (37,357 of 48,787) of the significant (FDR >0.05) variants. Variants in this QTL region explained up to 37% of the genetic variance of resistance to the disease. Interestingly the most significant variants were located within the *IFI27L2A* gene. Furthermore, our analyses showed remarkable association between *IFI27L2A* gene expression and VNN resistance in the brain and head kidney. Expression quantitative trait loci analyses further revealed that GWAS significant variants also had significant impact on the expression of *IFI27L2A* in both brain and head kidney. *IFI27L2A* is an interferon inducible gene demonstrated to have antiviral properties in other species. Together, our results provide a more refined insight into the potential causal genetic background of resistance to VNN that could further be utilized for enhancing genomic selection or genome editing to produce more resistant fish.

Key Words: European seabass, viral nervous necrosis, genomics, QTL, eQTL

P134 Atlantic salmon miRNAs associated with smolitification and sea-water adaptation. R. Andreassen*[1], A. Shwe[1], S. Ramberg[1], A. Krasnov[2], and T. Østbye[2], [1]*Oslo Metropolitan University, Oslo, Norway,* [2]*Nofima (Norwegian Institute of Food, Fisheries and Aquaculture Research), Ås, Norway.*

Smoltification is a developmental process transforming parr into saltwater-adapted smolt. It is induced by artificial photoperiod in Atlantic salmon (AS) production. It is highly energy demanding and associated with suppressed immune function. About 15% of farmed AS transferred to sea die, and mortality rate is high during the first months post seawater transfer (SWT). Suboptimal smoltification and disease are considered contributory factors to death shortly after SWT. miRNAs are small ncRNAs regulating gene expression at the post-transcriptional level. However, their involvement in regulation of smoltification and SW adaptation is less well studied, and here we aimed to gain a better understanding of miRNA's role in regulation of this developmental transition. Head kidney (n = 48), liver (n = 42) and gill (n = 42) samples from 6 time points: parr (T1), early and late light treatment, smoltified, one week post SWT, one month post SWT were small-RNA sequenced. DESeq2 was applied for differential expression analysis comparing each of the time points with T1. Heatmap2 (R-package gplots) was used for hierarchical clustering to group differentially expressed miRNAs (DE-miRNAs) with similar dynamic changes. Samples from each group (n = 6) were analyzed on the 44k Salgeno-2 microarray to identify differentially expressed genes (DEGs) that were used for in silico target gene predictions and enrichment analysis by use of the MicroSalmon GitHub repository and PANTHER Overrepresentation test (ORT). DE-miRNAs were revealed in head-kidney (n = 54), liver (n = 62) and gill (18). They were clustered into groups by increasing or decreasing expression patterns, commonly showing largest changes at smolt or post SWT stages. The ORT showed smoltification associated biological processes as enriched, e.g., regulation of hormone levels, stress response, and ion transport (head-kidney), lipid homeostasis, energy metabolism, circadian rhythm and growth (liver), immune system networks, extracellular matrix and lipid metabolism (gill). Collectively, this indicate that the DE-miRNAs are important post-transcriptional regulators of smoltification and SW adaptation.

Key Words: miRNA, Atlantic salmon, smoltification, small RNA sequencing

P135 Whole-genome sequencing data provide a landscape picture of genetic variability in sea cucumber species. F. Bertolini*[1], A. Ribani[1], V. Taurisano[1], A. Rakaj[2], A. Fianchini[2], F. Capoccioni[3], D. Pulcini[3], S. Bovo[1], and L. Fontanesi[1], [1]*Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Bologna, Italy,* [2]*Department of Biology, University of Rome Tor Vergata, Rome, Italy,* [3]*Centro di ricerca "Zootecnia e Acquacoltura," Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Monterotondo (Rome), Italy.*

Holothuria is a genus of marine invertebrates commonly called sea cucumbers, that belongs to the phylum Echinodermata. *Holothuria* species are deposit feeders, ingesting sediment with oral tentacles, extracting and digesting the organic matter with associated microorganisms, and voiding the sand through the anus. In this way they bioturbate significant areas of the seabed, playing a central role in the benthic habitats where these species live. The interest in *Holothuria* spp. for aquaculture began in Asia and soon reached the European market, making these species promising candidates to establish novel aquaculture production systems worldwide. The genus counts more than 160 species, many of which are uncharacterized at the genome level. Here, we provide a comparative genome analysis between 2 Mediterranean sea cucumber species, *Holothuria polii* (HP) and *Holothuria tubulosa* (HT) utilizing the reference genomes of both *Holothuria glaberrima* (HG) and *Holothuria scabra* (HS), the only 2 species of this genus for which a reference genome is available thus far. The HG and HS reference genomes are composed of 89,105 and 4,345 scaffolds, respec-

tively. Whole-genome sequencing data sets of 4 HP and 3 HT were produced, filtered and then mapped to the 2 reference genomes with standard options using BWA tool. The genome coverage of the sequencing data obtained for HT was 59.58% and 20.24% against the HG and HS genomes respectively, whereas the genome coverage obtained for HP was 55.62% and 19.16% against the same 2 genomes respectively. Variant calling was performed utilizing the HG-based alignment, retrieved more than 25 million high-quality SNPs. These SNPs were used for the study of population differentiation via the F_{ST} statistics; HP and HT that was 0.34 presented a F_{ST} index equal to 0.34. This information provided a first comparative analysis of genome information between HT and HP species and underlined the need to further expand genomic research in holothurian species. This information will be used to detect genomic regions of high genetic divergence that might differentiate HT and HP species.

Key Words: aquaculture species, *Holothuria*, SNPs, F_{ST}

P136 ISAG Bursary Award: Construction of a high-density genetic linkage map using 2b-RAD sequencing in dusky kob (*Argurosomus japonicus)*. T. Jackson and C. Rhode*, *Stellenbosch University, Stellenbosch, South Africa.*

A high-density genetic linkage map is a useful resource in genomic research, providing a means for quantitative trait loci (QTL) mapping, gene mapping, and comparative genomic analysis. Information which can be incorporated into current selective breeding strategies, leading to the increased success of finfish aquaculture. To provide the fundamental basis for future genomics research, this study constructed the first high-density genetic linkage map for dusky kob (*Argyrosomus japonicus*) using 2b-restriction site-associated DNA (2b-RAD) sequencing for genome-wide single nucleotide polymorphism (SNP) genotyping. This approach was performed using 3 full-sib F1 families, where a total of 21,515 SNPs were discovered and genotyped. Of which, 4,482 high-quality SNPs were assigned to 24 linkage groups (LGs), which agreed with the haploid chromosome number. The map spanned a length of 1,784 cM with an average marker space of 0.54 cM and a genome coverage rate of 96.3%. These results are consistent with the high-quality maps produced for the closely related species, *Argyrosomus regius* and *Larimichthys crocea*, apart from the male vs female recombination rates which have yet to be reported for these species. The recombination rate for this species was determined to be higher in females than males (an average female-to-male ratio of 1.2:1), coinciding with the previous research conducted on other teleost species. Overall, this study was able to produce a high-quality linkage map which will serve as an important tool and resource for detecting and fine mapping of QTLs, which will contribute considerably to the development and improvement of ongoing marker assisted selection (MAS) initiatives in the species.

Key Words: linkage map, 2b-RAD sequencing, single nucleotide polymorphism, aquaculture

P137 Withdrawn

P138 Withdrawn

P139 A technology for producing all-female progenies of the flathead grey mullet by selecting sex-reversed males. L. David*[1], G. Hirsch[1], I. Oz[1], D. Agiv[1], E. Marcos-Hadad[1], A. Bennet-Perlberg[2], A. Naor[2], and B. Ginzbourg[3], [1]*The Hebrew University of Jerusalem, Rehovot, Israel,* [2]*Israel Ministry of Agriculture and Rural Development, Dor, Israel,* [3]*Dagon Fish Hatchery, Kibbutz Maagan-Michael, Israel.*

Flathead gray mullet (*Mugil cephalus*) is a cosmopolitan marine food-fish, mainly fished in seas, but also increasingly farmed on-land. Aquaculture relies on capturing wild fry in estuaries and acclimating them to grow out in ponds. Capturing wild adults and fry puts pressure on natural populations. Natural populations yield irregular fry supply, hampering the development of mullet aquaculture. Mullet is a desirable aquaculture species, in which females grow faster than males. Only recently, the life cycle of mullet in captivity was closed allowing to produce fry in hatcheries. Breeding for desirable traits starts when hatchery production is in place. We developed a technology to genetically select brood fish producing all-female progeny. Sex was oftentimes evolutionarily shaped as a trait with only 2 phenotypes (female and male) and a fixed phenotypic segregation ratio of 1:1. Fish are a prime group to study sex determination as different species evolved different sex-de-

termination mechanisms. Mullet has a genetic mechanism influenced by hormonal manipulations. The technology involves a hormonal treatment producing sex-reversed mature males (milt producing males with a female sex genotype), which are then crossed to normal females for producing all-female progenies. While control groups had a 1:1 sex-ratio, in 3 treatment groups an excess of 63%, 74% and 84% males were identified, indicating that some males there were sex-reversed. Control groups were screened using ddRAD-seq to identify several thousands of SNP markers, from which 280 were significantly associated with sex and their mapping identified a single genomic position, suggesting a monogenic sex-determination system. Tightly linked markers had over 80% accuracy in determining the genetic sex of fish and allowed identifying sex-reversed males. Then, mature sex-reversed males were crossed with normal females to produce, for the first time, an all-females progeny group. Incorporating this technology into hatchery production will advance further development of mullet aquaculture and profitability of this industry.

Key Words: fish, genome-enabled breeding, DNA sequencing, candidate gene, aquaculture

P140 Population genetics of two critically endangered rhino rays from the Southwest Indian Ocean region. M. Groeneveld*[1], J. Klein[1], R. Bennett[2], M. Bond[3], D. Ebert[4,5], K. Gledhill[6], S. Jaquemet[7], J. Kiszka[3], A. Macdonald[8], B. Mann[9], J. Nevill[10], A. Price[1], M. van Staden[1], B. Wueringer[11,12], A. Bester-van der Merwe[1], [1]*Department of Genetics, Stellenbosch University, Stellenbosch, South Africa,* [2]*Wildlife Conservation Society, New York, NY,* [3]*Institute of Environment, Department of Biological Sciences, Florida International University, University Park, FL,* [4]*Pacific Shark Research Center, Moss Landing Marine Laboratories, Moss Landing, CA,* [5]*South African Institute for Aquatic Biodiversity, Grahamstown, South Africa,* [6]*Fish Ecology Lab, University of Technology Sydney, Broadway, Sydney, Australia,* [7]*UMR Entropie, Université de La Réunion, La Réunion, France,* [8]*School of Life Sciences, University of KwaZulu-Natal, Westville, South Africa,* [9]*Oceanographic Research Institute, Durban, South Africa,* [10]*Environment Seychelles, Mahé, Seychelles,* [11]*Sharks and Rays Australia, Bungalow, Queensland, Australia,* [12]*Department of Biological Sciences, Faculty of Science and Engineering, Macquarie University, Macquarie Park, New South Wales, Australia.*

Wedgefish (Rhinidae) are threatened by unsustainable trade globally and in the Southwest Indian Ocean (SWIO) due to their high-value fins. The whitespotted wedgefish *Rhynchobatus djiddensis* and the bottlenose wedgefish *Rhynchobatus australiae* are classified as critically endangered on the International Union for Conservation of Nature's Red List of Threatened Species, yet a lack of species-specific knowledge and taxonomic uncertainty still exists within this genus. Delineating populations and understanding the genetic connectivity of endangered and exploited species are important for their conservation management. Species identity of samples (n = 189) collected across the SWIO was confirmed based on the cytochrome oxidase c subunit 1 (*COI*) and nicotinamide adenine dehydrogenase subunit 2 (*ND2*) gene regions, where a ~98% sequence similarity match was considered reliable. The genetic diversity and population structure within and between species and sampling locations were investigated using a dual marker approach: (a) 2 concatenated mitochondrial gene regions, *COI* and the control region (n = 120), and (b) 9 nuclear microsatellite markers (n = 151). The overall genetic diversity was low, with an indication that different evolutionary forces are at play on a mitochondrial versus nuclear level. Analyses based on both marker types (haplotypes, *F*-statistics, multivariate and clustering analyses, with statistical significance defined at a 0.05 level) indicated clear differentiation of species. For *R. djiddensis*, the sampling populations from South Africa and Mozambique were generally homogeneous with no intraspecific genetic structure. For *R. australiae*, significant differentiation was found between the majority of sampling populations especially Australia, Reunion Island and Seychelles, whereas those from Madagascar and Tanzania were genetically the most similar. This information provides insights into the distribution range and population structure of the whitespotted wedgefish species complex that can support the sustainable management of wedgefish.

Key Words: conservation genetics, genetic identification, population structure, genetic markers, fisheries

Genetics of Immune Response and Disease Resistance

P141 A genome-wide association study for genetic susceptibility to *Corynebacterium pseudotuberculosis* infection in sheep. J. Kyselová*[1], L. Tichý[1], J. Marková[2], K. Kavanová[2], M. Beinhauerová[2], A. Gurgul[3], T. Szmatola[3,4], and Z. Sztankóová[1], [1]*Institute of Animal Science, Prague, Czechia,* [2]*Veterinary Research Institute, Brno, Czechia,* [3]*University of Agriculture in Krakow, Centre for Experimental and Innovative Medicine, Krakow, Poland,* [4]*National Research Institute of Animal Production, Balice, Poland.*

Caseous lymphadenitis (CL) is a contagious chronic bacterial disease of small ruminants of global relevance caused by *Corynebacterium pseudotuberculosis* (*Cp*). Unfortunately, little information is available on genetic resistance to this disease. The present study was conducted on the Suffolk breed kept in the Czechia. A total of 321 adult sheep (143 animals serologically positive and 178 serologically negative to *Cp*) belonging to 4 flocks were genotyped with the GGP Ovine 50K SNP array. A genome-wide scan was performed on the individual marker genotypes to identify polymorphisms associated with host susceptibility to CL. Genome coordinates for all SNP were based on the ARS-UI_Ramb_v2.0 ovine genome assembly (GenBank accession GCA_016772045.1). Genotyping data were quality-controlled using PLINK. Markers were removed from the analyses when they had a call rate below 97%, a minor allele frequency of less than 0.05, or showed a significant ($P < 0.00001$) deviation from Hardy-Weinberg equilibrium. The final data set contained 28 478 SNP marker genotypes. GWAS analyses were performed for 2 classes of phenotype (negative, i.e., less susceptible animals to CL, and positive, i.e., more susceptible animals to CL) using the mixed linear model association (*-mlma*) method in the GCTA software tool. Two SNPs on chromosomes 11 and 20 approached genome-wide significance (raw *P*-value $< 5 \times 10^{-5}$) and were considered to be suggestively significant. The most significant SNP was assigned to the *TRIM16* gene, which is involved in the positive regulation of cytokine production. Further following, 9 SNPs with raw $P < 5 \times 10^{-4}$ were revealed on different chromosomes. Three were located inside *LCLAT1*, *PDE4D*, and *SPIDR* genes encoding enzymes acting in phospholipid biosynthesis, signal transduction through T cell receptors, and DNA damage repair. These results provide a first insight into the genetic background of the immunological response to CL in sheep, but further research in larger populations is necessary. Estimating the contribution of SNP markers to genomic selection breeding strategies might enable the production of more disease-resistant animals.

P142 A sensitive and specific exonuclease III–assisted recombinase-aided amplification colorimetric assay for rapid detection of nucleic acids. C. Zhao*[1], Y. Zhou[1], J. Zhang[1], S. Zhao[1,2], and S. Xie[1,2], [1]*Huazhong Agricultural University, Wuhan, Hubei, China,* [2]*Hubei Hongshan Laboratory, Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, Hubei, China.*

The development of a contamination-free on-site nucleic acid detection platform with high sensitivity and specificity but low-cost for the detection of pathogenic nucleic acids is critical for infectious disease diagnosis and surveillance. In this study, we combined the recombinase-aided amplification (RAA) with the exonuclease III (Exo III)-assisted signal amplification into a platform for sensitive and specific detection of nucleic acids. The RAA-Exo III colorimetric assay is

based on the principle that the target DNA fragment is first amplified into dsDNA by isothermal RAA and then the dsDNA product is cleaved by Exo III, which removes single nucleotides in the 3′→5′ direction step by step. Given that Exo III cleaves several nucleotides at a time, allowing specific stem-loop DNA probe to rapidly bind the target ssDNA and to produce a 5′ overhanging dsDNA fragment that is subsequently cleaved by Exo III as a substrate, resulting in a blue to red color change or a positive signal band for lateral flow detection. We found that this platform enabled a naked eye visual detection of ASFV at a detection limit as low as 2 copies per reaction in 30 min. As expected, no cross-reactivity was observed with other porcine viruses. In addition, to avoid aerosol contamination, a one-tube RAA-Exo III colorimetric assay was also established for accurate detection of ASFV in clinical samples. Taken together, we developed a rapid, instrument-free, and low-cost Exo III-assisted RAA colorimetric assay-based nucleic acid detection platform.

Key Words: RAA-Exo III colorimetric assay, nucleic acid detection, stem-loop DNA probe, on-site

P143 Withdrawn

P145 Porcine epidemic diarrhea virus induces upregulation of kruppel-like factor 4 to promote its replication in porcine intestinal epithelial cells. H. Wang*, S. Wu, and W. Bao, *Yangzhou University, Yangzhou, Jiangsu Province, China.*

Porcine epidemic diarrhea virus (PEDV) is the main pathogen causing severe diarrhea in pig farms. Identification of regulators involved in PEDV infection is highly important for better understanding PEDV pathogenesis and for the prevention and control of PEDV. Herein, we performed transcriptomic analysis on the jejunum of pigs naturally infected with PEDV and identified 290 differentially expressed genes (DEGs). The kruppel-like factor 4 (KLF4) was identified as potential regulator of the DEGs, with higher binding motif occurrences in the promoter regions of DEGs. Quantification of KLF4 in PEDV-infected IPEC-J2 cells at varied time periods by qPCR showed that KLF4 expression was significantly upregulated at both mRNA and protein levels. To investigate the mechanisms of PEDV affecting KLF4 expression, we detected the core promote region of KLF4 by dual-luciferase assay. We then transfected vectors expressing the core promote sequence into IPEC-J2 cells and infected the cell with PEDV, and found that PEDV infection significantly enhanced the promoter transcriptional activity. The results indicated that PEDV can promote KLF4 expression by upregulating the promoter transcriptional activity. To explore the roles of KLF4 in PEDV infection, we knocked out KLF4 in IPEC-J2 cells using CRISPR/Cas9 technique and found that PEDV genome copies obviously increased in KLF4$^{-/-}$ cells compared with the KLF4$^{+/+}$ cells. RNA-seq were then conducted on PEDV-infected KLF4$^{-/-}$ and KLF4$^{+/+}$ cells, and 2295 DEGs were identified. The DEGs were enriched in pathways including cytokine-cytokine receptor interaction and intestinal immune network for IgA production. To further explore the regulatory roles of KLF4, we performed ChIP-seq on the DNA enriched by anti-KLF4 antibody. The binding motif of KLF4 was detected, and 95 genes within 5 kb of binding sites were identified. A subset of DEGs such as AQP1, LY6E, and MUC5B, were identified as the target of KLF4, indicating that KLF4 may regulate PEDV infection by regulating the expression of the target genes.

Key Words: porcine epidemic diarrhea virus, KLF4, virus replication, transcriptional activity

P146 ISAG Bursary Award: Characterization of the host-specific glycan responding to African swine fever virus infections. K. Han*[1], L. Sun[2,3], S. Wan[1], C. Cao[1], M. Lu[1], J. Yan[4], G. Peng[2,3], S. Zhao[1], and M. Yu[1], [1]*Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China,* [2]*State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China,* [3]*Key Laboratory of Preventive Veterinary Medicine in Hubei Province, The Cooperative Innovation Center for Sustainable Pig Production, Wuhan, China,* [4]*Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China.*

African swine fever virus (ASFV) is a devastating infectious disease in pigs, severely threatening the global pig industry. Resistance breeding is an important strategy for preventing virus infection. As a tool for targeted gene modification, gene editing technology has been applied for livestock disease-resistance breeding, which raises breeding efficiency. However, the lack of effective host genes associated with ASFV infection limits the application of gene editing technology in resistance breeding because of the unclear mechanisms of ASFV infection. Glycans are covalently attached to proteins to form glycoproteins, playing important roles in viral entry, replication, release, and immune escape. Glycan microarray technology is a high-throughput tool for studying the interactions of glycan with a variety of biomolecules. In this study, the glycan of ASFV binding specificity has been screened using glycan microarray analysis. Then, the glycoproteins modified by ASFV binding glycan were identified based on the data from ASFV host cells transcriptome sequencing, CRISPR screening, and protein expression patterns analysis. Finally, the glycan function in ASFV infection was elucidated by combining the competitive inhibition assay and glycan-protein interaction analysis. Thus, beyond our interpretation of the ASFV infection mechanism, this study identifies candidate targets for resistance breeding to prevent ASFV infection.

Key Words: swine, glycan, African swine fever virus (ASFV), disease resistance, glycan microarray

P147 Functional verification of key miR-223 for *Staphylococcus aureus*–induced bovine mastitis. X. Liu*[1,2], G. Dari[1], S. Mi[1], D. E.

MacHugh[2,3], and Y. Yu[1], [1]*College of Animal Science and Technology, China Agricultural University, Beijing, China,* [2]*UCD School of Agriculture and Food Science, University College Dublin, Dublin, Ireland,* [3]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland.*

Staphylococcus aureus (*S. aureus*)–induced mastitis is one of the most intractable problems for the dairy industry, causing significantly reduced milk yields and early slaughter of cows worldwide. MicroRNAs (miRNAs) can post-transcriptionally regulate gene expression and studies in recent years have shown the importance of miRNA-associated gene regulation in *S. aureus*–induced mastitis. In this study, to investigate the role of miR-223 in mastitis, we performed experiments to overexpress and suppress miR-223 in an immortalized bovine mammary epithelial cell line (MAC-T) infected with *S. aureus*. Overexpression of miR-223 in MAC-T cells repressed cell apoptosis and necrosis induced by *S. aureus* infection, whereas suppression of miR-223 had the opposite effect. Transcriptome expression profiling with weighted gene co-expression network analysis (WGCNA) and gene set variation analysis (GSVA) showed that miR-223 affects apoptosis and inflammation-related pathways. Furthermore, differentially expressed (DE) genes were evaluated, and genes exhibiting contrasting expression trends in the miR-223 overexpressed and suppressed groups were assessed as potential target genes of miR-223. Potential target genes, such as *PARP1*, *ZBP1*, and *GALNT18*, were then observed to be associated with the apoptosis and necroptosis pathways. Finally, through integrative analysis of GWAS data and the animal QTL database, we determined that target genes of miR-223 were significantly enriched in SNPs and QTLs related to somatic cell count (SCC) and mastitis. In summary, miR-223 has an inhibitory effect on *S. aureus*–induced cell apoptosis and necrosis by regulating *PARP1*, *ZBP1*, and *GALNT18*. The results from this work will improve our understanding of host-pathogen interaction and the immunobiology of *S. aureus* infection in cattle and will provide useful information for new veterinary interventions to control bovine mastitis.

Key Words: cattle and related species, microRNA, animal health

P148 Investigation on the interference effect of CRISPR-Cas13d system against porcine epidemic diarrhea virus. C. Zhao, X. Hu, and R. Zhang*, *China Agricultural University, Beijing, China.*

Porcine epidemic diarrhea virus (PEDV) is a coronavirus pathogen of the pig intestine, that causes acute diarrhea, vomiting, dehydration and high mortality in neonatal piglets, resulting in significant economic losses in swine industry. Cas13d is a small VI-D CRISPR effector that has shown high efficiency in targeting RNA viruses. Here, we designed 41 crRNAs targeting the M, E, N and ORF3 regions of PEDV and testing their interference efficiency using HEK293T cells, with an interference efficiency of up to 97.0%. We further constructed and screened efficient tandem crRNAs stably expressing Cas13d and different ORFs targeting PEDV, achieving a robust and long-lasting antiviral effect in Vero cells. Importantly, the stable transformed Vero cells showed almost complete cleavage of the PEDV virus. After 36–72 h of infection, the viral copy number was significantly reduced by 1,000-fold compared with the control group. Our study also confirmed that Cas13d can effectively interfere with different serotypes of PEDV, and off-target detection revealed almost no trans cleavage activity-induced gene differential expression. Overall, our findings provide experimental evidence for the potential of CRISPR-Cas13d-based system as a RNA-guided therapy against PEDV infection.

Key Words: CRISPR-Cas13d, RNA virus, porcine epidemic diarrhea virus (PEDV), disease resistance, crRNA

P149 Identification of porcine genes against pseudorabies virus infection by genome-wide CRISPR activation screening. A. Shangguan*, Y. Sun, Z. Liu, J. Jiang, and S. Zhang, *Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Education Ministry of China, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei Province, China.*

Pseudorabies is a highly contagious viral disease caused by the pseudorabies virus (PRV), and it is one of the most devastating diseases for the swine industry worldwide. In addition, reports of PRV infection in humans have emerged in recent years, posing a potential threat to public health. Therefore, there is an urgent need for the identification of PRV infection–related host genes and their interactions to provide theoretical basis and target genes for vaccine and drug development and breeding of PR-resistant pig breeds. To identify host factors that can rescue PK-15 cells from PRV infection, we used a genome-scale CRISPR activation screen. A total of 191 porcine resistance candidate genes of PRV infection were screened, among which we selected *MAPK12*, *RIPK1*, *ACTR1B*, *COX7A2*, *MCM5*, *TUSC1*, *OGFOD2*, *VASH2*, *SRP9*, and *PJA1* for further verification. Activated cells of these 10 genes were constructed separately and infected with PRV, and the inhibitory effect of gene activation on PRV infection was initially detected using RT-qPCR. The result showed that 9 genes (except *MCM5*) had inhibitory effect on PRV infection. We then performed flow analysis and found that 7 genes (*OGFOD2*, *COX7A2*, *MAPK12*, *RIPK1*, *TUSC1*, *SRP9*, and *PJA1*) had better effect. Two genes (*RIPK1* and *PJA1*) that inhibited PRV infection were further identified by RNAi technology. The results provide a better understanding of key host factors that protect cells from PRV infection and might assist in identifying novel antiviral targets.

Key Words: CRISPR activation, pseudorabies virus (PRV), genome-wide screen, *RIPK1*, *PJA1*

P150 Withdrawn

P151 ISAG Bursary Award: Functional diversity of toll signaling pathway in Czech Simmental cattle with respect to health and resilience traits. K. Samake*[1], T. Valcikova[2], M. Bjelka[3], and K. Novak[4], [1]*Charles University, Prague, Czech Republic,* [2]*Czech Uni-*

versity of Life Sciences, Prague, Czech Republic, [3]Breeding Company CHD Impuls, Bohdalec, Czech Republic, [4]Institute of Animal Science, Prague-Uhrineves, Czech Republic.

The work was aimed at the screening for and interpretation of functional polymorphism in the key members of the toll signaling pathway in the population of the Czech Simmental cattle. Focus was on the transcription factor NFkappaB as the main pleiotropic factor for phenotypic traits and to MyD88 as an interactor insufficiently studied until now. Hybrid resequencing with Illumina X-Ten WGS and PacBio amplicon sequencing yielded 22 and 13 SNPs in *NFkappaB1* and *NFkappaB2* genes, respectively, while over 30 SNPs were found in the *MYD88* gene. The PacBio amplicons were used for haplotype determination in the given population, resulting in 7 haplotypes for *NFkappaB1*, 6 haplotypes for *NFkappaB2*, and additional tens of SNPs in *MYD88*. Based on the functional prediction and haplotype assignment, a subset of candidate SNPs of interest was chosen for subsequent genotyping with the SNaPshot technique. The working sets of reactions included 8 and 11 SNPs in *NFkappaB1* and *-2* genes, respectively, and 18 SNPs in *MYD88*. The presence of a nonsynonymous mutation R474G in *NFkappaB1* allows to assume phenotypic effects due to the pronounced pleiotropy of this gene. The results from genotyping of individual animals were used for haplotype reconstruction in the given population and for the association study in the set of 164 bulls using haplotype data. The breeding values for milk production, health traits and female fertility were correlated with allelic forms of the 2 key genes along with the *TLR* gene series. The ongoing work includes the resilience trait of milk production as the indicator potentially affected by the toll pathway members. The project was supported by the Institutional Research Concept no MZE-RO0723 of the Ministry of Agriculture of the Czech Republic.

Key Words: cattle and related species, immunogenomics, genotyping, innate immunity, animal health

P152 Superior survivability of *GBP1* and *GBP5* heterozygous pigs undergoing porcine respiratory syndrome outbreaks. R. Pena*[1], K. Keutgens[2], and L. Fraile[1], [1]*Universitat de Lleida-AGROTECNIO Centre, Lleida, Spain,* [2]*PXL University of Applied Sciences and Arts, Hasselt, Belgium.*

The porcine respiratory disease complex causes respiratory signs leading to pneumonia in rearing pigs and failure to gain weight later in the finishing period. Although the etiology is complex, it usually involves coinfection of viral and bacterial agents. Typically, 30 to 70% of pigs will be affected during an outbreak, with a 4 to 6% mortality rate, depending on the secondary infections. In a screening experiment in production farms in Northeast Spain, we collected samples from transition and rearing farms with active respiratory outbreaks, with mortalities ranging from 13.8 to 71.4%. In each farm, samples were collected from 50 to 80 dead animals (CASES; outbreak) and 50–80 of the best growing pigs (CONTROLS; 2 weeks later). Microbiology and serology test confirmed that farms were infected with PRRSV and either *Streptococcus suis* or *Actinobacillus pleuropneumoniae*. Two SNP molecular markers at *GBP1* (rs80800372) and *GBP5* (rs340943904) genes were genotyped in an initial cohort of 478 samples (269 cases and 209 controls) from 3 farms undergoing PRRSV + *Streptococcus suis* coinfection. Results show that incidence of cases (pigs dead during the outbreak) was reduced by 7% in heterozygous pigs for either marker when compared with the incidence of cases in pigs homozygous for the major (susceptible) allele. Despite their close mapping on the pig chromosome 4, these 2 markers have a moderate linkage disequilibrium, with an r2 of 0.8. We are currently testing other molecular markers previously associated with resilience to infections in a larger cohort of approximately 900 pigs, including farms infected with PRRSV and *Actinobacillus pleuropneumoniae.*

Key Words: pigs and related species, disease resilience, infectious disease, genetic marker, animal health

P153 IUIS-VIC Travel Award 2: Due to their improved immunity, disease-resistant common carp fish are also less infective. B. Dorfman*, E. Marcos-Hadad, R. Tadmor-Levi, and L. David, *Department of Animal Sciences, R. H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Rehovot, Israel.*

The common carp (*Cyprinus carpio*) is among the most widely produced aquaculture species. Outbreaks of a disease caused by cyprinid herpes virus type 3 (CyHV-3) have been significantly damaging its production worldwide. Our group has been breeding for CyHV-3 disease-resistant strains. When infected, resistant fish control better the viral replication while susceptible fish cannot and consequently succumb to the disease. This resistance relies on improved host immunity. In this study, experiments using infection-by-cohabitation tested the infectivity of disease-resistant and susceptible fish and how the combination of resistance and infectivity differences affect mortality and disease spread. Disease resistant and susceptible fish played roles of shedders (infecting) and cohabitants (infected) in all 4 type-role combinations. Mortality rates were highest in groups of susceptible cohabitants infected by susceptible shedders and lowest in groups of resistant cohabitants infected by resistant shedders. However, fewer mortalities were found in susceptible cohabitants infected by resistant shedders compared with susceptible cohabitants infected by susceptible shedders. Additionally, viral loads in spleen of resistant cohabitants infected by resistant shedders were lower compared with the same resistant cohabitants infected by susceptible shedders. Finally, virus levels in water of tanks with susceptible shedders were higher than in tanks with resistant shedders. Taken together, we empirically and clearly demonstrated that because disease-resistant fish better control the virus replication they release less virus particles into the environment and, hence, infect other fish less than disease-susceptible fish. This study demonstrates that incorporating resistant fish benefits aquaculture production twice, by reducing both mortalities and disease spread.

Key Words: fish, immunology, qPCR, infectious disease, aquaculture

P154 Association of the *IRAK1* gene polymorphism with health, milk and exterior traits in cattle. L. Tichý*[1,2], V. Šteiger[1], L. Zavadilová[2], D. Schröffelová[1], J. Kyselová[2], M. Pribánová[1], L. Vostrý[2], J. Kucera[1], and Z. Sztankóová[2], [1]*Czech Moravian Breeders' Corporation, Hradištko, Czech Republic,* [2]*Institute of Animal Science, Prague-Uhríneves, Czech Republic.*

Interleukin-1 receptor-associated kinase 1, encoded by the *IRAK1* gene, is an essential enzyme in the Toll-like receptor signaling pathway. Although there is a lack of information about the *IRAK1* gene in cattle, it is possible to estimate the effect of the *IRAK1* gene in other animal species. Polymorphism in its coding region may cause changes, not only in immunity but also in metabolic processes. The objective of the study was to investigate rs110533802 polymorphism of *IRAK1* in different cattle breeds and estimate its functional impact. DNA microarray technology (Illumina Infinium HTS method) was used to determine the rs110533802 polymorphism in 10 492 cows of the 6 cattle breeds. The same allelic frequency of 40% of the mutant allele T was found in Czech (n = 4954) and Hungarian (n = 4268) Holstein populations. Beef breeds were represented by Charolais (n = 261), Simmental (n = 281), Limousine (n = 365), and Aberdeen Angus (n = 289) populations. The allelic frequency of the mutant allele T varied between 0 and 10% in them. The next genotyped population was the Czech Fleckvieh dual-purpose cattle (n = 74), with the mutant allele frequency of 7%. Significantly higher occurrence of the mutant allele in dairy cattle leads to the hypothesis that it may be associated with milk yield. The GLM statistical model with fixed effects of herd, year and season of calving, age at calving, age at evaluation and genotype of polymorphism rs110533802 was applied to estimate effects of polymorphism on the investigated traits in 1016 Holstein dairy cows. Health, milk yield and exterior traits were selected as dependent variables. Statistically significant results were found for somatic cell score ($P = 0.0436$), udder depth

($P = 0.0001$), udder texture ($P = 0.0202$), angularity ($P = 0.0450$), body condition score ($P = 0.0047$), and stature ($P = 0.0134$). According to the obtained results, the rs110533802 polymorphism of *IRAK1* gene may be considered a genetic marker of some exterior and mammary gland traits in Holstein cattle.

Key Words: cattle, functional genomics, microarray, innate immunity, milk production

P155 ISAG Bursary Award: IUIS-VIC Travel Award 1: Transcriptomic signatures of peripheral immune cells associated with immune competence traits in Australian Angus cattle. A. Wilson*[1], P. Alexandre[2], T. Legrand[2], S. Denman[2], T. Reverter[2], C. Stewart[1], and R. Farr[1], [1]*Commonwealth Scientific and Industrial Research Organization, East Geelong, VIC, Australia,* [2]*Commonwealth Scientific and Industrial Research Organization, St Lucia, QLD, Australia.*

Infectious disease incurs considerable cost for Australian beef cattle producers through loss in productivity, monitoring, and treatment. Immune competence (IC), a combined measure of cell-mediated (cell-IR) and antibody-mediated (Ab-IR) immune responses, is a generalized disease resilience trait that can be combined with production traits into a weighted selection index for the aim of breeding high-producing and disease resilient animals. In this study we characterized the molecular responses that are associated with IC trait in livestock, with a particular emphasis on the coding and non-coding transcriptome. In this study, 51 Australian Angus cattle were evaluated for cell-IR and Ab-IR at weaning. This was performed by measuring delayed-type hypersensitivity and antibody titers following intradermal and intramuscular vaccination with a multivalent clostridial vaccine, respectively. Total RNA isolated from peripheral blood mononuclear cells (PBMCs) collected before vaccination was profiled using next-generation sequencing. Whole transcriptome analysis was performed using the nf-core/RNaseq pipeline. Animals were ranked by their Ab-IR, Cell-IR and IC scores and split into quartiles. The lowest and highest quartiles were classified as low and high responders, respectively. Machine learning demonstrated that, for each of the 3 traits, a distinct 5-gene signature could accurately (>95%) classify animals as high or low responders. Differential gene expression analysis with DESeq2 comparing low and high responders found that several genes were differentially expressed (>1.5 FC, *P*-value <0.05) for each trait. A total of 2,014 long non-coding genes were found to be highly expressed in PBMCs, including 1,350 novel lncRNAs. Machine learning also revealed a that distinct 4-lncRNA signature can also accurately (>95%) distinguish high and low responders. Our ongoing research is now focused on understanding the interplay between coding and non-coding RNAs and the functional role that this plays in IC with the goal of improving immune resilience in beef cattle.

Key Words: immune competence, Angus cattle, whole transcriptome, machine learning

P156 ISAG Bursary Award: Assessment of haemagglutination titre and serum lysozyme concentration in Nigerian indigenous chicken genotypes. U. Akpan*, A. S. Adenaike, M. I. Takeet, A. A. Bello-Ibiyemi, and C. O. N. Ikeobi, *Federal University of Agriculture, Abeokuta, Ogun state, Nigeria.*

This research was conducted to assess the immune status of 3 indigenous Nigerian chicken genotypes, namely; Normal-feather, Frizzle-feather and Naked neck. The chickens were inoculated with sheep red blood cell (SRBC), a multi-determinant antigen. A total of 268 chickens from the 3 genotypes divergently selected for high and low response to SRBC and a random-bred control line, originating from the same base population were used for the study. The birds (8 weeks of age) were inoculated with 1 mL of 1% suspension SRBC via the jugular vein and antibody responses at 5, 10 and 15 d post-inoculation (dpi) were measured. Data collected on the haemagglutination (HA) titer and serum lysozyme concentration (SL) were analyzed using the Linear Model procedure of R version 4.0.2. Results showed that genotype did not significantly ($P > 0.05$) affect HA titer. However, the results obtained showed significant ($P < 0.05$) prevalence of antibody response at 5 dpi. There was significant ($P < 0.05$) interaction effect of genotype and sex for SL at 5 dpi, in which female Frizzle-feather had significant higher value when compared with female Normal-feather. The study therefore suggests that the Nigerian indigenous female Frizzle-feather chicken genotype has a good potential for serum lysozyme response and could be selected to improve such trait. Also, selections for immunocompetent traits such as HA titer and serum lysozyme concentration should be prioritized at 5 dpi.

Key Words: immune response, sheep red blood cell (SRBC), haemagglutination, serum lysozyme, indigenous chicken

P157 Withdrawn

P158 Exploring, evaluating, and quantifying the mammalian alveolar macrophage response to intracellular mycobacterial pathogens using an integrative multi-omics approach. T. J. Hall[1], M. Mittermite[2], J. A. Browne[1], G. P. McHugo[1], J. F. O'Grady[1], E. L. Clark[3], M. Salavati[3,4], S. V. Gordon[2,5], and D. E. MacHugh*[1,5], [1]*UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland,* [2]*UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland,* [3]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, Scotland, United Kingdom,* [4]*Dairy Research and Innovation Centre, SRUC South and West Faculty, Barony Campus, Parkgate, Dumfries, Scotland, United Kingdom,* [5]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland.*

Bovine tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium bovis*, which is responsible for significant economic losses in the livestock industry worldwide and can also cause TB disease in a range of other mammals, including humans. Alveolar macrophages are the primary cells targeted by the pathogen during early infection, and although they play a crucial role in controlling the infection, the exact nature of the host-pathogen interaction, and the genetic

and epigenetic factors that drive host tropism are not fully understood. Here, we have used RNA-seq, miRNA-seq, ChIP-seq, and ATAC-seq to examine the effect of *M. bovis* infection on the bovine alveolar macrophage (bAM) epigenome and transcriptome. In addition to this, we have also challenged bAMs with *Mycobacterium tuberculosis* (the primary cause of human TB), *M. bovis* BCG (the vaccine strain), and gamma-irradiated (killed) *M. bovis* to examine the bAM epigenomic and transcriptomic responses across multiple pathogenic insults. The results of this multi-omics comparison shed new light on the function of pivotal response genes and support the hypothesis that pathogen-driven epigenetic reprogramming of the host macrophage is key to bacterial survival and host tropism for *M. bovis* and other TB-causing mycobacteria.

Key Words: cattle, tuberculosis, macrophage, transcriptome, epigenome

P159 Withdrawn

P160 Genome-scale CRISPR screen identifies TRIM2 and SLC35A1 associated with porcine epidemic diarrhea virus infection. H. Liu[1], J. Wang[2], Z. Guo[1], X. Zeng[2], Y. Yang[1], S. Li[1], X. Li[1,3], S. Zhao[1,4], C. Wang[2], and S. Xie*[1,4], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Lab of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, Hubei, P. R. China,* [2]*Key Laboratory of Pig Molecular Quantitative Genetics of Anhui Academy of Agricultural Sciences, Livestock and Poultry Epidemic Diseases Research Center of Anhui Province, Anhui Provincial Key Laboratory of Livestock and Poultry Product Safety Engineering, Hefei, Anhui, P. R. China,* [3]*The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan, Hubei, P. R. China,* [4]*Hubei Hongshan Laboratory, Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, Hubei, P. R. China.*

CRISPR/Cas9 has been used extensively in pigs as one of the tools in disease resistance research. Porcine epidemic diarrhea (PED) is the most devastating disease in the global pig industry due to its high fatality rate in piglets. Existing vaccines have become ineffective against the epidemic PED virus (PEDV) variants. There is still a need to breed disease-resistant pigs to prevent and control these viruses. In this study, we designed a pooled African green monkey genome-scale CRISPR/Cas9 knockout (GeCKO) library containing 75,608 single guide RNAs targeting 18,993 protein-coding genes to identify key host factors facilitating PEDV infection and potential therapeutic targets in Vero E6 cells. We discovered several highly expressed but unreported genes associated with PEDV infection after challenging the Vero E6 cells with PEDV. For instance, knocking out the tripartite motif 2 (TRIM2) and the solute carrier family 35 member A1 (SLC35A1) significantly inhibited PEDV replication. Quantitative glycoproteomics showed that knocking out SLC35A1 interfered with glycoprotein expression, especially ADAM17, APNEP and ACE2, to reduce PEDV infection; rather than the entry-dependent cellular CMP-sialic acid. These findings provide a new perspective for a better understanding of host-pathogen interactions and candidate targets for the generation of gene-editing disease-resistant pigs. In addition, we also developed a deep learning strategy-based sgRNA activity prediction algorithm, and thus built an online sgRNA design platform, called sgRNAcas9-AI (http://123.57.239.141:8080/), which can help users to design highly active sgRNAs. In the future, we will use gene editing technology to produce PEDV-resistant pigs.

Key Words: pig, CRISPR screening, porcine epidemic diarrhea virus (PEDV), TRIM2, SLC35A1

P161 ISAG Bursary Award: LncRNA446 regulates tight junctions by inhibiting the ubiquitinated degradation of Alix after porcine epidemic diarrhea virus infection. Y. Xiao*, W. Qin, H. Wang, and W. Bao, *Yangzhou University, Yangzhou, Jiangsu, China.*

Porcine epidemic diarrhea (PED) is a highly contagious disease, caused by porcine epidemic diarrhea virus (PEDV), which causes huge economic losses. Tight junction-associated proteins play an important role during virus infection; therefore, maintaining their integrity may be a new strategy for the prevention and treatment of PEDV. Long noncoding RNAs (lncRNAs) participate in numerous cellular functional activities, yet whether and how they regulate the intestinal barrier against viral infection remains to be elucidated. Here, we established a standard system for evaluating intestinal barrier integrity and then determined the differentially expressed lncRNAs between PEDV-infected and healthy piglets by lncRNA-seq. A total of 111 differentially expressed lncRNAs were screened, and lncRNA446 was identified due to significantly higher expression after PEDV infection. Using IPEC-J2 cells and intestinal organoids as in vitro models, we demonstrated that knockdown of lncRNA446 resulted in increased replication of PED, with further damage to intestinal permeability and tight junctions. Mechanistically, RNA pulldown and an RNA immunoprecipitation (RIP) assay showed that lncRNA446 directly binds to ALG-2-interacting protein X (Alix), and lncRNA446 inhibits ubiquitinated degradation of Alix mediated by TRIM25. Furthermore, Alix could bind to ZO1 and occludin and restore the expression level of the PEDV M gene and TJ proteins after lncRNA446 knockdown. Additionally, Alix knockdown and overexpression affects PEDV infection in IPEC-J2 cells. Collectively, our findings indicate that lncRNA446, by inhibiting the ubiquitinated degradation of Alix after PEDV infection, is involved in tight junction regulation. This study provides new insights into the mechanisms of intestinal barrier resistance and damage repair triggered by coronavirus.

Key Words: long noncoding RNA, porcine epidemic diarrhea virus, intestinal barrier, tight junction, Alix

P162 Association of variants in antibacterial *TLR* genes with reproductive traits in Czech Simmental cattle. K. Novak*[1], K.

Samake[2], and M. Bjelka[3], [1]*Institute of Animal Science, Prague-Uhrineves, Czech Republic,* [2]*Charles University, Prague, Czech Republic,* [3]*Breeding Company CHD Impuls, Bohdalec, Czech Republic.*

The Toll-like receptor functions are reflected in various phenotypic traits in addition to the immune response itself. Therefore, we tested the *TLR* gene diversity present in the Czech Simmental (Czech Red Pied) cattle population for association with not only the traits of infection resistance, but also with the female reproductive traits. The starting bull set comprised 164 animals representing complete gene pool. Hybrid resequencing with Illumina X-Ten WGS and PacBio amplicon sequencing yielded 7 and 9 SNPs in the *TLR4* and *TLR5* genes, respectively. The SNPs were validated with primer extension assays and characterized by the assignment to haplotypes and by function prediction. The breeding values for the reproductive traits, namely incidence of cystic ovaries, early reproductive disorders (ERD), calving ease, maternal calving ease, production longevity and calf vitality index were used for the association study. Nominal *P*-values <0.05 for associations were detected in 18 combinations between 14 polymorphisms and 15 traits using one-way ANOVA. After Benjamini-Hochberg test, the *TLR4* variants g.610C > T (rs43578094) and g.10310T > G (rs8193072) in the reference AC000135.1 remained strictly associated with the index of ERD and maternal calving ease, respectively, at FDR <0.05. The *TLR4* variant g.9422T > C (rs8193060) was associated with as many as 4 traits. The permissive FDR interpretation for the *TLR5* variants indicated associations with cyst incidence, ERD and maternal calving ease. Positional matches with known QTLs for calving ease endorse the causative roles of *TLR4* and *TLR5*. The findings are consistent with the known effects of *TLR4* variation on reproduction in model species. Consequently, the Bayesian inference supports the role of bovine *TLR4* and *TLR5* in the formation of female reproductive traits. However, additional experiments discriminating between different mechanisms of action are necessary. The project was supported by the Institutional Research Concept MZE-RO0723 and by the grant QK22020280 of the Ministry of Agriculture of the Czech Republic.

Key Words: cattle and related species, immunogenomics, genotyping, innate immunity, disease resilience

P163 ISAG Bursary Award: Genomic markers associated with immune traits in Sasso chickens raised in Ethiopia. M. Girma*[1,2], M. Katrina[3], S. Kate[3], W. Esatu[2], B. Solomon[2], T. Dessie[2], P. Androniki[3,4], V. Lonneke[3], H. Olivier[2,5], B. Georgios[3,6], and M. Dikeledi[1], [1]*Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Florida, South Africa,* [2]*CTLGH-LiveGene, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [3]*Centre for Tropical Livestock Genetics and Health, The Roslin Institute, University of Edinburgh, Easter Bush Campus, Midlothian, UK,* [4]*The Royal Veterinary College, Hawkshead Lane, Hatfield, Hertfordshire, UK,* [5]*Cells, Organisms and Molecular Genetics, School of Life Sciences, University of Nottingham, Nottingham, UK,* [6]*Scotland's Rural College (SRUC), Animal and Veterinary Sciences, Easter Bush, Midlothian, UK.*

Newcastle disease virus (NDV) is a highly contagious avian pathogen that threatens poultry production. It is endemic to many areas and responsible of many epidemic events. Selection for antibody (Ab) response has potential to effectively improve the resistance to disease in chickens. However, the origin of the variation among chickens in Ab response to NDV remains unclear. Here, we aimed to identify the genes modulating Ab response to a viral pathogen such as NDV while under outdoor conditions. A genome-wide association study (GWAS) was performed. After NDV vaccination, Sasso T451A chickens were naturally exposed to environmental challenges at the ILRI poultry research facility in Addis Ababa, Ethiopia. Phenotypic and immune data from 1,022 chickens in 2 batches (batch 4 with 507 birds and batch 5 with 515 birds) were recorded. Genotyping information from low-pass sequencing was obtained from 935 chickens (2,676,181 SNPs). The results revealed that chickens from batch 4 show a stronger Ab response at 56 d old and a lower Ab response at 112 d old compared with batch 5 chickens. We used BioMart data mining and variant effect predictor tools to annotate the SNPs and candidate genes, respectively. Five significant SNPs (rs316795557 [*FOXP2*] – chr 1, rs313761644 [*CEP170B*] – chr 5, rs733628728 – chr 13 and 2 unnamed SNPs – chr 30 and chr 33) were associated ($P < 3.92E-7$) with chicken antibody response to NDV. These SNPs are in genome regions including several genes regulating the immune response. The results of this study pave the path for more investigation into the importance of the chicken immune response to NDV.

Key Words: antibody response, genome-wide linkage analysis, Newcastle disease, Sasso T451A, vaccine challenges

P164 Integration of information from multiple gene expression and genome-wide association studies on host resistance of cattle to infestation with *Rhipicephalus microplus* ticks. K. Chooyoung*, B. Mable, and N. Jonsson, *School of Biodiversity, One Health and Veterinary Medicine College of Medical, Veterinary and Life Sciences University of Glasgow, Glasgow, United Kingdom.*

The cattle tick *Rhipicephalus microplus* causes massive damage to cattle throughout the tropics and subtropics. Resistance to ticks is moderately heritable (h^2 ~0.4). Many studies have examined gene expression in the skin and blood of cattle of high and low resistance to ticks, and several genomic-wide association studies (GWAS) have been conducted. However, no genomics or phenotypic biomarkers for resistance are in commercial use. The objective of this study was to combine information from gene expression studies (GEXS) and genome-wide association studies (GWAS) on host resistance to infestation with *R. microplus*, to create a list of candidate biomarkers (genes or gene products) for which there were multiple sources of supporting evidence. From the literature, 16 GEXS (7 microsatellites studies, 9 NGS; 4 blood and 12 skin studies) and 12 GWAS (9 SNPs and 3 others) were identified that provided sufficient information to identify genes as significantly associated with tick resistance (as per authors' original declarations). This yielded 10,495 DEGs and 288 QTLs, which were then filtered to only those genes for which multiple studies showed consistent results. The final list included those QTLs significant in at least 2 independent GWAS (n = 11); DEGs significant in at least 4 skin (n = 6) or 2 blood (n = 10) GEXS; QTLs that were also significant DEG in at 1 blood or 2 skin GEXS (n = 10). The list of genes included 3 transcription factor genes, 12 genes associated with immune function, 3 genes with the extracellular matrix, 6 genes with the structural protein, and 13 genes in others. A total of 37 genes were identified for which multiple sources of evidence could be obtained and which are being further investigated for their value as biomarkers, either genomic or phenotypic.

Key Words: genome-wide association studies, gene expression studies, biomarkers, cattle and related species

Genome Edited Animals

P165 *Drosophila melanogaster* (fruit fly): A platform for anticancer drug discovery and development. S. Malindisa* and M. Ntwasa, *University of South Africa, Florida, Johannesburg, South Africa.*

Cancer is a complex disease whereby multiple genetic, epigenetic, metabolic and microenvironment modifications contribute to development of a tumor. In the traditional anticancer drug discovery, drug candidates are usually screened in vitro using 2- or 3-dimensional mammalian cell cultures. However, these methods fail to accurately mimic the human disease state, and therefore translatability to whole organisms is often challenging. *Drosophila melanogaster*, commonly known as the fruit fly, has emerged as a beneficial system for modeling human cancers. Fundamental research has shown the evolutionary conserva-

tion of key genes and signaling pathways between flies and humans. Moreover, *Drosophila* has a lower genetic redundancy in comparison to mammals. These factors allow for the generation of complex *Drosophila* genotypes and phenotypes. Numerous studies have successfully created *Drosophila* models for various cancers. These models have been used for high-throughput screening of drugs which led to the identification of compounds currently used for cancer treatment. In this presentation, we propose the application of *Drosophila* as a platform for anticancer drug discovery. Using the GAL4/UAS system, transgenic flies with specific genetic mutations in known cancer-causing genes such as *KRAS*, *p53* and *PTEN* genes will be generated. Current experimental work is ongoing for breeding transgenic flies. Following generation of offspring with cancer, several FDA-approved drugs will be tested for cytotoxicity. RNA, protein and metabolites will be extracted. Gene and protein expression will be measured by qPCR and Western blot respectively. The amount of drugs ingested by flies will be determined using HPLC-mass spectrometry. *Drosophila* can mediate high-throughput screening of compounds and can provide valuable information on drug bioavailability and toxicity. These models are inexpensive and efficient, thus reducing the cost and time of anticancer drug discovery. The success of the establishment of the drug screening platform using *Drosophila* will contribute toward advancing drug discovery research in under resourced nations.

Key Words: *Drosophila melanogaster*, cancer models, drug screening

P166 Withdrawn

P167 Withdrawn

P168 Withdrawn

P169 ISAG Bursary Award: Field-deployable nucleic acid detection with RAVI-CRISPR. D. Tao[1], B. Xu[1], S. Li[1], C. Zhao[1], S. Zhao[1,2], X. Li[1,3], and S. Xie*[1,3], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Lab of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, China,* [2]*The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan, China,* [3]*Hubei Hongshan Laboratory, Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, China.*

Rapid diagnosis based on naked-eye colorimetric detection remains challenging, but it could build new capacities for molecular point-of-care testing (POCT). By screening a range of candidate Ca-

s12a nucleases, here we identify novel Cas12a homologous nucleases with high activity, which could facilitate the CRISPR-based nucleic acid detection. Next, we evaluated the performance of 16 types of single-stranded DNA-fluorophore-quencher (ssDNA-FQ) reporters for use with CRISPR/Cas12a based visual colorimetric assays. Among them, 9 ssDNA-FQ reporters were found to be suitable for direct visual colorimetric detection, with especially very strong performance using ROX-labeled reporters. In addition, we developed a convolutional neural network algorithm standardize and to automate the analytical colorimetric assessment of images and integrated this into the MagicEye mobile phone software. Subsequently, a field-deployable assay platform named RApid VIsual CRISPR (RAVI-CRISPR) based on a ROX-labeled reporter with isothermal amplification and novel Cas12a/crRNA targeting was established. We deployed RAVI-CRISPR in a single tube toward an instrument-less colorimetric POCT format that requires only a portable rechargeable hand warmer for incubation. Our study demonstrates this novel RAVI-CRISPR system for distinguishing different nucleic acid targets with high specificity and sensitivity as the simplest-to-date platform for rapid pen-side testing.

Key Words: RAVI-CRISPR, Cas12a, MagicEye, point-of-care testing

P170 Rethinking the genetic basis of pregnancy recognition in ruminants: Pregnancy in type I interferon receptor (*IFNAR2*) knockout sheep. C. J. Davies*[1,2], E. K. Peterson[1,2], M. J. Brothers[1,2], A. J. Thomas[1,2], H. M. Rutigliano[1], Y.-M. Lee[1], and I. A. Polejaeva[1], [1]*Department of Animal, Dairy & Veterinary Sciences, Utah State University, Logan, UT,* [2]*Center for Integrated Biosystems, Utah State University, Logan, UT.*

Type I interferons (IFN) initiate immune responses to viruses. Their effects are mediated by the type I interferon receptor, IFNAR, a heterodimer of IFNAR1 and IFNAR2. One or both genes encoding the sheep IFNAR were disrupted in fetal fibroblast lines using CRISPR/Cas9 and lambs were produced by somatic cell nuclear transfer. Subsequently, a herd of about 30 *IFNAR2*$^{+/-}$ ewes was developed and bred to either *IFNAR2*$^{+/-}$ or *IFNAR2*$^{-/-}$ rams to produce *IFNAR2*$^{-/-}$ lambs. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) for IFN-stimulated gene (ISG) expression confirmed that IFNAR deficient sheep fail to respond to IFN-α (IFNA). The accepted mechanism for pregnancy recognition in ruminants involves a novel type I IFN, interferon-tau (IFNT), interacting with IFNAR. Because of the numerous publications on the role of IFNT in pregnancy recognition, we hypothesized that *IFNAR2*$^{-/-}$ ewes would be infertile. However, in December 2022 we bred 2 approximately 9-mo-old *IFNAR2*$^{-/-}$ ewes to an *IFNAR2*$^{+/-}$ ram and, to our surprise, both ewes became pregnant. Pregnancy in these ewes was confirmed by progesterone profiling, pregnancy specific protein B (PSPB) enzyme-linked immunosorbent assay (ELISA), and ultrasound exams. The ultrasound exams demonstrated the presence of a live fetus with a heartbeat in both ewes. At the time of their ultrasound exams, the ewes were at 46 and 50 d of gestation, which are well past the time of pregnancy recognition in sheep. The establishment of pregnancy in 2 *IFNAR2*$^{-/-}$ ewes demonstrates that the accepted mechanism of pregnancy recognition in ruminants, which involves IFNT interacting with IFNAR, is incorrect or is not required for establishment of pregnancy. Either there is another receptor for IFNT or an alternative, possibly redundant, mechanism for pregnancy recognition. Since *IFNAR1*$^{-/-}$ and *IFNAR2*$^{-/-}$ fetuses survive to term and are healthy at birth, and both *IFNAR2*$^{-/-}$ rams and ewes are fertile, unless the effects of IFNT are mediated by an alternative receptor, IFNT is not required for pregnancy recognition or the development of a healthy ruminant fetus.

Key Words: sheep, CRISPR-Cas9, pregnancy, fertility

P171 ISAG Bursary Award: sgRNAcas9-AI: A program for prediction of CRISPR/Cas9 and its variant sgRNA activity using deep learning. S. Li[1], X. Zhang*[2], S. Zhao[1,3], C. Zhao[1,4], and S. Xie[1,4], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Lab of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, China,* [2]*Institute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover, Hannover, Germany,* [3]*Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan, China,* [4]*The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan, China.*

The clustered regularly interspaced short palindromic repeats and associated proteins 9 (CRISPR/Cas9) system-mediated genome modification has been widely used in various species and cell types. In recent years, several mutated and modified Cas9 variants have emerged with the advantages of low-target and broadened protospacer adjacent motif (PAM) loci compared with the wild type. However, there is a lack of algorithms to accurately predict the cleavage efficiency and off-target effects of sgRNAs in Cas9 and its variants, which are critical in determining the efficiency of gene editing. Here, we developed a new algorithm for sgRNA activity prediction (sgRscore) using deep learning strategies. Public sgRNA activity data sets of 9 Cas9 variants, including spCas9, eSpCas9(1.1), HypaCas9, evoCas9, SpCas9-VRQR, Sniper-Cas9, SpCas9-HF1, SpCas9-NG and xCas9, were collected to train our model. Compared with the other 6 mainstream models, sgRscore showed the best prediction performance. The attention mechanism and lightGBM algorithm were applied to explore the interpretability of sgRscore. By combining sgRscore with Crisflash, we developed a new software named sgRNAcas9-AI for the calculation of sgRNA on-target activity and off-target effect of Cas9 and its variants. It has been deployed to online website.

Key Words: sgRscore, sgRNA activity prediction, deep learning, sgRNAcas9-AI

P172 Validation of the *PDGFD* gene function in sheep tail formation using base editing-induced start codon silencing. P. Kalds*[1,2], S. Zhou[1,3], S. Huang[1], K. Sun[1], Y. Gao[1], J. Han[4,5], Y. Chen[1], and X. Wang[1], [1]*Key Laboratory of Animal Genetics, Breeding and Reproduction of Shaanxi Province, College of Animal Science and Technology, Northwest A&F University, Yangling, China,* [2]*Department of Animal and Poultry Production, Faculty of Environmental Agricultural Sciences, Arish University, El-Arish, Egypt,* [3]*College of Veterinary Medicine, Northwest A&F University, Yangling, China,* [4]*CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China,* [5]*Livestock Genetics Program, International Livestock Research Institute (ILRI), Nairobi, Kenya.*

Sheep are one of the significant livestock species that have been used as a source of meat, milk, wool, and other byproducts. After domestication, the sheep tail phenotype has diverged into various morphological patterns. In modern breeding practices, the short and thin tail phenotype is most preferable. Recent genomic analyses highlighted the potential of the *PDGFD* gene as a significant candidate for the fat-tail phenotype. However, functional investigations are limited. In this research, we applied an adenine base editor (ABE)-induced start codon silencing to produce *PDGFD*-disrupted sheep. The editing efficiency at the cellular level was 56%. Using the microinjection approach, 14 lambs were generated, of which 9 (64.3%) were gene-edited. Targeted deep sequencing showed that the base conversion ranged from 35.06% to 98.30% in *PDGFD*-edited lambs. No bystander mutations were observed within the base editing window, and no off-target mutations were detected in the *PDGFD*-edited lambs. The phenotyping results showed a significant proportional reduction in tail volume of the *PDGFD*-edited group compared with the wild-type (WT) group ($P < 0.00$), but no significant difference between their tail lengths and body weights ($P > 0.05$). Hematoxylin and eosin staining of the tail adipose tissues did not support differences in adipocyte size between the 2 groups, suggesting a potential association of *PDGFD* with the adipocyte number rather than the adipocyte size. To further compare the transcriptomic profiles of the *PDGFD*-edited and WT groups, total RNAs were extracted from the tail adipose tissues. Among the 694 overlapped DEGs, 365 and 329 were up- and downregulated, respectively. The 329 downregulat-

ed DEGs were included in 49 significant GO terms and 40 significant KEGG pathways (adjusted $P < 0.05$). The most significant GO terms and KEGG pathways were relevant to lipid regulation. These results showed that targeting the *PDGFD* gene in sheep could generate a proportional reduction in the tail fat volume and affect the transcriptomic profiles of tail adipose tissues.

Key Words: sheep, genome editing, base editing, RNA-seq, fat tail trait

Horse Genetics and Genomics

P173 A genome scan for homozygous haplotype deficiency in the Thoroughbred horse identifies variants for normal embryogenesis. J. F. O'Grady[1,2], B. A. McGivney[1], D. E. MacHugh[2], and E. W. Hill*[1,2], [1]*Plusvital Ltd., Dun Laoghaire, Dublin, Ireland,* [2]*University College Dublin, Belfield, Dublin, Ireland.*

Thoroughbred horses have high levels of inbreeding risking exposure to the harmful effects of recessive alleles. Deleterious recessive haplotypes tagging rare genomic variants disrupting embryonic and neonatal development have been reported in livestock species but have not been reported in the Thoroughbred. Here, we tested the hypothesis that the Thoroughbred genome harbors deleterious recessive haplotypes carrying lethal/semi-lethal alleles segregating within the population that may disrupt normal embryonic and/or neonatal development. The objective was to catalog haplotypes absent or depleted in the homozygous state and to identify candidate genes and variants causative of their depleted homozygosity. n = 14,931 Thoroughbred horses were genotyped on the Illumina Equine SNP70 BeadChip (70K) or the Axiom Equine Genotyping Array (670K). An overlapping sliding window approach (0.25–10 Mb) was used to identify 3,212 significantly ($P < 0.05$) depleted homozygous haplotypes. An investigation was undertaken to further characterize 20 of these haplotypes in detail. Whole-genome sequence data from n = 29 Thoroughbred and n = 70 mixed-breed horses was used to identify 668 predicted high/moderate effect variants within +/−1 Mb of the haplotypes that were not observed in the homozygous state. Eight variants located within conserved sequences of 7 genes that have functions essential for normal embryogenesis were identified as candidate variants underlying the observation of depleted homozygosity. These mutations are located within haplotypes that occur at frequencies of 2.6–15.1% in the population. Experiments to ascertain deleterious effects of these variants will lead to a better scientific understanding of equine embryonic development. In practice, judicious breeding management decisions to avoid carrier × carrier matings are likely to have a positive impact on reproductive efficiencies in the Thoroughbred. This would reduce the overall frequency of unfavorable haplotypes in the population and alleviate the impact of harmful recessive alleles on pregnancy in Thoroughbred mares.

Key Words: horse breeding, lethal haplotype, pregnancy, deleterious variant, genome scan

P174 ISAG Bursary Award: Introgression within the horse genome. L. Johnson*[1], T. Kalbfleisch[1], E. Bailey[1], and K. de Silva[2], [1]*University of Kentucky, Lexington, KY,* [2]*University of Louisville, Louisville, KY.*

Introgression is the transfer of genetic material between 2 different species via repeated back crossing of the hybrid and one of the parent species. These are important events to understand in the study of molecular evolutionary relationships between species. Previous studies have found regions of introgression within the horse genome, likely hundreds of thousands to millions of years ago from a non-caballine equid that are present in current horse populations. Our hypothesis is, these regions are retained among horses because they are doing something functionally beneficial and are increasing the horse's fitness. After identifying these regions with maximum likelihood estimation in a sample thoroughbred population, the sample genomes were phased with BEAGLE. The resulting the variant call files (vcf) were viewed in Tassel 5, and a linkage disequilibrium plot was created. The phased genotypes were then analyzed, and the different haplotypes were extracted from the vcf, taking into consideration the linkage disequilibrium plot and allele frequencies. Three main haplotypes occurred in this sample population: the reference, or Twilight, haplotype, the non-caballine haplotype, and another haplotype of unknown origin. This pipeline presents the number of haplotypes in the sampled population and which polymorphisms are inherited together, which gives us a better understanding of the origin on the introgressed haplotype(s). The short-term goal is to identify which non-caballine equid is the most prominent donor of the non-caballine haplotype. Ultimately, we will develop software to automate this analysis and identify putatively impactful introgressed regions using recently published data from the Equine-FAANG project. Currently, we are finding what the introgressed regions code for in the context of the horse genome, either non-coding RNA, a gene, or a non-coding region, to narrow down the search when putting together phylogenetic trees. Overall, the more we understand introgression, the better we will be able to piece together evolutionary relationships, how they are essential for rapid adaptation historically, and how they impact gene function in modern populations.

Key Words: introgression, functional impact

P175 Identification of genetic variants frequency from RNAseq datasets and its use as a filtration tool to identify rare diseases in Arabian horse species. T. Szmatola[1,2], M. Stefaniuk-Szmukier[2], K. Piorkowska[2], T. Zabek*[2], and K. Ropka-Molik[2], [1]*University Centre of Veterinary Medicine, University of Agriculture in Krakow, Krakow, Poland,* [2]*National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

In recent years the advancements of next generation sequencing (NGS) technologies allowed for easy and efficient way of identifying genome wide mutations such as SNPs and INDELs. In humans, the usage of variant calling algorithms made it possible to prepare databases of known variants and their frequency in worldwide population. This allows for utilization of such databases as a filtration tool while searching for rare genetic variants which may be associated with specific disease. Such approach is not possible in the case of many animal studies; thus, here we present the results of variant calling derived from previously obtained RNaseq data of 30 Arabian horses. The Freebayes variant calling algorithm, after filtration of the results (minimal coverage set to 10 and quality to 30), allowed for identification of 111,655 genetic variants, of which 93,767 were located in gene regions. Moreover, 96% of all variants were labeled as known variants with only 6,661 accounted as novel. The average allele frequency was calculated as 0.45 (SD = 0.3) with a median of 0.39, while the mean coverage of variants was 241 (SD = 300). The selected 10 SNPs were validated by Sanger sequencing. Creation of such a database will allow for filtration of variants via allele frequency and thus make it easier to find rare mutations, possibly associated with a disease. It is noteworthy that RNaseq-derived variant calling, even though applying to a small part of the genome due to its origin, applies to the most important genetic variants that are positioned in exon of genes. This study was supported by 03-18-26-09.

Key Words: rare genetic variants, RNAseq, Arabian horses

P176 Is the Argentinean Polo Pony a horse breed? Genomic characterization and comparison with Thoroughbreds using SNP-array data. F. Azcona[1,2], A. Karlau[1,3], P. Trigo[1,2], R. Alvarez[1], and S. Demyda-Peyrás*[1,3], [1]*Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina,*

[2]*IGEVET, CONICET, La Plata, Buenos Aires, Argentina,* [3]*CONICET, La Plata, Buenos Aires, Argentina.*

Argentine Polo (AP) is a world-renowned horse breed created in the 80s for playing polo. Although it maintains an open registration policy, recent studies have shown that it has a high genetic influence from the Thoroughbred (TB). This study aimed to evaluate the genetic structure of AP horses and their relationship with TB using genomic data. We analyzed genotyping data (42,482 SNPs, Illumina GGP75K array) from 383 AP and 36 TB horses. Individuals were clustered by breed but also based on the number of known Polo ancestors in their pedigree: GEN0 and GEN3 (0 and 3 complete generations respectively). With these data, we analyzed the differences among populations using 3 different approaches: PCA, AMOVA, and F_{ST} indexes; but also at the genome level using F_{ST} q-values per SNP. PCA showed a slight but evident separation between AP and TB horses, which was more clear analyzing only GEN3. Similarly, AMOVA demonstrated that breed explains 4.3% of the variance, whereas the number of generations within AP explains 3%. The mean F_{ST} between AP and TB was 0.015, but it was higher by comparing GEN3 and TB (0.025) than GEN0 and TB (0.011; $P < 0.01$). In addition, 289 SNPs showed F_{ST} q-values significantly high (ranging from 0.17 to 0.92, q-value < 0.05) located all across the genome, depicting regions with large differences among breeds. Our data confirmed the close genomic relationship between AP and TB, produced most likely by a strong founder effect and an active gene flow among both breeds. Although the mean F_{ST} between the 2 breeds was relatively low, the differences detected comparing GEN0 and GEN3 with TB demonstrated a progressive differentiation over the generations. In addition, some genome regions with high genetic differentiation were found, suggesting the existence of incipient selection sweeps, which may be linked with traits or QTLS associated with sporting abilities. Overall, our results detected slight (but clear) genomic differences between AP and TB, in agreement with the breeding and selection process performed by AP breeders during the last 40 years.

Key Words: Argentinean Polo breed, SNP, horse genomics

P177 ISAG Bursary Award: Genomic analysis using massive sequencing data reveals genetic signatures that underlie breed features. Y. Wang*[1], X. Chai[1], H. Liu[1], J. Dou[1], Y. Liao[1], Z. Tang[1], J. Xu[1], S. Zhu[1], Y. Liu[1], X. Shen[1], D. Yin[1], L. Yin[1,2], X. Liu[1,2], M. Yu[1], Y. Fu[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei, PR China,* [2]*Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, Hubei, PR China.*

Since the domestication of the horses (*Equus caballus*; dating back to 5,000–6,000 years ago) in Eurasian steppes, domestic horses have been subjected to the combined effects of natural selection and human-driven artificial selection. This has resulted in diverse populations distributed across the world, and marked phenotypic diversity in several traits, including behavior, adaptability, body composition, sport ability, and coat color. Population genomic variations through diversifying selection have not been fully investigated. To address this issue, we collected whole-genome sequencing data from 539 horses of 55 breeds with an average coverage of up to 18.18-fold. After aligning ~37.21 Tb of high-quality data to the reference horses' genome, we identified ~32.35 million SNPs and ~3.37 million InDels, of which 51.1% were absent in the current horses dbSNP (Build 155) entries. The neighbor-joining phylogenetic tree and principal components analysis revealed the segregation of 55 breeds into 8 distinct clusters. To investigate the selection among the subpopulations, we identified a total of 2,629 candidate selected regions, including 1,878 candidate selected genes. These CSGs were mainly enriched in guanyl-nucleotide exchange factor activity (*P*-value = 1.1493E-5), GTPase regulator activity (*P*-value = 3.87045E-5), protein serine/threonine kinase activity (*P*-value = 1.184038E-4), and other pathways, which may be related to the long-term selection of traits such as disease resistance, adaptability, and responsiveness of horses. Further analysis with transcriptome data, we found *LCORL*, *MSRB3*, *Fam184B* and *DCAF16* genes that may be related to traits such as horse height, weight, hair, neck length and back length, among which *Fam184B* and *DCAF16* were newly discovered genes. In summary, our results illustrate how domestication has shaped patterns of genetic variation in horses and demonstrate that large-scale public data mining can provide valuable genetic resources that enable effective use of horses in agricultural production.

Key Words: horse, domestication, whole genome sequencing, artificial selection, selective sweep

P178 Association of the *mypn* gene with structural muscle fiber traits in the Purebred Spanish Horse by genome-wide association analysis. R. Álvarez-Quiñonez[1], M. Macri[2,3], A. Martinez[2,3], J. Rivero[1], and J. Vega-Pla*[4], [1]*Laboratory of Muscular Biopathology, Department of Comparative Anatomy and Pathology, School of Veterinary Medicine, University of Cordoba, Cordoba, Spain,* [2]*Department of Genetics, University of Cordoba, Cordoba, Spain,* [3]*Animal Breeding Consulting S.L, Cordoba, Spain,* [4]*Laboratorio de Investigación Aplicada, Cría Caballar de las Fuerzas Armadas, Cordoba, Spain.*

The Purebred Spanish Horse (PRE) is the most widespread and prestigious breed in Spain, and is also present in more than 65 countries. Genome-wide association studies (GWAS) have been performed for different phenotypes in equine breeds although the PRE horses have been little characterized in this regard. The objective of this study was to perform a GWAS analysis to identify associated genes considering 8 parameters of muscle fiber composition in PRE horses. Blood frozen samples stored from 160 horses were analyzed. Biopsy data of *m. gluteus medius* at 2-cm depth from the Muscular Biopathology Laboratory of the University of Cordoba were used. The samples were typed with the GeneSeek® Equine kit (Neogen). SNP filtering was performed with PLINK® v1.9, excluding SNPs with a MAF less than 0.05, missing genotype data higher than 0.01 and significant departure from HWE (*P*-value < 0.001). The GWAS was performed using GEMMA® v0.98.1. The *P*-values obtained were corrected using the FDR method. The rs57106192 SNP was associated with the IIX myosin heavy chain isoform. This SNP is located on ECA 1, next to the myopalladin (*MYPN*) gene (ENSECAG00000015157 EquCab3.0), which is a striated muscle-specific immunoglobulin protein that localizes to the Z-line and band I of the sarcomere, as well as to the nucleus and cardiac muscles, and has an important role in regulating actin organization in humans. Heterozygous mutations of the *MYPN* gene have been associated with overexpression in myocardial diseases in humans, meat quality traits in breeding and rearing cattle, and this gene has been the subject of positive selection and a significantly associated with height differences in some equine breeds. Our study demonstrates that GWAS analysis can be used to obtain important information about the genetic basis of phenotypic diversity in PRE horses. This research was partiality funded by Animal Breeding Consulting S.L., Cordoba, Spain.

Key Words: horses, genome-wide association, genotyping, athletic performance, muscle

P179 A de novo large ECAX partial deletion in a fertile Pura Raza Española mare detected using genomic data. Y. Pirosanto[1,2], A. Encina[3], G. Anaya[4], M. Valera[3], and S. Demyda-Peyrás*[1,5], [1]*Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina,* [2]*IGEVET, CONICET, La Plata, Buenos Aires, Argentina,* [3]*Departamento de Agronomía, ETSIA, Universidad de Sevilla, Sevilla, España,* [4]*Departamento de Genética, Universidad de Córdoba, Córdoba, España,* [5]*CONICET, La Plata, Buenos Aires, Argentina.*

Chromosomal abnormalities are a well-known source of infertility in the domestic horse. Nowadays, its detection rate is being increased, thanks to the development of SNP-based copy number aberration (CNA) analysis which can detect the loss or gain of DNA in the genome of an individual with increased accuracy. However, large chromosomal structural anomalies were barely reported in the species. Hereby, we report the case of a Pura Raza Española 5-year-old fertile mare bearing a large deletion in the ECAX p-arm. DNA samples from the mare and both parents were obtained from blood samples. All of

them were genotyped using the 75K GGP equine SNP-bead chip (Neogen, Scotland), and analyzed using a CNA bioinformatic pipeline developed by our group based on BAF and LRR results. In addition, the mare was examined by the veterinary services of the Pura Raza Español breeder's association. Results showed a large de novo heterozygous deletion in the ECAX, between positions 2,153,000 and 10,651,000 approximately, depicted by a hemizygous pattern in CNV analysis (BAF values close to 0 or 1 and a reduction of LRR to −0.5). Interestingly, no DNA loss was detected in the distal region of the p-arm, in which the pseudoautosomal region is located, nor was any sign of cell chimerism. In addition, the reproductive tract was normal, to the point that the mare foaled a healthy colt very recently. Finally, a follow-up in silico bioinformatic analysis revealed the presence of 38 genes included in the physical region affected. Among them, 13 were previously related to fertility, and 2 of them (OFD1 and TRAPPC2) to abortion in mammals. To our knowledge, this is the first report of a fertile mare bearing a large deletion in the ECAX. Further studies on the colt are being performed to determine if the offspring inherited the mare's deletion.

Key Words: CNA, SNP, horse, chromosomes

P180 Exterior features and DNA quality of the Kazakh horse of Zhabe type for 16S sequencing. S. Kassymbekova*[1], T. Assanbayev[2], A. Khamzina[1], and A. Ibadullayeva[1], [1]*Kazakh National Agrarian Research University, Almaty, Kazakhstan,* [2]*Toraighyrov University, Pavlodar, Kazakhstan.*

Aim in our work of describing the exterior features of the Kazakh horse of the Zhabe type, as well as evaluating the method of extracting genomic DNA from the upper respiratory tract, gut, and feces for 16S sequencing. Zhabe type are of great value for Kazakhstan, since they are distinguished by their exceptionally strong, often even rough physique, and excellent adaptability to semi-desert and steppe pastures. Domesticated horses live in different environments than their extinct wild ancestors, and therefore have different microbiomes. The first and most important step in sequencing is DNA extraction. Samples of the upper respiratory tract (n-16), gut (n-16) and freshly excreted feces (n-14) were taken and placed in special containers in the conditions of the Akshiman Agro farm in the Pavlodar region of North Kazakhstan. An information database was also created, which included information about the breed, sex, age, live weight and measurements of the main articles of the physique of experimental horses. Visual examination confirmed that all animals were clinically healthy without pathologies. The samples of swabs were transported in a thermal transport suitcase supplied with a refrigerant. The storage temperature was −20C. DNA extraction was performed using a commercial Purelink Microbiome Kit, according to the manufacturer's protocol. The mean DNA concentration from Zhabe type samples was 7.8 ng/mL by fluorometer. The highest concentration reaches 16 ng/µL (gut) and the lowest is 3 ng/µL (feces, and upper respiratory tract). According to the grading data, the live weight of mares from 5 to 7 years old averages 445.7 kg, which is 5.7 kg higher than the elite indicator. The stallion-producer exceeds the same indicator by 90 kg. The live weight of young fillies (3.5–4.5 years) averages 419.5 kg, which corresponds to the indicators of class 1. It is necessary to consider the fact that Zhabe type are finishing their growth and development by 5–7 years old. The color is bay for the whole herd. In general, the Zhabe type can be improvers of local Kazakh horses in terms of live weight and measurements.

Key Words: Kazakh horse, phenotype, DNA, extraction

P181 Genome-wide association study for microphthalmia in Warmblood horses. L. Chapard[1], N. Aerts[1], B. Van Mol[1,2], R. Meyermans[1], W. Gorssen[1], K. Hooyberghs[1], F. Pille[2], S. Janssens[1], and N. Buys*[1], [1]*KU Leuven, Center for Animal Breeding and Genetics, Department of Biosystems, Leuven, Belgium,* [2]*Department of Surgery and Anesthesiology of Domestic Animals, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*

Microphthalmia is a developmental eye disorder for which several cases has been recently reported in the Warmblood horse population. In this condition, one or both eyes are underdeveloped resulting in small and abnormal eye(s) and impaired vision. It can affect either the left, the right, or both eye(s), but most of the cases are unilateral. As common ancestors were traced back in the pedigree of affected animals, there seems to be an underlying genetic cause to this defect. To identify genomic regions that could be associated with this defect, a total of 47 cases (unilateral or bilateral) and 52 related controls (parents and/or siblings or half-siblings) were genotyped using the GGP Equine 70K. SNP positions were updated from EquCab 2.0 to EquCab3.0 with NCBI Genome Remapping Service. PLINK v1.9 was used to perform quality control (QC) on the 31 autosomes and the 99 horses. Individuals with a call rate ≤0.95, outlying heterozygosity, and duplicated individuals were removed as well as SNPs with a call rate ≤0.95, minor allele frequency ≤0.05, and Hardy-Weinberg equilibrium ≤0.0001. After QC, 54,443 SNPs and 96 individuals (45 cases and 51 controls) were retained. The genome-wide association analysis was performed using GCTA v1.94.1, and genetic relationships between individuals were accounted for using a genetic relationship matrix. After Bonferroni correction, one genome-wide significant SNP and one suggestive genome-wide significant SNP were identified for microphthalmia. These results are promising; however, additional analyses, a better phenotype definition, and collection of more samples are needed to further investigate the genetics underlying this developmental eye disorder in Warmblood horses.

Key Words: horses and related species, genome-wide association, single-nucleotide polymorphism (SNP), animal health

P182 Dissecting the genetic cause of myotonic dystrophy in horses. T. Simon*[1], D. Vélez-Irizarry[2], R. Naboulsi[1], A. Niazi[1], E. Bongcam-Rudloff[1], S. Valberg[2], and G. Lindgren[1,3], [1]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden,* [2]*Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University, East Lansing, MI,* [3]*Center for Animal Breeding and Genetics, Department of Biosystems, Leuven, Belgium.*

Myotonic dystrophy (MD) is an inherited neuromuscular disorder that leads to a wide range of clinical signs including stiffness, abnormal muscle relaxation, and muscle atrophy or hypertrophy. Affected muscles develop a prolonged contracture in response to percussion of the muscle and show waxing and waning complex repetitive discharges in electromyography (EMG). Two genetic mutations underlie the genetic basis for MD in humans that cause abnormal RNA processing. There are a limited number of case reports of MD in horses in the veterinary literature and its cause is unknown. By analyzing whole genome sequencing (WGS), RNaseq, and proteomics data from horses in our MD data set, we aim to identify the molecular mechanisms contributing to the condition in horses. Understanding the molecular mechanisms behind MD will lead to non-invasive diagnostic tests and potentially therapeutic strategies. Muscle samples have been obtained from 5 MD horses of Quarter Horse-related breeds (3 female, 2 male) 2 to 3 years of age. Signs of stiffness, muscle contractures, and muscle hypertrophy were present shortly after birth. EMG performed in 4 horses revealed complex repetitive discharge within hindlimb muscles and few abnormal discharges in forelimb muscles. Histopathologic analysis identified fiber size variation, internalized myonuclei, a preponderance of slow twitch type 1 fibers and fiber type grouping in gluteal and semimembranosus (SM) muscles of the MD foals but few abnormalities in triceps muscle consistent with EMG findings. MD horses and 7 age-, sex-, and breed-matched controls were low pass WGS to approximately 4× coverage. Fst values were calculated, and 2 chromosome regions on ECA 3 and 7 were significant across multiple window sizes. On chromosome 3 was found a candidate gene responsible for the facioscapulohumeral muscular dystrophy 1. Sequencing depth will be increased on the same horses to confirm the preliminary findings. The obtained results of this project may also provide insights into the related human MD disease, which affects an average of 1 out of 8,000 individuals.

Key Words: horses and related species, functional genomics, genome sequencing, muscle, animal health

P183 SNP-based genomic characterization of a top-performance population of Peruano de Paso horses. A. Karlau[1,2], F. Azcona[1,2], P. Trigo[1,2], A. Antonini[1], A. Molina[3], and S. Demyda-Peyrás*[1,2], [1]*Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina,* [2]*CONICET, La Plata, Buenos Aires, Argentina,* [3]*Universidad de Córdoba, Córdoba, España.*

The Peruano de Paso (PP) is a worldwide recognized horse breed known for its smooth ride, particular gait, and external beauty. Its breeder's association is located in Lima (Perú), where the most important functional competition is hosted. Hereby we characterized a group of top-performance PP horses, using genomic approaches. In addition, we compared this population with 5 additional foreign horse breeds. DNA was obtained from hair bulbs collected from 24 top-performance horses (and their relatives) at the Campeonato Nacional 2022 (Lima, Perú). Genotyping was performed using the 75K equine GGP SNP-bead chip (Illumina Inc.). Data curation (pruning by MAF (0.05) and LD (50, 5, 0.5)) was initially performed using PLINK v1.9, retaining 41,207 markers. The final data set comprised 109 horses, including 9 Arabians (AR), 14 Criollo Argentino (CA), 25 Argentinean Polo (AP), 26 Pura Raza Española (PRE), and 10 Spanish Trotters (ST). Finally, we characterized the population using 4 genomic different approaches: principal component analysis (PCA), F_{ST} indexes, F_{ROH}, and AMOVA. PCA results show a clear differentiation among the Peruano horses and the rest of the breeds, with the first 2 components responsible for ~11.5% of the total variation. F_{ST} differentiation indexes showed that PP horses were closer genetically to AC (0.08) and PRE (0.11), in comparison with the rest of the breeds (0.14 with AR, 0.12 with ST, and 0.16 with AP). Similarly, AMOVA results indicate that the variation between breeds was 12.03%, with the groups of individuals analyzed being very homogeneous. Finally, no differences were observed in F_{ROH} among breeds, but PP showed significantly higher values of recent F_{ROH} (3 generations only) than the rest of the breeds, depicting the existence of recent close matings. In this preliminary study, we analyzed for the first time a top-performance group of Peruano de Paso horses. We detected a genetically well-defined population, but also an increase of recent inbreeding. Both findings agree with the strong selection intensity applied in this population. Larger studies are still necessary to validate our findings.

Key Words: Peruano de Paso horse, horse genomics, characterization

P184 Evaluation of single nucleotide polymorphisms (SNPs) for parentage control in horse breeds in Korea. S. Y. Lee*[1] and G.-J. Cho[1], [1]*Korea Racing Authority, Racing Laboratory, Gwqcheon Si, Gyeonggi-do, South Korea.*

In this study, we aimed to evaluate the possibility of single nucleotide polymorphisms (SNPs) for parentage testing of horse breeds in Korea. The genotype was provided for 96 horse samples (38 Thoroughbred horses, 17 Jeju horses, 20 Quarter horses, and 21 American Miniature horses) with 15 microsatellite (Ms) markers (AHT4, AHT5, ASB2, ASB17, ASB23, CA425, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG10, LEX3 and VHL20) and 101 SNPs markers recommended by ISAG. SNPs analyzed QC, HomFLD, HetSO, HomRO, MAF, and H.W. *P*-value. The results of paternity test using Ms and SNPs for each foal of the Thoroughbred horse and Quarter horse are as follows. In the case of Ms, among the 15 markers, AHT5, ASB2, ASB17, ASB23, CA425, HMS7, HTG10, and LEX3 marker were contrary to Mendel's genetic law in Thoroughbred horse and Quarter horse, so paternity was not established. In the case of SNPs, among the 101 markers, 31 markers including MNEc_2_2_2_98568918_BIEC2_502451 in Thoroughbred horses, and 30 markers including MNEc_2_30_7430735_BIEC2_816793 in Quarter horses, respectively, were not established due to Mendel's genetic law in parentage testing. In conclusion, it is suggested that 101 SNPs analyzed in this study can be used for paternity test of horses. However, it is thought that research on more samples will be needed in the future.

Key Words: horses, microsatellite (Ms), SNPs, parentage testing, QC

P185 ISAG Bursary Award: Single-step genomic model improved reliability in conformation traits in the Pura Raza Español horse. C. Ziadi*[1], D. Perdomo-González[2], M. Valera[2], A. Encina[3], N. Laseca[1], S. Demyda-Peyrás[1], and A. Molina[1], [1]*Department of Genetics, University of Córdoba, Córdoba, Spain,* [2]*Department of Agronomy, ETSIA, University of Sevilla, Sevilla, Spain,* [3]*Asociación Nacional de Criadores de Caballos de Pura Raza Española (ANCCE), Sevilla, Spain.*

Conformation traits are very important in the Pura Raza Español horse (PRE). Although most morphological traits have medium to high heritability, there is a great interest in obtaining highly reliable assessments as quickly as possible. In this sense, genomic evaluation is a method for improving the reliability of breeding values' estimation. The aim of this study was to compare genetic parameters and reliabilities for conformation traits in PRE between classical approach with pedigree-based REML and single-step genomic REML. Measurements from 5 different zoometric traits were analyzed: scapular-ischial length (SiL), length of back (LB), dorso-sternal diameter (DsD), thoracic perimeter (TP), and perimeter of anterior cannon bone (PACB). The data set consisted of conformation records of 7,152 animals and 41,888 animals in the pedigree. A total of 2,916 animals were genotyped with 61,271 SNPs and included in the analysis. Genetic parameters, genetic (EBVs) and genomic (GEBVs) breeding values were estimated using, respectively, pedigree and genomic relationship matrices. Analyses were performed using a multivariate model with the HiBlup v1.3.1 software. The estimates of heritabilities were similar in both methodologies (0.64, 0.51, 0.34, 0.53, and 0.34 for SiL, LB, DsD, TP, and PACB, respectively). A significant gain in reliabilities for ssGREML over REML evaluations has been observed in all traits, with an overall increase oscillating between 7.74 and 27.83%, being greater in genotyped animals (14.97 to 41%) compared with non-genotyped (6.84 to 26.13%). Similarly, in genotyped stallions with more than 40 controlled foals, this increase was much lower (1.98 to 11.45%). Finally, in non-genotyped animals, the increase was close to zero in some traits, when the reliability of the sire was previously very high. This work demonstrated the effectiveness of the genomic approach for the routine genetic evaluation of conformation traits in the PRE breed.

Key Words: reliability, single-step, horse

P186 ISAG Bursary Award: Molecular inbreeding negatively affects the reproductive life of Pura Raza Española mares. N. Laseca*[1], D. Perdomo-González[2], M. Valera[2], A. Molina[1], P. Azor[3], and S. Demyda-Peyrás[1], [1]*Department of Genetics, University of Cordoba, Córdoba, Spain,* [2]*Department of Agronomy, ETSIA, University of Seville, Seville, Spain,* [3]*Asociación Nacional de Criadores de Caballo de Pura Raza Española, ANCCE, Seville, Spain.*

Fertility is a key factor for the economic success of livestock production systems. Livestock populations bred under strong selection processes tend to lose genetic variability. This may lead to an increase in inbreeding and, consequently, a decrease in fertility, due to the onset of inbreeding depression. In some horse breeds, increased inbreeding is a major concern, since it is affecting negatively reproductive traits in mares and stallions. In this study, we evaluated the effect of increased molecular inbreeding on mare reproductive performance by relating runs of homozygosity (ROH) genomic approach with the estimation of genetic breeding values (EBVs) for 3 reproductive traits (age at first foaling [AFF], age at last foaling [ALF], and foaling interval [A12]) in a population of 78,921 Pura Raza Española (PRE) mares. EBVs were estimated using an animal REML mixed model implemented in BLUPF90+, including animal effect, coat color, country of origin, and stud size as fixed effects. Molecular inbreeding values (FROH) were estimated individually as the percentage of the genome harboring ROH using 61,271 SNPs in a population of 2,055 genotyped PRE mares with the GGP Equine Array. Recent FROH values were also determined (6 generations). Finally, linear regression was used to estimate the incidence of FROH increase in ALF, AFF, and A12. Results showed a moderate to high FROH on average (0.139). Most of them was explained by

recent inbreed matings (0.079). Regression analysis demonstrated that a 10% increase in molecular inbreeding means an increase of 0.037% in the mare's age at first foaling and 0.389% in the foaling interval. Likewise, the ALF is shortened by 0.739% in the longevity of the mare. Similar results were obtained by analyzing recent FROH values (0.033% in AFF, 0.664% in ALF, and 0.386% in A12). Our findings demonstrate that increased molecular inbreeding results in a reduction of the average mare's reproductive life, an increment in the foaling interval, and consequently a reduction in the number of offspring obtained from the mare throughout her reproductive life.

Key Words: reproductive traits, molecular inbreeding, mares, reproductive life, genomics

P187 Reference genome of the native Finnhorse as a tool to study the adaptation of northern Eurasian horse breeds. K. Pokharel*[1], M. Honkatukia[1,2], C. Ginja[3], M. Weldenegodguad[1], J. Peippo[1,2], H. Lindeberg[4], T. Reilas[1], and J. Kantanen[1], [1]*Natural Resources Institute Finland, Jokioinen, Finland,* [2]*NordGen - Nordic Genetic Resource Center, Ås, Norway,* [3]*Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, Portugal,* [4]*Natural Resources Institute Finland, Maaninka, Finland.*

The Finnhorse is a native horse breed of Finland and has been an important part of Finnish culture, economy, and society in general. To better understand the genetic makeup of this breed and its evolution, we performed high-throughput sequencing and de novo assembly of the Finnhorse genome (a mare of the working horse breeding section of the Finnhorse herd book). We used a combination of Illumina and PacBio sequencing technologies to generate high-quality sequence data with an estimated coverage of 100×. Moreover, we sequenced RNA-samples (n = 30) of neck (taken from the neck, below the mane), metacarpal, and tail head adipose tissues of the Finnhorse, the Siberian Yakutian horse and the Portuguese Garrano breed to investigate adaptation of horses to northern and southern Eurasian biogeographic regions. Adipose tissues are known to play crucial roles in thermoregulation and insulation, which are important adaptations for animals living in cold environments. The final Finnhorse genome assembly consists of 2.37 Gb with an N50 scaffold of 83.77 Mb. We identified a total of 19,748 protein-coding genes in the Finnhorse genome using a combination of ab initio prediction and homology-based approaches. The high-quality genome assembly and annotation of Finnhorse will facilitate further investigation of the genetic basis of various phenotypic traits, as well as contribute to the development of new breeding strategies and management practices in conservation of horse genetic resources. Moreover, transcriptome study of adipose tissues provides valuable insights into how these tissues function and how they may have evolved to help horses adapt to the northern Eurasian environment.

Key Words: assembly, genetic resources, adipose tissue, gene expression

P188 ISAG Bursary Award: Identification of personality-related genes associated with tractability of handling in Thoroughbred horses. T. Yokomori*[1], A. Ohnuma[2], T. Tozaki[2], M. Ishimaru[3], F. Sato[3], Y. Hori[4], T. Segawa[1], and I. Takuya[1], [1]*Nihon University, Fujisawa, Kanagawa, Japan,* [2]*Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan,* [3]*Japan Racing Association, Urakawa, Hokkaido, Japan,* [4]*The University of Tokyo, Meguro, Tokyo, Japan.*

Recently, the focus in the horse racing industry has been on securing a second career for retired racehorses for the purposes of improving animal welfare and personality is expected to be an important trait. Previously, we discovered 15 novel candidate genes associated with personality in horses based on human personality-related genes and polymorphisms in the Thoroughbred population. The aim of this study was to investigate the association between behavior data and 5 of the candidates: *CDH13*, *HSD11B1*, *ANKK1*, *SLC6A4*, and *GABRA6*. Behavioral investigation was conducted using a 3-point-scale questionnaire consisting of 17 items to evaluate tractability by a consensus of 3 caretakers for 169 one-year-old Thoroughbred horses (80 colts and 89 fillies) between 2011 and 2013. The data tables of 3-point scores were transformed into a polychoric correlation coefficient matrix. Using the matrix, singular regression analysis was conducted by sex. One genotype per gene was used as explanatory variables and a column of principle component (PC) scores for each PC as objective variables. Additionally, the genotypes were analyzed with 3 genetic models: dominant, recessive, and additive. The results of PC analysis indicated that PC3 represented "tolerance for movement restrictions due to harnesses" and PC4 represented "tolerance for human and external changes." For PC3, colts with recessive homozygote on *CDH13* showed low scores (dominant model, $P < 0.05$). For PC4, fillies with heterozygote on *SLC6A4* showed high scores (additive model, $P < 0.01$), and fillies with at least one minor allele on *SLC6A4* also showed high scores (recessive model, $P < 0.05$). Among potential factors related to tractability of horses, these results suggested that *CDH13* is involved in reactivity to movement restriction of a part of the body in colts and *SLC6A4* in reactivity to movement restriction and changes in surroundings in fillies. These 2 genes are expected to be useful as one of the parameters for evaluating psychological tolerance in Thoroughbred horses.

Key Words: horses, statistical genetics, genotyping, behavior, animal welfare

P189 Changes in the gene expression profile of equine mesenchymal stem cells (MSC) after their allogeneic administration in horses matched or mismatched for the major histocompatibility complex (MHC). A. Cequier[1,2], E. Bernad[1], M. García-Martínez[1], B. Serrano[1], F. Vázquez[1,2], A. Romero[1,2], A. Vitoria[1,2], L. Barrachina[1,2], and C. Rodellar*[1], [1]*Laboratorio de Genética Bioquímica LAGENBIO, Instituto Agroalimentario de Aragón–IA2 (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.*

Musculoskeletal injuries have a great impact in equine industry. Conventional treatments have limitations that can be overcome by the anti-inflammatory and regulatory effects of MSC. Allogeneic MSC administration is advantageous but requires further knowledge on the interactions with the immune system in vivo. Such interactions can be influenced by donor-receptor MHC-matching/mismatching and MHC expression level, which changes upon MSC inflammatory exposure and differentiation. This study evaluated the expression of genes related to the immunomodulatory and immunogenic profiles of equine MSC after their in vivo administration under different conditions. Allogeneic MSC in basal conditions (MSC-B), inflammatory primed (MSC-P) or differentiated into chondrocytes (MSC-C) were administered to 18 MHC-matched/mismatched horses. MSC were encapsulated in alginate and 3 scaffolds/recipient placed subcutaneously and retrieved after 1, 3, and 6 weeks. The procedure was repeated to assess the effect of a second administration. After retrieval of each scaffold, the expression of genes related to immunomodulation (*VCAM1*, *IL6*, *COX2*, *iNOS*, *IDO*) and immunogenicity (*CD40*, *CD80*, *MHCI*, *MHCII*) of MSCs was assessed by RT-qPCR. MSC-C showed the highest immunomodulatory profile, and their administration in MHC-mismatched horses did not increase their immunogenic profile. In contrast, MSC-P showed lower immunomodulatory and higher immunogenic profile in all recipients regardless of the MHC haplotype. MSC-B administered in MHC-mismatched horses showed a higher immunogenic profile and a lower immunomodulatory profile. MHC haplotype, inflammatory exposure, and chondrogenic differentiation of equine MSC affect their immune profile in terms of gene expression. Interestingly, MSC-C may offer advantages for allogeneic cell therapy, contrary to previous in vitro findings. The role of MHC haplotype and its expression level in the interactions of MSC with the immune system needs to be further addressed in vivo to optimize cell therapies.

Key Words: horses and related species, cell biology, immunology, microsatellite, qPCR

P190 Whole-genome trio sequencing to reveal the genetics of equine microphthalmia. I. Shutava[1], B. Ekesten[1], C.-J. Rubin[2], S. Mäkeläinen[2], T. Bergström[1], J. Tetens[3], and S. Mikko*[1], [1]*Swedish University of Agricultural Sciences, Uppsala, Sweden,* [2]*Uppsala University, Uppsala, Sweden,* [3]*University of Göttingen, Göttingen, Germany.*

Microphthalmia is characterized by abnormally small eyes. It presents as a heterogeneous disorder that can be uni- or bilateral with variable penetrance. In humans, variants in at least 29 genes, are proposed to cause microphthalmia. Some of them are believed to be inherited as autosomal recessive, others autosomal dominant, and yet others as X-linked disorders. Environmental factors have been suggested as part of the etiology creating an even more complex picture. In horses, the knowledge about microphthalmia is poor, but there is an increasing number of reported cases. In this study, more than 50 equine microphthalmia cases were identified. More than 80% of them were females, although uni- and bilateral cases were distributed evenly between females and males. In all unilateral cases, the left eye was affected with the right eye being normal. Enucleated eyes from 3 cases were examined by light microscopy and diagnosed with severe microphthalmia. Pedigree analysis identified potential inheritance patterns, and ancestral founders in specific sire family lineages. Eight cases, and 12 of their carrier parents were whole-genome sequenced at 20–60×. Using the GATK best practices workflow for cohort analysis, we developed a general pipeline for genome variant detection and prediction of their effect. Bioinformatic tools predicted autosomal recessive, and X-linked variant effects, as well as putative protein functions. Candidate genes were examined, but, so far, no conclusive variant common to the 8 cases was yet discovered.

Key Words: horse, genome sequencing, functional genomics, bioinformatics, animal health

P191 ISAG Bursary Award: The epigenetic landscape of the satellite-free centromere of horse chromosome 11. E. Cappelletti*[1], F. Piras[1], L. Sola[1], S. Peng[2], A. Barber[3], M. Santagostino[1], J. Petersen[3], R. Bellone[2,4], C. Finno[2], T. Kalbfleisch[5], E. Bailey[5], S. Nergadze[1], and E. Giulotto[1], [1]*Department of Biology and Biotechnology, University of Pavia, Pavia, Italy,* [2]*University of California-Davis, School of Veterinary Medicine, Department of Population Health and Reproduction, Davis, CA,* [3]*Department of Animal Science, University of Nebraska–Lincoln, Lincoln, NE,* [4]*University of California-Davis, School of Veterinary Medicine, Veterinary Genetics Laboratory, Davis, CA,* [5]*University of Kentucky, Gluck Equine Research Center, Lexington, KY.*

Centromeres are essential chromosomal loci which are epigenetically specified by the histone H3 variant CENP-A. Although mammalian centromeres are typically associated with satellite DNA, the centromere of horse chromosome 11 is satellite-free. We previously demonstrated that the position of its CENP-A binding domain is not fixed but slides within an about 500 kb region in different individuals, giving rise to positional alleles. These epialleles are inherited as Mendelian traits but their position can slide in a generation. As members of the equine FAANG community, we recently proved that the ECA11 CENP-A binding domain is located in the same region in tissues from different embryonic origin from the same individual, suggesting that the position of the centromeric domain is maintained during development. By performing ChIP-seq experiments with an anti-H3K9me3 antibody on chromatin extracted from horse fibroblasts, we proved that the ECA11 centromeric domain is contained in an about 3 Mb domain of constitutive heterochromatin. Using RNA-seq, ChIP-seq and miRNA-seq data sets from different tissues of 4 horses produced by the FAANG equine consortium, we evaluated the transcriptional profile and the histone marks associated with active/permissive chromatin or facultative heterochromatin in the ECA11 centromeric region. Our findings showed that, in the majority of tissues, the ECA11 centromere is contained within a transcriptionally silent domain corresponding to the heterochromatic region previously identified in fibroblasts. However, transcription of the Carbonic Anhydrase 10 gene, which is partially overlapping the centromeric locus, can be detected in the nervous system. Pilot ChIP-seq experiments with an anti-H3K9me3 antibody on brain chromatin from the FAANG stallions revealed that constitutive heterochromatin is shifted from the centromeric domain, suggesting that that CA10 gene expression may represent a boundary for centromeric function. We are currently performing H3K9me3 ChIP-seq experiments on other tissues to investigate the interplay between centromeric function and chromatin remodeling.

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

P192 A missense mutation of BCHE promotes the butyrylcholinesterase activity in Chinese horses. Y. Zhang*[1], X. Liu[1,2], and L. Jiang[1], [1]*Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China,* [2]*Centre d'Anthropobiologie et de Génomique de Toulouse, Toulouse, France.*

It is widely accepted that cholinesterase inhibition is the primary mechanism for acute organophosphorus (OP) toxicity. Butyrylcholinesterase (BChE) is an enzyme which displays a high degree of structural homology to AChE. Due to its high affinity for binding chemical warfare nerve agents (CWNAs), and the fact that null-BChE yields no apparent health effects, exogenous BChE has been explored as a candidate therapeutic for OP intoxication. Animal models are imperative for evaluating the efficacy of OP medical countermeasures, and a thorough characterization of available animal models is important for translating results to humans. We detected the basal cholinesterase activity levels in the circulation of 663 blood samples from 10 different horse farms and pasture, and sequenced the genomes of 409 Chinese native horses from 8 breeds at an average depth-of-coverage of ~11.1×. Genome-wide association study revealed top-association between variation at the butyrylcholinesterase (BCHE) and the cholinesterase activity levels. Among them, a missense mutation (Asn104Lys) made BCHE protein more stable and significantly increased the cholinesterase activity (P = 1.55E-11). The BCHE overexpression results of wild type and mutant type also confirmed that the missense mutation increased the cholinesterase activity of horse, but reduced in human. Fine-scale analysis across an extended population of 729 individuals came from 24 breeds showed that the mutation is widely distributed in Chinese horse breeds. Re-analysis of ancient DNA data showed that the C allele (mutation), first occurred some ~5,000 years ago, and rose in frequency since. Thus, the objective of this study was to compare the circulating levels of each of the cholinesterases of Chinese native horses, as well as to explore the mutation Asn104Lys found in Chinese horses was promoted the biological activity of BChE derived from horse and inhibited the biological activity of BChE derived from human. This mutation, along with corresponding future efforts, may finally lead to a novel therapeutic and source to combat organophosphorus intoxication.

Key Words: Chinese native horse, butyrylcholinesterase, organophosphates, biological modeling

P193 Construction of genome-wide INDEL database, application to a parentage-test using INDELs for horse registration, and a gene-editing test for doping control. T. Tozaki*, A. Ohnuma, M. Kikuchi, T. Ishige, H. Kakoi, K.-i. Hirota, and S.-I. Nagata, *Genetic Analysis Department, Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan.*

Thoroughbreds are the most famous breed of racehorses worldwide and are currently of high economic value. To understand genomic variability in Thoroughbreds, we identified genome-wide insertions and deletions (INDELs) and obtained their allele frequencies in this study. INDELs were obtained from whole-genome sequencing data of 101 thoroughbred racehorses by mapping sequence reads to the reference genome. By integrating individual data, 1,453,349, 113,047, and 18 INDELs were identified in the autosomal (1–31) chromosomes, X chromosome, and mitochondrial genome, respectively, for a total of 1,566,414 INDELs. Diallelic INDEL sizes ranged from −286 to +476 bp, with the majority, 717,736 (52.14%) and 220,672 (16.03%), being 1-bp and 2-bp variants, respectively. INDELs have the advantage of having lower occurrence rates than SNPs and STRs. Therefore, from the identified INDELs, we selected 39 diallelic loci to construct a par-

entage and identification panel in horses. The panel showed 0.9994, 0.9855, and >0.9999 for PE1, PE2, and PE3, respectively, when analyzed by multiplex PCR of DNA from 67 thoroughbred horses. Registration of Thoroughbred racehorses requires parentage testing using the STRs recommended by ISAG. While parentage testing using SNPs is under development, this INDEL panel may serve as a complementary panel. The racing industry prohibits the generation of genetically modified racehorses for fair competition. We recently developed a gene editing test to detect such illicitly modified racehorses. This test uses the following criteria to identify artificial editing: identifying INDELs with homozygote of alternative alleles not found in current Thoroughbred populations. As this study analyzed and validated the types, sizes, locations, and frequencies of INDELs in the current thoroughbred population, these results will contribute to improving the gene-editing test.

Key Words: horses and related species, genome sequencing, genotyping, sequence variation, sport

P194 Genomics of gaits in Icelandic horses is more complex than DMRT3. H. Sigurdardottir*[1,2], E. Albertsdottir[3], T. Kristjansson[3], M. Rhodin[4], G. Lindgren[1,5], and S. Eriksson[1], [1]*Swedish University of Agricultural Sciences, Dept. of Animal Breeding and Genetics, Uppsala, Sweden,* [2]*Agricultural University of Iceland, Faculty of Agricultural Sciences, Hvanneyri, Borgarbyggð, Iceland,* [3]*The Icelandic Agricultural Advisory Centre, Reykjavik, Iceland,* [4]*Swedish University of Agricultural Sciences, Dept. of Anatomy, Physiology and Biochemistry, Uppsala, Sweden,* [5]*KU Leuven, Livestock Genetics, Department of Biosystems, Leuven, Belgium.*

The Icelandic horse has captured interest in many countries largely because of its unique ability to perform 5 gaits, including the lateral gaits tölt and flying pace. Despite the gait versatility characterizing the breed, the ability to perform flying pace varies widely between individuals and some seem to lack the ability to pace altogether. The discovery of a single base change in the DMRT3 gene had a key role in understanding the variability, as this single mutation alters the pattern of locomotion and in a homozygous form, enables the flying pace. However, there is a sizeable ratio of homozygous horses that do not perform pace, despite a favorable DMRT3 genotype. The quality of pace is assessed based on various features such as clarity of beat, speed, stride length and suspension phase. It is therefore likely that pacing ability and quality of the gait is influenced not only by the DMRT3 gene, but also other genetic and environmental factors. Hence, the aim of the present study was to further investigate the genetic background of pace in Icelandic horses by using a genome-wide association study. A total of 362 Icelandic horses with phenotypic records from breeding field tests were genotyped with the 670 K+ Axiom Equine Genotyping Array. Several SNPs on chromosomes 4 and 9 reached the suggestive genome-wide significance level ($P < 1.0 \times 10^{-5}$) and were identified to associate with the breeding score for pace. A haplotype analysis further revealed 2 opposite haplotypes on each chromosome having positive or negative effects on the pace score. The most frequent haplotype on chromosome 4 had favorable effect on pace score but unfavorable effects on breeding scores for tölt, trot, gallop, and canter. Likewise, the most frequent haplotype on chromosome 9 had favorable effect on pace score but unfavorable effect on scores for trot and gallop. This study revealed that there appear to be multiple regions of interest in relation to ability and quality of flying pace in Icelandic horses. Further studies of these regions are needed to better understand the genetic control of the gait.

Key Words: horses, animal breeding, genome-wide association, complex trait, quantitative trait locus (QTL)

P195 A resource for documenting and tracking genetic diversity in US Thoroughbred horses. J. L. Petersen*[1], T. S. Kalbfleisch[2], J. N. Cullen[3], and E. F. Bailey[2], [1]*University of Nebraska–Lincoln, Lincoln, NE,* [2]*University of Kentucky, Lexinton, KY,* [3]*University of Minnesota, Minneapolis, MN.*

Genetic diversity in US Thoroughbreds has recently gained interest. Currently, there are no public resources that allow the equine research community to measure genetic diversity of the US Thoroughbred population or track changes in diversity as a function of time. For this project, whole-genome shotgun sequence data sets have been generated for nearly 100 animals born between 1965 and 1986, and more than 100 animals born from 2000 to 2020. The horses born between 2000 and 2020 were selected to represent the diversity of the breed based upon pedigree relationships to result in a catalog of genetic variants present in this breed. The data also allow for genomic estimates inbreeding within each horse, providing information to evaluate changes in genomic diversity in recent decades. 20-fold coverage of the genome was targeted for each horse. The sequence was mapped to the EquCab3.0 reference genome, and variants called utilizing the Burrows-Wheeler Aligner, and GATK Best Practices respectively. Across all samples, 16.7 million variants (indels and SNPs) were identified. Estimates of individual inbreeding based upon runs of homozygosity (F_{ROH}) utilizing over 10 million bi-allelic variants were orders of magnitude (20 to 40-fold, on average) greater than estimates for the same horses based upon their 5-generation pedigree. Further, pedigree- and genomic-based estimates were weakly correlated ($R^2 < 0.3$). Individual inbreeding coefficients (F_{ROH}) of horses born between 2000 and 2020 were significantly greater than those of the horses born between 1965 and 1984/5; however, the rate of change in inbreeding did not differ between groups ($P = 0.80$). Ongoing analyses will further examine both genomic and pedigree-based estimates of diversity. Additionally, future data collection will involve genotyping of ~900 additional US Thoroughbreds utilizing low-pass sequencing, allowing for a more in-depth evaluation of genetic diversity of the breed. We anticipate releasing this initial data set to public sequence repositories in late 2023.

Key Words: genetic diversity, US Thoroughbreds

P196 Genomics of Thoroughbred stallion subfertility. C. Castaneda, R. Juras, B. W. Davis, and T. Raudsepp*, *School of Veterinary Medicine, Texas A&M University, College Station, TX.*

An idiopathic form of subfertility in Thoroughbred (TB) stallions, with normal physical and semen parameters, has been attributed to impaired acrosome exocytosis (IAE). According to a genome-wide association study, the latter is significantly associated with a certain double-homozygous A/A-A/A genotype in *FKBP6* exon5. The association was recently confirmed by comparison of breeding records of 150 TB stallions with their *FKBP6* genotype. The molecular causes of this association are unknown. Development of a TaqMan assay for *FKBP6* genotyping determined that the frequency of the A/A-A/A genotype in global horse breeds/populations and TBs separately is 4%. While this genotype is present in other breeds, it is associated with subfertility only in TBs, suggesting that *FKBP6* is only tagging a TB-specific haplotype and not the cause. The aim of this study was to identify this haplotype and search for candidate genes and variants for IAE. By TaqMan assay, we detected the *FKBP6* A/A-A/A genotype in 22 subfertile TB stallions, of which 14 have confirmed IAE. We generated short-read whole genome sequence data for 9 case TBs and aligned the data with the Equine Genome Variant Database (EGVD) comprising 428 horses from 46 breeds, including 55 TBs. We found *FKBP6* A/A-A/A genotype in 21 horses (9 TB cases, 1 EGVD TB, and 11 horses of other breeds) and showed that despite the same genotype, the sequence variant landscape in a 110 kb region around *FKBP6* is the same only across TBs and is different in other breeds. We inspected 8,447 single nucleotide variants (SNVs) in all TBs (10 A/A-A/A and 54 other), determined a 171-kb haplotype block specific to A/A-A/A TBs only, and identified 38 implicated SNVs in 5 genes for further investigation. Of these, a variant in one candidate gene is homozygous only in case TBs and of low allele frequency (6%) in EGVD horses. These findings strongly support our hypothesis that the A/A-A/A genotype in *FKBP6* exon5 is tagging TB- and case-specific haplotypes which are expected to contain genetic variants responsible for the subfertility phenotype and IAE. Ongoing studies involve PacBio and RNA sequencing for the discovery of candidate structural and/or regulatory variants.

Key Words: IAE, *FKBP6*, haplotype, TaqMan

P198 ISAG Bursary Award: Updated perspective on the genetic diversity, phylogeography and population dynamics of domestic pigs in Southeast Asia. J. K. Layos*[1], C. J. Godinez[2], and M. Nishibori[3], [1]*College of Agriculture and Forestry, Capiz State University, Mambusao, Capiz, Philippines,* [2]*Department of Animal Science, Visayas State University, Baybay City, Leyte, Philippines,* [3]*Graduate School of Integrated Sciences for Life, Hiroshima University, Hiroshima, Japan.*

The temporary connections between Southeast Asia's (SEA) insular and continental biodiversity hotspots, as well as the much-debated modern human migration events into Island Southeast Asia (ISEA), were believed to have influenced the diversity and distribution of the genus *Sus* in these regions. Using large-scale samples of complete mitochondrial DNA D-loop sequences, the phylogeography, population dynamics, and haplogroup-specific divergence of domestic pigs in SEA were investigated. We identified 149 haplotypes from 447 samples, with an overall higher genetic diversity (n = 0.976 ± 0.003) compared with other geographic locations. Vietnam and the Philippines had the highest genetic diversity, while Myanmar obtained the lowest, with all samples classified only under the D2 haplogroup. Phylogenetic trees and median-joining networks revealed 6 (6) well-supported clades, confirming a new divergent matrilineage (i.e., haplogroup D7) previously identified in the Indo-Burma Biodiversity Hotspots and the Philippines as a sister clade of the D2 haplogroup. Results also support the existence of 2 subclades of Type I Lanyu pigs, the Taiwanese Lanyu and the Philippine Lanyu subclades (i.e., Palawan, Bohol, and Northern Luzon haplotypes), which could be associated with the long-distance migration of islanders of Lanyu (Orchid Island) and the Batanes Archipelago. The prehistoric population history analyses supported the population and demographic expansion hypothesis that may have spread from mainland SEA to the rest of the ISEA and the Pacific region. The maximum clade credibility tree estimated that the oldest divergent event was dated to 2.574 Mya (95% HPD: 0.650–3.250 Mya), which is the split between the Asian and European major clades. The newly postulated haplogroup D7 diverged later, at 0.776 Mya (95% HPD: 0.210–0.800 Mya) than the D2 haplogroup, with a TMRCA of 1.176 Mya (95% HPD: 0.210–1.500 Mya). These findings provide new perspectives on the complex evolutionary history of pig dispersal in ISEA, crucial for creating conservation strategies for animal genetic resources.

Key Words: biodiversity, bioinformatics, conservation, evolutionary biology, pigs

P199 Using microsatellite markers to study the population structure and genetic diversity of the native Pulawska and three commercial pig breeds in Poland. A. Radko, A. Koseniuk, and G. Smolucha*, *National Research Institute of Animal Production, Balice, Poland.*

Microsatellites (STRs) are widely used molecular markers for the individual identification of animals and parentage verification. Also, these markers have been implemented for meat traceability. Consumers frequently choose pork as their meat of choice, so maintaining high standards of pork production, not just for commercial pigs but also for native pig breeds, is crucial. Native breed pigs can provide meat that is both high in quality and functional and local, primitive breeds give rise to native pig breeds. The purpose of this study was to assess genetic diversity and determine the population structure of the native Pulawska pig (PUL, n = 85), along with 3 commercial pig breeds: Polish Landrace (PL, n = 85), Polish Large White (PLW, n = 74) and Duroc (DUR, n = 84) with using 14 STRs recommended for pig identification and parentage verification. In the analysis, we selected 14 loci from the ISAG main panel of 15 markers: S0090, S0101, S0155, S0227, S0228, S0355, S0386, SW24, SW240, SW72, SW857, SW911, SW936, and SW951. The PCR reaction was amplified using the Type-it Microsatellite PCR Kit (Qiagen) and fluorescently labeled primers. The PCR products were analyzed using an ABI 3500xl capillary sequencer (Applied BioSystems) in the presence of a size standard of 500 LIZ (Thermo Fisher Scientific) and a reference sample. The results were analyzed using the GeneMapper® Software 4.0 (Applied Biosystems). Based on the principal coordinate analysis (PCoA) supported the classification of the populations into 4 clusters. The genetic Reynolds distances (Ow) showed a close relationship between PL and PLW breeds and the most distant for DUR and PUL pigs. The genetic differentiation values (FST) were lower between PL and PLW and higher between PUL and DUR. Our study demonstrates that a panel of STR markers recommended by ISAG may also be useful for pig breed prediction and, in the future, for meat traceability which is especially important for the population of native or local pigs, such as the Pulawska breed, which can provide meat that is both high in quality and functional.

Key Words: pig, microsatellites, STR, Pulawska, diversity

P200 History and genetic diversity of African sheep: Perpendicular contrasts of phenotypes and genomic diversity. A. Da Silva[1], A. Ahbara[2], S. Ben Jemaa[3], Y. Cao[4], E. Ciani[5], E. Dzomba[6], O. Hanotte[7], S. Mastrangelo[8], A. Missohou[9], A. Molotsi[10], A. Muchadeyi[11], J. Mwacharo[12], M.-L. Li[4], S. Hall[13], J. Lenstra*[14], [1]*PEREINE/E2LIM, Faculty of Science and Technics, Limoges, France,* [2]*Department of Zoology, Faculty of Sciences, Misurata University, Misurata, Libya,* [3]*Laboratoire des Productions Animales et Fourragères, Institut National de la Recherche Agronomique de Tunisie, Université de Carthage, Ariana, Tunisia,* [4]*CAS Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China,* [5]*Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari "Aldo Moro," Bari, Italy,* [6]*Discipline of Genetics, School of Life Sciences, University of KwaZulu-Natal, Scottsville, South Africa,* [7]*School of Life Sciences, University of Nottingham, Nottingham, UK,* [8]*Dipartimento Scienze Agrarie, Alimentari e Forestali, University of Palermo, Palermo, Italy,* [9]*Animal Production and Nutrition Unit, Inter-State School of Veterinary Science and Medicine (EISMV), Dakar, Senegal,* [10]*Department of Animal Sciences, University of Stellenbosch, Matieland, Stellenbosch, South Africa,* [11]*Agricultural Research Council, Biotechnology, Platform, Onderstepoort, South Africa,* [12]*International Centre for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia,* [13]*Department of Environmental Protection and Landscape, Estonian University of Life Sciences, Tartu, Estonia,* [14]*Faculty of Veterinary Medicine, Utrecht University, Utrecht, Utrecht, The Netherlands.*

Domestic sheep in Africa have adapted to contrasting and extreme climates and play an important role in the local community-based economies of several African countries. Here we review the Neolithic immigrations of thin-tailed sheep as deduced from archeological evidence, the later introduction of fat-tailed breeds, and the more recent history of sheep in Egypt, the Maghreb, West and Central Africa, East Africa, and Southern Africa, respectively. We present a comprehensive molecular survey on the basis of 50K SNP genotypes of 55 African breeds contributed by several laboratories. We propose that gene flow has a major influence of the relationships of breeds from different regions and has partially overwritten the diversity profile created by the initial migrations. A genetic contrast between sheep north and south of the Sahara is perpendicular to the west-east contrast of thin- and fat-tailed sheep. There are no close genetic relationships between African and Asian fat-tailed breeds, whereas we observe within Africa only a modest effect of the tail types on the breed relationships.

Key Words: sheep, population genomics, single-nucleotide polymorphisms (SNPs), breed diversity, heritage

P201 ISAG Bursary Award: The first *Rangifer tarandus* Y chromosomal phylogeny. E. Bozlak*[1,2], K. Pokharel[3], M. Weldenegodguad[3], A. Paasivaara[3], J. Kantanen[3], and B. Wallner[1], [1]*Institute of Animal Breeding and Genetics, University of Veterinary Medicine*

Vienna, Vienna, Austria, [2]Vienna Graduate School of Population Genetics, University of Veterinary Medicine Vienna, Vienna, Austria, [3]Natural Resources Institute Finland, Jokioinen, Finland.

Reindeer (*Rangifer tarandus*) are one of the intriguing mammals known for their closeness to present indigenous human populations as an important source of meat and materials. Reindeer, called caribou in North America, have a circumpolar distribution, and all extant populations belong to the same species. Studies based on autosomal microsatellites, mtDNA and genomic data have revealed insights into the species' population structure, history, and domestication process. Due to its uniparental inheritance without recombination, the Y chromosome is a potent locus for decoding the male population history of mammals. Here, we created the first *R. tarandus* Y phylogeny and draw conclusions on the male demography of species. We assembled 1,320 Y chromosomal contigs from whole genome sequencing (WGS) data, representing in total 1.3 Mb of single-copy Y region, which were used as reference for variant calling with short-read data. We next mapped 55 WGS males representing semi-domestic and wild individuals from Fennoscandian and Russian tundra reindeer, forest reindeer, Svalbard reindeer, and caribou. A maximum parsimony tree was created based on 450 polymorphic sites. We observed 2 early separated clades: Clade A containing samples from Arctic Islands, Eurasia, and a few samples from North America (so-called Eurasia+ clade) and Clade B formed only by caribou from North America. Most caribou and samples collected from Arctic islands in the Eurasia+ clade (A) formed long private branches. This can be interpreted as a signature of early population movements before the northern land/sea boundaries were formed after the Last Glacial Maximum. Within Eurasia+, there were haplogroups specific for some geographic regions. Furthermore, we detected at least 3 recent expansion events, mainly represented by semi-domestic samples in different regions. These findings might be related to independent domestication events of reindeer, as previously suggested by mtDNA patterns. This study places *R. tarandus* onto the list of species with resolved Y phylogenies. It further builds the base for Y-chromosomal haplotype screening for studying diversity, migration, and admixture among populations.

Key Words: reindeer, Y chromosome, sequence variation

P202 ISAG Bursary Award: Temporal changes in genomic diversity of the northernmost cattle populations in Europe. M. Weldenegodguad*[1], M. Kjetså[2], A. Blauer[3], A. M. Johansson[4], C. Sarmento[5], S. Guimarães[5], C. Ginja[5], M. Honkatukia[2], and J. Kantanen[1], [1]*Natural Resources Institute Finland, Jokioinen, Finland,* [2]*NordGen—Nordic Genetic Resource Center, Ås, Norway,* [3]*University of Turku, Turku, Finland,* [4]*Swedish University of Agricultural Sciences, Uppsala, Sweden,* [5]*BIOPOLIS-CIBIO-InBIO, Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, Portugal.*

In Northern Fennoscandian regions, cattle (*Bos taurus*) are one of the most important livestock species in terms of their economic, societal, and cultural values. Ancient DNA studies play a pivotal role in investigating the evolutionary and population history of domestic animals. Ancient DNA data can also help to examine temporal changes in the genetic diversity. To investigate the population history of Northern Fennoscandia cattle, we performed high-throughput sequencing and retrieved genome sequences of ancient cattle specimens from Finland, Norway and Sweden. The specimens were excavated in the northern regions of these countries. We used the existing whole-genome sequence data of the northern native cattle breeds and investigated genetic relationships between these and their ancient counterparts. We successfully extracted the DNA and constructed genomic libraries from 27 ancient specimens: 5 post-medieval samples (ca. 300 years old) from Finland; 10 medieval and post-medieval samples (ca. 700–300 years old) from Sweden; and 12 medieval and post-medieval samples (ca. 700–300 years old) from Norway. Of the total samples, 14 yielded sufficient autosomal genome coverages (0.01X to 0.29X) for population genomic analyses. Moreover, for 13 samples it was possible to collect mitogenomes (90% covered at over 3X) for a phylogenetic analysis. More than 1.6 million SNPs were detected in the ancient samples and, that may represent the unique genetic diversity of the Medieval Fennoscandian cattle populations.

Key Words: ancient DNA, cattle, genetic diversity, paleogenomic, population genomics

P203 An archaeogenomics study of Iron Age cattle from Althiburos, Tunisia. C. Ginja*[1], S. Guimarães[1], R. da Fonseca[2], R. Rasteiro[3], R. Rodríguez-Varela[4], L. G. Simões[5], C. Sarmento[1], M. Carme Belarte[6], N. Kallala[7], J. Ramon Torres[8], J. Sanmartí[9], A. M. Arruda[10], C. Detry[10], S. Davis[11], J. Matos[12,13], A. Götherström[4], A. E. Pires[1,14], and S. Valenzuela-Lamas[10,15], [1]*BIOPOLIS-CIBIO-InBIO, Universidade do Porto, Vairão, Portugal,* [2]*Center for Global Mountain Biodiversity, GLOBE Institute, University of Copenhagen, Copenhagen, Denmark,* [3]*Bristol Medical School, University of Bristol, Bristol, UK,* [4]*CPG, The Centre for Palaeogenetics, Stockholm University, Stockholm, Sweden,* [5]*Human Evolution, Department of Organismal Biology, Uppsala University, Uppsala, Sweden,* [6]*ICREA, Institut Català de Recerca i Estudis Avançats, Barcelona, Spain, and ICAC, Institut Català d'Arqueologia Clàssica, Tarragona, Spain,* [7]*INP, Institute National du Patrimoine, Tunis, Tunisia,* [8]*Consell Balear d'Eivissa, Eivissa, Balearic Islands,-Spain,* [9]*Departament de Prehistòria, Història Antiga i Arqueologia, Universitat de Barcelona, Barcelona, Spain,* [10]*UNIARQ, Centro de Arqueologia da Universidade de Lisboa, Faculdade de Letras da Universidade de Lisboa, Lisboa, Portugal,* [11]*LARC/DGPC, Laboratório de Arqueociências, Direcção Geral do Património Cultural, Lisboa, Portugal,* [12]*Unidade Estratégica de Investigação e Serviços de Biotecnologia e Recursos Genéticos, Instituto Nacional de Investigação Agrária e Veterinária, I.P., Oeiras, Portugal,* [13]*CE3C, Centre for Ecology, Evolution and Environmental Changes, Universidade de Lisboa, Lisboa, Portugal,* [14]*Faculdade de Medicina Veterinária, Universidade Lusófona, Lisboa, Portugal,* [15]*Archaeology of Social Dynamics, Consejo Superior de Investigaciones Científicas-Institució Milà i Fontanals d'Humanitats (CSIC-IMF), Barcelona, Spain.*

Cattle are one of the principal domesticated animals in Eurasia and Africa and, as attested by figurines, rock art and craftwork produced across many cultures, have high symbolic value. Genomic analyses of aurochs, the ancestors of cattle, and early domestic cattle confirmed independent events of introgression of wild stock. However, the origins of the divergent North African taurine cattle are still debated, and diachronic archaeogenomics data from this region are needed to test alternative demographic scenarios. The major aim of this study was to investigate the genomic composition of Iron Age cattle from the Maghreb, a key region for understanding the dynamics of cattle dispersal and admixture with local aurochs following their earliest domestication in the Fertile Crescent more than 10,000 years ago. We generated genome-wide high-throughput sequence data and mitogenomes for 4 archeological specimens of domestic cattle from the Eastern Maghreb, i.e., Althiburos, El Kef, Tunisia, a permanent settlement of local Numidian agropastoralists. We also obtained PCR-based sequence data for a fragment of the mitochondrial hypervariable D-loop region for these and a further 8 cattle specimens collected in Althiburos. Five specimens were directly radiocarbon dated to ~2,877–2,003 cal BP, i.e., 9th to 1st century cal BCE. Four of these yielded sufficient autosomal genome coverages (0.01× to 0.10×) for population genomic analyses. Principal component analysis and model-based clustering of autosomal data showed Althiburos cattle were genetically close to the pre-domestic Northwest African aurochs and shared ancestry with present-day N'Dama taurine cattle. Maternal lineages were assigned to the R and T1 haplogroups found in 2 and 10 Althiburos specimens, respectively. These Iron Age specimens from Althiburos are the oldest R-mitogenomes described so far in domestic cattle. Our results corroborate the introgression of aurochs females into the domestic stock of cattle from Althiburos, and renew the debate on whether there was a third domestication event of taurine cattle in North Africa.

Key Words: domestic cattle, aurochs, ancient DNA, population genomics, domestication

P204 Genetic structure of Criollo sheep populations with Iberian and African breeds. J. Cappello[1,2], M. Revidatti*[1,2], S. De la Rosa[1,2], V. Morales[1,2], E. Tejerina[1,2], BiOvis Consortium[2], and A. Martínez[2,3], [1]*Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste, Corrientes, Argentina,* [2]*Red CONBIAND, Córdoba, España,* [3]*Facultad de Veterinaria, Universidad de Córdoba, Córdoba, España.*

A set of 23 STR markers were analyzed in 1239 sheep belonging to 28 populations (America, Spain, and Africa): Criollo populations from Argentina (5), Mexico (1), Brazil (1), Paraguay (1), Ecuador (1), USA (2), Chile (1), Bolivia (1), Uruguay (1), Perú (1). We also included samples from 8 Spanish and 5 African breeds. The objective was to analyze interracial diversity between Criollo and Iberian/African breeds through the Bayesian clustering method. The genetic structure was determined by using Structure v. 2.3.4. The optimal K was estimated by using the Structure Harvester program, which turned out to be K = 26. When K = 4 was assumed, the 5 Argentinean, the Bolivian and the Paraguayan Criollo populations clustered together, and the 4 local Spanish breeds was added to this group. Another group was represented by Nigerian and Pelibuey. The third cluster grouped the Uruguayan and the USA; and the last one formed by Brazilian, Mexico and Balearic Islands breeds. At K = 7 the Argentinian Criollo separated from the other groups, while Mallorquina and Ecuadorian Pelibuey; the Nigerian breeds and Spanish Pelibuey; the USA and Uruguayan sheep; Brazilian, Mexico and Menorquina population; and Roja Mallorquina and Canaria formed the other 6 different clusters. At K = 10, breeds in the Criollo group generally showed different ancestry relative to each other with very few exceptions. The influence of Spanish breeds was detected in Mexican (by Menorquina) and one Argentinean population (by Roja Mallorquina). No influence could be detected from the African populations. At K = 16 the Criollas are grouped among themselves as follows: 2 of the Argentine populations; 2 from the USA; and a third cluster formed by Mexican and Peruvian breeds. The Criollo group did not show influence of Spanish or African breeds. Finally, at K = 26, each breed separated as a unique population, with a low percentage of admixture, except for the Nigerian ones, which remain in the same cluster. These results suggest that creole sheep constitute a different group, not necessarily composed of a high proportion of the supposed ancestral breeds. This work has been developed within the CONBIAND network.

Key Words: ewe, local breeds, molecular

P205 Genetic diversity of Clydesdale and Shire draft horses with implications for management. J. L. Petersen*, A. M. Barber, A. M. Fuller, and I. Grazian, *University of Nebraska-Lincoln, Lincoln, NE.*

The draft horses of the UK, the Scottish Clydesdale and British Shire, have been bred for their size and utility as work horses for over 200 years; formal breed registries were established in the late 1800s. Thousands of these popular horses were exported to North America and elsewhere near the turn of the 20th century. In the 1930s, however, draft horse populations were greatly reduced. Each breed is now considered "at risk" by the Rare Breed Survival Trust. Using varying data sets, the purpose of this study was to characterize the diversity of and evaluate population structure within and between breeds. Clydesdales born between 2016 and 2018 in the US (n = 34), Canada (n = 29), or Scotland (n = 31) were genotyped at ~72,000 SNP markers. Microsatellite genotypes (12 loci) were compiled from over 3,500 Shire horses born between 1971 and 2021, all registered with the American Shire Association. Finally, whole-genome sequence (WGS) was generated on 29 horses for comparisons of diversity. The data demonstrate evidence of differentiation between Scottish and North American Clydesdales with pairwise F_{ST} values between the Scottish sample and either North American sample 5-fold greater than that between the Canadian and US samples. Greater diversity determined by expected heterozygosity and allelic richness was identified in the North American Clydesdales compared with the Scottish sample. Individual inbreeding was significantly ($P < 0.001$) higher in the Scottish Clydesdales (avg $F_{ROH} = 0.34$) compared with the Canadian and US samples (avg $F_{ROH} = 0.30$ in each). Microsatellite data from the Shires demonstrated that allelic richness decreased between 1971 and 2021, although the reduction was not significant ($P = 0.34$). A similar reduction in expected heterozygosity was observed. Two breeds were differentiated by WGS, which also revealed that the Clydesdales had individual inbreeding coefficients that were, on average, 1.1× that of the Shires. These data can help breeders determine the best means to continue to breed quality horses while being mindful of genetic diversity.

Key Words: single-nucleotide polymorphisms, microsatellites, population genomics, inbreeding, equine

P206 Genetic characterization of deleterious alleles in traditional cattle populations in Europe and Africa. R. Crooijmans*[1], R. Gonzalez-Prendes[1], M. Derks[1], N. Ghanem[2], C. Ginja[3], D. Kugonza[4], L. Makgahlela[5], and K. Juha[6], [1]*Wageningen University and Research, Animal Breeding and Genomics, Wageningen, The Netherlands,* [2]*University of Cairo, Animal Reproduction Department, Cairo, Egypt,* [3]*University of Porto, Centro de Investigacão em Biodiversidade e Resursos Genéticos, Vairão, Portugal,* [4]*Makerere University, Animal Breeding and Genetics, Kampala, Uganda,* [5]*Agricultural Research Council, Animal Breeding and Genetics, Pretoria, South Africa,* [6]*Natural Resources Institute Finland, Jokioinen, Finland.*

Traditional local cattle breeds are under severe pressure of extinction worldwide due to their low production performance compared with commercial breeds, as well as their legislation. Breed replacement or crossbreeding with commercial transboundary cattle is a threat to these native breeds. One very big advantage of traditional local cattle breeds is the high adaptation to the ecosystems they have lived in. How did this adaptation shape the genome of these animals? Within the OPTIBOV project, we have sampled and sequenced over 500 cattle from 26 traditional breeds. Additionally, we established standardized protocols (SOPs) for phenotype collection in 6 different countries (Finland, The Netherlands, Portugal, Egypt, Uganda, and South Africa) for both traditional cattle breeds and one commercial dairy breed. The derived phenotypes are used in combination with whole genome sequence data of the same animals to try to find association to adaptation to different ecotypes. Each individual has a certain number of harmful mutations in its genome. These mutations can lower the fitness of the individual carrying them, dependent on their dominance and selection coefficient. Effective population size, selection, and admixture are known to affect the occurrence of such mutations in a population. We detect breed-specific variations, including SNPs and structural variants (SVs). To improve the detection of SVs, we create breed-specific long read Nanopore reference genomes. The relative roles of demography and selection are key to understanding the process of adaptation. In this study we will investigate the number of deleterious alleles. We hypothesize that the series of events of bottlenecks, introgression, inbreeding and strong artificial selection associated with domestication increased mutational load in these traditional cattle breeds.

Key Words: traditional cattle breeds, deleterious alleles, WGS

P207 ISAG Bursary Award: Admixed ancestry or independent race: A phylogenetic meta-analysis on the phylogeography of Philippine chickens. C. Godinez*[1,2], J. Layos[2,3], Y. Yamamoto[2], T. Kunieda[4], and M. Nishibori[2,1], [1]*Department of Animal Science, College of Agriculture and Food Science, Visayas State University, Visca, Baybay City, Leyte, Philippines,* [2]*Laboratory of Animal Genetics, Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima, Japan,* [3]*College of Agriculture and Forestry, Capiz State University, Burias, Mambusao, Capiz, Philippines,* [4]*Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Ehime, Japan.*

An important unresolved question is whether the founding lineages of chickens introduced into the Philippines were descended from wild endemic populations or domestic chickens from mainland Southeast Asia (MSEA) that had become feral. A total of 562 complete mtDNA control region sequences of Southeast Asian chickens (n = 223 ISEA, and n =

339, MSEA) were meta-analyzed in this study, along with 35 sequences of Pacific chickens, to elucidate the genetic relationship and population history of Philippine chickens with the rest of the chicken populations across Southeast Asia. The Bayesian phylogenetic tree revealed the distinct phylogenetic position of the diverse D-lineage, indicating that the Philippine-Pacific D1b subclade underwent in situ diversification in the Philippines with their founding population (sub-haplogroup D1a) before expanding eastward. Notably, red junglefowls from Palawan and Mindoro have been found to be related to the ancestral Hap-D2 lineage, implying that the Philippine red junglefowl descended from the MSEA founding population and became exoferal or admixed following colonization in the archipelago. There is also close genetic affinity between Philippine and Cambodian chickens, both of which belong to haplogroup D. Meanwhile, there is no discernible subgrouping of chicken populations in ISEA. This phylogenetic meta-analysis contributes to the general understanding of Philippine chicken phylogeography, allowing for better management of these genetic resources.

Key Words: evolutionary genomics, feralization, mitochondrial DNA, phylogeny, poultry and related species

P208 Genomic tools for the monitoring of genetic diversity. P. Boettcher*[1], R. Baumung[1], P. Burger[2], L. Colli[3], I. Curik[4], G. Leroy[1], C. Looft[5], A. Manunza[6], G. Mészáros[7], D. Ouedraogo[8], B. Rosen[9], A. Stella[6], Y. Utsunomiya[10], J. Windig[11], J. Soelkner[7], [1]*Food and Agriculture Organization of the UN, Rome, Italy,* [2]*University of Veterinary Medicine Vienna, Vienna, Austria,* [3]*Università Cattolica del Sacro Cuore, Piacenza, Italy,* [4]*University of Zagreb, Zagreb, Croatia,* [5]*University of Applied Science Neubrandenburg, Neubrandenburg, Germany,* [6]*IBBA-CNR, Milan, Italy,* [7]*BOKU, Vienna, Austria,* [8]*Joseph KI-ZERBO University, Ouagadougou, Burkina Faso,* [9]*United States Department of Agriculture, Beltsville, MD,* [10]*São Paulo State University, São Paulo, Brazil,* [11]*Wageningen University and Research, Wageningen, The Netherlands.*

Census population size is a key factor influencing the risk of extinction of animal populations. For the past 30 years, FAO has used this metric for monitoring the state of animal genetic resources, by using data provided by countries in the Domestic Animal Diversity Information System (DAD-IS). Indicator 2.5.2 of the UN Sustainable Development Goals is based on this criterion. Genetic variation of a population has impacts on reproductive fitness and adaptation to environmental changes, and thus also influences its extinction risk. Maintenance of the genetic diversity within populations of wild and domesticated species is therefore among the goals of the recently-adopted Kunming-Montreal Global Biodiversity Framework. FAO has worked with international experts to review approaches for the measurement of within-population diversity, has proposed effective population size (Ne) as a suitable indicator. Ne is generally straightforward in its interpretation. An additional advantage is the possibility of estimation with demographic, pedigree or genomic data. Ne has furthermore been proposed as an indicator for implementation of the Biodiversity Framework. For monitoring the Ne of livestock breeds, genomic data may be the most feasible option for most countries. At the same time, methods for estimating historical Ne are improving, facilitating monitoring of recent diversity loss. From the economic point of view, the current genotyping costs for estimating Ne for most breeds and species based on 100 genotyped individuals would be around USD 2,500/breed. Many countries have already demonstrated the technical capacity, scientific interest and political will to study their breeds on the molecular level. A literature review of 250 randomly selected breeds from a representative group of countries, revealed that genomic studies (SNP or whole genome sequencing) have already been undertaken on 39% of breeds when considering in-country research and 68% when accounting for international studies on transboundary breeds. This proportion increases to 75% when all molecular genetic studies are considered. Member countries have requested FAO to continue to review, develop and refine indicators for genetic diversity and propose related data fields for incorporation into DAD-IS.

Key Words: genetic diversity, breed, monitoring, genomics, effective population size

P209 ISAG Bursary Award: An insight into whole-genome resequencing data of Indian native goats with global breeds reveals high within-breed genetic diversity and distinct population structure. N. Balasubramaniam*[1,2], S. Dixit[2], S. Singh[2], S. Koloi[1,2], and I. Ganguly[2], [1]*ICAR-National Dairy Research Institute, Karnal, Haryana, India,* [2]*ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana, India.*

Indian goats possess varied levels of adaptability, productivity and disease resistance. Genomic diversity of goats surviving in extreme conditions hold answers to building a sustainable goat production system. We studied diversity of 11 Indian breeds (n = 102) compared with worldwide goats from 30 breeds and 5 outgroups (n = 101, public domain). Indian goat samples were sequenced using Illumina NOVASEQ6000 platform. Sequences were combined to generate a VCF file; annotation was done using the *Capra hircus* reference. Nucleotide diversity (p), inbreeding coefficient (F), observed (Ho) and expected heterozygosity (He), proportion of polymorphic SNP (PN), average pairwise genetic distance (DST), effective population size (Ne), linkage disequilibrium (LD, r^2) and LD decay were obtained. NJ tree, PCA and Admixture were carried out to assess population structure. 21.44 billion reads remained after quality control and alignment with ARS1. Average coverage was 99.2% and 17.2% duplication rate; depth of sequencing was ~9×. Kanni Adu (KAN) had 1.95M SNP while Jharkhand Black (JB) had 30.77K; average r^2 was highest in JB (0.859) and lowest in Jakhrana (JAK; 0.496). LD showed rapid decay ($r^2 < 0.2$) within 5 kb in all the breeds except JB, Sangamneri (SAN) and KAN. Changthangi (CHA; 0.379) and JAK (0.363) had the highest p, while KAN had the highest PN (0.340). Average Ho was lowest in JB (0.119) and highest in CHA (0.505); He was lowest in KAN(0.249) and highest in CHA(0.378). KAN, Tellicherry (T), SAN and JB were distinct in PCA. Admixture graph at k = 2–12 showed that k = 8 had minimum error (0.423). PCA revealed that other *Capra* species were distinct from *C. hircus*. Indian goats were widely distinct from exotic; 3 breeds from Pakistan clustered with Indian goats. Goats spread over the Indian subcontinent have high within-breed diversity (>94%), harboring substantial genetic variations to respond to future demands and climate change. Within-breed diversity, gene flow among indigenous animals and shared genomic regions are contributors for the admixed nature of animals.

Key Words: Indian, goats, diversity, admixture, PCA

P210 Withdrawn

P211 ISAG Bursary Award: Multiple origins and genetic diversity of Philippine native pigs. J. B. Banayo*[1,2], K. L. V. Manese[2], K. O. Furusho[2], A. J. Salces[2], and T. Yamagata[1], [1]*Nagoya University, Chikusa, Nagoya, Japan,* [2]*University of the Philippines Los Baños, Laguna, Philippines.*

The native pig has a niche among women-led smallholder farms in the Philippines. Considering this sociocultural importance and its crucial role in addressing the issue of household food security and livelihood in rural communities, this study was conducted to characterize the genetics and physical traits of native pigs in the Philippines (PhNP) to inform conservation management. We compared PhNP (n = 157 collected from 7 Philippine provinces in Luzon and Visayas regions) with other breeds using partial mtDNA D-loop sequence, 21 ISAG-FAO recommended microsatellite markers, and 18 physical traits. Phylogenetic analysis showed that PhNP have Asian wild boar (*Sus scrofa*) origins from multiple domestication centers, such as the East (D2), Southeast Asia (D7), and the Cordillera/Lanyu clade. We further show that D7 clustered within the general D2 clade, suggesting its D2 ancestry and formation due to a population bottleneck upon dispersal to Southeast Asia. Our results also dispel the local belief that the PhNP were domesticated from endemic wild pigs of the Philippines. Linear discriminant (LD) analysis of physical traits differentiated the pigs representing the D2 (North Luzon lowlands), and the D7 subclades (South Luzon and Visayan), but not the Cordillera clade (North Luzon highlands). Discriminating traits (coefficient of LD 27.0 to −46.0) were the ratios of tail to body length, ear to body length, and snout to head length, supporting the locally observed variation in ear and snout. On the other hand, microsatellite analysis showed pairwise *Fst* between 0.130 and 0.427, suggesting sufficient genetic differentiation between PhNP populations. The effective population size (Ne) was below 50, except Kalinga, which has 420. Kalinga is a predominantly indigenous community that uses wild pigs in PhNP production, highlighting hybridization benefits to PhNP. To mitigate low Ne, we propose to encourage breed use by incentivizing native pig farming as a conservation service. This study shows the diverse genetics of PhNP and contributes to our understanding of domestic animal diversity in an island country.

Key Words: pigs and related species, breed diversity, phylogeny, effective population size, physical trait

Livestock Genomics for Developing Countries

P212 ISAG Bursary Award: History and unique evolutionary adaptation of indicine cattle. N. Chen*[1], X. Xia[1], Q. Hanif[2,3], T. Hussain[4], N. A. Gorkhali[5], E. Terefe[6,7], G. Belay[6], A. Tijjani[7], T. Zegeye[8], M. G. Gebre[9], J. A. Lenstra[10], J. Han[3,11], O. Hanotte[11,12], Y. Jiang[1], C. Lei[1], [1]*Key Laboratory of Animal Genetics, Breeding and Reproduction of Shaanxi Province, College of Animal Science and Technology, Northwest A&F University, Yangling, China,* [2]*National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan,* [3]*CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China,* [4]*Department of Molecular Biology, Virtual University of Pakistan, Rawalpindi, Punjab, Pakistan,* [5]*National Animal Breeding and Genetics Centre, National Animal Science Research Institute, Nepal Agriculture Research Council, Khumaltar, Lalitpur, Nepal,* [6]*College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia,* [7]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [8]*Mekelle Agricultural Research Center, Tigray, Ethiopia,* [9]*College of Agriculture, Haramaya University, Haramaya, Oromia, Ethiopia,* [10]*Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands,* [11]*International Livestock Research Institute (ILRI), Nairobi, Kenya,* [12]*School of Life Sciences, University of Nottingham, Nottingham, UK.*

Indicine cattle, also referred as zebu *Bos taurus indicus*, play a central role in the livelihood of pastoral communities across a wide range of agroecologies, from the extreme hot semi-arid to the hot humid tropical regions. However, the adaptive genetic changes and its origin following their dispersal in East Asia, from their centers of origin on the Indian sub-continent, remain poorly documented. Here, using high-quality whole-genome sequencing data from 355 indicine cattle of 57 breeds/populations, including indicine lineages worldwide, we were able to characterize in fine details their genetic diversity and population structures and to show that their local environmental adaptation in Asia, benefit from the introgression from other bovine species. Genomic regions carrying morphology-, immune-, and heat-tolerance-related genes underwent according to the Asian agroecologies divergent selection. We identified distinct sets of loci that likely contributed to adaptations to the hot semi-arid and the hot humid tropical ecosystems. Our results allow us to propose that the rapid and successful adaptation of East Asian indicine cattle to the hot humid environments was a direct consequence of a small number of introgressed genome regions from banteng and/or gaur following initially a coastal rather than inland routes of dispersion. Our findings provide comprehensive explanatory insights into the unique successful global evolutionary adaptation of indicine cattle.

Key Words: cattle and related species, re-sequencing, adaptation, population genomics, introgression

P213 Journeying to a sustainable dairy breeding program in Tanzania. G. Gebreyohanes*[1], J. Ojango[1], L. Eliamoni[1], N. Kelay[1], K. Suzan[2], K. Daniel[3], C. Ekine[1], M. Raphael[1], and O. Mwai[1], [1]*International Livestock Research Institute, Nairobi, Kenya,* [2]*Green Dreams Tech, Nairobi, Kenya,* [3]*Tanzania Agriculture and Livestock Research Institute, Dodoma, Tanzania.*

Tanzania smallholder dairy is constrained by lack of access to high-yielding and locally adapted genetics. Artificial insemination (AI) is used as the tool for delivering improved dairy genetics. Uptake of AI services is variable across the country. Information on individual animals born through AI and their performance levels within the different environments are lacking, hence difficult to fully evaluate the performance of the progeny born from AI bulls. To develop a successful breeding program regular animal performance recording is necessary that will provide records for genetic evaluation and ranking of animals to be used as parents of future bulls. The African dairy genetic gains (ADGG) program in partnership with the Tanzania Livestock Research Institute initiated a national dairy performance recording, evaluation, and breeding program in 2016. This paper reports on the journey so far in building a breeding program at the national level and suggests a way forward for its sustainability. The program started with revamping the existing national animal identification system. This involved initiating farmer, herd, and individual animal registration, establishing a national digital database that enabled recording and genotyping of cows and bulls owned by farmers. From 2016 to 2023, 33,769 farms with 79,269 animals have been registered, and total of 260,827 test-day milk yields and 44,853 body weight records have been captured. About 7,458 animals have been genotyped and used to generate genomic breeding values. Results from the genomic evaluations were used to develop a selection index that improves the rate of milk production but keeps the body weight constant. Semen from the top bulls is being promoted

through AI to benefit thousands of dairy farmers. From the analysis of the production trends, an improvement of approximately 54% (from an average of 6.7 L to 10.34) of milk yield/cow/day, from 2016 to-date is reported. It can be concluded that the Tanzanian dairy industry can benefit from sustained breeding program that continuously identifies and delivers improved adapted and productive dairy genetics.

Key Words: Tanzania, dairy, database, animal registration

P214 ISAG Bursary Award: A genomic characterization of the SA Bonsmara breed using the BovineHD 777K array. D. Alberts*, S. F. Lashmar, and E. van Marle-Köster, *University of Pretoria, Pretoria, Gauteng, South Africa.*

The South African Bonsmara, a Sanga-derived composite breed, originally developed to be genetically composed of five-eighths Afrikaner (Sanga subspecies) and three-eighths Hereford and Shorthorn, is well-adapted with good growth and mothering ability. The breed has an open herd book where females are added based on phenotypic inspection of functional traits, thus implying an unknown base composition of the modern-day Bonsmara. In this study, high-density genotypes (777K SNP) of 10 founder animals were analyzed to assess the within-breed variation, inbreeding and admixture of the Bonsmara and 2 of its base breeds. A total of 4 Afrikaner, 4 Bonsmara, and 2 Hereford animals were genotyped using the Illumina® BovineHD 777K single nucleotide polymorphism (SNP) array as part of the Beef Genomics Programme (BGP). Following standard quality control, using Plink v1.09, a total of 735,239 autosomal SNPs was available for downstream analysis. The Hereford cattle demonstrated higher observed heterozygosity values (0.532) compared with the Afrikaner and Bonsmara (0.421) breeds, whereas the latter had comparable observed heterozygosity values with the Afrikaner (0.416). The average inbreeding coefficient (F_{SNP}) of the Bonsmara was estimated at −0.12, and the Afrikaner and Hereford −0.14 and −0.27, respectively. Genomic structure analysis indicated that the Bonsmara have an admixed genome, confirming the origin of the breed. This study forms part of a larger project which will be expanded with whole-genome sequencing data from founder animals and current high-impact animals that are geographically distributed throughout South Africa. A comprehensive genetic characterization of founder animals will aid in detecting genome-level changes in the historic versus modern genetic composition of the breed over time.

Key Words: cattle and related species, animal breeding, single-nucleotide polymorphism (SNP), breed diversity

P215 ISAG Bursary Award: Genotyping-by-sequencing: A powerful tool to reveal genomic relatedness and admixture in local Tunisian sheep breeds. I. Baazaoui*[1], S. Bedhiaf-Romdhani[1], K. G. Dodds[2], R. Brauning[2], R. Anderson[2], T. Van Stijn[2], A. McCulloch[2], and J. McEwan[2], [1]*National Agricultural Research Institute of Tunisia, Ariana, Tunisia,* [2]*AgResearch Limited Invermay Agricultural Centre, New Zealand.*

In developing countries, the molecular characterization of indigenous livestock resources largely depends on the use of simple and economical genomic technologies. High-throughput sequencing protocols for simultaneous discovery and genotyping of single nucleotide polymorphisms (SNPs) have underpinned the development of a genotyping-by-sequencing (GBS) method, that offers highly multiplexed, reproducible and cost-effective alternative to existing genotyping techniques. In Tunisia, small farmers living in rural areas with low incomes own 80% of national sheep flock. Around 4 million breeding ewes make up the national herd, composed by 1 dairy Sicilo-Sarde sheep and 4 meat breeds (Barbarine, Queue Fine de l'Ouest, Noire de Thibar, and D'man) with more than half is represented by unique fat-tailed Barbarine breed. In this study, we used GBS data to assess the population structure of 197 Tunisian sheep belonging to 5 local breeds and a crossbred population, to deepen our understanding of how different breeding practices affect the current genetic structure. After data quality control, we obtained 115,121 SNPs with 31% missing genotypes and a mean sample depth of 3.19 in 183 samples. The results of the hierarchical admixture and calculation of genomic relatedness matrix pointed out a genetic homogenization between fat-tailed Barbarine and thin-tailed Queue Fine de l'Ouest breeds and their crosses due to massive anarchic crossbreeding, which is a current practice in Northern African sheep breeds. We also highlighted a clear distinctiveness of Noire de Thibar and Sicilo-Sarde breeds, consistently with past introgression events of European sheep gene flow. Our findings demonstrate that GBS technology is a promising method to characterize local livestock populations in countries with limited funding, since it may produce a reasonably complete data set with thousands of SNP loci for a fraction of the cost of array-based genotyping.

Key Words: Tunisian sheep, single nucleotide polymorphism (SNP), genotyping-by-sequencing, genomic relatedeness, cost-effective

P216 ISAG Bursary Award: Genomic analysis reveals low level of inbreeding in Ugandan goat breeds. R. B. Onzima*[1], H. P. Doekes[2], R. Mukiibi[3], and R. P. M. G. Crooijmans[2], [1]*Faculty of Agriculture and Environmental Science, Muni University, Arua, Uganda,* [2]*Animal Breeding and Genomics, Wageningen University and Research, Wageningen, The Netherlands,* [3]*Roslin Institute, University of Edinburgh, Edinburgh, Scotland, United Kingdom.*

In farm animals, the coefficient of inbreeding has been traditionally obtained from pedigree records which is often lacking.in developing countries. However, the increasing availability of SNP data is providing avenues for computing inbreeding levels where pedigree data may be lacking. Using information based on runs of homozygosity (ROH) generated from 45294 autosomal SNPs, we evaluated genomic inbreeding coefficient, F_{ROH} in 6 Ugandan goat breeds: Boer (n = 13), Karamojong (n = 15), Kigezi (n = 29), Mubende (n = 29), Sebei (n = 29) and Small East African (n = 29). The average genomic inbreeding coefficient among the indigenous goat breeds was generally low ranging from 0.8% to 2.4% compared with 13.8% in exotic Boer. Short ROH predominated in the indigenous breeds except Karamojong goats. Meanwhile, the long ROH were predominant in the exotic Boer goats. The presence of short ROH in the indigenous breeds signifies more distant inbreeding events than in the present populations. The long ROH were predominant in Boer and Karamojong goat breeds indicating occurrence of a more recent inbreeding that could be attributed to more recent selection events to concentrate particular production traits of economic importance.

Key Words: genomic inbreeding coefficient, runs of homozygosity, goats

P217 ISAG Bursary Award: Anthropological events and environmental stress are shaping the genomes of Ethiopian indigenous goats. S. Belay*[1,2], G. Belay[2], H. Nigussie[2], A. Tijjani[3,4], A. M. Ahbara[3,5], T. Dessie[4], G. M. Tarekegn[6,7], H. Jian-Lin[8,9], S. Mor[4,10], H. S. Woldekiros[11], K. Dobney[12,13], O. Lebrasseur[4], O. Hanotte[3,4], and J. M. Mwacharo[14,15], [1]*Tigray Agricultural Research Institute, Mekelle, Ethiopia,* [2]*Addis Ababa University, Department of Microbial, Cellular and Molecular Biology, Addis Ababa, Ethiopia,* [3]*School of Life Sciences, University of Nottingham, Nottingham, UK,* [4]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [5]*Department of Zoology, Misurata University, Misurata, Libya,* [6]*Animal and Veterinary Sciences, Scotland's Rural College (SRUC) Staff Group, Roslin Institute Building, Easter Bush Campus, University of Edinburgh, Edinburgh, UK,* [7]*Institute of Biotechnology (IoB), Addis Ababa University, Addis Ababa, Ethiopia,* [8]*CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Beijing, China,* [9]*Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China,* [10]*University of Liverpool, Institute of Infection, Veterinary and Ecological Sciences, Liverpool, UK,* [11]*Department of Anthropology Washington University in St. Louis, St. Louis, MO,* [12]*University of Liverpool, Department of Archaeology, Classics and Egyptology, Liverpool, UK,* [13]*University of Sydney, Sydney, Australia,* [14]*Small Ruminant Genomics, International Centre for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia,* [15]*SRUC, Animal and Veterinary Sciences and Centre for Tropical Livestock Ge-*

netics and Health (CTLGH), Roslin Institute, University of Edinburgh, Edinburgh, UK.

Anthropological, biological, and natural processes/impacts have shaped livestock genomes over millennia and can be used to explain their current geographic distribution and infer genetic divergence. Here, we analyzed 57 Ethiopian indigenous domestic goat genomes alongside 67 equivalents of East, West and Northwest African, European, South Asian, Middle Eastern and wild Bezoar goats. ADMIXTURE (K = 4) and phylogenetic analysis reveal 4 genetic groups (African, European, South Asian and the wild Bezoar) with Middle Eastern goats an admixture of the 4. At K = 5, the West African Dwarf and Moroccan goats are clearly separated from the Eastern African ones (Kenya, Ethiopia). Likely historical legacies of goat arrival and migration into Africa with 2 entry points (coastal Mediterranean Sea and the Horn of Africa regions). Fst, XP-EHH, and *Hp* analysis reveal signatures of selection in Ethiopian goats overlaying genes for thermo-sensitivity, oxidative stress response, high-altitude hypoxic adaptation, reproductive fitness, pathogen defense, adaptive and innate immunity, pigmentation, DNA repair, modulation of renal function and integrated fluid and electrolyte homeostasis. Notable examples include *TRPV1* (a nociception gene); *PTPMT1* (a critical hypoxia survival gene); *RETREG* (a regulator of reticulophagy during starvation), and *WNK4* (a molecular switch for osmoregulation). These results indicate human-mediated translocations and adaptation to contrasting environments which (re)shapes indigenous African goat genomes in the Horn of Africa over millennia.

Key Words: goat, genetic diversity, selection signatures

P218 Post-GWAS functional annotation for tick count, growth traits, and skin thickness in F_2 Angus × Nguni crossbred cattle. N. Mkize*[1,2], A. N. Maiwashe[1], B. Dube[1], K. Dzama[2], and N. O. Mapholi[3], [1]*Agricultural Research Council, Centurion, Gauteng, South Africa,* [2]*Stellenbosch University, Stellenbosch, Western Cape, South Africa,* [3]*University of South Africa, Florida, Gauteng, South Africa.*

The understanding of the biological mechanism underlying economic traits in cattle is crucial for the improvement of the traits through genetic selection. The current study performed a post-GWAS functional analysis to understand biological mechanisms underlying tick count, growth traits, and skin thickness in the F_2 Angus × Nguni cattle population. Functional annotation, pathway, and cluster analysis were formed using DAVID and ShinyGo bioinformatics resources. Fisher's exact test with false discovery rate adjustment was used and the statistical significance was considered at P (DFR) < 0.05. For the tick count trait, the characterized genes included *ZNF398*, *ZNF789*, *U6*, *7SK*, GTPase, *GIMAP7*, *GIMAP8*, *GRM7*, *REPIN1*, *ATP6V0VE2*, and *RARRES2*. The tick count-associated enriched gene ontology terms and pathways were related to cellular processes, regulation of biological processes, and response to a stimulus. While for growth traits, the characterized genes included *RBFOX2*, *UQCRFS1*, *CMM5*, *CAVN14*, *TRPM8*, *MSANTD3*, and *TMEFF1.* The associated enriched ontology terms and pathways were related to the developmental processes, metabolic processes, and biological regulations. Moreover, the skin thickness enriched GO terms were related to immune response and transmembrane receptor protein. To conclude, biological mechanisms uncovered from this study hold promise for the genetic improvement of the traits of interest through selective breeding. However, these findings warrant validation through fine mapping analysis and further studies to explore molecular interaction for the identified genes.

Key Words: gene ontology, gene set enrichment, candidate gene

P219 ISAG Bursary Award: Low genetic diversity and population structuring of *Amblyomma hebraeum* and *Rickettsia africae* from coastal and inland regions in the Eastern Cape Province of South Africa. A. Pillay*[1], N. Nyangiwe[2], and S. Mukaratirwa[1,3], [1]*University of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa,* [2]*Döhne Agricultural Development Institute, Stutterheim, South Africa,* [3]*Ross University School of Veterinary Medicine, Basseterre, St. Kitts & Nevis.*

Amblyomma hebraeum is the main vector of *Rickettsia africae*, the causative agent of African tick bite fever in southern Africa. Because pathogen dispersal is known to be influenced by tick adaptations to climate or host species, this study aimed to analyze the genetic diversity of *A. hebraeum* and *R. africae* infection of ticks collected from cattle in the Eastern Cape province of South Africa. DNA was extracted, amplified, and sequenced for the *COI* and *ITS2* markers from *A. hebraeum* samples and the *17kDa* and *ompA* genes for rickettsial detection. Between 6 and 10 haplotypes were identified from 40 *COI* and 31 *ITS2* sequences; however, no population structuring was observed among sites (ΦST = 0.22, $P < 0.05$). All *A. hebraeum* isolates clustered with southern Africa GenBank isolates. *Rickettsia africae* was detected in 46.92% (95% CI = 41–53%, n = 260) of ticks. All *R. africae* isolates clustered with strain PELE and Chucks, which were reported previously from South Africa. These results confirm that *A. hebraeum* populations are undergoing a recent population expansion driven by cattle movement, facilitating local and long dispersal events across the Eastern Cape province. This is of great public health importance which may affect tourists visiting these regions and requires further long-term surveillance of ATBF patients, and *R. africae*-infected ticks.

Key Words: *Amblyomma hebraeum*, *Rickettsia africae*, population genetics, South Africa, tick-borne disease

P220 ISAG Bursary Award: Building genomic resources for cattle breeds at risk of extinction in Nigeria. O. Opoola*[1], M. Wheto[2], R. Mukiibi[3], R. Mrode[4,5,6], and A. Djikeng[1,5], [1]*Centre for Tropical Livestock Genetics and Health (CTLGH), The Roslin Intitute, University of Edinburgh, Easter Bush Campus, Edinburgh, UK,* [2]*College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta (FUNAAB), Abeokuta, Ogun State, Nigeria,* [3]*The Roslin Institute, University of Edinburgh, Easter Bush Campus, Edinburgh, UK,* [4]*Scotland's Rural College (SRUC), Edinburgh, UK,* [5]*International Livestock Research Institute (ILRI), Nairobi, Kenya,* [6]*The University Edinburgh, Scotland.*

Nigeria has considerable under-exploited animal resources including locally adapted beef breeds that could be utilized to ensure food security. Muturu cattle have been identified as a promising breed for beef production in Nigeria due to its hardiness and adaptation to the prevailing climate and environmental challenges. Compared with other cattle breeds, Muturu cattle have a large frame, which translates into higher meat-to-bone ratio. Recent reports revealed the breed to be under threat of replacement or crossbreeding with Zebu leading to low population of breeding individuals. The low population is further enthralled with farmer native beliefs of the breed being sacred, hence, most preferring on-farm mating leading to inbreeding. Additionally, only a few studies have been performed to phenotypically and genetically characterize the breed in Nigeria. Therefore this study aims to: (i) support breed conservation and establishment of improved Muturu cattle population in Nigeria, (ii) establish a framework for animal identification for experimental data collection and performance recording using approved animal ear tags and (iii) assess the genetic variations, population structure, diversity and ancestral origins to establish differences and/or similarities in the breed's genetic background. Currently, the project has undertaken animal identification using approved ear tags and engaged Muturu farmers about suitable breeding practices in January 2023. DNA materials (tail hair) from 500 Muturu cattle have been sampled from FUNAAB's research station and 429 farmers in the Southwest of Nigeria. Samples are being genotyped with Geneseek Genomic Profiler (GGP) 100K array at Neogen's Dairy School, UK. We anticipate that the resulting data will support the phenotypic and genetic characterization (and diversity) of Muturu cattle to inform the development of conservation strategies and sustainable utilization of the Muturu breed thereby contributing to food security in Nigeria and the surrounding

region. The existence of such a breeding strategy will help to overcome some of the native beliefs, leading to improved productivity.

Key Words: cattle, conservation genomics, genotyping, breed diversity, meat production

P221 Withdrawn

P223 ISAG Bursary Award: Molecular detection and phylogenetic analysis of lumpy skin disease virus (LSDV) from 2019 to 2022 outbreak in Bangladesh. A. Bhuyan*[1], J. Khanom[1], A. Bhuiyan[2], R. Rubaya[1], and J. Alam[1], [1]*National Institute of Biotechnology, Ashulia, Bangladesh,* [2]*Bangladesh Agricultural Research Council, Faridpur, Bangladesh.*

Lumpy skin disease (LSD), a deadly viral infection of cattle caused by the LSD virus (LSDV), has lately spread throughout South and East Asia. This disease first appeared in Bangladesh in July 2019, first in the Chattogram district, and spread throughout the country within a very short time. In this study, a total of 171 samples (blood, skin scraping, buccal swabs and nasal swabs) were collected from Natore, Kurigram, Dinajpur, Dhaka and Mymensingh districts of Bangladesh from 92 LSD-suspected cattle. From 85 collected serum samples, antibody was found ~61.99% positive for LSDV by indirect ELISA. To detect LSDV, DNA was extracted from the processed samples and conventional PCR was done using the primer designed for "G" gene. Virus were isolated successfully from PCR positive samples through the chorioallantoic membrane (CAM) route of chicken embryo and were further verified as LSDV by PCR. In this investigation, 26 out of 92 cattle were tested positive for LSDV. Only 29.17 percent of blood samples were tested positive for LSDV; however, 80% of skin samples tested positive, indicating that skin scraping could be a good specimen for PCR-based LSD diagnosis. Two more genes RPO30 and Ankyrin Repeat were used for the conformation. For molecular characterization 25 positive samples were partially sequenced and 19 sequence were submitted to NCBI. According to multiple sequence alignment and phylogenetic analysis Bangladeshi isolates shared a tight association with LSDV KSGP-0240 type Kenyan 1974 strains and LSD WD/JS 10-LT India 2019 strain. Hence it can be suspected that the circulating virus arrived in Bangladesh from India. Furthermore, all samples from the 5 research areas had almost identical sequences, indicating that a single strain is most likely circulating across the country. These findings emphasize the necessity of continuous monitoring the genetic makeup and molecular epidemiology of LSDV, which can be exploited to create future vaccines based on the strains that are now circulating currently.

Key Words: cattle, chicken embryo, PCR, sequence, multiple sequence alignment

P224 Genome-wide association study screens candidate genes for semen quality traits in selected Chinese and South African beef cattle bulls. M. Modiba*[1], K. Nephawe[1], J. Wang[2], C. Yuan[2], K. Mdladla[3], L. Wenfa[2], and B. Mtileni[1], [1]*Tshwane University of Technology, Department of Animal Sciences, Pretoria, Gauteng, South Africa,* [2]*Jilin Agricultural University, College of Animal Sciences and Technology, Changchun, Jilin, China,* [3]*Agricultural Research Council, Biotechnology Platform, Pretoria, Gauteng, South Africa.*

Selecting animals based on semen phenotypes may be challenging due low to moderate heritability of traits. Genome-wide association studies has confirmed the efficiency of single-nucleotide polymorphisms (SNPs) as genetic markers to identify genes that are associated with phenotypic traits in any breeding program. This study aims to identify genomic region and candidate genes associated with semen quality traits in selected Chinese and South African beef Cattle. A total of 144 semen samples were collected from South African Nguni (n = 28), Bonsmara (n = 21), Angus (n = 22) and Simmental (n = 25), and Chinese Belgian blue (n = 24) and Simmental (n = 24). Genomic DNA was extracted and genotyped using Bovine SNP 150K BeadChip. Plink v1.07 filtered data using: (1) remove SNPS with call frequency ≥90, (2) remove individuals with (MIND) ≤0.02, (3) SNPs with missingness (GENO) ≤0.02, (4) minor allele frequency (MAF) ≥0.02, (5) Hardy–Weinberg equilibrium (HWE >0.00001), related samples and samples below threshold were excluded. Association was conducted using FarmCPU model in R studio package (rMVP), databases NCBI identified gene IDs and gene ontology was used for functions of genes. After QC 110founders and 105 672 SNPs were remaining, 14 SNPs were significant with 9 candidate genes (*INHA*, *LOC784679*, *FREM1*, *DMRT1*, *DMRT2*, *DMRT3*, *CYP2C87*, *SLC16A12* and *LOC100139670*) associated to semen motility, 2 genes (*PAPPA* and *SPIN1*) associated to semen volume, one gene (*RASSF9*) associated to concentration, and 6 genes (*DOP1B*, *ACAD11*, *DNAJC13*, *PDE3A*, *GDF9* and *PDGFRB*) associated with progress motility. The current result shares genomic regions and candidate genes associated to semen traits in beef cattle bulls. Candidate genes in this study can be screened for causal mutations responsible for the genetic variance of semen traits and to be further studied. Furthermore, the study highlighted that for a successful association, sample size with sufficient statistical power is critical in detect causal genes.

Key Words: beef cattle, bulls, genome-wide association, candidate gene, FarmCPU

P225 ISAG Bursary Award: Low-coverage whole-genome genomic characterization of indigenous chicken ecotypes of Tigray, Ethiopia. G. G. Berhe*[1,2], G. B. Woldemichael[2], and M. Z. Kelkay[1], [1]*Tigray Agricultural Research Institute, Mekelle, Tigray, Ethiopia,* [2]*Addis Ababa University, College of Natural resource; Department of Microbial, Cellular and Molecular Biology, Addis Ababa, Addis Ababa, Ethiopia.*

The Tigray Region is the first entry route, around 400–800 BC, for the domestic chicken to Africa. Following its introduction, these chickens were exposed to the diverse agro-ecological conditions of the region with an altitude range from 600 to 4,000 m above sea level. We sampled 33 indigenous chickens from 3 agro-ecologies (lowland, midland, highland) to assess their genetic diversity. Low-pass whole-genome sequencing (0.5–1.0) was performed with imputation using a panel of African chicken genomes sequenced at high coverage

(Gencove). A total of 23 million SNPs unique to lowland (6.6), midland (8.4), and highland (6.9) were recorded. Population structures were assessed using principal component analysis (PCA) and admixture. PC1 and PC2 captured 13.91% of the genetic variation, with PC1 separating the chickens into 2 groups (lowland and highland/midland). The PC2 separated the highland and lowland ecotypes. ADMIXTURE analysis reveals 3 main ancestral genetic backgrounds, wild *Gallus*, Tigrayan highland chicken and Jarso chicken from Ethiopia. This may hint at the reality of the chicken introduction to Africa through the Tigray Region. We observed higher nucleotide diversity in midland chickens, followed by lowland and the highland ecotypes. The highland chicken displayed a higher genetic inbreeding coefficient of 0.18 compared with 0.04 and 0.06 for the midland and lowland chickens, respectively. The observed mean heterozygosities were between 0.24 to 0.29, with the higher value for the midland ecotypes. The results of this study may contribute to the conservation and utilization of locally adapted indigenous chicken in the Tigray Region (Ethiopia).

P226 ISAG Bursary Award: Population structure and admixture patterns in indigenous African cattle. M. K. Bitew*[1], G. Senczuk[1], M. Di Civita[1], C. Persichilli[1], S. Ben Jemaa[2], E. Ciani[3], J. M. Mwacharo[4,5], O. Hanotte[6,7], and F. Pilla[1], [1]*Department of Agriculture Environmental and Food Sciences, University of Molise, Campobasso, Italy,* [2]*Laboratoire des Productions Animales et Fourragères, Institut National de la Recherche Agronomique de Tunisie, Université de Carthage, Ariana, Tunisia,* [3]*Department of Biosciences, Biotechnologies & Environment, University of Bari Aldo Moro, Bari, Italy,* [4]*Small Ruminant Genomics, International Centre for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia,* [5]*Animal and Veterinary Sciences, Scotland's Rural College (SRUC) and Centre for Tropical Livestock Genetics and Health (CTLGH), Edinburgh, United Kingdom,* [6]*School of Life Sciences, University of Nottingham, Nottingham, United Kingdom,* [7]*LiveGene, International Livestock Research Institute, Addis Ababa, Ethiopia.*

With more than 150 autochthonous breeds, Africa is home to an important fraction of cattle genetic resources. However, many are at risk of extinction following uncontrolled crossbreeding and breed replacements with exotic breeds. In addition, due to their longstanding presence in the African continent together with the extreme heterogeneous environment, many cattle breeds have adapted to various local environments acquiring unique features. Herein, we examined the population structure, admixture patterns and selection signatures in 503 individuals from 29 indigenous cattle populations from 15 African countries using the Illumina bovine SNP50K BeadChip. After filtering for minor allele frequencies and missing genotypes, we obtained 19,405 SNPs. To explore genetic patterns, ADMIXTURE analysis, multidimensional scaling plot, and TreeMix were performed. To identify genomic regions putatively related to climate adaptation, we contrasted extended haplotype homozygosity profiles between cattle populations reared in different environments. The PCA and ADMIXTURE analyses grouped the study populations in to 3 groups: African indicine *Bos taurus indicus*, African taurine *Bos taurus taurus*, and their crossbreds. The 2 principal components differentiated the *B. t. indicus* from *B. t. taurus* while higher K values separated the crossbred animals. Several migration and gene exchange events are also highlighted by TreeMix. The strong phylogeographic structure observed in African cattle might be the outcome of a combination of adaptive processes driven by natural selection and historical admixture driven by human historical migratory dynamics. Using the R package rehh, we have searched footprint of selection in the African cattle breeds with known important functions of candidate genes related to environmental and biological roles. Differences in the length and distribution of run of homozygosity in the genome of the analyzed cattle breeds have been also observed.

Key Words: cattle and related species, animal breeding, bioinformatics tools, admixture, conservation

P228 Withdrawn

P229 Identification of recombination hotspots in selected South African indigenous beef cattle. N. A. Magagula*[1,2], A. A. Zwane[2], K. T. Ncube[3], and B. J. Mtileni[1], [1]*Tshwane University of Technology, Pretoria, Gauteng, South Africa,* [2]*Agricultural Research Council, Centurion, Gauteng, South Africa,* [3]*ZooOmics Indaba Biotechnical Industries, Pretoria, Gauteng, South Africa.*

Recombination is a fundamental process that facilitates variation among species. The frequency of recombination events that occur in hotspots tend to differ among individuals. The aim of this study was to identify recombination hotspots between 2 selected South African indigenous beef cattle, and determine the recombination rates among the breeds. A total of 200 samples from Bonsmara (n = 100) and Nguni (n = 100) were genotyped using the Illumina BovineSNP50 Beadchip. Haplotype phasing was done using FASTPHASE, using ARS-UCD1.3 SNP assembly coordinates. Recombination events were identified within half-sib families and both the number of recombination events and the recombination rate was calculated within each 0.5 Mb window of the genome. The 10% windows with the highest recombination rate on each chromosome were considered to be recombination hotspots. The preliminary results revealed a significant difference in estimated recombination rates between the 2 breeds, with Bonsmara having 25% to 10% higher recombination rates per meiosis than Nguni between the 2 breeds. 234 and 325 windows containing recombination hotspots were detected in Bonsmara and Nguni respectively. Recombination rate per 0.5-Mb window had a strong negative correlation with chromosome size and a strong positive correlation with GC content across the genome in both breeds. The results indicate that breed-specific features of detected recombination events and the control of recombination events is a complex polygenic trait even in South African indigenous beef cattle.

Key Words: indigenous cattle, DNA, haplotype, recombination, SNP

P230 ISAG Bursary Award: Autozygous regions, inbreeding, and effective population size in South African Afrikaner cattle. S. Lashmar* and E. van Marle-Köster, *University of Pretoria, Pretoria, Gauteng, South Africa.*

The Sanga cattle subspecies (*Bos taurus africanus*) have a genetic composition that is admixed between African and European taurine

as well as indicine in varying ancestral proportions. The South African (SA) Afrikaner breed is considered the oldest Sanga breed and has contributed to the development of many SA composite breeds (e.g., the Bonsmara). As an adaptive local genetic resource with declining population numbers, a genome-level quantification of within-breed inbreeding is necessary. In this study, inbreeding and autozygosity (i.e., genome-wide runs of homozygosity, ROH, patterns) as well as effective population size (Ne) was investigated. A total of 306 Afrikaner stud animals (169 females, and 137 males, originating from 32 breeders across SA and Namibia) were genotyped for a set of 126,936 post-quality control single nucleotide polymorphisms (SNPs). A total of 26,462 ROH (mean ± standard deviation ROH per animal = 86.48 ± 28.24) with a mean length of 3.95Mb and autosome-wise ROH coverage ranging from 0.069 (BTA2) to 0.249 (BTA12) were identified. The top 1% of SNPs occurring in ROH (threshold = 41.5% occurrence in population) were located on 4 autosomes (BTA5, 7, 12, and 13) in regions containing genes responsible for, among other biological processes, the *HSPH1* heat shock protein gene on BTA12, important for heat tolerance; and the *FBXL21* gene on BTA7 that plays a role in the mammalian circadian rhythm. The SNP-based inbreeding coefficient (F_{SNP}) indicated no inbreeding (mean ± standard deviation = −0.001 ± 0.106), however, the ROH-based coefficient was positive (F_{ROH} = 0.136 ± 0.060) with the majority of contributing ROH (0.709) being <4 Mb in size. The ROH length profile indicated that inbreeding was mostly due to ancient consanguinity. Despite this, the Ne has declined (Ne = 162 versus 527 for 12 compared with 120 generations ago) and is expected to be even smaller at present. The loss-of-diversity parameters estimated here suggest that the Afrikaner breed's diversity should be monitored for future sustainable and climate-smart breeding strategies.

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

P231 Withdrawn

P234 Structural variations and wild introgression in East Asian cattle genomes confer adaptation to local environments. X. Xia, F. Zhang, S. Li, X. Luo, L. Peng, W. Pang, C. Lei, and N. Chen*, *Northwest A&F Unversity, Yangling, Shaanxi, China.*

Structural variations (SVs) in individual genomes are major determinants of complex traits, including adaptability to environmental variables. The Mongolian and Hainan cattle breeds in East Asia are of taurine and indicine origins that have evolved to adapt to cold and hot environments, respectively. However, few studies have investigated SVs in East Asian cattle genomes and their role in environmental adaptation, and little is known about adaptive introgressed SVs in East Asian cattle to date. In this study, we examined the roles of SVs in the climate adaptation of these 2 cattle lineages by generating highly contiguous chromosome-scale genome assemblies with a scaffold N50 at 104 Mb. Comparison of the 2 assemblies along with 18 Mongolian and Hainan cattle genomes obtained by long-read sequencing data provided a catalog of 123,898 nonredundant SVs. Several long-read-based SVs overlapping with specific genes were associated with epidermal differentiation, skin barrier, and bovine tuberculosis resistance. Genotyping in 95 whole-genome sequences from 9 breeds by short-read sequencing identified 5,563 SVs that were differentiated along a north–south gradient, which overlap with genes that are enriched in pathways related to environmental adaptation. We identified 5,046 Chinese indicine-stratified SVs that possibly originated from banteng. Our findings highlighted the unique contributions of SVs in East Asian cattle to environmental adaptation and disease resistance.

Key Words: structural variation, genome assembly, long-read sequencing, East Asian cattle

P235 Recent selection and adaptive introgression facilitated adaptation to high altitude in QTP cattle. Y. Lyu, X. Xia, F. Wang, N. Chen*, and C. Lei, *Northwest A&F University, Yangling, Shaanxi, China.*

Cattle on the Qinghai-Tibet Plateau (QTP) develop a unique phenotype and production performance under hypoxia, UV radiation, and cold conditions. However, the genetic mechanisms underlying the survival of QTP cattle warrant further investigation. Here, we analyzed 258 cattle genomes, including 84 QTL cattle representing 5 populations living at continuous altitudes from 3,400 to 4,300 m, revealing that QTP cattle had a phylogeographic trend with taurine and indicine ancestries. We found recently selected genes of QTP cattle related to body size and cold adaptation. We also identified signals of sympatric introgression from yak into QTP cattle at different altitudes, covering 0.35% to 1.86% of their genomes, including several adaptative yak introgressed genes involved in the hypoxia response and UV radiation. Our results revealed that recently selected and adaptative yak introgressed genes contribute to the adaptation of QTP cattle to high-altitude environments.

Key Words: re-sequencing, population genomics, recent selection, introgression

P237 Molecular and serological prevalence of corridor disease (buffalo-associated *Theileria parva*) in cattle populations at the livestock/game interface of KwaZulu-Natal province, South Africa. S. Mbizeni*[1,2], B. J. Mans[1,3], and A. A. Latif[2], [1]*University of South Africa, Johannesburg, Gauteng, South Africa,* [2]*University of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa,* [3]*Agricultural Research Council, Pretoria, Gauteng, South Africa.*

Theileria parva are obligate intracellular protozoal parasites responsible for 3 disease syndromes in cattle, known as East Coast fever (ECF), corridor disease (CD), and Zimbabwean theileriosis. The parasites are mainly transmitted by *Rhipicephalus appendiculatus* and *Rhipicephalus zambeziensis*. Although ECF has been eradicated and Zimbabwean theilerioses has never been reported in South Africa, CD outbreaks still occur in cattle and game interface in endemic areas. The increase in the reports of CD outbreaks in recent years raised questions about the probability of adaptation of buffalo-derived cattle *T. parva*

strains in cattle herds adjacent to game reserves. A cross-sectional study was conducted in cattle herds in the CD controlled area of KwaZulu-Natal province. Blood samples were collected from 1,137 cattle from 14 herds and analyzed by quantitative real-time PCR (qPCR) and indirect fluorescent antibody test (IFAT) to establish *T. parva* prevalence. A total of 484 samples from 4 of the 14 herds were further tested on qPCR for the presence of *Theileria taurotragi* infections. The data were analyzed using descriptive statistics and a chi-squared test was used to assess for association between variables. The overall prevalence of *T. parva* was 1.3% (95% CI: 1–2%) and 19.9% (95% CI: 17–22%) on qPCR and IFAT, respectively. The qPCR positive samples were only detected in late summer and autumn, and IFAT positive samples were detected in all seasons sampled, with higher numbers during summer months. The Pearson chi-squared test showed that the *T. parva* prevalence rates on both qPCR and IFAT were positively associated with herds with a previous history of CD outbreaks ($\chi^2 = 8.594$, $P = 0.003$; 69.513, $P < 0.001$, respectively). The overall prevalence of *T. taurotragi* was 39.4% (95% CI: 35–44%) with the herd-level prevalence range between 35.0% and 43.4%. Possible cross-reaction to *T. parva* IFAT was detected on a few samples that were positive for *T. taurotragi* infections, but there was no positive association between *T. taurotragi* infections and IFAT positivity ($\chi^2 = 0.829$, $P = 0.363$). This study assisted to demonstrate the extent of occurrence of subclinical carriers and the level of exposure to *T. parva* infections in cattle populations at the livestock/game interface of KwaZulu-Natal province. The molecular and seroprevalence rates were low when compared with other areas where cattle-adapted *T. parva i*nfections are endemic. The adaptation of buffalo-derived *T. parva* in cattle population resulting in cattle-cattle transmissions seem to be unlikely under the current epidemiological state.

Key Words: *Theileria parva*, *Theileria taurotragi*, corridor disease, prevalence, livestock-game interface

P238 ISAG Bursary Award: The development of a 61K Illumina SNP chip for dromedaries under the frame of the 2019 Agricultural Greater Good (AGG) initiative. M. Di Civita[1], G. Senczuk[1], S. Bruno[2], V. Landi[3], S. Brooks[4], F. Almathen[5,6], B. Faye[7], S. B. S. Gaouar[8], M. Piro[9], K. S. Kim[10], H. Dadi[11], P. C. Iglesias[12], H. Al-Haddad[13], M. Al-Abri[14], F. Pilla*[1], X. David[15], A. Eggen[15], P. Burger[16], and E. Ciani[2], [1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy,* [2]*Department of Biosciences, Biotechnologies and Environment, University of Bari "Aldo Moro," Bari, Italy,* [3]*Department of Veterinary Medicine, University of Bari "Aldo Moro," Valenzano, Bari, Italy,* [4]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [5]*Department of Public Health, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia,* [6]*Camel Research Center, King Faisal University, Al-Ahsa, Saudi Arabia,* [7]*CIRAD-ES, UMR SELMET, Montpellier, France,* [8]*Department of Biology, Abou Bakr Belkaid University of Tlemcen, Tlemcen, Algeria,* [9]*Department of Medicine, Surgery and Reproduction, Institut Agronomique et Vétérinaire Hassan II, Rabat BP, Morocco,* [10]*Department of Animal Sciences, Chungbuk National University, Chungbuk, Korea,* [11]*Ethiopian Biotechnology Institute (EBTi), Addis Ababa, Etiopia,* [12]*Department of Genetics, Faculty of Veterinary Sciences, University of Córdoba, Córdoba, Spain,* [13]*Department of Biological Sciences, Kuwait University, Kuwait City, Kuwait,* [14]*Department of Animal and Veterinary Sciences, Sultan Qaboos University, Muscat, Oman,* [15]*Illumina, Agrigenomics, Evry, France,* [16]*Research Institute of Wildlife Ecology, Vetmeduni, Vienna, Austria.*

Dromedary camels represent one of the most important domestic animals in terms of meat, milk, leather and transportation in many low-income countries, also contributing to many social and cultural aspects. In addition, dromedaries are so fascinating also from a biological point of view, being among the few big mammals to have evolved specific adaptations to extreme environmental conditions. Under the frame of the 2019 Illumina® Agricultural Greater Good (AGG) initiative program, which is aimed at supporting studies on sustainability, productivity, and nutritional density of agriculturally important crop and livestock species, a total of 179 dromedaries from the entire geographic distribution range were whole-genome sequenced (WGS). Raw reads were mapped against the dromedary reference genome (CamDro3) and the variants were called using the Illumina® Dragen germline platform. From a total of 13,560,911 biallelic SNPs, after a multistep filtering approach aimed at ensuring SNPs evenness across chromosomes and removing SNPs with less than 0.05 minor allele frequency and 0.1 missing call rate, a subset of 61,208 SNPs was selected. The panel included 59,069 autosomic SNPs with an average distance of 32 kb, 1,230 SNPs on X chromosome and 77 mitochondrial SNPs. In addition, about 1,000 loci from 47 genes with known functional relevance were enriched. The linkage-disequilibrium (LD) decay graph indicated that at r^2 value ranging from 0.3 to 0.5 we found pairs of loci separated 50 kb apart. This value resulted higher than that reported in other cattle commercial breeds and even higher than that observed in sheep breeds. This result confirms that the selection of SNPs with an average distance of 32 kb will perform well in linkage disequilibrium mapping approaches such as in looking for selection signatures or in genome-wide association studies. The panel is currently being validated and we are confident that will represent a further relevant step toward the understanding of dromedary genomics.

Key Words: Old World camelids, genome sequencing, genotyping, single-nucleotide polymorphism (SNP)

P239 ISAG Bursary Award: Genome-wide scan for selection signatures in South African indigenous goat ecotypes. A. M. Magoro*[1,2], A. Zwane[2], K. Hadebe[3], and B. Mtileni[2], [1]*Tshwane University of Technology, Pretoria, South Africa,* [2]*Agricultural Research Council-Animal Production, Pretoria, South Africa,* [3]*Agricultural Research Council-Biotechnology Platform, Pretoria, South Africa.*

Whole-genome scan of signatures of selection contributes to the identification of genomic regions that are functionally important for agricultural production. Indigenous goats have adapted to different environments for survival, breeding and production. Their genomes are likely to have acquired unique alleles for various traits of economic importance. The objective of this study was to investigate signatures of selection in indigenous goat ecotypes from 4 provinces of South Africa. Non-descript goat ecotypes representing populations from the Free State (n = 18), Gauteng (n = 24), Limpopo (n = 28), North West (n = 30) provinces and a conservation flock from the Agricultural Research Council (ARC)-Animal Production (n = 25) were genotyped using Illumina Goat SNP65K BeadChip. Quality control was performed using PLINK v1.9. A total of 43,726 autosomal SNPs remained for downstream analyses. Pairwise F_{ST} of the populations was observed to be lower in non-descript populations (FS, GP, LP and NW) and higher in the ARC population. The R package rehh was used to detect selection signatures implementing 2 complementary approaches: integrated haplotype score (iHS) and cross-population-extended haplotype homozygosity (XP-EHH). The most significant regions were identified at a threshold of -log (iHS) >4 ($P < 0.0001$). A total of 161 top selection signature regions were identified across all the populations with some genes relating to adaptation (*PRKCB*), reproduction process (*BMPR2*, *HOOK1*, *ROR2*), feed efficiency (*DACH1*), response to stress (*HSMCE1*, *ALS2*, *GTF2I*, *ROR2*), and skeletal system development (*ROR2*, *BMPR2*, *HOOK1*) based on the iHS analysis. The XP-EHH analysis identified 215 regions, with several genes relating to cell development (*ABCA12*, *Figure 4*, *CASP8*, *IL4R*) and response to growth factors (*BMPER*, *VWC2L*). The genes identified in this study are highly significant to the biological function and traits of economic importance in goats. The results indicate that various selection pressures have influenced the genome of indigenous goats in South Africa and provide new knowledge important for breeding and conservation of these indigenous goat ecotypes.

Key Words: adaptation, genomic regions, non-descript goat

P240 Withdrawn

P241 Virginia Tech research education programs: Models for increasing STEM participation in middle- and low-income countries. E. Smith*, *Virginia Tech, Blacksburg, VA.*

Middle- and low-income countries have the same challenges faced by the United States of America and other high-income countries: engaging, recruiting, and training students from low-income backgrounds in STEM and related fields. We describe training programs and their statistics, and suggest they can be used to train the next generation of aspiring animal geneticists from developing countries. Our training programs are based on the hypothesis that a cohort approach to graduate education have better outcomes than the professor-driven tradition of the land grant system. A total of 227 trainees have participated in these research education programs. The undergrad institutions of origin have been national and the US territories and Puerto Rico. The training plan has included innovative approaches such as an ombudsperson for graduate education, retrospectives by scientists as "scientific journeys" on YouTube, peer and near-peer mentoring, and a committed engagement with alumni for their lived experiences and to continue impacting their careers. Additional required courses include Effective Grant Writing and Scientific Writing. The courses help us to not only provide additional training in "soft skills," but to stay in regular contact with trainees in the early months of the PhD. Together, these efforts have resulted in 91 PhDs, 3 MDs, 30 MS, and 1 DPharm. Trainees are pursuing diverse careers including 13 in tenure-track academic positions, one of whom was recently tenured. Our recent rate continues to be above 75%. Beyond research skills, our trainees acquire networking, mentoring, and communication (written and oral) skills that have contributed to their successes. Our creativity as scientists, I will argue, has made these successes more likely in a department not generally considered biomedical or behavioral. Our future efforts with these research training programs will include the use of "lived experiences" by our distinguished alumni as a training tool.

Key Words: research education, STEM, developing countries, average students

P242 ISAG Bursary Award: Population genomics of indigenous African cattle inferred from 537 whole-genome sequencing. A. Tijjani[1,2], S. Kambal*[3,4], K. Marshall[5], O. Hanotte[1,3,6], and on behalf of the African Cattle Genomics Consortium[1], [1]*Centre for Livestock Genetics and Health (CTLGH), International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [2]*The Jackson Laboratory, Bar Harbor, ME,* [3]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [4]*University of Khartoum, Khartoum, Sudan,* [5]*International Livestock Research Institute (ILRI), Nairobi, Kenya,* [6]*School of Life Sciences, University of Nottingham, University Park Campus, Nottingham, UK.*

The escalating climate change crisis challenges cattle well-being and sustainable production across Africa. However, the ability of indigenous cattle to thrive in stressful conditions (e.g., high temperature, high altitude, high infectious disease prevalence), as well as limited feed and water availability, provide opportunities to mitigate the consequence of climate change. Here, a large data set of cattle populations was collated for genome-wide SNP discovery and population genomic analysis. We analyzed 537 whole-genome sequences representing 41 indigenous breeds from 12 sub-Saharan African countries, comprising newly generated and publicly available data. A total of ~33 million SNPs were discovered across all the cattle populations, with ~3.5 million of these being novels. Predicted non-synonymous mutations account for around 31% of annotated SNPs in coding genes. Furthermore, we found 31,677 SNPs potentially causing loss or gain of function compared with the European taurine cattle genome of reference (Hereford, ARS-UCD1.2), including 1,478 stop codon gain, 175 stop codon loss, and 258 start codon loss mutations. The population structure and admixture analyses separate non-admixed African taurine *Bos taurus taurus* from African indicine × taurine crosses. We observe a high genetic differentiation between longhorn and shorthorn taurine with a higher proportion of shared Muturu ancestry (shorthorn), compared with N'Dama (longhorn), among African zebu, sanga, and zenga crossbreds. However, these proportions decrease from West to East. Our finding yields a comprehensive insight into African cattle population structure and variation profile, emphasizing the need for fine characterization to inform sustainable genetic improvement toward healthy and well-adapted cattle populations.

Key Words: African cattle, adaptation, single-nucleotide polymorphism (SNP), population genomics

P243 Structural variant calling using ONT long-read whole-genome sequencing of indigenous Zulu sheep. N. Nxumalo*[1], A. Molotsi[1], C. Rhode[1], and N. Kunene[2], [1]*Stellenbosch University, Stellenbosch, Matieland, South Africa,* [2]*University of Zululand, Empangeni, Kwadlangezwa, South Africa.*

Livestock genetic variation has been based on genetic markers such as single nucleotide polymorphisms, indels (<50 bp) and short tandem repeats. Even though structural variants (SV) have been reported as a significant constituent underpinning livestock phenotypic variation, their exploration has been minimal. Structural variant calling requires long-read sequencing reads that covers the whole length of the variant. The aim of this study was to explore Zulu sheep breed genetic divergence through SV calling using long-read sequencing. Blood samples were collected from 2 purebred Zulu sheep kept at the University of Zululand; high molecular weight DNA was extracted and used for whole genome sequencing. Sequencing was done using Oxford Nanopore technology at the Central Analytical Facility at Stellenbosch University. After raw reads quality control, competent reads were mapped to ARS-UI_Ramb_v2.0 sheep reference genome (96.08 mapping percentage) using minimap2/2.1. Structural variants were called using sniffles/2.0.7 and included duplication (12), inversions (33), breakend (166), deletions (9,541) and insertions (15,986). Variant Effect Predictor (VEP) was then used to predict their possible genomic effect. There were 62,014 identified SVs that overlapped genes and 19,749 transcripts. Some identified SVs may significantly affect Zulu sheep breed phenotypic uniqueness based on their functional genomic location and may underpin consequences on intergenic variants, intron variants (HDAC4, SCLY, UBE2F, MLPH, AGAP1), and feature elongation (ZMYM4, AGO3, SH3D21, MANEAL, UTP11), thus altering gene regulation mechanisms, splicing site recognition, and extended transcriptions and translations. Further investigation on the identified SVs using genome-wide association studies may assist to understand genomic bases of Zulu sheep phenotypic unique characteristics.

Key Words: genetic heterogeneity, structural variation, whole-genome sequencing, Zulu sheep

P244 ISAG Bursary Award: Whole-genome sequencing of Landim pigs of Mozambique reveals a close relationship with Angolan native pigs and suggests selection for immune response. F. Teixeira*[1,2], P. Sá[1], D. Santos[1], C. Garrine[3], R. Zimba[4], L. Souza[3], H. Chiaia[2], A. Leitão[1], J. M. Cordeiro[2], L. T. Gama[1], and A. J. Amaral[1,5], [1]*Centre for Interdisciplinary Research in Animal Health and Associate Laboratory for Animal and Veterinary Sciences, Faculty of Veterinary Medicine, University of Lisbon, Alto da Ajuda, Lisbon, Portugal,* [2]*Faculty of Veterinary Medicine, University José Eduardo dos Santos, Huambo, Angola,* [3]*Faculty of Veterinary Medicine, University Eduardo Mondlane, Maputo, Mozambique,* [4]*Escola Superior de Desenvolvimento Rural de Vilankulo, University Eduardo Mondlane, Maputo, Mozambique,* [5]*Escola de Ciências e Tecnologia Universidade de Évora, Évora, Portugal.*

Landim pigs, a population of native pigs from Mozambique, are currently threatened by the recent introduction of European exotic breeds. Like most African pig populations, Landim pigs have never been characterized at the genome level and are well adapted to harsh conditions which include adaptation to endemic diseases. In this study, we provide a comprehensive genetic characterization of Landim pigs using whole-genome sequencing (WGS). We generated genomes from Landim pigs (n = 6) and compared these genomes to local pigs from Angola and European and Asian domestic pigs and wild boars currently in the public domain (n = 78). Analyses of population structure showed that Angolan local and Landim pigs are closely related, and both are more closely related to European than Asian breeds. Preliminary results suggest that Landim pigs display a duplication in Chr4 that has been reported only in Chinese domestic pigs, overlapping the *TBX19* gene that is associated with development and growth. The functional analysis of missense SNPs in Landim pigs shows that these occur in genes related to immune system response. The integrated haplotype score (iHS) analysis revealed candidate regions under selection overlapping genes also related to immune response. This study represents the first assessment of the genetic background of native pigs from Mozambique and opens the path to understanding the dynamic and interlinked history of African pig populations. The present study further reports unique genetic attributes related to an immune response that should be further explored.

Key Words: pigs and related species, genome sequencing, population structure, adaptation, conservation

P245 Genetic differentiation of *Camelus bactrianus* from Kazakhstan. K. Dossybayev*[1,2], D. Ualiyeva[1], M. Amandykova[1,2], T. Kapasuly[1,2], A. Mussayeva[1], Z. Orazymbetova[1], G. Shaltenbay[1,2], and B. Bekmanov[1,2], [1]*Laboratory of Genetics and Cytogenetics, Institute of Genetics and Physiology, CS MSHE RK, Almaty, Kazakhstan,* [2]*Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan.*

The Bactrian camel represents an Old World camel that is well adapted to the cold and dry deserts of Middle and Central Asia. It is used to be the main source of food and logistics for the nomadic tribes of people since ancient times. Nowadays camels are bred worldwide for meat and dairy products. Recently, in Kazakhstan camel farming has been growing rapidly, particularly, in 2022 there were registered around 272,000 camels. The successful development of animal husbandry mainly depended on the genetic characteristics of farm breeds. Mitochondrial DNA is an optimal molecular marker which, due to the high mutation rate, allows for tracing the evolutionary history of matrilines as well as determining the speciation process. Nowadays, the genetic differentiation of local camels is poorly understood. Thus, to investigate the evolutionary relationships of domesticated Bactrian camels from Kazakhstan with extant populations spread worldwide, we determined the sequences of mitochondrial D-loop region from 13 camels of the Almaty population. Totally, the analysis involved 50 samples including the sequences from GenBank. The targeted mitochondrial region consisted of a total length of 321 bp, which was analyzed by the Sanger method. The phylogenetic analysis recovered 2 main clusters representing the basal position of the monophyletic clade of Kazakhstani Bactrian camels with Arabian Dromedary camels, and a polyphyletic clade of *Camelus ferus* and *Camelus bactrianus* from Eastern Central Asia (China, Mongolia). These results were supported by the haplotype network analysis as well with detection of 3 haplogroups. The obtained results suggest the possible past admixture and origin of a common ancestral form of the Central Asian population from the Arabian Peninsula. The current research may play a crucial role in the future investigations of the evolutionary history of the species. This research was funded by grant no. AP14870678 of the Ministry of Sciences and Higher Education of the Republic of Kazakhstan.

Key Words: *Camelus bactrianus*, Kazakhstan, mtDNA, phylogeny

P246 Whole-genome diversity of dromedary camels from the entire geographic distribution range. G. Senczuk*[1], S. Bruno[2], M. Di Civita[1], V. Landi[3], S. Brooks[4], F. Almathen[5,6], B. Faye[7], S. B. S. Gaouar[8], M. Piro[9], K. S. Kim[10], H. Dadi[11], C. Iglesias Pastrana[12], H. Al-Haddad[13], M. Al-Abri[14], C. Persichilli[1], F. Pilla[1], P. Burger[15], and E. Ciani[2], [1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy,* [2]*Department of Biosciences, Biotechnologies and Environment, University of Bari "Aldo Moro," Bari, Italy,* [3]*Department of Veterinary Medicine, University of Bari "Aldo Moro," Bari, Italy,* [4]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [5]*Department of Public Health, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia,* [6]*Camel Research Center, King Faisal University, Al-Ahsa, Saudi Arabia,* [7]*CIRAD-ES, UMR SELMET, Montpellier, France,* [8]*Department of Biology, Abou Bakr Belkaid University of Tlemcen, Tlemcen, Algeria,* [9]*Department of Medicine, Surgery and Reproduction, Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco,* [10]*Department of Animal Sciences, Chungbuk National University, Chungbuk, Korea,* [11]*Ethiopian Biotechnology Institute (EBTi), Addis Ababa, Ethiopia,* [12]*Department of Genetics, Faculty of Veterinary Sciences, University of Córdoba, Córdoba, Spain,* [13]*Department of Biological Sciences, Kuwait University, Kuwait City, Kuwait,* [14]*Department of Animal and Veterinary Sciences, Sultan Qaboos University, Muscat, Oman,* [15]*Research Institute of Wildlife Ecology, Vetmeduni, Vienna, Austria.*

During human history, dromedaries have played a central role in many essential aspects including commercial transportation and for human sustenance in terms of meat, milk and leather. Still now, dromedaries represent an essential social and cultural element in many Arabian and Asiatic countries. This has been possible mainly due to the dromedary's strong adaptation to extreme environmental condition in marginal agro-natural zones. Under the frame of the 2019 Illumina® Agricultural Greater Good initiative, the whole-genome sequencing of 336 dromedaries from the entire geographic distribution range has been carried out. Another 22 WGS samples were included from public databases. All samples were mapped against the reference genome (CamDro3), and a variant calling analysis was performed using the Illumina® Dragen germline platform. A total of 505,662 SNPs were retained after filtering for minor allele frequency and missing call rate. The overall variability was explored by using genetic diversity indices (H_o, pairwise F_{ST} and F_{ROH}), while the population genetic relationships were assessed by the use phylogeographic inference tools (PCA, ADMIXTURE and Neighbor-net). The overall H_o values were higher than those observed for African cattle and sheep breeds, while all F_{ROH} values were positive with the highest values observed in Australian and Kenyan populations. Both PCA and ADMIXTURE analyses revealed a weak genetic structure; however, a phylogeographical pattern was observed. At the best K value (K = 5), a first separation involved samples from Ethiopia and Kenya following a subsequent split between African and Asian populations. A genomic cline from East to West was also evident, suggesting a possible dominant role of ancient caravan routes along the Sahara Desert in molding the observed genetic pattern. The topology inferred by the Neighbor-net graph basically confirms the genetic repartition into 5 main groups. Interestingly, the proportion of ancestral components to-

gether with the Neighbor-net basal position, would seem to confirm the Southern Arabian Peninsula as an important cradle of domestication.

Key Words: Old World camelids, evolutionary genomics, population genomics, biodiversity

P247 ISAG Bursary Award: Differential proteomics revealed the impact of heat stress on milk whey proteins in indigenous Deoni (*Bos indicus*) and Holstein Friesian (*Bos taurus*) crossbred cows. E. Rana*[1,2], K. P. Ramesha[1], N. Azharuddin[1], M. A. Najar[3], M. K. Sinha[1], S. Jeyakumar[1], L. Gopalakrishnan[3,4], P. Nag[1], S. Mall[1], M. Ashokan[1], M. Dasgupta[1], A. Kumaresan[1], D. N. Das[1], and T. S. K. Prasad[3], [1]*Southern Regional Station, ICAR—National Dairy Research Institute, Bangalore, India,* [2]*Livestock Development Department, Government of Chhattisgarh, Chhattisgarh, India,* [3]*Center for Systems Biology and Molecular Medicine, Yenepoya Research Centre, Yenepoya (Deemed to be University), Mangalore, India,* [4]*Institute of Bioinformatics, International Technology Park, Bangalore, India.*

Heat stress is a significant financial threat to animal husbandry. However, indigenous (*Bos indicus*) cattle can survive and perform better under heat stress conditions as compared with exotic (*Bos taurus*) breeds or their crossbreds. Still, the mechanisms at a molecular level associated with heat tolerance among these cattle are ill-understood to date. Proteins are the phenotypic product of genes that explain the effect of direct environment in a better way. In the present study, high-throughput milk whey proteomics was performed to identify the subtle changes occurring at protein level compared between normal (temperature humidity index, THI = 66.6) and heat stress (THI = 82.2) conditions in Deoni and Holstein Friesian crossbred cows. A total of 412 proteins were identified in milk whey samples by LC-MS/MS technique coupled with bioinformatics analysis. Differential milk whey proteomics revealed that 27 and 53 proteins were upregulated (>1.5-fold), whereas 10 and 8 proteins were downregulated (<0.6-fold) during heat stress as compared with normal condition in indigenous and crossbred cows, respectively. It was observed that the upregulated proteins were mainly related to defense response, metabolic process and response to external stimuli. The Gene Ontology analysis showed 38.09 and 48.15% of the enriched biological processes were related to the defense mechanism in indigenous and crossbred cows, respectively. The study was validated by ELISA method which revealed that the expression of haptoglobin, an acute phase protein, was highly significant during heat stress conditions, thus, could act as a potential biomarker associated with thermo-tolerance of the animal. Collectively, it is concluded that a definite difference exists in the milk whey protein profile of dairy cows and the information on their relative abundance during heat stress conditions would act as an aid to select and propagate thermo-tolerant dairy cows. This study would serve as a foundation for further proteomics studies on milk.

Key Words: cattle and related species, proteomics, mass spectrometry, gene ontology, biomarker

P248 Tracking the adaptive history of African cattle using low-coverage genomes. S. I. Ng'ang'a*[1,2], J. A. Ward[3], G. V. Smith[4], S. Rossiter[2], C. Faulkes[2], D. G. Bradley[5], O. Hanotte[6,7], D. E. MacHugh[8], and L. A. F. Frantz[1,2], [1]*Palaeogenomics Group, Department of Veterinary Sciences, Ludwig Maximilian University, Munich, Germany,* [2]*School of Biological and Chemical Sciences, Queen Mary University of London, London, United Kingdom,* [3]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Dublin, Ireland,* [4]*SilverStreet Capital, London, United Kingdom,* [5]*Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland,* [6]*International Livestock Research Institute, Addis Ababa, Ethiopia,* [7]*School of Life Sciences, University of Nottingham, Nottingham, United Kingdom,* [8]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland.*

Africa is home to more than 150 distinct breeds of domestic cattle, with rich phenotypic diversity reflecting adaptation to a wide range of agro-ecological conditions. The genetic basis of cattle adaptations to the myriad of African ecosystems, however, is poorly understood, and many breeds are endangered, due to uncontrolled crossbreeding and replacement by non-native breeds. Low-coverage sequencing (LCS) can improve our knowledge of genomic diversity in African cattle and pave the way for cost-effective genome-enabled breeding programs. The LCS approach requires high-coverage genome sequence data to generate a reference panel that is then used to impute low-coverage genomes. Importantly, for accurate imputation, the LCS reference panel should include sufficient genomic representation of the cattle breeds that are to be imputed to a whole-genome scale. Previous studies have shown that this is difficult to achieve in African cattle due to a lack of existing genomic information. To address this issue, we developed an imputation pipeline based on a large reference panel combining 150 newly generated high-coverage genomes from multiple African cattle populations, together with publicly available genome data, which comprises over 3,200 cattle genomes from 133 cattle populations. Through imputation of down-sampled high-coverage genomes from various cattle populations, we show that our imputation pipeline provides genotype imputation accuracies >99% for common variants (minor allele frequency [MAF] ≥5%; compared with 75% in previous studies) and between 92% and 98% for rare variants (0.5% ≤ MAF < 1%; compared with 30% in previous studies) in 0.5× coverage of African cattle genomes. The accuracy of our LCS imputation pipeline, however, was lower for Asian cattle of primarily *Bos indicus* ancestry, reflecting the need to sequence more cattle from Asian countries. We then used this reference panel to impute >1,000 low-coverage African cattle genomes. Population genomic analyses from this data set will provide a new perspective on how natural selection and human-mediated breeding has shaped the genetic composition of African cattle.

Key Words: cattle and related species, genome-enabled breeding, imputation, adaptation

P249 Poultry genomics within the Centre for Tropical Livestock Genetics and Health. J. Smith*[1], A. Gheyas[1], A. Trujillo[1,2], A. Kebede[3], G. Gebru[4,5], N. Seboka[5,6], M. Rachman[2], T. Dessie[7], and O. Hanotte[2,7], [1]*Centre for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, University of Edinburgh, Edinburgh, UK,* [2]*University of Nottingham, Nottingham, UK,* [3]*Amhara Regional Agricultural Research Institute, Bahir Dar, Ethiopia,* [4]*Tigray Agricultural Research Institute, Mekelle, Tigray, Ethiopia,* [5]*Addis Ababa University, Addis Ababa, Ethiopia,* [6]*Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia,* [7]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia.*

The Centre for Tropical Livestock Genetics and Health (CTLGH; https://www.ctlgh.org/) is a collaboration between the Roslin Institute (RI; UK), Scotland's Rural College (SRUC; UK) and the International Livestock Research Institute (ILRI; Kenya/Ethiopia). The ethos behind the center is tackling global challenges through genetic improvements in tropical livestock. Working as part of CTLGH we use genomics to identify ways to improve productivity in indigenous chickens. Our main interest is in understanding the tolerance of these birds, which are resilient to environmental stresses such as temperature, altitude and food/water scarcity. Our main aim is to understand the genes controlling these resilience traits so that favorable characteristics can be used in breeding programs to produce birds which are not only environmentally adaptable, but are also healthier and more productive. This would benefit livelihoods of both smallholder farmers and larger farming enterprises across low-to-middle income countries (LMICs). As our global climate continues to change it is important that we are able to produce livestock that can withstand that change. We have used whole-genome sequencing of hundreds of birds from different areas across Ethiopia and Nigeria to identify candidate genes for environmental adaptation (e.g., heat stress). Ecological niche modeling has also allowed for definition of ecotypes and creation of environmental suitability maps. Investigation of the MHC in these birds has also identified many novel haplotypes, with implication for disease resistance. As we now enter a new phase of research within CTLGH, we look to investigate new populations of birds across different African countries and use differ-

ent genomic approaches (transcriptomics, epigenetics) to gain deeper insight into our candidate genes and regions. Our data are also included in efforts by the Chicken Diversity Consortium, bringing together sequence data from thousands of chickens representing ancient, commercial, research, exotic, and indigenous breeds from around the world, to enable pan-genomic analysis of the chicken genome.

Key Words: chicken, genetics, environmental adaptation, genomics

Microbiomes

P250 Withdrawn

P251 High-throughput metagenomic characterization of the fecal microbiota of peste des petits ruminants–infected West African Dwarf goats. I. Muritala*[1], B. O. Sodimu[1], M. N. Bemji[1], M. A. Busari[1], G. F. Farayola[1], S. Saleem[2], N. Kumari[3], S. Jaiswal[3], M. A. Iquebal[3], S. M. Ahmad[2], A. O. Sonibare[4], M. Wheto[1], and E. M. Ibeagha-Awemu[5], [1]*Department of Animal Breeding and Genetics, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria,* [2]*Division of Animal Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, Jammu and Kashmir, India,* [3]*Division of Agricultural Bioinformatics, ICAR—Indian Agricultural Statistics Research Institute, New Delhi, India,* [4]*Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria,* [5]*Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada.*

The gut microbiota (GM) is known to play vital roles in digestion, immunity and health maintenance in livestock and human. Microbiota dysbiosis has been associated with several human and livestock diseases, but its role in the pathogenesis of peste des petits ruminants (PPR) disease in goat is not known. Thus, this study characterized the microbiota of feces of PPR infected (PPR-inf; n = 19) and non-infected (CTL; n = 14) West African Dwarf (WAD) goats. Next-generation amplicon sequencing of the 16S rRNA gene in fecal microbial DNA from PPR-inf and CTL goats was accomplished with Illumina MiSeq system. Bioinformatics analysis of generated microbial sequences was accomplished with QIIME 2 and other standard tools. Microbiota diversity was significantly ($P < 0.05$) higher in CTL goats with predominant bacterial genera such as *Ruminococcaceae* UCG-010 and UCG-005, *Akkermansia*, *Prevotella*, *Fusobacterium*, etc., compared with PPR-inf group. Meanwhile *Campylobacter*, *Treponema*, *Moraxella*, *Bacteroidetes* and *Bacillus* were more abundant ($P < 0.05$) in PPR-inf goats compared with CTL goats. *Campylobacter* and *Moraxella* have been implicated in campylobacteriosis and lower respiratory tract infections, respectively, in human and livestock. Functional prediction indicated that genes associated with transport system, substrate-binding, and multiple antibiotic resistance were prominent in PPR-inf goats, while genes associated with general secretion pathway and GntR family transcriptional regulators were prominent in PPR-inf and CTL groups ($P < 0.05$). In conclusion, there were marked differences in the bacterial composition between PPR-inf and CTL goats. Our data suggest that microbiota alterations or dysbiosis could be responsible for diarrheic symptoms observed in PPR disease, and thus implicate the microbiota in PPR pathogenesis.

Key Words: fecal microbiota, microbial richness, goat, *Campylobacter*, *Treponema*, *Moraxella*

P252 ISAG Bursary Award: Nasal microbiome diversity in West African Dwarf goats with peste des petits ruminants viral infection. I. Muritala*[1], M. N. Bemji[1], M. A. Busari[1], B. O. Sodimu[1], S. M. Ahmad[2], A. Negi[3], S. Jaiswal[3], M. A. Iquebal[3], B. Bhat[2], M. O. Ozoje[1], O. L. Ajayi[4], and E. M. Ibeagha-Awemu[5], [1]*Department of Animal Breeding and Genetics, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria,* [2]*Division of Animal Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, Jammu and Kashmir, India,* [3]*Division of Agricultural Bioinformatics, ICAR—Indian Agricultural Statistics Research Institute, New Delhi, India,* [4]*Department of Pathology, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria,* [5]*Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada.*

Peste des petits ruminants (PPR), regarded as an economically important disease of small ruminants (especially goats), is a highly contagious disease characterized by oculo-nasal discharge and diarrhea, among others. Diarrheic episodes indicate microbiota changes during PPR, and even though the microbiota has been implicated in the etiology of many infectious diseases, little is known about its role in PPR in goats. Thus, this study characterized the nasal microbiome in PPR-infected West African Dwarf (WAD) goats using 16S rRNA metagenomics sequencing. A total of 33, 8- to 12-mo-old WAD goats, consisting of 19 PPR-infected (PPRG) and 14 non-infected (CTLG) goats were studied. Microbial DNA extracted from nasal samples was subjected to high-throughput next generation 16S-rRNA amplicon sequencing using Illumina MiSeq system. Bioinformatics analyses of generated sequences were done with QIIME 2 and other standard tools. The mean reads in CTLG (27,472.50) were significantly ($P < 0.05$) lower than in PPRG (45,520.13). Microbial diversity was higher ($P < 0.01$) in CTLG than in PPRG. The core bacterial genera *Corynebacterium* 1, *Dietzia*, *Brevibacterium*, *Brachybacterium*, *Kocuria*, *Micrococcaceae*, *Rikenellaceae* RC9 gut group, *Salinicoccus*, *Staphylococcus*, *Christensenellaceae* R-7 group, *Ruminococcaceae* UCG-010, and *Akkermansia* were significantly more abundant ($P < 0.01$) in CTLG than in PPRG. Meanwhile *Haemophilus*, *Mannheimia*, *Moraxella*, and *Mycoplasma* were more abundant ($P < 0.05$) in PPRG than in CTLG, suggesting roles in PPR. Many

species of *Haemophilus*, *Mannheimia*, *Moraxella*, and *Mycoplasma* are known to cause several human and livestock diseases. Our data suggest that susceptibility to PPR could be associated with a shift in the normal nasal microbiome composition, which should be further studied for the development of management strategies for PPR control.

Key Words: metagenomics, infectious disease, microbial diversity, *Haemophilus*, *Mannheimia*, *Moraxella*, *Mycoplasma*, goat

P253 Links between gut microbiome functions and feed efficiency in growing pigs fed a conventional or a high-fiber diet. A. Cazals[1], O. Zemb[2], V. Déru[2,3], J. Bidanel[4], H. Gilbert[2], and J. Estellé*[1], [1]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [2]*Université de Toulouse, INRAE, ENVT, GenPhySE, Castanet-Tolosan, France,* [3]*France Génétique Porc, Le Rheu, France,* [4]*IFIP-Institut du Porc, Le Rheu, France.*

The gut microbiota plays a major role in the digestive process in pigs and has been associated with feed efficiency, a major economic trait for pig industry. At the same time, pig production has to address social and environmental challenges, such as the choice of sharing land and natural resources between production for animal feed or for human consumption, and the environmental impact of meat production. Increasing the fiber content in the diets of pigs is an alternative to explore. It is therefore important to study the impacts of diet modulations on both feed efficiency traits and the composition and function of the microbiota. In this study, we predicted the relative abundances of the KEGG Orthologs (KO) functions from 16S sequencing data using PICRUSt2 software from 1,170 fecal samples of Large White pigs, fed with conventional (CO) or a high-fiber (HF) diet. On a subset of 48 samples, a whole meta-genome sequencing (WMS) was performed. Increasing the fiber content did not change the functional richness of the microbiota, but 189 KO functions were differentially abundant between the diets. Since over 6,000 KO functions were detected in the samples, the impact of HF diet on gut microbiota functional potential was relatively modest. Combining animals from both diets, in a model including "diet" as a fixed co-factor, residual feed intake (RFI) values for each pig were used to analyze the correlation between microbiota functions abundances and feed efficiency. With this approach, differential analysis revealed over 1,600 KO differentially correlated to RFI value, and 24 enriched pathways. In the WMS data set containing much fewer individuals, no significant DA KO were found, but 68 enriched pathways were identified by using the "gage" algorithm. Interestingly, some KO pathways such as "flagellar assembly" or "Ribosome" were highlighted by using both 16S and WMS data sets. Overall, this study provides new insights to better understand how feed impacts the gut microbiome functions, which in turn contribute to the variability of feed efficiency in pigs.

Key Words: pig, gut microbiota, feed efficiency, residual feed intake, microbiota functions

P254 Comparative metagenomic along the gut biogeography of indigenous chicken. A. Tangomo Ngnintedem*[1,2], E. Machuka[3], B. Waweru[3], J.-B. Domelevo Entfellner[3], M. Gitau Gicheha[4], J. Maina Kagira[4], R. Pelle[3], A. Djikeng[5], and C. Keambou Tiambo[6], [1]*Biotechnology and Bioinformatics Research and Training Unit, Department of Animal Science, FASA, University of Dschang, Dschang, Cameroon,* [2]*Department of Molecular Biology and Biotechnology, Pan-African University Institute of Basic Sciences, Technology and Innovation, Nairobi, Kenya,* [3]*Biosciences Eastern and Central Africa–International Livestock Research Institute (BecA–ILRI) Hub, Nairobi, Kenya,* [4]*Department of Animal Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya,* [5]*Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Easter Bush Campus, Edingburgh, UK,* [6]*Centre for Tropical Livestock Genetics and Health (CTLGH), ILRI Kenya, Nairobi, Kenya.*

The gut microbiome modulates the host immune system, metabolism, and adaptability and affects food safety. Unfortunately, information on the gut microbiome of African indigenous chicken remains scanty despite the contribution of this genetic resource to food and nutrition security in Africa. Therefore, our capacity to accurately quantify gut microbiome diversity is extremely relevant to formulate host-specific nutrition requirements, novel veterinary therapy, adaptability and productivity strategies. In the current study, 16S rRNA gene high-throughput Illumina sequencing was used to explore the metagenomic diversity of 4 gastrointestinal tract regions (crop, gizzard, jejunum, cecum) and liver in chicken. 80 biopsies were collected from 16 individual chickens fed on the same diet and reared under an intensive farming system for a 14-week period. The α diversity indexes exhibited similar pattern distribution within-sample and tend to increase along the GIT, with a pic noted in the jejunum. The β diversity showed that the cecum microbiota forms a distinct cluster from other GIT microbiota. Analysis of the GIT content and liver revealed heterogeneous microbiota taxonomy dynamic along the GIT organ and liver. Venn diagram and heatmap also demonstrate that each organ sections form a unique ecosystem on its own, each associated with a particular physiological function. These results confirm the suitability of using the 16S rRNA gene high-throughput Illumina sequencing pipeline to accurately quantify gut metagenome. Further, the non-invasive sampling strategies from a single gut biogeography cannot give a clear map of the gut microbiome diversity because each organ section forms a particular ecosystem on its own.

Key Words: gut biogeography, comparative metagenomic, indigenous chicken, 16S rRNA gene, Illumina Miseq

P255 Preliminary results: Bacterial abundance in the microbiome from South African beef fecal samples through 16s rRNA targeted sequencing. O. P. Monchusi[1,2], K. P. Montso[2], C. N. Ateba[2], A. A. Zwane[1], and M. M. Makgahlela*[1], [1]*Agricultural Research Council, Irene, Centurion, Gauteng, South Africa,* [2]*North-West University, Mahikeng, South Africa.*

The gastrointestinal tract (GIT) of cattle harbors a complex microbial community. These microbes are crucial in animal nutrition, physiology, and health. Therefore a better understanding of microbial diversity in the GIT of beef cattle is imperative. This study aims to determine the microbial communities in different cattle breeds using 16S rRNA metagenomics sequence analysis. The Divisive Amplicon Denoising Algorithm 2 (DADA2) software package was used on RStudio for metagenomics analysis, and SILVA at 99% full-length trained classifier was used as a reference tool for taxonomic classification. A total of 40 fecal samples were collected from 4 free-grazing breeds (Tuli, Nguni, Afrikaner, and Holstein) comprising male and lactating cows from different provinces of South Africa. The results revealed that operational taxonomic units at the phyla level showed a similar distribution (approximately 50%) of *Firmicutes* across each breed. Followed by the Tuli breed, 34% of *Proteobacteria*, 30% of *Bacteroidota* in the Holstein, and 35% of *Planctomycetota* in both the Afrikaner and Nguni breed. From observations, there were similarities in the distribution of family, with an increased portion of *Planococcaceae*, *Clostridiaceae*, followed by *Ruminococcaceae*. However, female fecal samples were observed to have an increased number of *Lactobacillaceae* and *Carnobacteriaceae* with a variety of minimal abundance that was not identified in other breeds. In conclusion, cattle rumen fecal samples have an equal distribution in *Firmicutes* despite ecological region but exhibit a distinctive abundance of *Proteobacteria*, *Bacteroidota*, and *Planctomycetota*. These preliminary results show consistency in certain phyla and suggest a significant difference within certain abundant microbes in a specific breed. Most female breeds have an increased number of *Lactobacillaceae*, a diverse family of lactic acid bacteria found in the gut microbiota of animals. Since 16s rRNA is a targeted metagenomics analysis, more studies should be conducted investigating the rumen microbiota at the species level.

Key Words: 16s rRNA, gastrointestinal tract, microbiome, fecal

P256 Impact of the vaginal microbiota on the pregnancy rate by artificial insemination in three Spanish sheep breeds. E. L. Reinoso[1,2], F. Puente-Sánchez[3], C. González[1], J. H. Calvo[4], M. Serrano[1], and M. Saura*[1], [1]*INIA-CSIC, Madrid, Spain,* [2]*ETSIAAB Universidad Politécnica de Madrid, Madrid, Spain,* [3]*Swedish University of Agricultural Sciences, Uppsala, Sweden,* [4]*CITA-IA2, Zaragoza, Spain.*

Artificial insemination (AI) is an essential tool in ruminant breeding programs for dairy aptitude. Notwithstanding, its efficiency in sheep is low, which results in economic losses and delays selection efficiency. Recent studies in humans suggest that microbial communities residing in the female reproductive tract seem to be involved in reproductive failure and pregnancy complications. In this study, we have analyzed the composition and abundance of the reproductive tract microbiota in sheep to investigate its relationship with fertility. For that, vaginal exudate samples were taken from 332 ewes from 4 different flocks belonging to 3 breeds (Latxa, Manchega with 2 flocks, and Rasa Aragonesa). Microbial DNA was extracted from the samples and the V3–V4 regions of the microbial 16S ribosomal RNA gene were sequenced using Illumina MiSeq technology. The sequences were analyzed by identifying the amplicon sequence variant (ASV) with the Dada2 package of the R software (35,577 ASVs identified). Beta diversity studies were carried out using principal components (PCA) and PERMANOVA to determine the factors associated with the composition of the microbiota. Finally, differential abundance analysis was performed between pregnant and non-pregnant ewes. A linear model corrected by flock and linear models for each independent flock were fitted. Differentially abundant ASVs were found for the different models fitted, but no ASVs common to all breeds were detected. However, when grouping the ASVs at the taxonomic level of phylum/class, most of the significantly differential abundant taxa in pregnant ewes belonged to *Proteobacteria*/*Gammaproteobacteria* groups and those in non-pregnant belonged to *Fusobacteria*/*Fusobacteriias*. These results agree with previous studies in cattle and humans, and suggest that the composition of the vaginal microbiota depends mainly on the flock and can be greatly affected by elements related to management, rather than the genetics of the breed.

Key Words: amplicon sequence variant (ASV), fertility, microbiome, ribosomal RNA 16S, sheep

P257 Using a Snakemake workflow for metagenomic analysis of sheep rumen microbiome divergently selected for methane emissions. B. Perry, A. Kim, H. Henry, T. Bilton, A. McCulloch, K. McRae, S. Clarke*, P. Janssen, J. McEwan, and S. Rowe, *AgResearch Limited, Lincoln, Canterbury, New Zealand.*

The objective of this project was to develop a bioinformatic pipeline for the analysis of metagenomic sequencing data generated from sheep rumen content. The pipeline was built using the popular Snakemake workflow language. Raw data in the form of 250-bp paired-end reads in fastq.gz format were trimmed, quality filtered and profiled in terms of taxonomy and function using open source software kraken2, bracken and humann3. Rumen microbiomes were compared between sheep of high and low methane selection lines. It was observed that biodiversity of the rumen microbiome was significantly higher in the high methane line, possibly due to a high prevalence of archaeal genera. Methanogens were highly associated with the high-methane line while the low-methane line were associated with producers of butyrate and propionate, which are products of the fermentation pathway that are not converted to methane. Future work should consider adapting this pipeline to handle and analyze metatranscriptomic data and running the functional profiling step of the pipeline against alternative reference databases and additional functional ontologies.

Key Words: microbiome rumen methane sheep metagenomics

P258 Bacterial diversity associated with feeding Boschveld chicken with the South African red sorghum variety. N. Nemukondeni*[1], C. A. Mbajiorgu[1], A. N. Sebola[1], O. M. Letsoalo[1], T. Mafuna[2], and M. Mabelebele[1], [1]*University of South Africa, Florida, South Africa,* [2]*University of Johannesburg, Auckland Park, South Africa.*

The objective of this study was to investigate the effect of feeding diets formulated with red sorghum variety containing low tannins grown in South Africa on the gut microbes of Boschveld indigenous chickens. A trial was conducted for 90 d using unsexed Boschveld indigenous chicken fed diets formulated with the inclusion of the red sorghum type at 5 inclusion levels (0, 25, 50, 75, 100%) replicated 4 times. Sample collections were done on d 60 and 90 of the trial, where ceca from 2 chickens per treatment per replicate were collected and instantly stored in a tube containing 90% ethanol and kept in ice for further analysis. All collected samples were sent for sequencing at Inqaba Biotechnical Industries in Pretoria, where genomic DNA samples were PCR amplified using a universal primer pair 341F and 805R, targeting the V3 and V4 region of the bacterial 16S rRNA gene. The resulting amplicons were purified and end-repaired, and Illumina-specific adapter sequences were ligated to each amplicon (NEBNext Ultra II DNA library prep kit). The amplicons were sequenced on Illumina's MiSeq platform using a MiSeq v3 (600 cycles) kit. Analysis was done using in-house python scripts version 3.6.1. kronaTools and the Rstudio software following phyloseq package R version 3.5.0. The findings of this study revealed different bacterial communities present in the gut of the studied indigenous chickens and varied more as chickens grew. However, common bacteria found regardless of the inclusion levels were *Firmicutes*, *Bacilli*, *Lactobacilli*, *Lactobacillus*, *Proteobacteria*, *Betaproteobacteria*, and *Streptococcaceae.* However, *Burkholderiales* bacteria were only present in the gut of chickens that were offered red sorghum diet at a 50% inclusion level. It can be concluded that feeding Boschveld chickens diets formulated with red sorghum at any inclusion level up to 100% does not have any adverse effects on gut health.

Key Words: microbiota, unisex, amplified, indigenous chickens

P259 Analysis of the gut microbiome sheds insights into breed resilience to challenges of antimicrobial resistance in Dohne Merino sheep. A. Khwela*[1,2], E. F. Dzomba[2], R. Pierneef[1], and F. C. Muchadeyi[1], [1]*Agricultural Research Council, Biotechnology Platform, Onderstepoort, Gauteng, South Africa,* [2]*Discipline of Genetics, School of Life Sciences, University of KwaZulu-Natal, Scottsville, KwaZulu-Natal, South Africa.*

Dohne Merino is one of South Africa's leading sheep breeds, which is also reared in Australia, New Zealand, and other European countries. In South Africa, sheep and other livestock populations are exposed to multiple diseases and parasites. The efforts to manage diseases and infections while maintaining high productivity has led to a high usage of antimicrobials in sheep production. This has resulted in a high prevalence of antimicrobial resistance (AMR), which is a major global concern that demands surveillance and action. The gut microbiome is of importance to the well-being of ruminant livestock by contributing to nutrition and health of the animals. The goal of this study was to investigate the gut microbial environment of South African Dohne Merino sheep by metagenomic sequencing of the rumen, reticulum, omasum, and abomasum of ewes (n = 6). We assessed relationships between microbiome composition and AMR prevalence across the 4 gut compartments. The members of the microbial population were fully characterized, and the resistome of the gut was analyzed. The microbial population was analyzed at phylum, class, order, genus, and species level. A total of 18 phyla were detected, with *Bacteroidetes* (54%) and *Firmicutes* (25%) being the most abundant. Members of the archaeal domain made up 16,7% of the overall population. A total of 1,769 species were detected in all the samples, with uncultured species dominating. A total of 12 AMR genes were identified in the gut and were found to confer resistance to 15 antimicrobials. A high prevalence of resistance to tetracycline, macrolide, nitroimidazole, and lincosamide was observed across all 4 compartments. Tetracycline genes were most abundant, making up 49% of the total AMR genes. The findings reveal that microbial population is influenced by each compartment's physiological conditions and function. The observed antimicrobial resistance

profiles reveal breed resilience and are likely selected for by the usage of antimicrobials as feed additives and in the treatment of diseases.

Key Words: Dohne Merino, gut microbiome, AMR, metagenomics

P260 Exploring links between porcine genome copy number variants and the diversity and composition of pig gut eukaryote and prokaryote microbial communities. M. Ballester*[1], D. Crespo-Piazuelo[1], J. Morata[2], L. Ramírez[1], O. González-Rodríguez[1], C. Sebastià[3,4], A. Castelló[3,4], A. Dalmau[5], S. E. Ramos-Onsins[3], K. Alexiou[3], J. M. Folch[3,4], R. Quintanilla[1], and Y. Ramayo-Caldas[1], [1]*IRTA, Caldes de Montbui, Spain,* [2]*CNAG-CRG, Barcelona, Spain,* [3]*CRAG, Campus UAB, Bellaterra, Spain,* [4]*UAB, Bellaterra, Spain,* [5]*IRTA, Girona, Spain.*

Recent evidence suggests that genetic variation in the pig genome partially controls the composition of porcine gut microbiota. However, since previous studies have been focused on the contribution of single nucleotide polymorphisms, little is known about the putative links between other sources of genetic variation such as copy number variants (CNVs). The main goal of this study was to assess the association between porcine genome CNVs and the diversity and composition of pig gut prokaryotic and eukaryotic microbial communities. For this purpose, we used whole-genome sequencing data to undertake a comprehensive identification of CNVs, followed by a genome-wide association analysis between the estimated CNV status and 52 microbial traits including 3 diversity indexes, and the relative abundance of 43 bacterial, 5 protists, and 1 yeast genus. We identified associations between CNVs and the relative abundance of 3 bacterial genera (*Faecalibacterium*, *Oscillospira*, and *Phascolarctobacterium*), 1 eukaryotic (*Kazachstania*), the richness and Shannon α-diversity of the bacterial communities. The CNV linked to the diversity index partially harbor *ABCC2-DNMBP* loci and was in silico predicted as gain. We validated by real-time quantitative PCR with a precision of 95.83% the gain of copies of this CNV. Further, its segregation and positive association with bacterial diversity was confirmed in an unrelated F_1 (Duroc × Iberian) cross. In summary, we report the first study exploring associations between porcine CNV and gut microbial traits. These results advise the relevance of considering the role of structural variants as host-genetic factors modulating porcine gut microbial communities and open the possibility of including CNVs in selection programs to simultaneously improve microbial traits and gut health.

Key Words: pigs, genome-wide association, microbiomics, copy number variation (CNV), qPCR

P261 Possible coevolution of balanced polymorphisms in the pig host and its intestinal microbiome. C. Hupperts*[1], M. Mni[1], W. Coppieters[1,2], C. Charlier[1], and M. Georges[1], [1]*Unit of Animal Genomics, GIGA-R and Faculty of Veterinary Medicine, Liège, Belgium,* [2]*GIGA—Genomics Platform, University of Liège, Liège, Belgium.*

A major effect of ABO genotype on the abundance of a genus of *Erysipelotrichaceae* (p.75.a5) in colon and feces was recently reported in the pig: animals with a functional ABO galactosyltransferase (A allele) have higher abundance of p.75.a5 than animals with a loss-of-function mutation (O allele) in this gene (Huang, 2022). The porcine O allele was shown to be at least 3.5 million years old and to likely be maintained by balancing selection. It was shown that this microbiome QTL was mediated by differences in colonic GalNAc concentrations between AA, AO and OO animals. It was also shown that the affected bacteria were capable of using GalNAc as carbon source. Members of the p.75.a5 genus are not the only intestinal bacteria that use GalNAc. Why then do members of this genus appear to be the only bacteria affected by ABO genotype in the pig? A possible hint came from comparing the organization of the GalNAc operon in ABO sensitive versus insensitive bacterial species. It appears that the GalNAc operon is not or less inducible in ABO sensitive bacteria when compared with ABO insensitive bacteria (including *Escherichia coli*), and that this may be due to the absence of a operon repressor in the former. Our aim is to understand the molecular mechanisms that underpin the sensitivity of the p.75.a5 genus to the ABO genotype of the host. Preliminary analyses suggest (i) that the p.75.a5 genus harbors a balanced polymorphism in the GalNAc operon that conditions the sensitivity to ABO, and (ii) that this polymorphism is in linkage disequilibrium with other segregating variants supporting extensive sexual exchange within the genus. We are exploring the possibility to use single-cell DNA sequencing with ONT to further explore the population genomics of p.75.a5 and other intestinal bacteria, and to test the hypothesis of the coevolution of balanced polymorphisms in the host and its intestinal microbiota. Latest results will be presented.

Key Words: pigs and related species, metagenomics, other method, linkage disequilibrium, animal health

P262 Withdrawn

P263 Genetic selection of the host drives gut microbiota enterotypes across generations. J. Estellé*[1], C. Larzul[2], M. Borey[1], F. Blanc[1], G. Lemonnier[1], Y. Billon[3], M. Thiam[4], B. Quinquis[4], N. Galleron[4], D. Jardet[3], J. Lecardonnel[3], F. Plaza-Oñate[4], and C. Rogel-Gaillard[1], [1]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [2]*Université de Toulouse, INRAE, ENVT, GenPhySE, Castanet-Tolosan, France,* [3]*INRAE, GenESI, Surgères, France,* [4]*Université Paris-Saclay, INRAE, MGP, Jouy-en-Josas, France.*

The gut microbiota associated to animals has been linked to many phenotypes essential for livestock production. In parallel, an increasing number of studies aims at understanding how the host genetics influences the microbiota composition and its physiology. Indeed, gut microbiota displays inter-individual variability even in highly controlled and homogeneous environments, and there is a lack of knowledge on the role of host genetics. We previously showed that the gut microbiota of 60-d-old pigs raised in similar conditions could be structured into 2 enterotypes, enriched either in *Prevotella* and *Mitsuokella* genera ("PM" enterotype), or *Ruminococcus* and *Treponema* ("RT" enterotype). To assess the host genetics' influence on gut microbiota composition, we are studying 2 divergent pig lines, named HPM and HRT, selected for gut microbiota enriched in genera pairs specifying each enterotype. Re-

sponse to selection over 3 generations revealed, per line, an increase in the prevalence of the selected enterotype and average relative abundances of directly and indirectly selected bacterial genera. Estimated heritabilities were significant for 62 genera abundances. Whole-metagenome sequencing refined differences between enterotypes at bacteria species levels, illustrating different functional potentials also. Overall, we experimentally demonstrated the influence of host genetics on gut microbiota, highlighting holobionts as units of selection and pigs as important biological models. The HPM and HRT divergent pig lines will potentially contribute to better understand the combined impact of host genetics and gut microbiota on a range of phenotypes relevant for sustainable livestock systems, from growth and feed efficiency to health and welfare.

Key Words: microbiota, gut, host genetics, pig, selection

P264 Differential miRNA profile in response to dietary treatment and their possible impact in the host-microbiota genetic regulation. T. Porto[1], T. Cardoso[2], J. Bruscadin[1], L. Conteville[2], P. Oliveira[1], G. Mourao[3], L. Coutinho[3], A. Zerlotini[4], J. Reecy[5], and L. Regitano*[2], [1]*Post-Graduation Program of Evolutionary Genetics and Molecular Biology, Federal University of São Carlos, São Carlos, SP, Brazil,* [2]*Embrapa Southeast Livestock Research Center, São Carlos, SP, Brazil,* [3]*Department of Animal Science, University of São Paulo, Piracicaba, SP, Brazil,* [4]*Embrapa Digital Agriculture, Campinas, SP, Brazil,* [5]*Department of Animal Science, Iowa State University, Ames, IA.*

MicroRNAs (miRNAs) are key post-transcriptional regulators of gene expression of both host and microbiota, and thus have the potential to influence microbiota composition and functionality. This project aims to identify the expression profile of miRNAs expressed in the rumen wall from Nelore (*Bos indicus*) bulls under nutritional intervention and to study the interaction between host miRNAs and ruminal microbiota. Two groups of Nelore bulls were submitted to different diets, i.e., conventional high-grain diet (n = 26) and agricultural co-products diet (n = 22). Rumen wall samples were collected, and total RNA was extracted. The sequencing of the miRNA libraries was performed on an Illumina Hiseq 2500 platform and yielded an average of 3.42 million reads per sample. Reads were mapped to the *Bos taurus* genome ARS-UCD1.2 with the software miRDeep2, and a differential expression analysis was performed using DESeq2. A total of 528 miRNAs were identified, 9 of them differentially expressed (DE; FDR ≤0.1); 7 miRNAs were upregulated and 2 were downregulated in the group fed agricultural co-products. To investigate evidence of microbiota regulation by host miRNAs, we analyzed in silico the potential interaction between the DE miRNAs and ribosome binding sites (RBS) annotated in 913 publicly available metagenome-assembled genomes (MAGs) from bovine ruminal contents. All 9 DE miRNAs had predicted targets in MAG genes. A total of 35 bacterial MAGs (25 of them classified as *Clostridiales*) presented predicted targets for the DE bta-miR-223, affecting 13 annotated bacterial genes. This miRNA has been previously identified as DE in bovines divergent for residual feed intake and thus has the potential to be an important regulator of this trait. The results from both analyses suggest that host miRNAs are affected by diet and may regulate genes of microorganisms found in the bovine rumen. Further analysis may indicate which metabolic pathways are influenced in this process, as well as whether this regulation can be modulated in different nutritional interventions.

Key Words: cattle and related species, genome regulation, non-coding RNA, microRNA, RNA-seq

P265 ISAG Bursary Award: Study of gut microbes and body metabolism function between Dorper and Tan sheep. Y. Ma*[1], X. Yang[1], G. Hua[1], G. Cai[1], X. Li[2], D. Feng[2], and X. Deng[1], [1]*Key Laboratory of Animal Genetics, Breeding, and Reproduction of the Ministry of Agriculture and Beijing Key Laboratory of Animal Genetic Improvement, China Agricultural University, Beijing, China,* [2]*Department of Animal Science and college of Agriculture, Ningxia University, Ningxia Hui Autonomous Region, China.*

Gut microbes interact with peripheral organs in the form of axis, affecting the growth of the host. Dorper and Tan sheep are known for their excellent growth performance and higher fat content, respectively. This study aims to establish microbial and host metabolic networks that provide for breeding against the gut microbiota. We collected 8-mo-old Dorper and Tan sheep (4 male, 4 female) rumen, cecum, colon intestinal contents and liver, hindgut organs tissues reared in the same environment. We then used 16S rRNA sequencing methods to obtain microbial signatures and combined with metabolomic analysis to mine the intestinal metabolic network. RNA-Seq technology was used to identify differentially expressed genes and pathways in liver and intestinal tissues. In our study, Dorper intestinal tissue has strong glycine serine metabolism (*PHGDH*, *PAST*), and through the one-carbon cycle, the production of NAD+ is increased, and more nucleotides are produced (cytosine, uracil). *Lactobacillus*, *Pseudomonas*, and other bacteria are involved in the nucleotide metabolism process. Meanwhile, the kynurenine pathway in the tryptophan metabolism (*ACMSD*, *KYNU*) increased the production of quinoline, indole and xanthine, which mediated the increase of NAD+ in the intestinal of Dorper. In Tan sheep, the decrease of bile acids in the intestine is related to the process of bile transport in the liver. *NTCP* and *ABCB11* genes promote the reabsorption and transport of intestinal bile acids, decrease the content of bile acids in the intestine, and reduce the digestion of fat in the intestine. In contrast, the lipid oxidation gene (*PPARGC1B*, *LPL*) decreased lipid accumulation in Dorper. In conclusion, our study confirms that increased nucleotide and NAD+ in Dorper promotes intestinal and host growth. Enterohepatic circulation and fat genes promote lipid accumulation in Tan sheep. This work was supported by the China Ningxia Agricultural Breeding Project (NXNYYZ20150103).

Key Words: nucleotide metabolism, enterohepatic circulation, lipid metabolism, NAD+

P266 Optimizing metagenomic sequencing: A comparative study of ONT adaptive sampling strategies to improve microbial DNA recovery. E. L. Reinoso-Peláez*[1,2], M. Saura[1], C. González[1], F. Puente-Sánchez[3], and M. Serrano[1], [1]*INIA-CSIC, Madrid, Spain,* [2]*ETSIAAB, Universidad Politécnica de Madrid, Madrid, Spain,* [3]*Swedish University of Agricultural Sciences, Uppsala, Sweden.*

One important constraint in metagenome studies is the large amount of host DNA recovered when extracting microbial DNA. To improve this limitation, Oxford Nanopore Technology (ONT) has developed a method called adaptive sampling (AS), which is based on the depletion of sequences with respect to a user-specified genome. The aim of this study was to compare the efficiency of AS versus conventional sequencing (CS) to determine the most efficient strategy for retaining the highest amount and quality of microbial DNA. For that, we sequenced microbial DNA from vaginal exudates of 12 ewes in a GridION device, under both sequencing strategies (AS and CS) and different quality thresholds (Q) for the basecalling: (i) AS and fast basecalling (AS-fast, Q = 8), (ii) AS and high-accuracy basecalling (AS-high, Q = 9), (iii) AS and super-accuracy basecalling (AS-sup, Q = 10), and (iv) CS and high-accuracy basecalling (CS-high, Q = 9). During AS, sequences were mapped against the sheep genome (GCA_002742125.1) to reject host sequences from the nanopores. After basecalling, *minimap2* software was additionally used to remove sequences assigned to the host. The quality filtering was assessed using NanoPlot software. The remaining sequences were processed with SqueezeMeta v. 1.6.0 software to evaluate the potential to recover microbiota reads. The number (and total size) of reads obtained with the different strategies were 211.9 K (94.3 Mb), 98.1 K (47.5 Mb), 91.4 K (42.5 Mb), and 28.4 K (13.9 Mb) for AS-fast, AS-high, AS-sup and CS-high, respectively. AS-fast yielded the highest recovery but also the highest number of unclassified reads (68.4%). Regarding the reads assigned to bacteria, AS-fast, AS-high, and AS-sup showed the highest recovery, being 3.30, 3.38,

and 3.74 times more than the CS-high, respectively. In summary, the strategy providing the highest bacterial enrichment was AS-sup, with 36.5 K reads (compared with 9.8 K for CS-high), thus proposing this strategy as the most efficient approach to recover the greatest amount of microbial sequences from biological samples contaminated with host DNA.

Key Words: adaptive sampling, metagenome, DNA recovery, ONT

P267 Host genomic regions associated with ewes' vaginal microbiota. M. Ramon*[1], E. Reinoso-Pelaez[2], M. Saura[2], O. González-Recio[2], C. Gonzalez[2], R. Arias[1], M. Pérez-Guzman[1], I. Beltrán de Heredia[3], J. Calvo[4], and M. Serrano[2], [1]*CERSYRA-IRIAF, Valdepeñas, Ciudad Real, Spain,* [2]*INIA-CSIC, Madrid, Spain,* [3]*NEIKER, Arkaute, Spain,* [4]*CITA-ARAID-IA2, Zaragoza, Aragón, Spain.*

Fertility is a trait of great economic importance in livestock breeding programs. The outcome of artificial insemination (AI) depends on several factors, making it difficult to identify the causes of low fertility. One of the factors known to influence fertility is the composition of the vaginal microbiota at the time of AI. The characterization of the microbiota can help to identify which microorganisms may be in overabundance in sub-fertile animals so appropriate treatments can be considered. In addition, this characterization can be used to study the role that the host genome may play in the composition of its vaginal microbiome. This work aims to conduct an initial exploration of the relationships between host genotype and vaginal microbiota in sheep. For that, vaginal samples from 288 ewes were collected before AI. From them, DNA was extracted and bacterial 16S ribosomal RNA hypervariable regions V3 and V4 were sequenced. Bioinformatics pipelines using qiime2 and SILVA nr99_v138.1 database for taxonomic annotation were used to obtain feature abundance matrices. In addition, genomic data (Illumina Ovina HD 680K AgResearch chip) from the same 288 ewes was available, and a QC analysis removing samples with a call rate below 95% and markers with a call rate below 95% and a MAF <0.01 was carried out prior to the analysis. A genome-wide association study (GWAS) was conducted using the mixed linear model approach in GCTA to look for associations between host genome and the microbial abundance for some important families known to affect fertility, such as *Actinomycetaceae*, *Fusobacteriaceae* and *Mycobacteriaceae*. Within the regions of the genome found to be significantly associated with the abundance for these families, we found genes involved in the immune response such as interleukins (ILs), FSHB and FSHR, GNRHR related to the follicle-stimulating and gonadotropin hormones, OXT and OXTR related to the oxytocin hormone, and THRA, THRB, TSHR and TRHR related to thyroid hormone. Further study of the role of these and many other identified genes will help us to learn more about the relationships between the host genome and the vaginal microbiota of ewes and its role in the fertility outcome

Key Words: microbiomics, genome-wide association, sheep and related species, fertility

Pig Genetics and Genomics

P268 Study on BMPR1B gene affecting endometrial cell growth and development to regulate high reproductive performance in Taihu pigs. Z. Liu[1,2], H. Zhang[1,2], D. Wang[1,2], J. Wang[1,2], T. Zeng[1,2], and K. Wu*[1,2], [1]*Department of Animal Genetics and Breeding, National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, China,* [2]*Key Laboratory of Animal Genetics, Breeding and Reproduction of the Ministry of Agriculture and Rural Affairs, College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Dynamics in endometrial epithelial cells (EECs) growth and development are vital factors for affecting the uterine environment and implantation. *BMPR1B* has been identified as a candidate gene for reproductive traits in pigs. Our previous study in Taihu pigs found that a unique haplotype in the first intron of *BMPR1B* promoted its' expression in endometrium. However, a 15-bp InDel was located on the haplotype, and little is known about the mechanism by InDel regulated the expression of *BMPR1B* and function of *BMPR1B* on the growth and development of endometrium. This study aimed to investigate the genetic structure and biological function of *BMPR1B* in porcine endometrium. Unique InDel of Taihu pigs in *BMPR1B* gene was identified by PCR and PAGE. Transcription factor binding sites of InDel were predicted at the AnimalTFDB3.0. Binding efficiency of InDel to ESR1 detected by dual luciferase activity. A BMPR1B overexpression immortalized Meishan pig EECs was isolated and established by lentiviral vector. The effect of *BMPR1B* on EECs proliferation, apoptosis, and migration were detected by CCK-8, EdU, Tunel, Wound-Healing, and Transwell assay. Here, a 15-bp InDel (AGCCAGAAAGGAGGA) was identified as a unique variation in Taihu pigs, and was shown to be responsible for the binding of ESR1 to the haplotype using dual-luciferase assays. After overexpression in EECs, the *BMPR1B* gene significantly decreased cell proliferation ($P < 0.01$). *BMPR1B* gene had no significant effect on late apoptosis. The *BMPR1B* gene significantly reduced migration ($P < 0.01$). In summary, our results identified a 15-bp InDel in the Taihu haplotype and provide a foundation for the functional regulation of *BMPR1B* in EECs. The finding contributed to the unique breeding prolificacy characteristics of Taihu pigs. Acknowledgments: This work was supported by the National Key Research and Development Program of China (2021YFF1000603) and (2022ZD0115704).

Key Words: Taihu pig, BMPR1B, endometrial epithelial cells, cell growth and development, prolificacy

P269 Withdrawn

P270 Effects of genetic markers on yield and meat quality traits is influenced by diet energy content in Iberian pigs. C. Óvilo*[1], L. Calvo[2], Y. Núñez[1], D. Menoyo[3], A. Rodríguez[2], C. López-Bote[4], and M. Muñoz[1], [1]*Departamento Mejora Genética Animal, INIA-CSIC, Madrid, Spain,* [2]*Incarlopsa, Tarancón, Cuenca, Spain,* [3]*Departamento de Producción Agraria, ETSIAAB, UPM, Madrid, Spain,* [4]*Departamento de Producción animal, Facultad de Veterinaria, UCM, Madrid, Spain.*

Identification of genes and markers associated with productive traits provide tools for animal breeding and contribute to the knowledge of the biological basis of relevant traits and physiological processes. The effects of genetic markers can be influenced by environmental factors as the diet. The objective of this work was to evaluate a panel of DNA markers for traits related to growth, yield, fatness and meat quality in an Iberian pig commercial population, as well as testing potential nutrigenetic effects conditional on the energy level of the diet. We designed a custom SNP panel including potentially causal SNPs located on biological and positional candidate genes. Fifty-four SNPs were genotypedin 358 Iberian crossbreds from a commercial population. Animals were divided in 2 groups receiving either a high (H, n = 193) or a low (L, n = 165) energy diet. Phenotypic records included productive, yield data and muscle and fat composition. The results of the association study confirmed the effects of known SNPs in candidate genes (*LEPR*, *ACACA, SCD, ELOVL6, PPARG* on fatness, growth and FA composition) and also provided associations for novel SNPs in scarcely studied genes (*AGPAT5*, *SOWAHB*, *PRKDC*, *NLE1*). In addition, interesting association results were observed for new positional candidate genes not explored so far, derived from a previous GWAS study in Iberian pigs. Besides, several significant interactions genotype × diet were detected, for genes such as *MYORG*, *ATRNL1*, *SCD*, *LEPR* or *PPARA*. An interesting qualitative interaction was observed for *MYORG*, with opposing effects of the SNP depending on the diet. For other genes the nutrigenetic effects were quantitative and due to the clearer effect of the SNP in the animals receiving L diet. This finding may explain non-concordant results obtained from some of these markers in different populations. The results provide valuable tools and tips for the future implementation of marker-assisted selection strategies and contribute to a better understanding of the genetic architecture of relevant traits and the interaction of genetics and nutritional factors.

Key Words: Iberian pig, SNP, candidate gene, association study, nutrigenetics

P271 Research on the classification model of selective sweep for different lines of Yorkshire pigs based on genome information. Y. Ma*, H. Song, S. Zhang, X. Li, and S. Zhao, *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, Hubei, China.*

Selection signal, as genomic characteristics, was formed in the process of population adaptive evolution, which is of great significance to the study of phenotypic polymorphism and its genetic basis. In general, selection signals are divided into negative selection, balanced selection and positive selection according to the change direction of the frequency of selected alleles. Here, we proposed a novel selective signal detection method and classifier based on machine learning model. Based on extensive simulations in this study, we discuss the statistical properties of the different established selection signature statistics, including a representative deep learning method partialS/HIC, a machine learning method Trendsetter, and 5 traditional statistical methods CLR, ω, his, Tajima`D and nSL. Totally, we found that the methods based on machine learning model have higher power than the traditional methods, and the novel method shows better robustness in all scenarios. In addition, we further explored the selection singles in different Yorkshire lines. We found that the genetic relationship between different Yorkshire lines was closer than that between other populations, but there were still significant differences. The analysis of parallel selection signals among populations found that 1034 parallel selection regions were identified among the 5 Yorkshire pig lines. All parallel selection regions contain 787 genes, which are mainly involved in development, immunity, and reproduction. Among these 32 regions, selection signals can be identified in all lines, and multiple genes in these 32 regions are related to reproductive traits. The analysis of inter-population-specific selection signals found that the genes in the specific selection signals of different lines all participated in many biological function pathways, and many functions can be enriched in multiple line-specific genes at the same time, indicating the genetic basis of polygenes for economic traits.

Key Words: pig, genomics, machine learning, selective sweep

P272 ISAG Bursary Award: Genome selection based on multiple artificial intelligence approaches boosting prediction accuracy. L. Wei*, D. Zhu, X. Hu, and Y. Wang, *State Key Laboratory for Agro-Biotechnology, China Agricultural University, Beijing, China.*

The generation of high-throughput sequencing data is promoting the transformation of breeding technology from traditional "experience breeding" to "precision breeding." Artificial intelligence (AI) algorithms do not require pre-defined rules, and they are good at fitting nonlinear complex relationships in data through autonomous learning of data and features, which is more advantageous for handling massive genotype data. In this study, we employed sequencing or microarray SNP data from 4 species (pig, chicken, horse, maize) and analyzed 22 phenotypes with different genetic architecture. A total of 45 combinations of genomic predictions were performed using 5 genotype data dimensionality reduction methods (low coverage sequencing data, LD, PCA, phate, and mutual information) combined with 9 different machine learning models (LR, ELNET, KNN, SVM, GBDT, LightGBM, XGBOOST, CATBOOST, RF). The results were also compared with traditional GBLUP and BayesR algorithms. The accuracy was defined as Pearson correlation between GEBV and true phenotype. It was found that the machine learning method showed better overall performance in cases with low heritability, polygenic inheritance, and a lower proportion of additive effects. Each trait has its optimal predicted streamline and about 5–10% improvement in accuracy were achieved compared with the traditional methods, as well as a significant improvement in speed. The above algorithms are integrated to form a prediction toolkit to automatically select the optimal dimensionality reduction strategy and machine learning model for traits with different genetic architecture, and finally obtain a trait-specific genomic AI prediction model. It is important to further improve the accuracy of important phenotypic breeding value estimation, accelerate genetic progress, take advantage of multi-threading and parallel computing, and accelerate the breeding process.

Key Words: artificial intelligence, dimensionality reduction, machine learning, prediction toolkit, breeding value estimation

P273 Genome-wide analysis of allele-specific circular RNAs in pigs and their role in cell proliferation. Y.-J. Li[1,2], H. Liu[1,3], Y.-D. Zhang[1,2], A. Li[4], L.-X. Pu[5], S.-R. Zhang[1], N. O. Otecko[1,3], M.-S. Peng[1], D. M. Irwin[6], W. Xie[7], Y. Qin[8,9], Z. Wang[9,10], H.-J. Wei[11,12], Z.-Y. Zhou*[1], Y.-P. Zhang[1], [1]*State Key Laboratory of Genetic Resources and Evolution, and Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China,* [2]*State Key Laboratory for Conservation and Utilization of Bio-resource in Yunnan, Yunnan University, Kunming, Yunnan, China,* [3]*Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan, China,* [4]*Shaanxi Key Laboratory for Network Computing and Security Technology, School of Computer Science and Engineering, Xi'an University of Technology, Xi'an, Shanxi, China,* [5]*State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu, China,* [6]*Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada,* [7]*Center for Stem Cell Biology and Regenerative Medicine, MOE Key Laboratory of Bioinformatics, THU-PKU Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing,*

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Circular RNAs (circRNAs) are a large class of RNAs with covalently closed continuous loop structures that are widely expressed. However, it is currently unknown if circRNAs shows allele-specific expression, as are the consequences of genetic variation on their circularization efficiency and subsequent biological function. Here, we identified and analyzed allele-specific circRNAs from reciprocal crosses between 2 highly divergent strains in pig. We also found that variation in intronic sequence affects circularization efficiency of circRNAs. Our evidence suggests that some allele-specific circRNAs influence cell proliferation through differences in miRNA sponge activity. Our study provides new insight into molecular mechanisms impacted by variation in genome sequence in the origin of pig phenotype.

Key Words: pig, circRNA, allele-specific circRNA, intronic mutation

P275 Effects of maternal antioxidant supplementation on the gut health of the offspring. A. Heras-Molina*[1,2], H. Laviano[1], G. Gomez[3], Y. Nuñez[2], F. Sanchez-Esquiliche[4], A. Gonzalez-Bulnes[5], A. Rey[1], C. Lopez-Bote[1], M. Muñoz[2], and C. Óvilo[2], [1]*UCM, Madrid, Spain,* [2]*INIA-CSIC, Madrid, Spain,* [3]*IRIAF, Toledo, Spain,* [4]*Sánchez Romero Carvajal, Huelva, Spain,* [5]*UCH-CEU, Valencia, Spain.*

Perinatal period is critical for the correct development of the piglet. At this stage, an important redox challenge takes place which can have important implications for the animals. Thus, antioxidant substances are being tested in sows during gestation and lactation to improve litter's health and productivity. The study of the nutrigenomics and metagenomics effects of these diets would allow us to ascertain their biological mechanisms. The aim of this work was to evaluate trans-generational effects of vitamin E (VE) and hydroxytyrosol (HT) given to sows during the perinatal period on the intestinal health of the piglets. For that, the piglets' transcriptome of the duodenum and the microbiome composition of the feces were studied. Thirty Iberian pregnant sows from the *Deheson del Encinar* farm were fed differing in the VE or HT supplementation levels from d 85 of gestation to weaning: Control (C): 30 mg VE/kg; VE:100 mg VE/kg; VEHT:100 mg VE/kg + 1.5 mg HT/kg. Five days after weaning, 10 male piglets from each group were sampled. Feces samples from all the animals were collected, whereas duodenum samples were taken from 5 animals/group. The microbiome was analyzed by using the 16S approach and transcriptome quantification was performed by using RNaseq. *Bacteriodota* and *Firmicutes* were the most abundant phyla, whereas *Prevotella, Alloprevotella* and *Lactobacillus* were the most abundant genera. Treatments had no effect on α nor β diversity, and microbiota differential abundance analyses showed no differences between groups. However, 50 differentially expressed genes (DEGs) were found in duodenal transcriptome when comparing C and VEHT piglets, and 202 between VE and VEHT groups. Routes related to carbohydrate metabolism were activated in group C. Also, genes linked to immunity and digestion (like *RNASE1*) were overexpressed in VEHT when compared with VE. In this comparison, VEHT piglets also showed activation of routes related to higher inflammatory status. In summary, maternal supplementation with different antioxidant did not modify the microbiome composition but affected duodenal transcriptome.

Key Words: pigs and related species, nutrigenomics, digestive system, animal nutrition

P276 Exploring the genetic basis of fetal development in Iberian pigs using liver RNA-seq data. P. Vázquez-Ortego[1], A. López-García[1], Y. Núñez[1], C. García-Contreras[1], M. Vázquez-Gómez[2], S. Astiz[1], A. Heras-Monina[2], B. Isabel[2], A. González-Bulnes[1], C. Óvilo[1], and M. Muñoz*[1], [1]*INIA-CSIC, Madrid, Spain,* [2]*UCM, Madrid, Spain.*

Iberian sows have a low uterine capacity leading to high variability in piglet birth weight in case of increased prolificacy. The differences in fetal development can determine their long-term postnatal growth. Liver is the major organ for the control of metabolic homeostasis and its functionality is compromised in restricted intrauterine growth events. In previous studies, we observed that fetus weight has a higher impact on the liver transcriptome in Iberian purebred pigs than in Large-White x Iberian crossbred. Therefore, in the present study we analyzed the genetic variants which would be potentially involved in the fetal growth and development of the Iberian breed. Liver RNA-seq data from 16 Iberian purebred and 16 crossbred fetuses at d 77th of gestation were used to identify genetic variants and analyze their allele frequency differences between genotypes and weights. Variant calling was performed using GATK pipeline. Those with a minor allele frequency lower than 0.05 and call rate lower than 0.5 were excluded. A total of 157,214 variants were detected and annotated with Variant Effect Predictor. Variants associated with differentially expressed genes (DEGs) between Iberian and crossbred fetuses (276 DEGs, 3,575 SNPs and 244 INDELs) or between high- and low-weight Iberian fetuses (824 DEGs, 10,226 SNPs and 634 INDELs) were retained. About 8.5% of the SNPs detected were missense and 8.1% of INDELs were annotated as frameshift variants. Among the variants mapped in the DEGs between high- and low-weight Iberian fetuses, 10 SNPs showed allelic frequency differences ranging from 0.6 to 0.9. The SNP with the highest allelic frequency difference (0.83) maps in the 5′UTR region of the *Cyclin L2* gene, which encodes a regulator of cell cycle. Moreover, another interesting variant (allelic frequency difference = 0.75) is located in the 3′UTR region of the *Glycogen Synthase 1* that plays a relevant role in the metabolism of glycogen. This work provides relevant insight regarding the genetic basis underlying fetal development in Iberian pigs.

Key Words: pigs and related species, functional genomics, RNA-seq, development

P277 Strategic decision-making within Iberian pig breeding programs through simulation approaches. M. Revilla*[1], B. Perez[1], E. Alcázar[2], A. González[2], J. Requejo-Puerto[1], J. Sánchez[2], and A. Huisman[1], [1]*Hendrix Genetics, 5830 AC Boxmeer, the Netherlands,* [2]*Ibéricos Vallehermoso S.L., Carretera la Solana a Villanueva de los Infantes, km. 9, 13248 Alhambra, Ciudad Real, Spain.*

Historically, Iberian pig production has been developed with purebred varieties. Iberian pigs were born and raised extensively, taking advantage of the '*Dehesa*' environment in southwestern Spain. The perfect adaptation of the Iberian breeds to this traditional system has resulted in high quality pork products. The Iberian breeds are characterized by lower yields compared with, more selected, commercial breeds, both productive and reproductive. The Iberian pig undergoes several certification processes, that aim to assure certain authentic characteristics of the finished products. These regulatory norms allow for a more intensive production system that use Duroc boars to improve specific traits in the crossbred offspring. The sow always is purebred Iberian animal. With the aim to improve both productive and reproductive traits, many traditional production systems have been substituted with intensive production systems. The objective of this study was to modify the existing breeding program of Ibéricos Vallehermoso, S.L. company to optimize as much as possible the reproductive and productive performance of the purebred Iberian and F_1 hybrid animals. To this end, a pipeline was built using the AlphaSimR package to perform stochastic simulations of all the processes involved in the genetic program. Parameters used for the simulation were based on historical phenotypes and pedigree data from the Iberian pig population. Ten generations of the genetic pro-

gram were simulated (15 replicates). We have investigated impacts of the precision of phenotypes (availability ranging 0.9–1.0) and weights used for traits in the selection index on genetic gains obtained. Results were calculated and compared between the high (GGP animals) and average (PS animals) performer animals of the population. The performance of the crossbred animals was also tested. Results obtained from this simulation helped us to compare the efficiency of different breeding strategies and optimize the selection stages. Overall, we illustrate here how simulation approaches could serve as a valuable tool for strategic decision-making within genetic programs.

Key Words: pig, animal breeding, imputation, fertility, genetic improvement

P278 Interactions of cortisol and sex-steroids in the regulation of porcine oviduct epithelium functions: insights from transcriptomic profiling. N. Trakooljul*[1], S. Du[2,1], E. Murani[1], J. Schoen[2,1], K. Wimmers[1], and S. Chen[2,1], [1]*Institute of Genome Biology, Research Institute for Farm Animal Biology (FBN), Dummerstorf, MV, Germany,* [2]*Department of Reproduction Biology, Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, BE, Germany.*

Maternal stress and elevated cortisol levels are associated with negative effects on animal reproduction. The oviduct creates the ideal microenvironment for fertilization and development of the gametes and embryo. During the estrous cycle, the oviduct epithelium undergoes transitions primarily orchestrated by sex-steroids, estradiol (E2) and progesterone (P4). This study aims to unravel molecular pathways underlying the action and interaction of cortisol, E2 and P4 in the oviduct. We treated air-liquid interface cultures of porcine oviduct epithelium (n = 6) with 250nM cortisol, 220pM E2 and 95nM P4 as single hormone and mixtures (cortisol+E2/cortisol+P4). Epithelial structure and barrier properties were monitored. Transcriptomic profiles (RNA-Seq) were analyzed at 12h and 72h post treatments. We identified differentially expressed genes (DEGs) by DESeq2 in the E2 (2851), P4 (1288) and cortisol (75) groups compared with the vehicle control (*p*-adjusted <0.05). E2 and P4 shared 427 DEGs. Of these, 75% (324) exhibited an inverse regulation. We detected 28 common DEGs in the E2vsCortisol comparison and 60 in the P4vsCortisol. Of these, 53% (15) show an inverse regulation in the E2vsCortisol, while almost all (59 DEGs) are directionally regulated in P4vsCortisol. Compared with the hormone mixture treatments, similar results were observed. Gene Ontology (GO) enrichment analysis using DAVID reveals terms associated with the E2 and P4 such as programmed cell death, body fluid secretion, cellular metabolic process, ion transmembrane transport and chemokine-mediated signaling pathway. We found 22 DEGs present in all cortisol, E2 and P4 treatments. Cortisol regulated all these DEGs in the same direction as P4, while it counteracted the E2 regulation of 59% (13/22). GO associated with the inverse regulated DEGs includes cellular response to stimulus/ stress, regulation of cell death/ proliferation and homeostatic process. Our findings provide insights into molecular interplays between maternal stress and sex-steroid hormones that may have implications for reproductive physiology and biology.

Key Words: RNA-seq, oviduct epithelium, cortisol, E2, P4

P279 Effect of chicory flour on inflammation and gut permeability in weaned piglets. T. Kulkarni[1,2], P. Siegien[1], P. Lemal[1], E. Arévalo Sureda[3], J. Wavreille[4], B. Cudennec[2], A. Lucau[2], N. Everaert[3], R. Ravallec[2], and M. Schroyen*[1], [1]*ULiège, Gembloux, Namur, Belgium,* [2]*ULille, Lille, Hauts-de-France, France,* [3]*KU Leuven, Leuven, Brabant, Belgium,* [4]*CRA-W, Gembloux, Namur, Belgium.*

Dysfunction of the host-microbial balance together with an impaired intestinal barrier, often found in weaned piglets, can challenge the immune system by initiating inflammatory mechanisms and increasing the risk of antigen penetration. Inulin, a well-known prebiotic with a positive effect on gut integrity, is commonly extracted from chicory root. The objective of this study was to compare the effect of chicory flour to inulin on porcine gut health at the few weeks after weaning. In this study, 2 in vivo experiments (E1 and E2) were performed, consisting of 72 male piglets each, weaned at D21 and subsequently divided in 3 groups: control (Ctrl), inulin (IN) and chicory flour (CHI). Along with *ad libitum* feed, daily supplementation was done by oral force-feeding with increasing 'inulin content' weekly (W) (W1: 0.5g/day, W2: 1g/day, W3: 1.5g/day) (E1) or double this dose (E2). On D29 and D43, 8 piglets per treatment were euthanized for blood and colonic tissue collection. Xylose was fed to the piglets 1 h before blood collection. For both experiments, 48 genes (inflammatory and barrier integrity genes) in the colon were analyzed by high-throughput qPCR. For both, E1 and E2, only on D43, IN and CHI piglets showed significantly lower serum Xylose concentration together with an upregulation of MUC2 (E1) and MUC1 (E2) in IN and CHI on D43. In E1, on D43, IN significantly upregulated the TLR2, TLR4, IFNα, and IL1β genes which explains that neither CHI nor IN had a positive effect to lower the inflammation at this dosage. Doubling the dose in E2 showed significant downregulation of TANK, IFNγ, and CXCL10, at D29, while at D43 both CHI and IN proved to be beneficial at this level of dosage, as the inflammation signaling and inflammatory targets genes NFκB1, DEFβ4A, TLR2, and IFNα were significantly downregulated. Since the inulin content was equated in both groups, and only CHI induced lower expression for most of the proinflammatory genes as compared with IN, other components than inulin present in CHI must have affected the inflammatory pathways. Therefore, CHI might be an interesting alternative to IN to improve gut health in weaned piglets.

Key Words: pigs, animal health, nutrigenomics, qPCR

P280 Enhanced prime editor by prolonging its expression and affecting strands discrimination in mismatch repair via harnessing episomal element. X. Han*[1], G. Zhao[1], Y. Xiong[1], R. He[1], Y. Su[1], S. Li[1], Y. Liu[1], C. Zhao[1], X. Xi[1], X. Wang[1], H. Wang[1], S. Xie[1], X. Li[1,2], J. Ruan[1,3], S. Zhao[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, Hubei, China,* [3]*Hubei Hongshan Laboratory, Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, Hubei, China,* [2]*The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan, Hubei, China.*

The ability to introduce desired mutations with minimal risk into human genome is critical for gene editing therapy of human diseases. The newly reported prime editor 2 (PE2) possess the ability to install all types of targeted DNA base pair substitution, small insertions/deletions (indels) without requiring double-strands breaks (DSBs) or donor DNA templates, but its applications are limited by low editing efficiency. DNA mismatch repair (MMR) was found to impede prime editing and promotes undesired indel byproducts. Here, we report the development of episomal prime editor 2 (epiPE2) in which prolongs the temporal expression of PE and affects strand discrimination in MMR by altering the methylation level of CpG sequence downstream of the targeting region with an episomal element EBNA1/Orip. epiPE2, in comparison with canonical PE2 (cPE2), enhanced the editing efficiencies by 2.0- to 12.2-fold while preserving low indel rates, and with regard to different editing types, the epiPE2 showed an average of 4.8×, 3.4×, 5.3× increase for small deletion, point mutation, and small insertion edits in HEK293T cells, respectively. And in HeLa cells, the epiPE2 system outperformed cPE2 with improved editing efficiencies by an average of 8.7-fold. Besides, we also compared epiPE2 with PE4max system, and the results showed epiPE2 enhanced the efficiency by an average of 3.2-fold than those of PE4max. Notably, cPE4max has the same relative low editing efficiency with cPE2 in longer insertion (HEK3+1Flagins, insert 24bp) while epiPE2 remain high editing efficiency in the same edit. What's more, efficient multiplex epiPE2 mediated precise gene editing, up to 10 genetic loci, were achieved in human cells. Overall, the study presents an efficient prime editor may enable applications in animal breeding, complex biological research and therapeutics.

Key Words: prime editing, episomal element, methylation

P281 Genetic improvement of litter size for dam line of Korean swine improvement network system. S. S. Lee*, S. M. Lee, and H. B. Yoon, *National Institute of Animal Science, Cheonan, Chungnam, South Korea.*

The Korean pig breeding industry consist of small and medium-sized breeding farms, with variable population sizes and inbreeding frequencies. It is crucial to assess the genetic parameters to understand their genetic potential. From 2016, the National Institute of Animal Science (NIAS) has organized a "National swine improvement network for dam line" with 12 nucleus farms and implemented a nationwide pig breeding program for dam lines of Landrace and Yorkshire. The aims of this project are selection of superior breeding stock suitable for Korean breeding objectives and keeping the genetic connectedness sustainably across the breeding farms by allowing exchange of genetic resources. This study aimed to investigate the genetic parameters of the litter size of Korean Landrace and Yorkshire breeds and to assess the performance of shared semen of selected animals. Pedigree records and performance data from 1989 to 2022 were collected from Korean breeding stock registration system. Two reproduction traits, namely total number of piglets born (TNB) and number of piglets born alive (NBA), were analyzed. Genetic evaluation has been done using multiple traits animal model with BLUPF90 program to estimate inbreeding coefficient, phenotypic value (PV) and estimated breeding value (EBV). After selection, total 58 selected sires were sent to 4 AI centers and then semen has been propagated to joining farms from 2018 to 2022. In 2022, the average PVs of TNB and NBA in shared semen population in Yorkshire were 15.1 and 14.2, whereas the average PVs of TNB and NBA in farm-based population were 14.6 and 13.1, respectively. The total average EBVs of TNB and NBA in shared semen population in Landrace were 0.66 and 0.72, whereas the total average EBVs of TNB and NBA in farm-based population were 0.48 and 0.50, respectively. The result shows that the nationwide-selected semen has better PV and EBV of litter size traits than farm-base selected semen. This research was supported by National Institute of Animal Science, Korea (No: PJ01670305).

Key Words: pig, Landrace, Yorkshire, litter size, estimated breeding value

P282 Estimation of variance components and GWAS for individual birth weight in Duroc pigs. S. M. Lee*, S. S. Lee, and H. B. Yoon, *Animal Genetics & Breeding Division, National Institute of Animal Science, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

In this study, the variance components of individual birth weight (IBW) in Korean Duroc pigs was estimated, the efficiency of genomic prediction and GWAS using SNP genotyping panels was investigated. A total of 20,070 IBW phenotypic records were used to identify a statistical genetics model to estimate variance components, and 1,029 genotypes (Illumina Porcine SNP60K v2: 818 and Affymetrix Axiom 650K: 211) were found to match with these records. Genotyping panels were imputed Illumina Porcine SNP60Kv2 using FImpute V3 (Sargolzaei et al., 2014), finally were used to analyze 43,861 SNPs in 923 genotype records. To estimate the genetic parameters, a linear animal model considering fixed effects (gender, sibling group, and parity) was analyzed with ASREML4.1 (Gilmour et al., 2015) software. In addition, deregressed estimated breeding values (DEBV), which includes parental effects, were re-estimated to be used as response variables in GWAS using estimated genetic parameters, estimated breeding value (EBV) and accuracy (ACC). GWAS used Bayesian methods (BayesB and BayesC), to estimate SNP marker effects. The average and standard deviation of IBW were 1.53 ± 0.3 kg, and the heritability was estimated to be 0.33. ACC was estimated to be the highest at 0.354 from the response variable DEBV of BayesC (π = 0.99). Upon examination of the excavated region in GWAS, the most significant region was discovered in the 25 Mb region of SSC16. Informative SNPs contained within this region were identified as ASGA0072766, ALGA0089851, and ALGA0089849, and the explanatory power of this region was estimated to be 2.83, 2.81, and 3.87% at π values of 0.50, 0.75 and 0.99 in BayesB with DEBV. The PTGER4 gene located in the SNP region, which is included in the results. Therefore, the PTGER4 gene, which is estimated to have a high genetic variance ratio in all phenotypes of the Duroc pig, is expected to have a significant impact on the selection of the Duroc pig's genome for IBW. This research was supported by National Institute of Animal Science, Korea (No: PJ01670306) and RDA Research Associate Fellowship Program.

Key Words: Bayesian method, Duroc pig, GWAS, individual birth weight, variance components

P283 Individual and population diversity of 20 representative olfactory receptor genes in pigs. M. Kang, B. Ahn, S. Youk, and C. Park*, *Department of Stem Cell and Regenerative Biotechnology Graduate School of Konkuk University, Seoul, Republic of Korea.*

Understanding the influence of genetic variations in olfactory receptor (OR) genes on the olfaction-influenced phenotypes such as behaviors, reproduction, and feeding is important in animal biology. However, our understanding of the complexity of the OR subgenome is limited. In this study, we analyzed 1,120 typing results of 20 representative OR genes belonging to 13 OR families on 14 pig chromosomes from 56 individuals belonging to 7 different breeds using a sequence-based OR typing method. We showed that the presence of copy number variations, conservation of locus-specific diversity, abundance of breed-specific alleles, presence of a loss-of-function allele, and low-level purifying selection in pig OR genes could be common characteristics of OR genes in mammals. The observed nucleotide sequence diversity of pig ORs was higher than that of dogs. To the best of our knowledge, this is the first report on the individual- or population-level characterization of a large number of OR family genes in livestock species.

Key Words: pigs and related species, DNA sequencing, sequence variation, olfactory receptor

P284 Withdrawn

P285 Wild boar and domestic pig distinguishing using SNP markers – preliminary studies. A. Piestrzynska-Kajtoch*, M. Natonek-Wisniewska, A. Koseniuk, A. Bieniek, B. Kleczek, J. Wolkowicz, P. Krzyscin, and A. Radko, *National Research Institute of Animal Production, Balice, Malopolska, Poland.*

The domestic pig is a meaningful source of global meat production and very important farm animal. Wild boar is the domestic pig ancestor. It is believed that multiple domestication events occurred separately in Asia and Europe. Moreover, crossbreeding between pigs and wild boar was documented—intentional for hunting purposes (hybrids) and accidental mixing of local populations of these 2 subspecies. All that makes those 2 subspecies evolutionarily, historically and genetically closely related. Distinguishing between animal species and subspecies is important from the economic and socio-religious point of view, but also for reasons of food safety, quality and meat adulteration. Distinguishing domestic pig and wild boar based on genetic markers is a challenge for laboratories around the world. The aim of our study is to create the SNP panel for these 2 subspecies distinguishing. To achieve the goal, we investigated 60 SNP markers, chosen on the basis of previous studies and scientific literature. By using reference sequences from GenBank and Ensembl, we designed custom TaqMan MGB genotyping assays (*Applied Biosystems*). We have analyzed SNPs using TaqMan OpenArray genotyping plates and QuantStudio 12K Flex Real-time PCR System (*Applied Biosystems*). We used the DNA of wild boars and several pig breeds from Poland. Some markers seemed to be very promising, e.g., markers located in genes *NR6A1, MC1R, lcnRNA, ACER1, SAF2B, GHRL, PRKAG3* and *OPRK1*. *NR6A1* marker clearly distinguished wild boar from domestic pigs. Allele 1 (T) in *ACER1* marker had the frequency only about 3.8% in wild boar and between 74 and 99% in different pig breeds. Similar differences in allele frequencies between subspecies were observed for markersin *SAF2B*, *lcnRNA, GHRL, OPRK1*. Amplification failed completely for 2 markers. Preliminary analysis of the data showed that some of the selected SNP markers were monomorphic across the entire study group and several SNPs showed low variability or clustering problems. Concluding, some of the markers could be potentially used in SNP panel for wild boar and domestic pig distinguishing.

Key Words: domestic pig, wild boar, SNP, OpenArray, subspecies distinguishing

P286 Detection of porcine testicular cells using ATAC-Seq and gene expression profiles. Y. Lian[1], S. Lukassen[2], J. Liebig[2], A. Sanchez[1,3], E. Rodriguez-Sierra[4], C. Lewis[4], C. Conrad[2], and A. Clop*[1,5], [1]*Centre for Research in Agricultural Genomis CRAG (CSIC-IRTA-UAB-UB), Cerdanyola del Valles, Catalonia, Spain,* [2]*BIH at Charité-Universitatsmedizin Berlin, Berlin, Germany,* [3]*Autonomous University of Barcelona, Cerdanyola del Valles, Catalonia, Spain,* [4]*PIC Europe, Sant Cugat del Valles, Catalonia, Spain,* [5]*Consejo Superior de Investigaciones Cientificas, Barcelona, Catalonia, Spain.*

Spermatogenesis is a complex developmental process involving multiple germline and somatic cell types that occurs in the testicle and leads to spermatozoa. The function, morphology and fertility of the sperm cell directly depend upon each of the molecular events that sequentially occur in each of the involved cell types, throughout spermatogenesis. Thus, the characterization of the transcriptomic and gene regulatory landscapes at a genomic level of the different cell types involved in spermatogenesis could provide a highly detailed understanding of the genetic and molecular basis of semen quality and male fertility. Using a protocol based on citric acid buffer, we isolated nuclei from flash frozen testicular samples from 4 adult Large White boars and sequenced their transcriptome and accessible chromatin using single cell multiome ATAC + Gene Expression technology. Data were preprocessed and quality filtered according to number of genes, number of counts, and mitochondrial read percentage. We obtained useful data from a total of 13,982 cells. We identified cell clusters containing between 1792 (spermatids) and 72 (T-lymphocytes) cells. Spermatogonia, spermatocytes, spermatids, Sertoli and Sertoli-like cells grouped in 2 distinct clusters each while, fibroblasts, endothelial, endothelial-like, Leydig, myoid, myeloid and T-cells, formed a separate cluster respectively. The 2 spermatid clusters showed the lower mean number of genes (983 and 1221) while Sertoli displayed the highest transcriptome complexity with a mean of 4674 genes. Further analyses are conducted to finally decipher porcine testicular cell populations.

Key Words: pig, testicle, single cell multiome, RNA, ATAC

P287 ISAG Bursary Award: A GWAS and RNA-Seq based analysis to shed light into the molecular and genetic basis of sperm cryo-tolerance in swine. Y. Lian*[1], M. Godia[1,2], J. E. Rodriguez-Gil[3], A. Castello[1], M. Yeste[4], S. Balasch[5], X. Barrera[6], C. Lewis[7], A. Sanchez[1,3], and A. Clop[1,8], [1]*Centre for Research in Agricultural Genomics, Cerdanyola del Vallès, Catalonia, Spain,* [2]*Wageningen University & Research, Wageningen, the Netherlands,* [3]*Autonomous University of Barcelona, Cerdanyola del Vallès, Catalonia, Spain,* [4]*University of Ginora, Girona, Catalonia, Spain,* [5]*Grup Gepork S.A., Les Masies de Rada, Catalonia, Spain,* [6]*Semen Cardona S.L., Cardona, Catalonia, Spain,* [7]*PIC Europe, Sant Cugat de Valles, Catalonia, Spain,* [8]*Consejo Superior de Investigaciones Cientificas, Barcelona, Catalonia, Spain.*

Sperm cryopreservation is very useful for animal breeding as it allows both storing genetic resources for long periods of time and transport to remote locations. In swine, this technology is not widely used, in part due to the high inter-individual variability on sperm cryotolerance, which has, in turn, been linked to several factors such as oxidative stress, DNA damage and impaired calcium homeostasis or mitochondrial function. Sperm cryodamage has a negative effect on acrosomal function and motility. We undertook a GWAS and RNA-Seq with the aim to characterize the molecular basis of sperm cryotolerance in pigs. We measured sperm viability, acrosome integrity and motility after a 5- and a 90-minute incubation at 37°C, in fresh and paired cryopreserved ejaculates. Cryotolerance was evaluated as the ratio between the cryopreserved and the paired fresh samples for each of these semen parameters. The GWAS, done on 99 samples and 371,209 SNPs, showed 2 genomic regions with chromosome-wise significant association with the ratio of total motility at 5-min incubation. One region harbored *TP73*, a candidate gene related to oxidative stress, spermatogenesis and fertility. The RNA-Seq from 20 ejaculates, revealed correlation between the RNA levels of 754 genes and at least one ratio. Genes showing the strongest or largest number of correlations included some linked to mitochondrial function (*BNIP3*, *PLD6*, *SDHAF2*), acrosome integrity (*IQCD*, *MROH2B*), calcium homeostasis (*ATP2A2*) and DNA damage (*ATRIP*). We then generated a SNP co-association and a gene co-expression gene network and kept only the nodes and edges present in the 2 networks. Using this information, together with RNA:ratio correlations, additional expression and correlation filters and the functional relevance of the genes, we built a RNA regression model that included 11 genes and predicted 8 ratios. The strongest R-square involved the ratio of progressive motility measured after 90-min incubation (R-square = 0.87; $P = 0.002$). Our results provide support for a larger study to identify molecular markers to predict sperm cryotolerance in pigs.

Key Words: boar, sperm, cryo-tolerance, GWAS, RNA

P288 Genomic regions harboring signatures of selection associated with QTLs in South African pigs from different breeds and production environments. N. Hlongwane*[1,2], E. Dzomba[2], M. Van Der Nest[1], K. Hadebe[1], and F. Muchadeyi[1], [1]*Agricultural Research Council - Biotechnology Platform, Private Bag X5, Onderstepoort, 0110, South Africa,* [2]*Discipline of Genetics, School of Life Sciences,*

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South Africa has diverse populations ranging from intensively raised and artificially selected commercial breeds to indigenous and village pigs that are reared under low input extensive production systems. Information on signatures of selection is valuable and can be used to improve production and adaptability. The aim of this study was to identify signatures of selection in South African villages, commercial, indigenous, wild and Vietnamese Potbelly pigs using SNP genotypes generated from the Porcine SNP60K beadchip. We used the F_{ST}, *iHS*, *XP-EHH* and *HapFLK* methods to unravel the effects of selection. F_{ST} per marker analysis revealed SNPs associated with traits such as meat quality, glucose metabolism, growth factors, cytoskeletal and muscle development between both domestic populations as well as between wild and domestic breeds. The *iHS* showed some genomic regions harbouring selection footprints within the village pigs on chromosomes 1, 7, 8, 16, and 14 associated with traits of economic importance (growth, meat and carcass quality, reproduction) and adaptation (disease resistance, immune response) that were predominantly under natural selection. *XP-EHH* method revealed QTLs associated with meat and carcass quality, diseases, adaptability, reproductive, morphological and growth traits in the different pairs of populations. *XP-EHH* analysis between Warthog and village pigs revealed signatures mostly coming from the Warthog associated with *AMBP* (chr 1), *NPW* (chr 3), *PSD* (chr 14), and *TMRSS* (chr 9) genes. *HapFLK* method detected genes on chromosomes 2 (*CAT*) and 14 (*IDE*) harbouring diverse QTLs for traits such as age at slaughter, meat to fat ratio, body weight, teat number and litter size. The strong signals toward meat and carcass quality traits points to both natural and artificial selection pressures to improve these traits in the South African pigs. Overall, the results of this study demonstrate the role that selection provides in shaping the genomic landscape of domestic and wild pigs and enhances our understanding of the relevance of the phenotypic and genetic diversity in these populations.

Key Words: porcine, genes, traits

P289 Analysis of the genetic variation in mitogenome sheds light on the ancestry of Tanzanian indigenous pigs. G. M. Msalya*[1], A. C. Adeola[2,3], L. Ajuma[2,3], Z. F. Cai[2], D. Mauki[4], T. T. Yin[2], M. S. Sheng[2,3], and Y. P. Zhang[2,3], [1]*Department of Animal, Aquaculture, and Range Sciences (DAARS), Sokoine University of Agriculture (SUA), Morogoro, Tanzania,* [2]*State Key Laboratory of Genetic Resources and Evolution and Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China,* [3]*Sino-Africa Joint Research Centre, Chinese Academy of Sciences, Kunming, China,* [4]*Center for Cancer Immunology, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences (CAS), Shenzhen, China.*

The population structure and evolutionary history of African indigenous pigs are of particular interest and fundamental for understanding their genetic diversity. Studies to evaluate phylogenetic relationships among Tanzania indigenous pigs (TIPs) may be lacking or extremely rare. Herein, we analyze complete mitogenome sequences of 67 indigenous pigs from selected geographic regions in Tanzania. The mitogenome assembly was done by the extraction of mitochondrial reads from whole genome sequence data. The resultant bam files were then sorted and reconverted to Fastq files using both SAMtools and bedtools programs, respectively. The mitogenome contigs were extracted through MEGAHIT software. Additional editing and assembly of the sequences was done using both Bio edit and Aliview programs, respectively. For phylogenetic analysis study, additional mitogenomes of 54 wild and domestic pigs from Far East, Near East, and Europe were downloaded from public database. The maximum likelihood phylogenetic tree for nucleotide alignments were constructed. Evidence from phylogenetic analysis showed that most of the Tanzanian indigenous pigs clustered in clade D, which represents wild and domestic pig from East/South-east Asia. Other individuals clustered in clade E with European domestic pigs. This further confirms the East/South-east Asian ancestry for domestic pig population in East Africa. Our study provides insights into Tanzanian indigenous pig's ancestry and diversity that would aid in future selection, breeding, and conservation programs.

Key Words: pig, mitogenome, phylogeny, genomic diversity, Tanzania

P290 Detecting copy number variation in a Korean composite pig breed, Woori-Heukdon populations. E. Cho[1], Y. Kim[1], H. Seong[1], S. Ha[2], H. Baek[2], J. Kim[2], S. Kwon[2], W. Park[1], D. Kim[3], D. Seo*[3], and J. Choi[2], [1]*Swine Science Division, National Institute of Animal Science, Rural Development Administration, Cheonan, South Korea,* [2]*Department of Animal Science, College of Animal Life Sciences, Kangwon National University, Chuncheon, South Korea,* [3]*TNT Research Institute, Jeonju, South Korea.*

One of the Korean synthetic pig breeds, Woori-Heukdon was developed by crossbreeding Korean Duroc (DUC), Korean native pig (KNP), and their crossbred populations (F1 and F2). In this study, we detected copy number variations (CNVs) with PennCNV algorithm, using the Illumina porcineSNP60 BeadChip array data of a total of 2,112 pigs including the crossbred and the parental populations. For further analysis, CNVs with consecutive SNPs ≥3 and CNVs length ≥10 Kb were retained, while 287 out of a total of 2,112 pigs were filtered out; consequently, a total of 1,825 pigs were used in further identifying CNVs. Overlapped CNVs were defined as CNV region (CNVR) manipulated with CNVruler. As a result, we retrieved a total of 1,717 CNVRs (261 gains, 1,304 losses and 152 mixed) from 10,375 CNVs (3,092 gains and 7,283 losses). Of those CNVs, there were more loss-CNVRs observed than the other ones among all populations except F1 population. The highest number of CNVRs were exhibited in DUC (828 CNVRs), followed by WRH (558 CNVRs) and F2 (114 CNVRs), and the lowest number of CNVRs in F1 (23 CNVRs). The average size of the CNVRs showed that DUC was the largest at 213.89 Kb and F1 was the smallest at 98.32 Kb. The number and length of CNVRs decreased in F1. Among all the pig autosomes, the DUC had the highest coverage on SSC14 (12.40%) and KNP and F1 on SSC5 (3.09%) and SSC11 (0.39%), respectively. Furthermore, KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (gene ontology) were assessed using DAVID. The KEGG pathway analysis showed that that CNVRs were various pathway including thyroid cancer, Nicotinate, and nicotinamide metabolism, and GO pathway showed the highest fold enrichment in locomotion function in biological process (BP), nuclear periphery function in cellular component (CC) and molecular function (MF). Our finding CNVs in the Korean pig populations would enrich CNV map for porcine species, and these results might be useful for a better understanding of phenotypic and genomic characteristics that are associated with economic traits in porcine.

Key Words: Woori-Heukdon, Korean native pig, Illumina PorcineSNP60 BeadChip, copy number variation (CNV), crossbreeding

P291 PIG-PARADIGM Host Pillar: Toward elucidating the interactions between the intestinal microbiome and host factors to determine their separate and combined influence on intestinal health in pigs. P. Karlskov-Mortensen*, J. P. Nielsen, M. K. Morsing, B. Guldbrandtsen, C. B. Jørgensen, and M. Fredholm, *University of Copenhagen, Frederiksberg, Denmark.*

PIG-PARADIGM is a cross disciplinary project established among Aarhus University, Denmark, Wageningen University, the Netherlands, UC Davis, USA, and University of Copenhagen, Denmark. The project encompasses 4 research pillars focused on the host, the microbiome, nutrition, and data integration with the overarching aim of reducing the need for antibiotics and the risk of AMR pathogen emergence and spread in pork production. Here we introduce the Host Pillar of the project. The pillar is divided into 3 research themes: One focuses on in-depth clinical characterization of the pigs from birth until 30 kg of live weight. By clinical diagnostics, qPCR diagnostics, and microbiome sequencing, we will develop a score, which differentiates between pigs with superior and inferior intestinal robustness. In the second theme, we integrate information from immunological and molecular phenotypes like the metabolome, transcriptome and metagenome with clinical phe-

notypes established in theme 1. This will identify host factors influencing intestinal health and clarify the individual and combined impact of host factors and the metagenome on intestinal and systemic health. The last theme relies on samples collected from pigs sacrificed at different ages during the trial. We will perform in-depth characterization of the interaction between the intestinal barrier and the microbiome in robust and non-robust pigs. This includes studies of pathology studies glycosylation pattern of mucins to elucidate whether differences in therein explain differences in susceptibility toward specific pathogens. This theme feeds into the Microbiome Pillar by delivering intestinal samples for characterization of the development of the metagenome in robust and non-robust pigs. Altogether, this pillar will elucidate the complex interactions between the intestinal microbiome and host factors and determine their separate and combined influence on intestinal health and resilience.

Key Words: pig, microbiome, antimicrobial resistance, multi-omics, host-pathogen interaction

P292 Copy number variation in porcine KIT locus affecting coat color detected with read depth analysis and digital PCR. M. Zorc*[1], M. Candek-Potokar[2], U. Sivka[3], N. Toplak[3], A. Tansek[1], and P. Dovc[1], [1]*University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia,* [2]*Agricultural Institute of Slovenia, Ljubljana, Slovenia,* [3]*Omega d.o.o., Ljubljana, Slovenia.*

KIT (Tyrosine kinase receptor) is an important regulatory molecule involved in the development and homeostasis of various cell systems. KIT is involved in the process of melanocyte colonization of the developing epidermis, and several mutations in the KIT gene associated with pigmentation have been detected in humans, mice, cattle, horses, cats, dogs, and pigs. It was suggested that a 4.3-kb duplication located approximately 100 kb upstream of the KIT locus in Hampshire pigs was the causative mutation for the belted phenotype. Later whole genome sequencing revealed a 450 kb duplication (DUP1) encompassing the entire KIT gene, a 4.3 kb duplication (DUP2) approximately 100 kb upstream of the KIT locus, a 23 kb duplication (DUP3) 100 kb downstream of KIT, and another 4.3 kb duplication (DUP4) within DUP3. Here we report the results of read depth analysis (Illumina short read sequencing) of breed-specific DNA pools and digital PCR analysis (Applied Biosystems QuantStudio Absolute Q Digital PCR System) of individual samples to identify copy number variants associated with the KIT locus in white-belted Slovenian autochthonous Krskopolje pigs, white-belted Swäbisch-Hall pigs, pigs representing the white commercial pig line, and wild boar. We identified DUP1–4 in white and white-belted pigs, but not in wild boars. However, using a highly sensitive dPCR method, 2–8 copies of DUP2 and DUP4 were detected in white and white-belted pigs, confirming the structural complexity of the KIT locus in pigs.

Key Words: KIT locus, copy number variation, digital PCR, pig, coat color

P293 A deeper screening of Bazna pigs genome revealed a significant contribution of Mangalitza pigs to their genetic background. V. A. Balteanu*[1], T. Figueiredo Cardoso[2], A. Zsolnai[3], and M. Amills[2], [1]*University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Institute of Life Sciences, Cluj-Napoca, Cluj, Romania,* [2]*Center for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB), Campus Universitat Autònoma de Barcelona, Bellaterra, Catalonia, Spain,* [3]*NARIC-Research Institute for Animal Breeding, Nutrition and Meat Science (ÁTHK), Herceghalom, Budapest, Hungary.*

Many local European pig breeds experienced in the last century a sustained demographic decline or disappeared due to the progressive abandonment of traditional pig production systems. An increased market demand for cured-ham was a turning point in Mangalitza breed survival. In Romania, an old Red Mangalitza population of around 200 pigs still survives. A similar decline was faced by Bazna pigs, a Romanian fatty pig breed with a black coat and white belt. Despite its similar appearance with other belted breeds, it is presumed to originate in 1872 crosses between Mangalitza and Berkshire. They were subsequently improved with British (ex. Wessex or Large White) and German (ex. Angeln Saddleback) breeds and officially recognized in 1958. Therefore, a fundamental conservation question arises *i.e.* which white belted pigs are the real Bazna? To verify this admixed origin theory, which is an essential step to implement reliable conservation programs, we first sequenced the *MT-CYB* gene in Bazna (n = 48) and Red Mangalitza (n = 48) from the Romanian population versus 48 Red, Blond and Swallow-belly Mangalitza pigs with Hungarian origin and 10 Vietnamese pigs. Several *MT-CYB* sequences available from Berkshire, Hampshire, Landrace, Large White, Pietrain and Meishan pigs were also used. Additionally, a Porcine SNP60 BeadChip data set was used to compare the autosomal background of Bazna pigs versus Mangalitza and British breeds or Romanian wild boar. Mitochondrial DNA variation was low in Mangalitza. In contrast, a higher diversity was noticed in Bazna. The NJ tree evidenced 2 main clusters, European (Mangalitza and wild boar) and Asian (Vietnamese and Meishan). In the European cluster few Bazna pigs clustered within the Mangalitza group, suggesting Mangalitza maternal contribution. Other Bazna pigs formed distinct sub-clusters close to some British pigs. Several Bazna pigs carried Asian haplotypes introgressed via British breeds. The autosomal SNP data analysis revealed an important contribution of Mangalitza colored varieties and British breeds to Bazna pigs genetic makeup, in agreement with mtDNA data and historical records.

Key Words: Bazna pig, admixed origin

P297 Withdrawn

P298 Withdrawn

P299 ISAG Bursary Award: Enhancer-promoter interaction map in the maternal-fetal interface during implantation reveals important regulatory regions and variations in pigs. Y. Sun*[1,2], R. Liu[1,2], H. Liang[1,2], K. Han[1,2], F. Wang[1,2], J. Cao[1,2], and M. Yu[1,2], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei, China,* [2]*College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China.*

Litter size, one of the most important reproductive traits of pigs, has pronounced effects on the profit of husbandry enterprises' enthusiasm of breeders. In addition, the litter size of pigs was directly affected by embryo implantation failure. Genome-wide association studies (GWAS) have successfully identified genetic variants associated with complex traits and diseases in the past decade. However, ~88% of those variants from GWAS studies are in non-coding regions of the genome and have been challenging to interpret. This study conducted RNA-Seq, ChIP-Seq, BL-HiC, and BL-HiChIP on luminal epithelium cells (LE) and endometrial tissue at d 12 (GD12) and d 15 of gestation (GD15). First, the differentially expressed genes (DEGs) were identified. Then, we identified active promoter regions and potential enhancer regions on the genome by H3K4me3 and H3K27ac modification. Next, the enhancer-promoter interactions were defined by combining Hi-C and Hi-ChIP data. The genes regulated by enhancer-promoter interactions were identified, including 642 DEGs between GD12 and GD15. Subsequently, we used HOMER to identify TF motif enrichment at the loop anchors within the 642 DEGs. Key TFs, such as C/EBP-β and NR4A1, were highly enriched in the loop anchors in the upregulated genes. Finally, we identified SNPs that locate in the transcription factor motifs and affect gene expression by altering the transcription factor binding. In conclusion, the high-resolution enhancer-promoter interaction map of pig endometrial tissue was constructed, and the key regulatory elements were identified. These findings provide insights in identifying the mechanisms of litter size in pigs.

Key Words: pig, litter size, embryo implantation, cis-regulatory elements, SNP

P300 Analysis of the genetic diversity of swine leukocyte antigen 1-linked olfactory receptor genes and analysis of correlation with reported porcine testicular expression levels. M. Kang*, B. Ahn, S. Youk, and C. Park, *Department of Stem Cell and Regenerative Biotechnology Graduate School of Konkuk University, Seoul, Republic of Korea.*

A large number of olfactory receptor (OR) genes are highly polymorphic and present in multiple clusters in the mammalian genome. They are mainly expressed in the olfactory epithelium but also expressed in several other tissues. The major histocompatibility complex (MHC) consists of the fastest evolving genes in the genome and maintains the highest inter-individual genetic diversity for specific immune reactions against a large number of foreign antigens. Although OR gene clusters are widely spread across the genome, some OR clusters have close linkage associations to MHC genes in mammals. To study the effect of evolutionary impact of MHC diversity on the evolution of other linked genes, we compared the genetic diversity between 8 MHC linked and 21 unlinked OR genes together with their reported levels of expression in pig testes. Genetic diversity of 4 highly expressed ORs (OLF42–1, OLF42–3, LOC100156552, LOC100514111; FPKM >0.05), 4 low-expression ORs (LOC100516811, LOC100522686, LOC100157348, LOC100516618; FPKM <0.01), and swine leukocyte antigen 1 (SLA1) encoding a MHC classical class I gene were assessed using polymerase chain reaction-sequence based typing (PCR-SBT) for 32 pigs of 6 different breeds. A total of 73 alleles with an average of 9.13 alleles per locus were identified from 8 MHC-linked OR genes which is higher than those of 21 MHC unlinked OR genes with an average allele number of 6.33, suggesting the presence of the possible effects of the linkage disequilibrium (LD) with MHC. Interestingly, a highly expressed OR, OLF42–3, showed LD in all analyzed breeds (n = 5), and further analyses is ongoing with more individuals for other OR genes. MHC-linked OR might play a role in reproduction considering that MHC-linked OR genes are also expressed from seminiferous tubule, sperm, and oocyte cumulus cells although their functions are unclear. OR genes linked to the MHC region may also contribute to the maintenance of specific MHC haplotypes and MHC-OR relationships.

Key Words: pigs and related species, DNA sequencing, linkage disequilibrium, olfactory receptor, MHC

P301 ISAG Bursary Award: Integrated analysis of genome-wide association studies and 3D epigenomic characteristics reveal the *BMP2* gene regulating loin muscle depth in Yorkshire pigs. S. Wan*[1], Y. Miao[2], Y. Zhao[1], S. Zhao[1], X. Xu[1], and T. Xiang[1], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture, Huazhong Agricultural University, Wuhan 430070, Hubei Province, China,* [2]*Research Institute of Agricultural Biotechnology, Jingchu University of Technology, Jingmen 448000, Hubei Province, China.*

The lack of integrated analysis of genome-wide association studies (GWAS) and 3D epigenomics restricts the deep understanding of genetic mechanisms of meat-related traits. With the application of such techniques as ChIP-seq and Hi-C, the annotations of *cis*-regulatory elements in the pig genome have been enriched, which offers a new opportunity to elucidate the genetic mechanisms and identify major genetic variants and candidate genes that are significantly associated with the important economic traits. Among these traits, loin muscle depth (LMD) is an important one as it largely affects the lean meat content. In this study, we integrated *cis*-regulatory elements and genome-wide association studies (GWAS) to identify the key gene and genetic variants regulating LMD. Five single nucleotide polymorphisms (SNPs) located on porcine chromosome 17 were identified to be significantly associated with LMD in Yorkshire pigs by GWAS. A 10 kb quantitative trait locus (QTL) was identified as a functionally important region by integrating linkage disequilibrium and linkage analysis (LDLA) and high-throughput chromosome conformation capture (Hi-C) analysis. Based on the results of GWAS, Hi-C, and *cis*-regulatory elements, the *BMP2* gene was identified as the major gene

regulating variation in LMD. Furthermore, through using dual-luciferase assays and electrophoretic mobility shift assay (EMSA), 2 SNPs, SNPs rs321846600 and rs1111440035 were identified as candidate SNPs that may be functionally related to the LMD QTL in Yorkshire pigs. Our results shed light on the advantage of integrating GWAS with 3D epigenomics in identifying major genes for quantitative trait. This study is the pioneering work to identify the major genes and related genetic variants regulating one key production trait (LMD) in pigs by integrating genome-wide association studies and 3D epigenomics.

Key Words: GWAS, epigenomics, integrated analysis, loin muscle depth, pig

P302 Methods to predict lameness in sows. G. A. Rohrer*[1], L. Ostrand[2], L. A. Rempel[1], T. Schmidt[2], and B. Mote[2], [1]*USDA-ARS US Meat Animal Research Center, Clay Center, NE,* [2]*University of Nebraska, Lincoln, NE.*

The objective was to identify traits recorded on gilts at 5 mo of age predictive of future lameness. Mobility was measured using a pressure-sensing mat (GAIT4) and 7 d of video recorded daily activity (NUtrack). Gilts (n = 3659) were assigned codes to describe their lifetime soundness. Animals retained for breeding and never detected with mobility issues were recorded as sound (SND), while retained animals that became lame were coded as lame sow (LSW). Culled gilts were in 3 categories: culled for leg structure (STR), visibly lame gilt (LGT) and other reasons (CLL). GAIT4 system creates a series of measurements for each foot related to pressure, duration and step length of each foot and a lameness score for each foot. Traits used to predict an animal's mobility status summarized values for all 4 feet: average step length, average stance time, standard deviation of stance time, variance of lameness score and variance of total scaled pressure. NUtrack measurements were rotations, velocity, distance walked, and times spent eating, sitting, standing, lying sternal, and lying lateral. Mixed model analyses were conducted in R fitting fixed effects of breed of sire, contemporary group and soundness score, with animal fit as a random effect. Heritability was estimated using animal effects from R models as phenotypes in WOMBAT, with 3 generations of pedigree. Analyses of GAIT4 measures found LGT and STR gilts had longer average stance time, greater variance of lameness scores and took shorter steps; estimates of heritability ranged from 0.23 to 0.28. NUtrack measurements predictive of soundness score were time eating, time standing, time lying lateral, distance walked and rotations. LGT and STR were less active and spent more time lying lateral than other animals. In addition, SND animals had more rotations and tended to have greater distance than LSW. Estimates of heritability for NUtrack measurements ranged from 0.21 to 0.31. Overall, NUtrack traits at 5 mo of age predicted soundness beyond gilt status and were heritable providing producers with traits to select gilts and improve mobility of future generations of pigs. USDA is an equal opportunity employer.

Key Words: pig, lameness, prediction, heritability

P303 Identification of genomic regions associated with fatty acid metabolism across four tissues in pigs. J. Liu*[1,2], C. Sebastià[1,2], T. Jové-Juncà[3], R. Quintanilla[3], O. González-Rodríguez[3], M. Passols[1,2], A. Castelló[1,2], A. Sánchez[1,2], M. Ballester[3], and J. M. Folch[1,2], [1]*Plant and Animal Genomics, Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB Consortium, Bellaterra, Spain,* [2]*Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain,* [3]*Animal Breeding and Genetics Program, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Torre Marimon, Caldes de Montbui, Spain.*

Fatty acids (FAs) are components of lipids and have important roles such as structural components of cell membranes, cellular fuel sources, and precursors of signaling mediators. This study aims at identifying potential genomic regions associated with FA profiles in several tissues and explores their role on whole body metabolism in pigs. A total of 432 commercial Duroc pigs were employed in the present work. Samples of blood were collected at 60 ± 8 d of age to extract the plasma. In addition, samples of adipose tissue (backfat), liver, and *gluteus medius* muscle were collected after slaughter (180–200 d of age). All animals were genotyped with the *GGP Porcine HD Array* (*Illumina*). Genotypes were imputed from the whole-genome sequences of 100 animals and SNPs with MAF <5% or missing genotypes >10% were removed. GWAS was performed between the 9,751,141 resulting SNPs and FA composition traits by the *fastGWA* tool of *GCTA v1.94.0*. The genomic regions containing at least 3 significant consecutive SNPs with distances <1 Mb were selected for gene annotation. The GWAS results showed a common interval at SSC2: 7.56–14.92 Mb associated with the Desaturase 5 activity in liver, backfat and muscle. Another interval located at SSC14: 103.81–115.64 Mb was identified for backfat and muscle FA composition, including UFA, SFA, C18:0, C18:1n7, C16:1n7/C16:0, and C18:1n9/C18:0. In addition, backfat-specific intervals were identified at SSC6: 146.07–148.36 Mb for MUFA, SFA, UFA, C18:1n9, and C18:1n9/C18:0 and at SSC4: 2.53–14.55 Mb for C14:0, C20:1n9, C20:2n6, C20:3n3, and C16:0/C14:0. In SSC15, a region at 86.99–101.29 Mb was associated with liver C18:4n3/C18:3n3. Finally, for plasma, the specific region SSC14: 118.92–124.75 Mb was associated with C18:0/C16:0. The current results increase our knowledge of the genetic architecture of FA-metabolism traits and will be useful in selection programs to improve health and energy metabolism in pigs. This study is part of the METAPIGEN (PID2020-112677RB-C21-22) and H2020 GENE-SWitCH (grant agreement n° 817998) projects.

Key Words: pig, genome-wide association, lipid, genetic marker, candidate gene

P304 Initiative for African indigenous pig genome project. A. C. Adeola*[1,2], X. Shi[1], X. Liu[3], O. F. Olaniyan[4], C. A. M. S. Djagoun[5], G. Msalya[6], D. H. Mauki[7], N. K. Wanzie[8], G. Niba[9], P. D. Luka[10], S. C. Olaogun[11], V. M. O. Okoro[12], S. Zhao[13], J.-L. Han[14], M.-S. Peng[1,2], Y.-P. Zhang[1,2], [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, Yunnan, China,* [2]*Sino-Africa Joint Research Centre, Chinese Academy of Sciences, Kunming, Yunnan, China,* [3]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China,* [4]*West Africa Livestock Innovation Centre, Banjul, the Gambia,* [5]*Laboratory of Applied Ecology, Faculty of Agronomic Sciences, University of Abomey-Calavi, Cotonou, Benin,* [6]*Sokoine University of Agriculture, Morogoro, Tanzania,* [7]*Center for Cancer Immunology, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences (CAS), Shenzhen, China,* [8]*Department of Zoology, University of Douala, Douala, Cameroon,* [9]*National Centre for Animal Husbandry, Veterinary and Halieutic Training, Jakiri, Cameroon,* [10]*National Veterinary Research Institute, Vom, Nigeria,* [11]*Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria,* [12]*Department of Animal Science and Technology, School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Nigeria,* [13]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China,* [14]*International Livestock Research Institute, Nairobi, Kenya.*

African indigenous pig's history and adaptation to environmental and human selection pressure underlies their remarkable diversity. Assessing this diversity is an important step toward understanding the genomic basis of productivity and adaptation fitness under the African farming systems. At the Kunming Institute of Zoology – Chinese Academy of Sciences, we have commenced international collaborative projects to improve our knowledge of African indigenous pig genomics. We collected about 1000 African indigenous pig samples and wild suids from 6 countries in sub-Saharan Africa and sequenced the genomes of 454 pigs. Population genomic analyses revealed large differences between East and West African populations, and gene flow from Eurasian populations was detected in African indigenous pigs. As this project progresses, we will focus on exploring the possibilities of introgression

from wild suids into African indigenous pig genomes which may provide insights into local adaptation including candidate genes associated with adaptation to diverse African local environments. We also plan to incorporate additional samples from other parts of Africa. Through the generation of African indigenous pig genomic data combined with the existing multi-omics data for subsequent analyses, this collaborative project aims to gain in-depth insights into the demographic history and adaptive evolution of African indigenous pigs. Therefore, we hope to establish a collaborative network of African scientists from multidisciplinary fields to work together and contribute to this important project to conserve and rationalize animal genetic resources in Africa

Key Words: African indigenous pig, population genomics, population structure, adaptation, genomic selection

P305 Multi-breed, multi-tissue, and multi-omics aiding the quest for key porcine regulators. D. Crespo-Piazuelo[1], A. Reverter[2], Y. Ramayo-Caldas[1], R. Quintanilla[1], H. Acloque[3], M.-J. Mercat[4], M. C. A. M. Bink[5], A. E. Huisman[5], and M. Ballester*[1], [1]*Animal Breeding and Genetics Program, Institute of Agrifood Research and Technology (IRTA), Torre Marimon, Caldes de Montbui, E08140, Spain,* [2]*CSIRO Agriculture and Food, St. Lucia, Brisbane, Queensland 4067, Australia,* [3]*INRAE GABI, Domaine de Vilvert, 78350 Jouy-en-Josas, France,* [4]*IFIP-Institut du porc and Alliance R&D, La Motte au Vicomte, 35651 Le Rheu, France,* [5]*Hendrix Genetics, P.O. Box 114, 5830 AC Boxmeer, the Netherlands.*

This study aims at identifying breed and tissue-specific key regulators in pigs by using co-expression and co-association gene network analysis. For that purpose, duodenum, liver, and muscle samples were obtained at slaughter from 300 pigs of 3 different breeds: Duroc, Landrace, and Large White (n = 100 each). Whole-genome sequencing and RNA-seq were performed on the Illumina NovaSeq6000 platform. RNA counts were quantified by RSEM/1.3.0 and normalized by TMM (trimmed mean of M-values). Lowly expressed genes and those missing in more than 20% of the animals were removed, remaining 13,891 genes expressed in duodenum, 12,748 genes in liver and 11,617 genes in muscle. Genetic variant calling, conducted with GATK/4.1.8.0 HaplotypeCaller, resulted in 44,127,400 polymorphisms (SNPs and indels) among all the individuals. After removing those variants with a minor allele frequency below 5% and more than 10% missing genotype data on each breed, 25,224,146 polymorphisms were kept among the 3 breeds. eGWAS were conducted using the fastGWA tool from GCTA/1.93.2. Gene co-expression networks were inferred using the PCIT algorithm and the list of genes encoding transcription factors (n = 1,109) and co-factors (n = 869), and the 100 most expressed genes in each tissue. Thus, a total of 2,248 genes remained to generate regulatory gene networks. Across breeds the transcription factors *CTCF*, *SP3*, and *TOX4* and the cofactors *CHTOP*, *ELOB*, and *NCL* were identifiedas the highest connected regulators in the duodenum, liver and muscle co-expression gene networks, respectively. Within breeds, *KHSRP*, *TOX4*, and *CSDE1* transcription factors and *HNRNPU, CRK*, and *CRK* co-factors showed the highest connectivity in Duroc, Landrace and Large White, respectively. Our results identified putative key regulatory genes that will be reassessed by the eGWAS analysis. These findings bring us closer to understanding gene expression regulation in porcine key tissues and will allow us to improve genomic evaluation procedures by considering this functional information. This study is part of the H2020 GENE-SWitCH project (grant agreement n° 817998).

Key Words: pig, genome regulation, network analysis, system genetics, RNA-Seq

P306 Identification of new transcription factors using eGWAS in four porcine tissues. S. Hosseini[1], M. Gòdia[1], M. Derks[1], B. Harlizius[2], O. Madsen[1], and M. Groenen*[1], [1]*Wageningen University & Research, Wageningen, the Netherlands,* [2]*Topigs Norsvin Research Center, Beuningen, the Netherlands.*

Efforts on porcine eGWAS have been mostly focused on a single tissue type, and although new meta-studies are now available, they use several porcine breeds coming from different genetic backgrounds, thus still masking regulatory regions of interest. In this study, we performed RNA-seq on 100 sows from a cross between 2 commercial pig breeding lines. RNA-seq was performed for 4 different tissues: liver, spleen, lung, and muscle, selected for their key roles in metabolism and immune response. The same animals were genotyped with the high-density (660K markers) Axiom Porcine Genotyping Array. With an average of 44 M reads per sample, we identified 12,680, 12,650, 13,310, 12,595 genes in liver, spleen, lung and muscle expressed with at least 1 CPM. After filtering the genotype data, 535,896 SNPs were kept for the eGWAS analysis. We identified several eQTL regions that included 2 or more significant SNP associations. We found 4,293, 10,630, 4,533, and 6,871 eQTLs for liver, lung, spleen, and muscle, respectively. As expected, a minority, 12, 6, 18, and 5% respectively, were classified as *cis*-eQTLs (<1 Mbp of their associated gene). Some of the most significant eGWAS peaks included *RDH16* in liver, involved in vitamin A metabolism and playing an important role in the regulation of feed efficiency in pigs by affecting energy metabolism, *TF* in lung, which plays a role in acute respiratory distress syndrome, and *OPLAH* in muscle associated with the regulation of energy metabolism in skeletal muscle. Interestingly, some *cis*-eQTL also had many trans eQTL effects and these *cis*-eQTL were often associated with transcription factors, indicating likely target genes. That is the case for *ZNF577* in liver, that despite its unknown function, belongs to a zinc finger protein family that is associated with adipogenesis and hepatic lipogenesis. Another example is *Erf* in spleen, that has been found to be required throughout hematopoietic (blood cells) development. In conclusion, our results show that eGWAS can help annotating new transcription factors involved in complex phenotypes of interest such as behavior, health, and robustness.

Key Words: pig, eQTL, RNA-seq, gene expression

P307 ISAG Bursary Award: Sequence based GWAS identifies novel loci influencing growth and reproduction traits in pigs. A. Boshove*[1], M. F. L. Derks[1,2], B. Harlizius[1], E. F. Knol[1], M. S. Lopes[3], M. van Son[4], and C. A. Sevillano[1], [1]*Topigs Norsvin Research Center, Beuningen, the Netherlands,* [2]*Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands,* [3]*Topigs Norsvin, Curitiba, Brazil,* [4]*Norsvin SA, Hamar, Norway.*

Genome-wide association studies (GWAS) based on large scale sequence data provide opportunities to map recessive deleterious variants in livestock populations using a non-additive model. Most deleterious variants segregate at relatively low frequency and therefore high sample sizes are required to identify these variants. In this study we report one of the largest sequence-resolution screens in pigs to date, with a total of 117,000 Large White animals imputed to sequence using a reference population of approximately 1,100 whole genome sequenced pigs. We imputed a total of 22,000,000 SNPs with high accuracies (R^2 > 0.9) even for low frequency variants (>1–5% minor allele frequency). Using this sequence data we performed both an additive and non-additive GWAS for several production and reproduction traits. We observe a clear difference in the QTLs found by the additive and non-additive models. We fine mapped known QTLs to identify causal variants using the additive model and revealed a new, relatively low frequent variant on chromosome 2 with a large effect on back fat. The non-additive model especially yielded novel low frequency variants affecting the fitness of animals (i.e., reduced growth, smaller litter size). One of the most notable deleterious variants we found is located on chromosome 2 with major impact on both growth and back fat in homozygous individuals. Additionally, we identified several independently segregating haplotypes with strong effects around the *MC4R* locus on chromosome 1, spanning only 3MB in size (1:59–62) and affecting both growth and back fat. Together we present a large-scale sequence-based association study that provides a key resource to identify novel variants for breeding and to further reduce the frequency of deleterious alleles.

Key Words: pigs and related species, genome-wide association, imputation, quantitative trait locus (QTL)

P308 ISAG Bursary Award: Allele-specific expression in pig genomic makeup and phenotypic implications. W.-y. Yao*[1,2], L. Bai[2], K. Li[2], L. Fang[3], M. A. M. Groenen[1], and O. Madsen[1], [1]*Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands,* [2]*Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China,* [3]*Center for Quantitative Genetics and Genomics (QGG), Aarhus University, Aarhus, Denmark.*

Allele-specific expression (ASE) is the imbalance in expression between parental alleles at the same locus, which can be identified and quantified by RNA sequencing. ASE is often associated with the cis-regulation of expression quantitative trait loci (eQTL). Therefore, identifying ASE variants can provide insight into the transcriptomic control of complex traits in pigs. This work, based on PigGTEx, has 3 objectives: (1) establish an atlas of ASE profiles among different breeds and tissues. (2) study the correlation between ASE and pig-eQTLand, (3) evaluate functional implications for specific sites and traits (e.g., introgression sites and GWAS). We selected 6,655 RNA samples, consisting of 23 pig breeds and 42 pig tissues from the Pig-GTEx RNA sampling. First, we developed a standardized computational pipeline to reduce reference mapping bias. The pipeline is based on the WASP method and provides accurate ASE profiling for large and complex RNA-seq data sets. We then used phASER software to identify the vast profile of ASE in different breeds and tissues as well as the haplotype level based on phASER Gene AE 1.2.0. The magnitude of the imbalance was quantified by allelic fold change(aFC), and the statistical significance of the imbalance was evaluated using binomial-based statistics. We detected a median number of 11,846 detectable expressed sites and 504 significant ASE sites for these RNA samples. We found many variations in ASE across tissues and breeds, ranging from 2.04% to 34.48%, suggesting complex genomic regulation of allelic expression. The highest ASE diversity was observed in the embryo and reproduction-related tissues. For future analyses, we will correlate the ASE with eQTL to decipher the effect of ASE. The identified ASE profiles in the breeds or tissues studied provide a valuable foundation for identifying the molecular regulatory codes driving complex traits and improving genomic prediction in pig breeding programs.

Key Words: pig, genome regulation, allele-specific expression, system genetics (eQTLs), complex trait

P309 ISAG Bursary Award: Comprehensive identification of functional DNA elements and 3D chromatin interaction map in the pig genome. D. Wang*[1], M. Hu[1], Y. Guo[1], R. Kuang[1], H. Zhou[1], R. Ma[1], Z. Han[1], L. Li[1], H. Peng[1], Z. Xu[1], Y. Zhang[1], M. Zhu[1,3], C. K. Tuggle[4], Y. Zhao[1], S. Zhao[1,2], [1]*Key Lab of Agricultural Animal Genetics, Breeding, and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei, China,* [2]*Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan, Hubei, China,* [3]*The Cooperative Innovation Center for Sustainable Pig Production, Wuhan, Hubei, China,* [4]*Department of Animal Science, Iowa State University, Ames, IA.*

As an important species in the livestock industry and biology research, the continuous development of pig genome studies provided a valuable resource to fundamentally enrich cis-regulatory element annotation and extend ENCODE and Roadmap Epigenomics projects in the research field of large animals. However, in contrast to the human and mouse ENCODE phase III, which further revealed landscapes of the 3-dimensional (3D) organization of chromatin and expanded into different developmental stages. On bias of that, we performed RNA-seq, ATAC-seq, ChIP-seq (H3K27ac and H3K4me3), in situ Hi-C and in situ ChIA-PET (RNA polymerase II and CTCF) in 11 diverse tissues of 2-week and 180-d Large White (LW) pigs (biological repeats n = 2), including longissimus muscle, backfat, heart, liver, spleen, lung, kidney, duodenum, pancreas, cerebrum and cerebellum. In total, 137 data sets were generated at present. For Hi-C experiments, in total, more than 19.45 billion paired-end reads were sequenced across all samples, providing more than 343 × coverage of the pig genome. Approximately 720 million paired-end reads sequenced for each sample. After filtering potentially artificial reads, we obtained about 5,318 million unique and valid contact reads, among which 3, 970 million reads were cis-contacts. We further explored the 3D structure of the pig genome and identified an average of 2,358 topologically associating domains (TADs) and 22,412 loops in each tissue of adult LW pigs. Based on chromatin interaction analysis of ChIA-PET data, we detected 31,719–70,939 long-range interactions between chromatin loci in the heart, spleen, muscle, and other tissues of 180-d LW pigs. Our research explored and revealed a comprehensive epigenomics map of gene expression, chromatin accessibility, cis-regulatory elements and chromatin interactions in 11 diverse tissues of LW pigs at different developmental stages, providing high-quality reference information for pig genome research.

Key Words: pig, regulatory elements, 3D chromatin interaction, epigenetics

P310 African swine fever infection enhances the host transcriptional regulation of membrane protein-encoding genes mediated by changes in chromatin state. X. Qi*[1], Y. Xiang[1], L. Sun[3,4], L. Xing[3], S. Zhang[1], Q. Zhao[1], L. Zhang[1], J. Li[1], P. Zhou[1], Z. Zheng[1], X. Li[1], L. Fu[1,2], G. Peng[3,4], and S. Zhao[1,2], [1]*Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education and Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China,* [2]*The Cooperative Innovation Center for Sustainable Pig Production, Wuhan, China,* [3]*State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China,* [4]*State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China.*

African swine fever virus (ASFV) is a virulent infectious virus with an extreme ability to infect primary porcine alveolar macrophages (PAMs). However, nothing is known about the host membrane proteins involved in ASFV infection. Here, we present a multi-omic epigenetic atlas of ASFV-exposed PAMs through profiling of 3D chromatin architecture and single-nucleus chromatin accessibility landscapes(sn-ATAC). ASFV infection leads to a rearrangement of active chromatin signaling in the cis-regulatory region of the host genome, which is associated with the activation of immune cells and transcription of membrane protein-encoding genes in the macrophage activation pathway. Specifically, the host genome employs histone H3 lysine 27 acetylation-mediated enhancer-promoter interactions to boost the transcriptional activity of membrane protein-encoding genes, thereby contributing to macrophage activation. Moreover, comparing the macrophages carrying viral DNA identified by snATAC-seq with wild-type macrophages provides a more reliable collection of membrane protein-encoding genes associated with infected macrophage activation. Different dimensions data indicates a co-occurrence of these membrane protein expression with susceptibility, for instance, inhibiting the expression of membrane proteins such as *CD244* and *CD206* can significantly decrease the host's susceptibility to ASFV. Collectively, the data provide a new insight to the regulation of host gene expression during ASFV infection and highlighted the genes encoding membrane proteins associated with macrophage activation.

Key Words: African swine fever virus, primary porcine alveolar macrophages, 3D chromatin architecture, single-nucleus chromatin accessibility landscapes, gene expression regulation

P311 Toward identification of new genetic determinants for postweaning diarrhea in piglets. E. Ibragimov, E. Ø. Eriksen, J. P. Nielsen, C. B. Jørgensen, M. Fredholm, and P. Karlskov-Mortensen*, *University of Copenhagen, Frederiksberg, Denmark.*

Postweaning diarrhea in pigs is a considerable challenge in pig farming industry due to its effect on animal welfare and production costs, and also due to the large volumes of antibiotics, which are used to treat diarrhea in pigs after weaning. Previous studies have revealed loci on SSC6 and SSC13 associated with susceptibility to specific diarrhea causing pathogens. However, postweaning diarrhea is a complex syndrome, which can be caused by a multitude of pathogens and, additionally, inherent factors in the pig may affect its overall susceptibility

to diarrhea. Hence, this study aimed to identify new genetic loci for resistance to diarrhea based on phenotypic data. In depth clinical characterization of diarrhea was performed in 258 pigs belonging to 2 herds during 14 d postweaning. The daily diarrhea assessments were used for classification of pigs into case and control groups. Genome-wide association studies (GWAS) and metabolomics association analysis were performed to identify new biological determinants for diarrhea susceptibility. With the present work we have revealed a new locus for diarrhea resistance on SSC16 specific to one of the studied herds. Furthermore, studies of metabolomics in the same pigs revealed one metabolite associated with diarrhea.

Key Words: pig, postweaning diarrhea, AMR, GWAS

P312 Combined targeted and untargeted metabolomics in pigs coupled with genomic information: toward a comprehensive genetic characterization of the pig metabolome. S. Bovo[1], G. Schiavo[1], F. Fanelli[2], A. Ribani[1], F. Bertolini*[1], M. Gallo[3], G. Galimberti[4], S. Dall'Olio[1], P. Martelli[5], R. Casadio[5], U. Pagotto[2], and L. Fontanesi[1], [1]*Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Bologna, Italy,* [2]*Department of Surgical and Medical Sciences, Endocrinology Unit, University of Bologna, Bologna, Italy,* [3]*Associazione Nazionale Allevatori Suini, Roma, Italy,* [4]*Department of Statistical Sciences "Paolo Fortunati," University of Bologna, Bologna, Italy,* [5]*Biocomputing Group, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy.*

Understanding the biological mechanisms governing the pig metabolism is fundamental for the development of new applications aimed at improving pig production efficiency. Production and reproduction traits are the final result of the molecular mechanisms taking place in an organism, that is the interplay within and between the biological layers encompassing the genome, proteome and metabolome spaces. Thus, the study of each layer allows to deconstruct such complex phenotypes in their small components providing new insights into their biology and additional new simpler phenotypes. Here, we characterized the genome and metabolome spaces to understand the genetic architecture governing the pig metabolism. To this purpose, targeted and untargeted metabolomic platforms were combined to analyze the abundance of more than 1000 plasma metabolites in about 1300 heavy pigs, including 900 Italian Large White and 400 Italian Duroc pigs, that were genotyped with a high-density SNP panel. For each breed, metabolomics profiles were used to study the metabolite-metabolite relationships via a network approach. The networks reconstructed for both breeds resulted similar, though differences emerged, with poorly interconnected modules. Then, metabolomics and genomics data were coupled to study the effect of genome variability over the metabolome via genome-wide association studies (GWAS). Different genomic scans were carried out, including single-marker and haplotype-based analysis of both single metabolites and metabolite ratios. Moreover, whole-genome sequencing data were used for the identification of putative causative mutations. GWAS analyses allowed to detect several quantitative trait loci, most of them including genomic regions carrying enzyme-encoding genes known to control the analyzed metabolites. Overall, we obtained for the first time a comprehensive catalog of genes and variants linked to the pig metabolism, opening new scenarios for the improvement of pig production systems.

Key Words: functional genomics, genome-wide association, metabolomics, pigs and related species

P313 ISAG Bursary Award: On the genetic basis of porcine semen traits: a large-scale genome-wide study on a synthetic line. P. Sá*[1], R. Godinho[2], M. Gòdia[1], C. Sevillano[2], B. Harlizius[2], O. Madsen[1], and H. Bovenhuis[1], [1]*Wageningen University and Research, Wageningen, the Netherlands,* [2]*Topigs Norsvin Research Center, Beuningen, the Netherlands.*

The pig industry is highly dependent on the production and commercialization of high-quality pig semen. Commercial boars are subject to regular collections and routine evaluations of semen quality, resulting in massive phenotypic data sets. The combination of automated phenotyping (CASA) and genomic tools allows for novel and unique opportunities to study the genetic background of boar semen characteristics. In this study, we estimated variance components for 14 semen traits to determine the extent to which these characteristics are influenced by systematic environmental factors and conducted a large-scale genome-wide association study to identify variants associated with these traits. Among others, total number of sperm cells and single morphological abnormalities were considered. Ejaculates were collected between 2007 and 2022 and evaluated using CASA system. The complete phenotypic data set included records from a total of 465,598 ejaculates collected from 5,758 commercial synthetic line boars, averaging 80 ejaculates per boar. Pedigree information included 17,701 animals spanning across 24 generations. Genetic parameters were estimated following a repeatability model. Genotype data included a total of 3,010 boars, with genotypes imputed to 660K SNP data. Preliminary results indicate moderate heritabilities; 0.20 for total number of cells, 0.18 for total motility and total abnormality rate. Repeatability estimates were 0.41 for total number of cells, 0.56 for total motility and 0.48 for total abnormality rate. The age of the boar at collection and the interval between collections were the most striking environmental effects. AI Station – Year – Season (AYS) of collection and temperature at collection, among others, were also found to be significant. The GWAS revealed several highly significant genomic regions that contained genes related to spermatogenesis and embryo development. Our results introduce new insight into the genetic nature of semen traits in pigs.

Key Words: pigs and related species, genome-wide association, single-nucleotide polymorphism (SNP), heritability, environment

Ruminant Genetics and Genomics

P314 ISAG Bursary Award: Association between host genetics of sheep and the rumen microbial composition. S. Mani*[1,3], O. Aiyegoro[2], and M. Adeleke[3], [1]*Agricultural Research Council – Anima Production, Agricultural Research Council – Anima Production, Pretoria, Gauteng, South Africa,* [2]*North West University, North West University, Potchefstroom, North West, South Africa,* [3]*University of KwaZulu Natal, University of KwaZulu Natal, Westville Campus, Durban, KwaZulu Natal, South Africa.*

A synergy between the rumen microbiota and the host genetics has created a symbiotic relationship, beneficial to the host's health. In this study, the association between the host genetics and rumen microbiome of Damara and Meatmaster sheep was investigated. The composition of rumen microbiota was estimated through the analysis of the V3-V4 region of the 16S rRNA gene, while the sheep blood DNA was genotyped with Illumina OvineSNP50 BeadChip and the genome-wide association (GWA) was analyzed. Sixty significant SNPs dispersed in 21 regions across the *Ovis aries* genome were found to be associated with the relative abundance of 7 genera: *Acinetobacter, Bacillus, Clostridium, Flavobacterium, Prevotella, Pseudomonas* and *Streptobacillus.* A total of 84 candidate genes were identified, and their functional annotations were mainly associated with immunity responses and function, metabolism, and signal transduction. Our results propose that those candidate genes identified in the study may be modulating the composition of rumen microbiota and further indicating the significance of comprehending the interactions between the host and rumen microbiota to gain better insight into the health of sheep.

Key Words: host genetics, rumen microbiota, sheep, genome-wide association studies, candidate genes

P315 Genome-wide association for functional longevity in Rubia Gallega beef cattle breed using a censored threshold model. M. Martínez-Castillero[1], D. López-Carbonell[1], H. Srihi[1], J. Altarriba[1], P. Martínez[2], M. Hermida[2], and L. Varona*[1], [1]*Universidad de Zaragoza, Zaragoza, Spain,* [2]*Universidad de Santiago, Lugo, Spain.*

The Rubia Gallega population is located in the Autonomous Region of Galicia and is one of the most important local beef cattle breeds in Spain. Functional longevity is included as one of its selection objectives, and it is recorded as the number of parities that a cow delivers in its productive life. The objective of this study was to estimate the heritability of functional longevity and to identify the genomic regions associated with its additive genetic variation. The phenotypic data set included the number of calving achieved from 54,933 cows. The average number of parities was 5.28 with a standard deviation of 3.38. Among them, 39,553 were culled cows, and 15,380 were still alive and its record was considered as lower censored. The pedigree was composed of 72,238 individual-sire-dam entries and 4,439 individuals were genotyped with the Axiom_BovMDv3 chip. After a standard quality control, 42,867 autosomal SNP were used. A ssGWAS was performed by backsolving the output of ssGBLUP under a censored threshold model, that was implemented with a Gibbs sampling using a single long chain of 500,000 iterations after discarding the first 25,000. The posterior mean estimate of the heritability was 0.17 with a posterior standard deviation of 0.03. The results of the ssGWAS indicated that the most relevant genomic region was located at BTA2, surrounding the *myostatin* (MSTN) gene that is associated with double muscling. Additionally, other genomic regions located at BTA 3, 7, 11, and 29 also explain a relevant proportion of the additive genetic variance. Within them, some interesting genes can be highlighted as potential candidate genes associated with functional longevity, such as *follicle Stimulating Hormone Receptor* (FSHR), *luteinizing hormone/choriogonadotropin receptor* (LHCGCR), and p*regancy-associated glycoproteins* (PAG). These genes confirm the central role of reproduction in the determination of cattle functional longevity.

Key Words: cattle, functional longevity, GWAS, censored thershold model

P316 Evaluation of Gal-3bp expression and modulation in cow blood and milk. M. Worku*[1] and B. Mulakala[2], [1]*North Carolina A&T State University, Greensboro, NC,* [2]*University of Vermont, Burlington, VT.*

Galectin-3 binding protein (Gal-3bp) is a glycoprotein encoded by LGALS3BP. It is involved in cellular interactions, pathogen binding and regulate proinflammatory cytokine production in response to lipopolysaccharide (LPS). Increased Gal-3bp has been observed in the circulation in patients infected with SARS-CoV-2. The expression and secretion of Gal-3bp in cows is largely unknown. The objective of the study was to evaluate the expression of LGALS3BP gene in cow's blood and milk. Further, the impact of plant phytochemicals on Gal-3bp gene expression in blood in the presence and absence of LPS was evaluated. Holstein Friesian cows (n = 3) from the North Carolina Agricultural and Technical State University Dairy herd were used. Aseptically collected milk was centrifuged to separate cells and whey. Blood collected from the jugular vein was centrifuged to harvest cells and plasma. Blood (1 mL) was treated with 10ng/mL of either Locust bean gum (LBG) or Modified Citrus Pectin (MCP) in the presence or absence of LPS or in Phosphate Buffered Saline (37°C for 30 min). Total RNA was isolated from milk and blood cells using Trizol. Expression of LGALS3BP was evaluated using real-time PCR. Housekeeping genes RPLP0 and UCHL5 served as internal controls. Fold change was determined using the Livak method (Fold change >2 was considered significant). The LGALS3BP gene was expressed in milk and blood, but it was expressed more in the blood. The expression of LGALS3BP gene remained unchanged in samples treated with LBG compared with the control. Locust bean gum in the presence of LPS upregulated LGALS3BP gene expression 3.7 folds compared with control. Expression of LGALS3BP gene remained unchanged with MCP treatment alone, but in the presence of LPS, expression was downregulated 5.8 folds compared with the control. The results of this study demonstrated that LGALS3BP gene is expressed in cow blood and milk. Further, LPS and plant phytochemicals modulate the mRNA expression of LGALS3BP in blood. The results of this research aid in the development of plant phytochemicals-based therapeutics to target LGALS3BP gene expression and LPS induced immune responses.

Key Words: cattle, LGALS3BP, Gal-3bp, expression, modulation

P317 The novel RNA-RNA activation of H19 on MyoD transcripts promoting myogenic differentiation of goat muscle satellite cells. L. Li*, C. Qin, Y. Chen, W. Zhao, Q. Zhu, D. Dai, S. Zhan, J. Guo, T. Zhong, L. Wang, J. Cao, and H. Zhang, *Farm Animal Genetic Resources Exploration and Innovation Key Laboratory of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan, China.*

Introduction Myogenesis is a complex process precisely orchestrated by the interaction of various coding and noncoding factors. LncRNA H19 was identified in the same selective screen with MyoD transcripts and exhibited a similar enrichment pattern to MyoD. However, the close interaction between MyoD and H19 remains largely unknown. Materials and methods Goat skeletal muscle satellite cells (MuSCs), mouse C2C12 myoblasts, and HeLa cells were used, and methods employed included RACE, mRNA-seq, RNAScope assay, ChIP-qPCR, MS2-RIP, RIP, and RNA pulldown. Results and Discussion Two isoforms generated from the H19 gene were identified in neonatal goat skeletal muscle (KY242301.1). They were highly identical to ruminants, including cattle (NR_003958.2, 93% identity) and sheep (AY091484.1, 92% identity). We expectedly found that H19 positively associated with the abundance of myogenic markers like MyoD, Myomaker, and Myomerger, coinciding with the elevated MyHC+ cells. Based on PolyA+ RNA-seq data, we found only the DEG downregulated by siH19 were enriched in muscle-related GO terms and harbored MYOD-binding motif GCANCTGNY. Moreover, ectopic MyoD efficiently neutralized the decrease of myogenic gene transcripts caused by H19 deficiency. H19 and MyoD transcripts overlapped with a doubled degree in differentiated cells. Furthermore, vectors containing the mutated and deleted H19-MyoD base-pairing region dramatically decreased their overlap; the association between truncated H19 and the MyoD transcripts was related to their affinity. Moreover, we confirmed that H19 stabilizes MyoD mRNA. We also identified the transcriptional activity of MyoD on the promoter of H19 in a MyoD-ChIP assay and A dual-luciferase reporter assay in mouse C2C12 cells and MuSCs. Together, we unexpectedly identified that H19 is highly affiliated with and stabilizes MyoD mRNA significantly via RNA-RNA interaction, extending the reservoir of H19 regulatory mechanisms.

Key Words: long noncoding RNA H19, myogenic differentiation 1(MyoD), RNA-RNA interaction, skeletal muscle satellite cells (MuSCs), goat

P318 ISAG Bursary Award: eQTL mapping in beef cows to identify genetic variants underlying fertility. N. Kertz*[1], P. Banerjee[1], J. Afonso[2], P. Dyce[1], and W. Diniz[1], [1]*Auburn University, Auburn, AL,* [2]*Embrapa Pecuária Sudeste, São Carlos, SP, Brazil.*

The cow-calf industry's sustainability and profitability depend on the reproductive success of the herd. Genomic information has provided insights into the quantitative trait loci (QTLs) underlying cow subfertility. Integrating functional information into the genomics regulatory layer can provide new markers for improving fertility. This study aims to identify gene expression regulatory polymorphisms in uterine luminal epithelial cells and investigate their effect(s) on fertility-related genes. We retrieved RNA-Seq data from uterine epithelial cells (GSE171577) of recipient cows (n = 18 non-pregnant – NP and n = 25 pregnant – P) sampled on d 4 before embryo transfer. Raw data quality control was performed, and read mapping was carried out using the multi-sample 2-pass mapping procedure from STAR. Gene normalization was performed using the log2CPM function from edgeR. Variant detection was

based on the uniquely mapped reads using the GATK software. After quality control, 43 samples, 203,404 SNPs, and 15,029 genes were used for eQTL analysis (expression quantitative trait loci). Using an additive linear model from the MatrixEQTL R-package, we identified 25,946 cis-eQTLs for 1,823 genes (FDR <0.05). Chromosomes 18 and 19 harbored the greatest number of cis-eQTLs with 2,392 and 2,283, respectively. A total of 50 cis-eQTLs were previously reported as differentially expressed genes, including *CD37, CXCL3, PILRA*, and *PPP6R1* genes. We also identified fertility-related genes such as *PLOD3, HDHD3*, and *LY6G6E,* which were modulated by 106, 86, and 65 SNPs, respectively. Functional analysis of cis-eQTLs retrieved immune-related biologic processes, including antigen processing and presentation of peptide antigen via MHC class Ib and defense response to Gram-positive bacterium (FDR <0.05). Additionally, fatty acid degradation and metabolic pathways were among the over-represented KEGG pathways. Our findings show that eQTLs influence key biological pathways and genes affecting fertility. Additionally, it provides novel functional regulatory mechanisms that may lead to the identification of causative mutations.

Key Words: system genetics (eQTLs), fertility, genetic improvement, RNA-seq

P319 Genome-wide estimation of ROH, linkage disequilibrium, haplotype block structure and past effective population size in Hanwoo cows. S. Oh* and D. Yoon, *Department of Animal Science and Biotechnology, Graduate School, Kyungpook National University, Sangju, Korea.*

This study was performed to understand genomic characteristics such as genetic diversity and genomic architecture using genome-wide SNP information for an efficient breeding program of Hanwoo cows. A total number of 7,273 genomic DNA samples were extracted from Hanwoo hair roots and genotyped by customized-Hanwoo SNP50K BeadChip. For analysis, 7,205 samples and 41,765 SNPs covering 2500.1 Mb of the genome were selected through a QC. The mean of observed heterozygosity, expected heterozygosity and MAF across autosomes was 0.359 ± 0.126, 0.358 ± 0.125, and 0.268 ± 0.132, respectively. The ROH (Runs of Homozygosity) was assessed, and a total of 2,702 ROH were detected. The average number of ROH per animal was 93 ROH. In addition, using D' and r^2, all SNP pairs on the same chromosome were used to estimate LD across the autosomes. On the autosomes, the D' was 0.388 ± 0.319, and r^2 was 0.055 ± 0.125. The average of D' and r^2 for SNP pairs at 0–50 kb apart were 0.705 ± 0.333 and 0.229 ± 0.287, respectively. The total number of haplotype blocks was 1,159, and the total length of the haplotype block was 61.22 Mb (2.44% of autosome lengths). The haplotype block with 2 SNPs was the most common, with 637 blocks. Furthermore, the past effective population size was 2,159 heads in 302 generations ago but decreased to 289 heads in 13 generations ago. The results of this study can provide genomic information for various studies such as QTL mapping, GWAS and GS of Hanwoo cows and be useful for breeding programs.

Key Words: cattle and related species, SNP, effective population size, homozygosity, linkage disequilibrium

P320 Withdrawn

P321 Analysis of the impact of *DGAT1* p.M435L and p.K232A variants on pre-mRNA splicing in a full-length gene assay. N. Gaiani, L. Bourgeois-Brunel, D. Rocha, and A. Boulling*, *Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France.*

DGAT1 is playing a major role in fat metabolism and triacylglyceride synthesis. Only 2 *DGAT1* loss-of-function variants altering milk production traits in cattle have been reported to date, namely p.M435L and p.K232A. The p.M435L variant is a rare alteration and has been associated with skipping of exon 16 which results in a non-functional truncated protein. The p.K232A variant is commonly found and is responsible for a loss of DGAT1 enzyme activity. A recent study has however brought to light an association between the p.K232A-containing haplotype and some modifications of the splicing rate of several *DGAT1* introns. In particular, the direct causality of the p.K232A variant in decreasing the splicing rate of the intron 7 junction was validated using a minigene assay in MAC-T cells. As both these *DGAT1* variants were shown to be spliceogenic, we developed a full-length gene assay (FLGA) to re-analyze p.M435L and p.K232A variants in HEK293T and MAC-T cells. Qualitative RT-PCR analysis of cells transfected with the full-length *DGAT1* expression construct carrying the p.M435L variant highlighted complete skipping of exon 16. On the other hand, quantitative RT-PCR analyses of cells transfected with the same construct carrying the p.K232A variant did not show any modification on the splicing rate of introns 1, 2, and 7. In conclusion, the *DGAT1* FLGA confirmed the p.M435L impact previously observed in vivo, but invalidated the hypothesis whereby the p.K232A variant strongly decreased the splicing rate of intron 7. An effect of the p.K232A variant on other *DGAT1* junctions cannot be excluded and remains to be determined.

Key Words: splicing variant, functional analysis, DGAT1

P322 ISAG Bursary Award: Genomic differentiation within the South African Hereford reference population. C. Croucamp* and E. van Marle-Köster, *University of Pretoria, Pretoria, Gauteng, South Africa.*

The Hereford cattle breed has been farmed in South Africa under various climatic conditions for more than 100 years. In this study, genomic diversity and runs of homozygosity (ROH) were estimated to gain insight into potential genomic regions under selection in the various production environments. Genotypes generated using 3 commercial SNP genotyping arrays were available for 811 South African Hereford cattle representing 7 climatic regions across South Africa. Following quality control using PLINK v 1.9, 692 animals and 35 692 autosomal SNPs were retained for downstream analyses. Genomic diversity pa-

rameters were estimated, followed by a principal component analysis (PCA) and ROH and the frequency thereof (F_{ROH}) per autosome using a consecutive runs approach with the R package detectRUNS. The expected heterozygosity was 0.379 with a mean F_{IS} of 0.005 ± 0.037 and LD = 0.425 with a small positive value of F_{IS} indicating slight inbreeding. Genomic diversity is comparable to other SA beef breeds and strong LD levels are indicative of larger haplotypes shared among individuals within this population. The PCA indicated 2 distinct clusters confirming the polled and horned ancestry. Identified ROH were found across all autosomes; 1 and 6 contained the highest number of SNPs per run. ROH varied between 4.99 Mb and 7.1 Mb in length. Mean FROH values per autosome ranged from 0.10 (autosome 5) to 0.17 (autosome 28). The lengths of the ROH segments found are indicative of both ancient and recent inbreeding, with recent inbreeding most likely due to intensive selection for certain production traits.

Key Words: genomic differentiation, Hereford, ROH, genetic diversity, South Africa

P323 ISAG Bursary Award: Genes near the Celtic POLLED variant are differentially expressed between horned and polled bovine fetuses at 58 days of development. J. Aldersey*[1], Y. Ren[1], W. Low[1], K. Petrovski[1], J. Williams[1,2], and C. Bottema[1], [1]*Davies Livestock Research Centre, University of Adelaide, Roseworthy, South Australia, Australia,* [2]*Department of Animal Science, Food and Technology, Università Cattolica del Sacro Cuore, Emilia Parmense, Piacenza, Italy.*

The presence of horns in ruminants has financial and welfare implications for the farming of domestic ruminants worldwide. The genetic interactions that lead to horn development are not known. Hornless, or polled, cattle occur naturally but the associated DNA variants (Celtic, Friesian, Mongolian, and Guarani) are in intergenic regions on bovine chromosome 1, and therefore, their functions are unknown. The leading hypothesis is that the POLLED variants affect the expression of nearby genes early in fetal development. The aims of this study were to 1) identify genes that may directly be affected by the Celtic variant, and 2) identify genes and pathways important for horn development. Twelve horned (p/p) and 12 polled (P/P) Hereford heifers were inseminated with semen from horned (p/p) and polled (P/P) Hereford bulls. The horn bud, frontal skin and forebrain skin regions were biopsied in horned (n = 4) and polled (n = 3) fetuses at 58 d of development. RNaseq was conducted for differential gene expression analyses and pathway analyses. Near the POLLED region, 3 genes (*C1H21orf62*, *SON*, and *EVA1C*) and one lincRNA (*LOC112447120*) were differentially expressed between horned and polled fetuses. Previously identified candidate genes, *RXFP2*, *TWIST2*, *ZEB2*, and *HOXD1* were also differentially expressed. New candidates for the horn development pathway were proposed based on the analyses (*MEIS2*, *PBX3*, *FZD8*, *CTNNB1*, and *LEF1*). Pathway analyses suggested that differentially expressed genes have functions in axon guidance, cytoskeletal structure, and the extracellular region, and therefore, these pathways may be vital for horn development. This study further validates some original candidate genes and suggests new candidates involved in bovine horn ontogenesis.

Key Words: horn, polled, headgear, cattle, Celtic

P324 ISAG Bursary Award: Multiple-trait joint genetic evaluation improves accuracy of prediction in South-African and Kenyan Holstein cattle population. I. Houaga*[1,2], R. Mrode[3,4,11], O. Opoola[1], M. Chagunda[5], M. Okeyo[4], J. E. O. Rege[6], V. E. Olori[7], O. Nash[8], C. B. Banga[9], T. O. Okeno[10], and A. Djikeng[1], [1]*Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Easter Bush, Edinburgh, United Kingdom,* [2]*The Roslin Institute, University of Edinburgh, Easter Bush, Edinburgh, United Kingdom,* [3]*Scotland Rural College (SRUC), Easter Bush, Edinburgh, United Kingdom,* [4]*International Livestock Research Institute (ILRI), Nairobi, Kenya,* [5]*University of Hohenheim, Hohenheim, Stuttgart, Germany,* [6]*Emerge Centre for Innovations-Africa (ECI-Africa), Nairobi, Kenya,* [7]*Aviagen Limited, Newbridge, EH28 8SZ, Edinburgh, United Kingdom,* [8]*Centre for Genomics Research and Innovation, National Biotechnology Development Agency, Abuja, Nigeria,* [9]*Agricultural Research Council (ARC), Pretoria, 0002, South Africa,* [10]*Department of Animal Sciences, Egerton University, Egerton, Kenya,* [11]*The University Edinburgh, Scotland.*

The African livestock sector plays a key role in improving the livelihoods of more than 800 million people through the supply of food, improved nutrition and consequently the health. However, its impact on the economy of the people and contribution to national GDP is generally below its potential and highly varied. This study was conducted to assess the effects of joint genetic evaluation on the accuracy and rate of genetic gain that could be achieved in livestock. Thus, a joint genetic analysis of 305 d milk yield (MY305), Age at first calving (AFC) and the first calving interval (CI1) was conducted in South African and Kenyan Holstein cattle population. The results showed that the accuracies of prediction in multi-traits joint genetic evaluation were higher than the accuracies of within country genetic evaluation for MY305 (0.7 vs 0.56) and AFC (0.78 vs 0.49) in Kenya and for AFC in South Africa (0.78 vs 0.76). Regardless of proportion of selected sires (Top 5 to 100 sires), selection based on across-country genetic evaluation resulted in higher and favorable gains for MY305 in Kenya and for AFC in both Kenya and South Africa. African countries need to put in place enabling policies, the necessary infrastructure and funding for national and across country collaborations for a joint genetic evaluation which will revolutionize the livestock genetic improvement in Africa.

Key Words: across country genetic evaluation, Holstein cattle, South Africa, Kenya

P325 A two-stage F_{ST} prioritization approach in the presence of high-density marker panels: a simulation study. S. Toghiani*[1], S. Aggrey[2,3], and R. Rekaya[2,4], [1]*USDA, Agricultural Research Service, Animal Genomics and Improvement Laboratory, Beltsville, MD,* [2]*Institute of Bioinformatics, The University of Georgia, Athens, GA,* [3]*Department of Poultry Science, The University of Georgia, Athens, GA,* [4]*Department of Animal and Dairy Science, The University of Georgia, Athens, GA.*

High-density SNP panels and sequence data have revolutionized the study of complex traits. Yet, increasing the number of markers can reduce the statistical power and increase false positives, impacting genomic selection (GS) accuracy. The fixation index (F_{ST}), a measure of variation in allele frequencies among populations, can pinpoint genome regions under selection pressure that can be prioritized in the association analysis. This study aimed to assess the impact of different F_{ST} prioritization scenarios on estimated heritability and GS accuracy. QMSim software was used to simulate a trait with a heritability of 0.4, assuming 500 QTL with effects drawn from a normal distribution and an effective population size of 200. The simulated population consisted of 30K animals from the last 2 generations of a 10-generation selective breeding program, with all animals genotyped using a 600K SNP marker panel. Accuracy of GS was evaluated by correlating true and estimated breeding values of 5K randomly selected animals in the last generation. The top and bottom 5% of genotyped animals in the 9th generation were clustered into 2 subpopulations based on their phenotype distribution and F_{ST} scores were calculated for each SNP. Three different scenarios were investigated: (1) selecting the 1% SNPs with the highest F_{ST} scores (S1; 6000 SNPs), (2) selecting the top 12 SNPs surrounding each QTL based on F_{ST} scores (S2; 5962 SNPs), and (3) selecting 12 random SNPs surrounding each QTL based on F_{ST} scores (S3; 5976 SNPs). Using all 600K SNPs, the accuracy and estimated heritability were 0.78 and 0.37, respectively. However, when SNPs were prioritized based on F_{ST}, the accuracy (estimated heritability) using GBLUP was 0.60 (0.28), 0.88 (0.34), and 0.89 (0.36) for S1, S2, and S3 scenarios, respectively. These findings suggest selecting a small number of SNPs surrounding a QTL is more effective in increasing the accuracy of genomic selection even when prioritizing SNPs with the highest F_{ST} scores. It is worth investigating the performance of the different scenarios when the number and positions of QTL are not completely known.

Key Words: SNP prioritizing, genomic selection, high-density

P327 ISAG Bursary Award: Molecular investigations on cryptorchidism in German Holsteins. F. Krull*[1], W. Wemheuer[1], T. Melbaum[2], and B. Brenig[1], [1]*University of Goettingen, Institute of Veterinary Medicine, 37077 Goettingen, Germany,* [2]*Bullseye-Genetics GmbH, 48341 Altenberge, Germany.*

Cryptorchidism is hereditary and the most common male malformation in mammals. The prevalence in bulls is about 1% and lower than in other livestock or humans (up to 12%). The absence or incomplete descent of the testes into the scrotum favors pathologies such as torsion or carcinogenesis and leads to significantly reduced sperm quality. Testosterone production remains unaffected, which leads to undesirable male behavior or inedible meat in pigs. Cryptorchidism related genes such as *INSL3*, *insulinprotein3*, or *RXFP2*, *relaxin-family peptide-receptor2*, are relevant for the efficacy of sex hormones in the prenatal organism and have only been described in a few species. For livestock, the genetic etiology of cryptorchidism remains unclear. We have identified 63 uni- or bilateral cryptorchid Holstein bulls. 62 were born in 2011 or later. The presumed common ancestor was born in 2008, but only 21 bulls could be traced back to this sire. All 63 bulls could be traced back to an ancestor born in 1983. However, this is certainly not the founder for cryptorchidism in the Holstein population, as he was widely used in Holstein breeding. To investigate the genetic background of cryptorchidism in German Holsteins we performed pedigree analyses, GWAS, whole genome resequencing and protein network analyses. EDTA-blood samples were collected of 37 of the 63 affected sires and genotyped using the EuroG_MD BeadChip. GWAS showed 4 significantly associated SNPs in a 2.6Mb region on chromosome 6 (BTA6) harboring 7 genes. However, no genes potentially associated with cryptorchidism were found in this region. Approximately 9Mb downstream to this region *tachykinin receptor* 3 (*TACR3*) is located, which is involved in Kallmann syndrome/hypogonadism. Using whole genome resequencing of 6 affected sires, 8 disruptive variants in 6 genes were identified that were however outside of the associated region. Protein network analyses were performed to identify additional potential candidate genes and metabolic pathways. To date, all molecular studies performed indicate a complex genetic origin of cryptorchidism in Holstein cattle.

Key Words: cattle and related species, genome sequencing, candidate gene, animal health

P328 ISAG Bursary Award: Genome-wide association analysis reveals polygenic regulation of ovine high-altitude adaptability. C. Li[1,2], B. C. Chen*[1], Y. J. Wu[3], J.-L. Han[4,5], Y. L. Chen[1], P. Zhou[6], H. Pausch[2], and X. L. Wang[1], [1]*Northwest A&F University, Yangling, Shaanxi, China,* [2]*ETH Zürich, Zürich, Switzerland,* [3]*Tibet Academy of Agricultural and Animal Husbandry Sciences, Lhasa, China,* [4]*Chinese Academy of Agricultural Sciences, Beijing, China,* [5]*International Livestock Research Institute, Nairobi, Kenya,* [6]*Xinjiang Academy of Agricultural and Reclamation Sciences, Shihezi, China.*

Sheep were domesticated in the Fertile Crescent and then spread across the world, where they have been encountering various environmental conditions, including extreme temperatures, pathogens, and high altitudes. More than 20 million sheep live on the Qinghai-Tibetan Plateau. They have played an essential role in facilitating permanent human occupation of this harsh high-altitude region. Here, we analyzed Illumina short reads of 994 whole genomes representing around 60 breeds/populations of domestic and wild sheep inhabiting areas from 0 to 5,000 m above sea level, PacBio HiFi reads of 13 sheep representing different breeds, and 104 transcriptomes from 13 organs of high- and low-altitude sheep. Association testing between the inhabited altitudes and 34,298,967 autosomal variants was conducted in 450 sheep to investigate the genetic architecture of altitude adaptation. Highly accurate HiFi reads were used to complement the current ovine reference assembly at the most significantly associated β-globin locus and validated the presence of the 2 haplotypes A and B among the 13 breeds. Of which, the haplotype A carried 2 homologous gene clusters: the first of 4 genes in the order of *HBE1*, *HBE2*, *HBB*-like, and *HHBC*; and the second of *HBE1*-like, *HBE2*-like, *HBB*-like, and *HBB*; while the haplotype B lacked the first cluster. Genotyping of these 2 haplotypes revealed that they are significantly differentiated between the high-altitude sheep with highly frequent or nearly fixed haplotype A and low-altitude sheep dominated by the haplotype B. Both *HBBC* and *HBB* were transcribed in the high-altitude sheep, while the transcription of *HBB* was particularly higher in muscle, lung, and heart of the high-altitude than the low-altitude sheep. Another highly associated genomic region contained the *EGLN1* gene which showed a reduced expression in muscle of high-altitude sheep. Our results provide evidence that rapid adaptive evolution of advantage alleles played an important role in shaping the environmental adaptation of Tibetan sheep.

Key Words: environmental adaptation, high altitude, hypoxia, selection signature, sheep

P330 Search for new mutations in cattle by systematic whole genome resequencing. M. Boussaha[1], C. Eché[2], C. Escouflaire[3], C. Grohs[1], C. Iampietro[2], A. Capitan[1], D. Milan[2,4], C. Gaspin[5,6], S. Fritz[3], C. Donnadieu[2], and D. Boichard*[1], [1]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France,* [2]*INRAE, US 1426, GeT-PlaGe, Genotoul, France Genomique, Université Fédérale de Toulouse, Castanet-Tolosan, France,* [3]*Eliance, 75012 Paris, France,* [4]*GenPhySE, Université de Toulouse, INRAE, INPT, ENVT, Castanet-Tolosan Cedex, F-31326, France,* [5]*Université Fédérale de Toulouse, INRAE, BioinfOmics, GenoToul Bioinformatics facility, 31326, Castanet-Tolosan, France,* [6]*Université Fédérale de Toulouse, INRAE, MIAT, 31326, Castanet-Tolosan, France.*

Systematic whole genome sequencing provides a rapid and powerful method to identify recent novel mutations on a cattle population scale. It helps farmers to detect early carriers of new genetic anomalies and more generally to boost genomic selection. Regarding genetic defects, the aim is to identify new candidate mutations that may have deleterious potential before they are widely spread in the population to inform the breeders. A major drawback is the high rate of false positives, ie of variants with a strong annotation but without any effect. Therefore, the annotation is the most critical step. We focused only on still unknown variants (ie de novo or likely very recent mutations) in highly conserved sequences in other species and/or with effects predicted to be similar to those described in OMIM or MGI databases. We applied this strategy on whole genome sequences on 571 artificial insemination bulls from to 14 dairy and beef breeds. In large breeds, recently marketed bulls were selected. In smaller breeds, influential ancestors not yet sequenced were chosen. In total, we identified 1548 novel genomic variants with a potential link to certain quantitative traits of interest or possible genetic abnormalities. These variants were investigated by searching for carrier or homozygous descendants, and by adding these variants on the custom part of the EuroGenomics SNP chip for an easy screening of the population. This information is also returned back to bull's owners, with a specific alert only for the few most critical variants. Although the annotation still lacks accuracy, we believe that whole genome sequencing of all artificial insemination bulls is an early, rapid and always cheaper method for identifying genetic defects before they disseminate in the population. The SeqOccIn project was funded by the Occitanie region, FEDER, and Apis-Gene.

Key Words: cattle, WGS, mutations, annotation, phenotypes

P331 Genomic partitioning to identify hidden heritability using multi-omics data set in Hanwoo cattle. Y. Kim*[1], D. Lee[2], D. Lee[2], Y. Chung[2], J. Kang[2], S. Lee[2], and S. Lee[2], [1]*Quantomic Research & Solution, Yuseong-gu, 34134, Daejeon, Republic of Korea,* [2]*Chugnam National University, Yuseong-gu, 34134, Daejeon, Republic of Korea.*

A phenotypic variation would be formed by complex biological mechanisms which involve massive genomic variants, other structural variants, and biological big data such as transcriptome. Therefore, it is important to understand the underlying processes to acquire more accurate knowledge for the prediction of breeding value in animal breeding. However, identifying biological schemes that are linked to the regulation of complex traits is a difficult work. Currently, the calculation of

genomic estimated breeding value (GEBV) relies on SNP genotypes and has proven to be effective in breeding programs. Nevertheless, a more efficient method of increasing the accuracy of breeding value estimation would require additional information such as multi-omics data. Therefore, investigating the multi-layer processes with multi-omics data would explain more genetic variance which means more accurate estimation of breeding value. This study investigated if multi-omics data would be useful to identify a causal mutation and would have a direct benefit for explaining genetic variance in Hanwoo cattle. To estimate genomic variance for SNPs, eQTL, and copy number variation (CNV), we used phenotypic data from 16,900 Hanwoo steers with genomic and multi-omics data.To estimate genetic variance for eQTL of marbling score, 25 animals were used in this study. The heritability of marbling score was estimated 0.4659 ± 0.0137. The result of genomic partitioning of multi-omics data, the heritabilities of SNPs, eQTL, and CNVs were estimated 0.4288 ± 0.0139, 0.0332 ± 0.0077, and 0.0039 ± 0.004, respectively. In addition, the heritabilities of CNVs for carcass weight, backfat thickness, eye muscle area, and marbling score were estimated 0.032 ± 0.006, 0.007 ± 0.005, 0.02 ± 0.006, and 0.016 ± 0.005, respectively. In conclusion, this study decomposed genomic variance of multi-omics data such as eQTL and CNV in breeding target traits in Hanwoo.

Key Words: Hanwoo cattle, multi-omics, heritability

P332 ISAG Bursary Award: The first gapless complete T2T Y-chromosome assemblies of cattle and sheep uncover their genomic architectures. T. Olagunju[1], B. Rosen[2], T. Smith[3], T. Hadfield[4], S. Koren[5], H. Neibergs[6], N. Cockett[4], and B. Murdoch*[1], [1]*University of Idaho, Moscow, ID,* [2]*USDA, ARS, Animal Genomics and Improvement Laboratory, Beltsville, MD,* [3]*USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE,* [4]*Utah State University, Logan, UT,* [5]*National Human Genome Research Institute (NHGRI), NIH, Bethesda, MD,* [6]*Washington State University, Pullman, WA.*

The Y-chromosomes of mammals contain most of the genes that are critical for sex determination and play vital roles in spermatogenesis and male fertility. Chromosome Y evolved through the degeneration of the genes of an autosome and has been difficult to assemble due to the high number of repeats and palindromic sequences spanning more than half of its length. Even the human Y-chromosome was less than half complete until recently. No species in the *Bovidae* family have a complete Y-chromosome assembly; the most complete assemblies reported to date are found in cattle and sheep and span 16Mb and 10.6Mb, respectively. This information gap results in a poor understanding of the structure and variation of this vital chromosome. We present the first gapless complete Y-chromosomes of cattle and sheep from draft telomere-to-telomere (T2T) assemblies, spanning 59Mb and 25Mb for cattle and sheep, respectively. *De novo* and homology-based annotations were used to identify and characterize the pseudo-autosomal region (PAR), male-specific Y (MSY), X-degenerate, ampliconic, and the previously hard-to-reach heterochromatic regions of these Y-chromosomes. Our T2T assemblies included the genomic structures comprising the most up-to-date assemblies of the cattle and sheep Y-chromosomes, adding some notable structures, including the satellite arrays and transposable elements, which are important landmarks that define critical chromosomal structures such as the centromere and the ampliconic regions. A cross-comparison of the 2 species revealed the structural differences and similarities between these 2 members of the *Bovidae* family. This research provides the most comprehensive information on the structure and organization of the previously unknown highly repetitive heterochromatic and ampliconic regions of the cattle and sheep Y-chromosomes. The findings in this work open new opportunities for further studies exploring the structural evolution and variation of cattle and sheep Y-chromosomes and comparisons of their genomic architectures with other mammalian species.

Key Words: chromosome Y, cattle, sheep, genome assembly, telomere-to-telomere (T2T)

P334 Milk productivity of different selection Holstein cows at Ayna dairy farm. A. Daulet*, B. Saule, U. Rashit, and N. Dinara, *Saken Seyfullin Kazakh Agrotechnical Research University, Astana c., Republic of Kazakhstan.*

The development of the dairy industry has become a priority for Kazakhstan. In this regard, the Government of the Republic of Kazakhstan allocated more than 200 million US dollars to support dairy farming, thanks to which, since 2011, more than 50 thousand heads of dairy and beef cattle have been delivered to the Republic of Kazakhstan. At the same time, most of the imports were the livestock of the Holstein breed, the number of which in Kazakhstan today has reached more than 40 thousand heads with an average milk yield of 7200 kg per lactation. The objective was to study the indicators of milk productivity of cows of various selections. The studies were carried out at Ayna Dairy farm, Akmola region of the Republic of Kazakhstan. The object of research were cows of the Holstein breed of 3 different selections. To determine the milk productivity of Holstein cows of different lines, groups of animals were formed: the first group - daughters of the bull HENDEL ALTAMARDON 011HO10580 of the Ues Ideal 1933122 line, the second group - the daughters of the bull DAVINCI DE0115528603 of the Montvik Chifshtein 95679 line, the third group - the daughters of the bull SHORTY NL421891351 of the Reflection Sovering CA line 198998. Milk productivity was studied according to the results of control milkings once a month. As a result, it was found that the group of daughters of the bull Shorty NL421891351 had the highest milk yield of 6797 ± 342.9 kg, and was higher than that of the daughters of the bull Davinci DE0115528603 by 430 kg or 6.8% ($P < 0.95$), also higher than the group of daughters of the bull HENDEL ALTAMARDON 011HO10580 by 1015 kg or 17.6% ($P < 0.99$). The average milk yield for the herd was 5763 kg. Thus, the milk productivity of the daughters of the bulls Shorty NL421891351 and Davinci DE0115528603 was higher than that of the daughters of HENDEL ALTAMARDON 011HO10580, which indicates a better adaptation of animals of European selection in the conditions of the Republic of Kazakhstan. On the basis of which recommendations were made to breed the Holstein breed of European selection in this farm

Key Words: cattle and related species, milk production

P335 ISAG Bursary Award: Development of a rapid SNP genotyping assay for novel SNPs associated with BLV-induced lymphoma. S. Watanuki*[1], R. Matsuura[1], C.-W. Lo[1], S. Saito[1], Y. Miyazaki[2], Y. Matsumoto[1,3], S.-n. Takeshima[4], and Y. Aida[1], [1]*Laboratory of Global Infectious Diseases Control Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan,* [2]*Livestock Improvement Association of Japan, Inc., Gunma, Japan,* [3]*Laboratory of Global Animal Resource Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan,* [4]*Department of Food and Nutrition, Jumonji University, Saitama, Japan.*

Enzootic bovine leukosis (EBL) is a B-cell lymphoma caused by bovine leukemia virus (BLV), leading to significant economic losses worldwide. Therefore, early detection of high-risk cows for EBL is crucial. However, the detailed mechanism of BLV-induced leukemogenesis remains unclear because EBL is a multifactorial disease caused by a complex interplay between host genetic factors and environmental factors in addition to viral factors. Here, we aim to identify novel SNPs associated with BLV-induced lymphoma using genome-wide association analysis (GWAS) and to establish a novel rapid SNP genotyping assay. The 120 BLV-infected but clinically normal Holstein cattle and 120 BLV-infected Holstein cattle with lymphoma in Japan were genotyped using a GWAS with the Illumina BovineHD BeadChip (770K). After quality control, the 594810 SNPs were used for an association study based on Fisher's exact test. In all, one SNP was found on an intronic region of the gene encoding a protein involved in the endoplasmic reticulum stress response pathway on bovine Chr9 ($P = 7.8 \times 10^{-8}$), by applying a Bonferroni correction for multiple testing. Furthermore, by the moderate threshold ($P < 1.0 \times 10^{-5}$), the 21 suggestive SNPs were detected. Next, we selected 4 SNPs out of 21 SNPs and optimized the

PCR conditions for a rapid SNP genotyping assay based on Real-time PCR System using MGB probes. Three DNA polymerases and 2 PCR instruments were examined in this study. As a result, we specifically detected the SNPs within one hour using the THUNDERBIRD Probe qPCR Mix (TOYOBO) and LightCycler480 IIqPCR system (Roche). This is the first report to identify SNPs associated with BLV-induced lymphoma using GWAS and establish a novel rapid diagnostic method. This diagnosis system is an innovative method that allows for simultaneous analysis of SNP genotyping and measurement of BLV proviral load by using the LightCycler480. Our results will be helpful to understand the mechanisms of BLV-induced leukemogenesis by clarifying the expression and function of the genes that induced lymphoma were present around the SNP.

Key Words: bovine leukemia virus, SNP, genotyping, GWAS, cattle

P336 New loci for milk production traits in German Black Pied cattle (DSN) using whole-genome sequencing data. P. Korkuc*[1], G. B. Neumann[1], D. Arends[2], K. May[3], S. König[3], and G. A. Brockmann[1], [1]*Humboldt-Universität zu Berlin, Berlin, Germany,* [2]*Northumbria University, Newcastle upon Tyne, United Kingdom,* [3]*Justus-Liebig-Universität Gießen, Gießen, Germany.*

German Black Pied (DSN, "Deutsches Schwarzbuntes Niederungsrind") is an endangered dual-purpose cattle breed from Germany consisting of around 2,500 herdbook cows. It was almost entirely replaced by Holstein due to their higher milk yield. Today, DSN is maintained as a genetic reserve because of its high milk fat and protein content and its relation to Holstein as an ancestor breed. To maintain DSN in the long term, it is important to improve its milk production while preserving its dual-purpose character and breed-specific characteristics. This study aimed at fine-mapping genomic loci that had been associated with milk production traits before. Therefore, we investigated genome-wide associations for milk production traits in 2,160 DSN cows using 11.7 million DNA variants from whole-genome sequencing data of 304 DSN cattle. We identified 1,980 associated variants ($-\log_{10}(p) \geq 6.8$) in 13 genomic regions on 9 chromosomes. The locus with the highest significance ($-\log_{10}(p) = 11.93$) on milk fat content was identified on chromosome 5 at 86.5 Mb. The top marker was located in the gene *MGST1*. In contrast, *DGAT1* did not show any association since it was fixed (0.97) for the alanine protein variant coding for high milk and protein yield in DSN. *CSN1S1* and *GNG2* were identified as key genes affecting protein content ($-\log_{10}(p) = 8.47$) and protein yield ($-\log_{10}(p) = 10.48$), respectively. We also suggest *FGF12* for protein and fat yield, *HTR3C* for milk yield, *TLE4* for milk and protein yield, and *TNKS* for milk and fat yield. The selection of favorable alleles can help to improve milk yield and composition in DSN, but caution is necessary with respect to potential unfavored linkages to genes affecting muscularity. This is particularly important for maintaining the dual-purpose type of DSN. Such linkages need to be carefully investigated before applying milk-associated variants for selection in this small population.

Key Words: GWAS, Holstein, MGST1, association study, milk yield

P338 ISAG Bursary Award: A cell atlas across longissimus dorsi muscle from early embryo to aging goats and trajectories of myogenic progenitor/stem cells. Y. Chen*, C.-H. Huang, H.-P. Zhang, and L. Li, *Institute of Animal Genetics, Breeding and Reproduction, College of Animal Science and Technology, Sichuan Agricultural University, Chengdu, Sichuan, China.*

From embryonic stage to aging, the structure and function of muscle cells undergo great changes, including the proliferation and differentiation of muscle cells, the fusion and maturation of muscle fibers, and the activation and regeneration of satellite cells. The developmental trajectory of skeletal myogenesis and the transition between progenitor and stem cell states are unclear. Analyzing the process of animal muscle development can further explore the key genes of muscle development and find a breakthrough for improving mammal's muscle quantity and quality. To characterize this as yet unstudied dynamic process, here we used single-cell RNA sequencing to construct cell atlas of goat Longissimus dorsi muscle at a total of 14 time points from embryo to old age. A total of 120,944 single cells were used for analysis and comparison. All cell populations were analyzed by seuret and differential genes, and finally divided into 14 cell types according to specific genes identified in each cluster and known cell type markers. We identified differences in gene expression patterns across developmental stages and cell types, co-regulatory gene networks, and potential TF candidate genes that may regulate developmental stages. The potential synergies and signal exchange between cells were revealed by ligand analysis. In addition, we aimed to further understand the transitions between myogenic stem cell subtypes during development and the key genes involved in regulating these processes. We pooled myogenic stem cells at all stages and identified the differentiation trajectory and development trends among multiple subgroups. The ability of proliferation and differentiation of MuSCs at different stages was further compared. In conclusion, our study maps how cell types and gene networks change with muscle development, helps us understand the modes of action of different cell types during muscle development. This work provides a resource for any laboratory to map the characteristics of muscle single muscular-derived progenitor cells or stem cells that develop myogenesis.

Key Words: scRNA-seq, skeletal muscle, myogenic stem cells, myogenic progenitor cell

P339 MicroRNA levels in newborn calves before and after colostrum ingestion. H. T. Do[1,2], T. Chen[1], J. L. Williams[1,3], K. Petrovski[1], K. Ren[1], W. Y. Low[1], T. D. Van[1], and C. D. K. Bottema*[1], [1]*Davies Livestock Research Centre, School of Animal & Veterinary Sciences, University of Adelaide, Roseworthy Campus, Roseworthy, SA 5371, Australia,* [2]*Faculty of Animal Science, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi, Vietnam,* [3]*Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, 29122 Piacenza, Italy.*

MicroRNAs (miRNAs) are short non-coding RNAs that are present in very high concentrations in colostrum at d 0 postpartum. These miRNAs may play a critical role in newborn calf health or metabolism if they are similar to immunoglobulins and absorbed from the colostrum into the neonate calf blood. To determine if the miRNAs are absorbed, the miRNA profiles in calf blood were analyzed before and after colostrum ingestion. Calves received either dam colostrum or pooled colostrum plus milk. Total RNA was extracted from the colostrum within 2 h postpartum, pooled colostrum and from d 0 calf blood (before feeding colostrum) and d 1 calf blood (after feeding colostrum). The miRNA from the blood and colostrum was sequenced and the data analyzed using a bioinformatic pipeline. MicroRNAs that had increased levels in the d 1 calf blood may have been absorbed from the colostrum, and consequently, their concentrations were confirmed by RT-qPCR. The miRNA profiles in calf blood were complex at d 0 as there was a total of 1,163 miRNAs detected (997 known and 186 novel miRNAs). Of these miRNAs, 97% were also detected in the d 1 calf blood. Interestingly, only 22 miRNAs had significantly different levels between d 0 and d 1 calf blood. Three of the 22 miRNAs, miR-1260b, let-7a-3p, and miR-12042, had higher levels in the calf blood on d 1 after 2 colostrum feedings, suggesting that these miRNAs may have been absorbed from the colostrum. However, the results from the RT-qPCR only confirmed that the miR-1260b level was significantly higher on d 1. In addition, the miRNA levels in the calf blood were not correlated with the levels in the corresponding colostrum. There were also no differences in the miRNA levels if the calves received different volumes of colostrum in the first 24 h (4 L dam colostrum vs 2 L pooled colostrum + 2 L milk). Therefore, it is likely that very few, if any, of the miRNAs in the colostrum are absorbed by the calves.

Key Words: bovine, cattle, miRNA, milk, blood

P340 Withdrawn

P341 Population genomics of South American Creole cattle using high-resolution genome-scale SNP data. J. A. Ward*[1], S. I. Ng'ang'a[2,3], I. A. S. Randhawa[4], G. P. McHugo[1], J. F. O'Grady[1], J. A. Browne[1], A. M. Pérez O'Brien[5], T. S. Sonstegard[5], D. G. Bradley[6], L. A. F. Frantz[2,3], and D. E. MacHugh[1,7], [1]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Dublin, D04 V1W8, Ireland,* [2]*Palaeogenomics Group, Department of Veterinary Sciences, Ludwig Maximilian University, Munich, 80539, Germany,* [3]*School of Biological and Chemical Sciences, Queen Mary University of London, London, E1 4NS, United Kingdom,* [4]*Faculty of Science, The University of Queensland, Gatton, QLD 4343, Australia,* [5]*Acceligen, Eagan, MN,* [6]*Smurfit Institute of Genetics, Trinity College Dublin, Dublin, D02 PN40, Ireland,* [7]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, D04 V1W8, Ireland.*

The term *Creole* was applied to livestock that had been brought over to the American colonies of Spain and it can be seen in breed names today, indicating those animals that trace their origins to the Iberian Peninsula. Cattle are a recent addition to the Americas, having been first brought over at the end of the 15th century on Columbus' second voyage. In the intervening centuries, there have been further introductions of cattle from different regions (*Bos taurus*, *Bos indicus*, and hybrid populations) that have shaped the genomic landscape of South American cattle. The degree to which African cattle contributed to admixed Creole cattle, and when this contribution occurred is contentious, with suggestions that African cattle ancestry came indirectly to the New World via slave trading networks; however, direct African importation cannot be discounted. Over subsequent centuries these cattle populations have developed into locally adapted breeds with unique traits that have evolved in the tropical humid environments of countries such as Columbia and Venezuela. A notable example is the "slick" trait, found in several Creole breeds, which manifests as multiple physiological adaptations to heat stress. More recently, there have been introductions of zebu cattle directly from India, due to their suitability for the agroecological conditions in Brazil and neighboring countries. Zebu genetic ancestry expanded rapidly across the continent, primarily through breeding with existing Creole cattle populations. This introduction of zebu cattle has resulted in a situation where many of the locally adapted Creole breeds have been subject to genetic erosion or have been marginalized to areas where small numbers persist. Using a previously published global data set and newly generated whole-genome sequence data for Creole and African cattle, we evaluated the three-way admixture that is characteristic of these cattle and investigated, at genome-wide scale, selection signatures that reflect the unique adaptations of Creole cattle.

Key Words: selection scan, selection, adaptation, population genomics, admixture

P343 Study on cattle genomic selection for low-carbon beef production. D. Shin*[1], J.-E. Park[2], J. Heo[1], H.-K. Lee[1], S. Son[1], and J.-M. Kim[3], [1]*Jeonbuk National University, Jeonju-si, Jeollabuk-do, Korea,* [2]*Jeju National University, Jeju-si, Jeju-do, Korea,* [3]*Chung-Ang University, Anseong-si, Gyeonggi-do, Korea.*

According to past research and several reports, cattle contribute 8% of anthropogenic greenhouse gas emissions (Total livestock: 14%). With a growing concern for rapid climate change, efforts to monitor and mitigate emissions have become necessities for sustainable beef production. Various methods were being developed to reduce carbon emissions in beef production, and according to this global trend, we studied breeding method to reduce carbon emissions. In previous study, we could identify beef emission intensity highly correlated with annual carcass gains in FAO statistic data. And we could estimate individual cattle emission intensity from slaughtered and identification data. We thought that this method could be applied to select and produce low-carbon beef cattle. Because we have already been using genomic selection methods for genetic improvement of other slaughter traits, we can use emission intensity as new trait improved traits and select low-carbon cattle individuals. In this study, Korean cattle reference data contained 18,114 individuals with complete genomic (SNP chip), pedigree, and phenotypic data. The heritability of cattle emission intensity was 0.333, and it was proved that improvement could be possible if breeding value for emission intensity was used. In addition, we proved that faster improvement could be possible if reference data contained genomic data. This study showed that breeding could play an important role in reducing carbon emissions in livestock industry.

Key Words: cattle, beef, carbon, emission, genomic selection

P344 Identification of biomarkers for residual feed intake in dairy cows using targeted serum metabolomics. D. Hailemariam*[1], M. Hashemiranjbar[1], G. Manafiazar[1,2], P. Stothard[1], and G. Plastow[1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [2]*Animal Science and Aquaculture Department, Faculty of Agriculture, Dalhousie University, NS, Canada.*

Residual feed intake (RFI) is a measure of feed efficiency and defined as the difference between actual feed intake and expected feed requirements for maintenance and production. The objective of this research was to identify biomarkers for residual feed intake using a targeted serum metabolomics approach. RFI was calculated for 72 lactating Holsteins and low- and high-RFI animals were identified based on deviation from the mean (RFI < −0.5 SD for low and RFI >0.5 SD for high). Serum samples were collected at 50, 150 and 240 d in milk (DIM) and analyzed using direct injection and tandem mass spectrometry (DI-MS/MS). Serum metabolite concentrations were compared between low- (n = 20) and high- RFI (n = 20) groups at each of the 3 time points during lactation. The results indicated that in early lactation (50 DIM) serum concentrations of 5 acyl carnitines, 2 organic acids, 2 glycerophospholipids, and an amino acid were significantly different ($P < 0.05$) between RFI groups. Mid-lactation (150 DIM) was characterized by the differential concentration ($P < 0.05$) of 10 acyl carnitines, 8 glycerophospholipids, 4 biogenic amines, 2 amino acids and an organic acid. At late lactation (240 DIM), 4 amino acids, 3 glycerophospholipids, 2 organic acids and choline were significantly ($P < 0.05$) different between the groups. Receiver operating characteristic curve analysis identified candidate biomarkers with area under the curve values of 0.82, 0.93 and 0.76 at early, mid and late lactation stages, respectively. A multiple linear regression analysis at early lactation identified a set of 9 metabolites that predicted individual RFI phenotypes with validation R^2 (R^2val) of

0.54, and root mean square error (RMSE) of 1.92. At mid-lactation, a set of 10 metabolites predicted RFI with R^2val = 0.68, RMSE = 1.78. Similarly, at late lactation 6 metabolites predicted RFI with R^2val = 0.64, RMSE = 1.65. Overall, candidate biomarkers that can classify RFI categories and sets of metabolites that can predict individual RFI phenotypes were identified from serum samples collected at early, mid and late lactation stages.

Key Words: dairy cows, metabolomics, biomarkers, feed efficiency

P345 ISAG Bursary Award: A bovine GWAS reveals determinants of mobilization rate and dynamics of endogenous retroviruses. L. Tang*[1], B. J. Swedlund[1,2], C. Harland[1,3], K. Durkin[1,4], M. Artesi[1,4], G. C. M. Moreira[1], S. Dupont[1], J. Dejong[5], L. Karim[5], M. Deckers[5], E. Mullaart[6], W. Coppieters[1,5], M. Georges[1], and C. Charlier[1], [1]*Unit of Animal Genomics, GIGA-R, University of Liège, Liège, Liège, Belgium,* [2]*Keck School of Medicine, University of Southern California, Los Angeles, CA,* [3]*Livestock Improvement Corporation, Research & Development, Hamilton, New Zealand,* [4]*Laboratory of Human Genetics, GIGA-R, Liège, Liège, Belgium,* [5]*GIGA-Genomics Platform, University of Liège, Liège, Liège, Belgium,* [6]*CRV, Research & Development, Arnhem, the Netherlands.*

We have previously shown that a family of endogenous retroviruses (ERVKs) is still active in the cattle germline through detection of de novo transposition (*dn*T) events in whole-genome sequencing data of 127 extended cattle trios. Here, we refined a method to directly capture de novo insertion events and reproducibly quantify the ERVK transposition rate within the male germline of individual bulls. By applying this method to 430 bulls, we demonstrated that the *dn*T rate (*dn*TR) of ERVKs exhibits profound inter-individual variation. To investigate whether this inter-individual variation has a genetic basis, we performed a genome-wide association study (GWAS) and found that 8 loci have significant positive effects on *dn*TR. Polymorphic ERVKs underlie 4 out of 8 GWAS peaks. These ERVKs present intact proviral open reading frames, suggesting they are able to encode the machinery for ERVK mobilization. However, many de novo inserted ERVKs could only trace back to defective donors, illustrating that *dn*T can happen by *trans*-complementation from a competent locus. Further, we demonstrate that the *dn*TR of an individual is defined by the number of competent copies within its set of polymorphic ERVKs, explaining jointly 33% of the variance within our data set. Altogether, we provide the first comprehensive study of the genetic basis of inter-individual variation of a family of highly active transposable elements within the germline of a mammalian species.

Key Words: cattle, genome-wide association, other method, gamete, animal health

P346 High genetic diversity is maintained in the small endangered breed of German Black Pied cattle. G. A. Brockmann*[1], G. B. Neumann[1], P. Korkuc[1], M. J. Wolf[2], K. May[2], and S. König[2], [1]*Humboldt-Universität, Berlin, Germany,* [2]*Justus-Liebig Universität, Giessen, Germany.*

German Black Pied cattle (DSN, *Deutsches Schwarzbuntes Niederungsrind*) contributed to the development of Holstein, the high-yielding and most-widely used dairy breed in the world. Different from Holstein, DSN is a dual-purpose breed. The breed has been replaced almost entirely because of its low milk yield, which is 2,500 kg less than in Holstein cattle. Currently, DSN has only about 2,500 herdbook animals. To better place DSN on the market, breeding goals are improving milk production while maintaining the dual-purpose characteristics, robustness and genetic diversity. For accessing the genome of DSN, we sequenced 304 DSN animals and developed the DSN-specific bovine DSN200k SNP chip representing 182,154 sequence variants of DSN (173,569 SNPs, 8585 indels). Using this chip and whole genome sequencing information, we investigated the diversity of DSN and performed genome-wide association analyses. DSN showed a close genetic relationship with breeds from the Netherlands, Belgium, Northern Germany, and Scandinavia. The nucleotide diversity in DSN (0.151%) was higher than in Holstein (0.147%) and other breeds. The F_{Hom} and F_{RoH} values in DSN were among the lowest. Genomic regions with high F_{ST} between DSN and Holstein, significant XP-EHH regions, and RoH islands detected in both breeds harbor candidate genes that had been reported for milk, meat, fertility, production, and health traits, including one QTL detected in DSN for endoparasite infection resistance. Using whole genome sequencing data in genome-wide association studies, we identified 13 significant genomic loci for improving milk production. Interestingly, DGAT1 was fixed (0.97) for the alanine protein variant for high milk and protein yield. The data provide evidence for the excellent diversity management within DSN. Despite the small population size, DSN has a high level of diversity and low inbreeding. The genomic analyses provide information on potential gene pools that could be used to maintain diversity and to genetically improve the breed.

Key Words: endangered breed, diversity, inbreeding, selection

P347 X-linked genes influence various complex traits in dairy cattle. M. Sanchez*[1], C. Escouflaire[2], A. Baur[2], F. Bottin[1], C. Hozé[2], M. Boussaha[1], S. Fritz[2], A. Capitan[1], and D. Boichard[1], [1]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [2]*Eliance, Paris, France.*

The search for quantitative trait loci (QTL) affecting traits of interest in mammals is frequently limited to autosomes, with the X chromosome excluded because of its hemizygosity in males. This study aimed to assess the importance of the X chromosome in the genetic determinism of 11 complex traits related to milk production, milk composition, mastitis resistance, fertility, and stature in 236,496 cows from 3 major French dairy breeds (Holstein, Montbéliarde, and Normande) and 3 breeds of regional importance (Abondance, Tarentaise, and Vosgienne). Estimates of the proportions of heritability due to autosomes and X chromosome (h^2_X) were consistent among breeds. On average over the 11 traits, h^2_X = 0.008 and the X chromosome explained ~3.5% of total genetic variance. GWAS was performed within-breed at the sequence level (~200,000 genetic variants) and then combined in a meta-analysis. QTL were identified for most breeds and traits analyzed, with the exception of Tarentaise and Vosgienne and 2 fertility traits. Overall, 3, 74, 59, and 71 QTL were identified in Abondance, Montbéliarde, Normande, and Holstein, respectively, and most were associated with the most-heritable traits (milk traits and stature). The meta-analyses, which assessed a total of 157 QTL for the different traits, highlighted new QTL and refined the positions of some QTL found in the within-breed analyses. Altogether, our analyses identified several functional candidate genes, with the most notable being *GPC3*, *MBNL3*, *HS6ST2*, and *DMD* for dairy traits; *TMEM164*, *ACSL4*, *ENOX2*, *HTR2C*, *AMOT*, and *IRAK1* for udder health; *MAMLD1* and *COL4A6* for fertility; and *NRK*, *ESX1*, *GPR50*, *GPC3*, and *GPC4* for stature. This study demonstrates the importance of the X chromosome in the genetic determinism of complex traits in dairy cattle and highlights new functional candidate genes and variants for these traits. These results could potentially be extended to other species as many X-linked genes are shared among mammals.

Key Words: dairy cattle, X chromosome, genes, genetic variants

P348 Nanopore long read sequencing for genome-wide cattle sperm methylation profiling. M. Gòdia*, R. P. M. A. Crooijmans, A. C. Bouwman, M. P. L. Calus, and M. A. M. Groenen, *Wageningen University & Research, Wageningen, the Netherlands.*

Recent advances in base and methylation calling using the Oxford Nanopore Technologies (ONT) open up possibilities to jointly study genomic and epigenomic variation using long reads. Contrary to other techniques, ONT can sequence native DNA sequences without harsh chemical treatments or PCR amplifications. Methylation is the most studied epigenetic base modification: a methylation of cytosine in a 5′ CpG 3′ context. Methylation of CpGs (CpGme) can affect gene expression in a variety of manners and has also been associated with reduced sperm quality or infertility. In this study, we aimed to profile the cattle sperm methylome at single nucleotide resolution and compare

the methylation profile of sperm with samples from blood and testis. For this, cattle DNA was extracted from different samples including: 10 cryopreserved sperm ejaculates, and as a control: 1 testis and 1 blood sample and were sequenced in a promethION (ONT) device. On average, we obtained a sequencing depth of 18x, with N50 reads ranging from 7 to 29 kb length. Using a filter of 10 reads and at least, 10% with a GpGme, an average of 58.2% of the CpGs were found methylated in sperm, 51% in testis and 63.3% in blood. Surprisingly, the sequencing coverage was correlated with the number of CpGme. The proportion of CpGme in genomic features (e.g., Transcription Start Site, Coding DNA Sequence, intergenic) was found similar across the tissues studied. Interestingly, when setting a threshold for which at least 30% of the reads should be methylated, thus 'stricter repressive mode', we found that CpGme in sperm were overlapping in higher levels with Transposable Elements (33% SINE; 11.5% LINE), compared with testis (29.1% SINE; 9.9% LINE) or blood (27.7% SINE; 9.8% LINE). This suggests, that high DNA methylation in sperm of these regions is a safeguard against the mobilization of transposable elements to ensure genomic stability. In conclusion, analysis of the cattle sperm methylome showed a precise CpGme genome-wide landscape clearly divergent from other tissues and with non-methylated CpGs gene's promoters enriched for biological processes as spermatogenesis and embryonic development.

Key Words: cattle, epigenomics, gamete

P349 Sequence-based GWAS meta-analyses for beef production traits. M. Sanchez*[1], T. Tribout[1], N. Kadri[2], P. Chitneedi[3], S. Maak[3], C. Hozé[4], M. Boussaha[1], R. Philippe[5], M. Spengeler[6], C. Kuehn[3], Y. Wang[7], C. Li[7,8], G. Plastow[8], H. Pausch[2], D. Boichard[1], [1]*Université Paris Saclay, INRAE, AgroParisTech, GABI, Jouy en Josas, France,* [2]*ETH, Zürich, Switzerland,* [3]*Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [4]*Eliance, Paris, France,* [5]*INRAE, USC1061 GAMAA, Université de Limoges, Limoges, France,* [6]*QualitasAG, Zug, Switzerland,* [7]*Lacombe Research and Development Centre, Agriculture and Agri-Food Canada, Lacombe, Canada,* [8]*Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada.*

When performed at the whole genome sequence level, the meta-analysis (MA) of within-population GWAS results can be powerful and accurate for identifying causal variants for complex traits. One objective of the H2020 BovReg project is to perform MA at the sequence level for various dairy and beef cattle traits. For beef production, 5 partners from France, Switzerland, Germany, and Canada contributed with 54,782 animals from 15 purebred populations Charolais, Montbéliarde, Normande, Limousine, Blonde d'Aquitaine, Brown Swiss, Original Braunvieh or crossbred Charolais x Holstein, and Angus, Charolais and beef composite. Sequence-based GWAS results for 4 growth, 9 morphology, and/or 15 carcass traits were combined in 16 MA done using both fixed effects and z-score methods. This study demonstrates the value of MA, as a complement to within-population GWAS, in identifying a larger number of QTL, a smaller number of genomic variants in QTL and candidate variants located more frequently in genes. By applying here the most commonly used MA methods for GWAS, we confirm that the fixed effects method appears to be more powerful in detecting QTL, although MA combined substantially different traits in the present study. MA directly pointed out variants in genes, including *MSTN*, *LCORL*, *ARRDC3*, and *PLAG1*, previously associated with morphology and carcass traits in different studies. For example, the Q204X mutation, ranked 1st in the QTL peaks at the proximal end of BTA2 and causing a premature stop codon in the gene encoding myostatin (*MSTN*), was reported as one of the polymorphisms responsible for the double-muscled phenotype in several cattle breeds. We have also identified dozens of other variants located in genes whose function may be related to meat production traits (e.g., *COL3A1collagen type III α 1 chain*). By better identifying candidate genes and causal variants associated with beef production traits in cattle, MA appears to be of great interest in deciphering the biological mechanisms underlying these traits. The BovReg project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 815668.

Key Words: GWAS, meta-analyses, cattle, beef production

P350 ISAG Bursary Award: Size and composition of haplotype reference panels impact the accuracy of imputation from low-pass sequencing in cattle. A. Lloret-Villas*, H. Pausch, and A. Leonard, *ETH Zürich, Universitätstrasse 2, 8092, Zürich, Switzerland.*

Millions of cattle are genotyped every year for the purpose of genomic prediction. Low-coverage whole-genome sequencing (lcWGS) followed by genotype imputation is a cheap alternative to routine microarray-based genotyping. We assessed the impact of haplotype reference panel composition and sequencing coverage on the accuracy of lcWGS imputation in a target population consisting of cattle from the Brown Swiss (BSW) breed. We showed that GLIMPSE can accurately impute sequence variant genotypes into cattle genomes sequenced at low coverages. For instance, a same-breed haplotype panel consisting of 75 sequenced samples enabled us to genotype more than 13 million sequence variants in animals sequenced at 0.5-fold sequencing coverage with F1 scores greater than 0.9. Overall, same-breed haplotype reference panels with n = 150 sequenced samples outperformed multi-breed panels for sequencing coverages lower than 1-fold, including low allele frequencies. In absence of an adequately sized breed-specific panel (e.g., when less than 30 animals with sequence data are available), F1 scores of 0.9 could also be accomplished either by increasing the sequencing coverage of the target samples or by enlarging the reference panel with distantly related samples from other breeds. Nevertheless, since suboptimal haplotype reference panels lack variants private to the target breed, the resulting imputed lcWGS data are depleted for this type of variation.

Key Words: cattle, lcWGS, imputation, genotyping, variant calling

P351 Puberty changes the transcriptome of epiphyseal growth plates in *Bos indicus* heifers. M. Fortes*[1,2], T. Daro[1], J. Afonso[3], M. Tahir[4], and L. PN[5], [1]*The University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, Queensland 4072, Australia,* [2]*Queensland Alliance for Agriculture and Food Innovation, Brisbane, Queensland, Australia,* [3]*Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil,* [4]*Agriculture Victoria, AgriBio Center, Melbourne, Victoria, Australia,* [5]*CSIRO, Agriculture and Food, Queensland Bioscience Precinct, Brisbane, Queensland, Australia.*

Late puberty onset in *Bos indicus* heifers may result from selective pressure for environmental adaptation over reproduction (Utsunomiya et al., 2019). Late puberty often means delayed first calving (Burns, Fordyce, Holroyd, 2010) with related productivity losses. Puberty is a critical life stage triggered when the hypothalamic-pituitary-gonadal (HPG) axis loses sensitivity to the negative feedback of steroid hormones and the hypothalamus increases GnRH secretion. The endocrine crosstalk of the HPG axis affects the transcriptome of multiple tissues. In this study, we focus on the transcriptome of the epiphyseal growth plate (EGP), comparing 5 pre- and 5 post-pubertal heifers. The aim was to further our understanding of the mechanisms of bone growth during puberty. The EGP is a cartilage section found at the end of long bones, which contributes to pubertal bone growth. Pubertal release of the growth hormone, insulin-like growth factor, and sex steroids cause adolescent bone development. Skeletal growth increases at the start of puberty and plateaus after the "growth spurt." The transcriptome data obtained by RNA sequencing was used to identify differentially expressed (DEx) genes, predict co-expression networks, and detect transcription factors (TF) that potentially regulate EGP dynamics during puberty. RNA sequencing analysis followed a standard workflow (i.e., STAR aligner followed by DESEq2). As a result, 187 transcripts were considered DEx (P-value <0.05 and $\log_2$ fold change ≥1.3). The majority of DEx transcripts (142) had higher expression

pre-puberty. The promoter regions of DEx transcripts were mined for TF binding motifs and 41 TF were identified as potential regulators. Nine of these TF were expressed in the EGP, including the TF known as *Egr-1* and *Egr-2* (early growth response 1 and 2). The network of DEx transcripts was enriched for the terms *"extracellular space," "signal"* and *"secretion."* It is possible that the EGP secretes signals that feedback to the HPG and contributes to pubertal reproductive development. This working hypothesis requires further investigation.

Key Words: puberty, cattle, bone growth, RNA, gene expression

P352 International Sheep Genomics Consortium: providing underpinning resources for the sheep research community. S. M. Clarke*[1], R. Brauning[1], and International Sheep Genomics Consortium[2], [1]*AgResearch, Mosgiel, Otago, New Zealand,* [2]*sheephapmap.org, International Sheep Genomics Consortium.*

The long-term goals of the International Sheep Genomics Consortium (ISGC) are to develop underpinning resources for the sheep research community. This has resulted in continued improvement of the sheep genome assembly and development of low, medium and high-density Illumina SNP chips. The ISGC members have continued to make available whole genome sequence data to the community; this has been captured to produce Run3 of the ISGC "1000 genome" project. Through the application of a single harmonized pipeline for read QC, mapping, variant detection and annotation, the ISGC makes available variant collections derived in a standardized manner. Run3 has seen ~3500 animals analyzed with variant collections positioned on the Rambouillet v2 genome assembly with the aim to provide users with variants defined by chromosomal location, functional annotation, animals of interest, breeds of interest. In addition to Run3, an alternative to array-based genotyping has been investigated through use of Gencove's low-pass sequencing plus imputation platform. To bring together sequence and array based genotyping results, converting results from SNP chips into VCF format is being investigated. An update of the ISGC's activities will be presented.

Key Words: sheep, genomics, variants, low-pass sequencing

P353 The automated genetic ability evaluation system based on genomic information using Hanwoo reference cattle. Y. Kim*[1], D. Seo[1], D. Kim[1], O. Kwon[1], E. Hong[1], S. Yu[2], S. Lee[3], J. Kim[4], and M. Park[1], [1]*TNT Research Co, Ltd., Jeonju, South Korea,* [2]*Korea Institute for Animal Products Quality Evaluation, Sejong, South Korea,* [3]*Chungnam National University, Daejeon, South Korea,* [4]*Yeungnam University, Gyeongsan, South Korea.*

Recently, the Korean Hanwoo beef market is experiencing a double whammy of a price drop due to oversupply and an increase in production costs due to rising international grain prices. In addition, consumer trends for beef preference are changing, and unlike in the past, the improvement goal of Korean beef needs to change. To meet the diversifying needs of consumers and establish a rapid improvement system, a genetic ability evaluation system was developed using Korean cattle genomic information. As a reference group for estimating Hanwoo gEBV, genomic information was collected from 27- to 32-mo-old Korean cattle castrated between 2017 and 2022 using the Illumina Hanwoo 50K SNP Array. The carcass traits performance of the reference population and pedigree information were collected by sharing information with the Korean Institution for Animal Products Quality Evaluation (KAPE). A basic Hanwoo cattle reference population consisting of about 20,000 steer bulls was established through the project of the Animal Molecular Genetic Breeding Project of the Next Generation Biogreen 21 Project. This evaluation model was loaded into the web application system. Currently, it constitutes a reference population of 30,000 animals through the update of 10,000 animals in 2022. Using the established reference population and evaluation model, the accuracy of gEBV for carcass traits of Hanwoo was over 73.2%. In the analysis of 1,000 heads, the time was reduced by about 2.7 times compared with the existing analysis method, confirming that it is an optimized system that can evaluate Hanwoo in real time at industrial sites and provide information to farms.

Key Words: Hanwoo, genomic selection, gEBV, genetic ability

P354 ISAG Bursary Award: Long read based chromosome-level reference genome that encounters complex repetitive sequences in Alpaca (*Vicugna pacos*). M. Mendoza*[1], K. Munyard[2], T. Raudsepp[1], and B. Davis[1,3], [1]*Department of Veterinary Integrative Biosciences, School of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX,* [2]*Faculty of Health Sciences, Curtin Medical School, Curtin University, Perth, Australia,* [3]*Department of Small Animal Clinical Sciences, School of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX.*

A reference genome is the most essential asset to contemporary genetics research and necessary to study the health and structure of populations, evolution, morphological variation, and heritable disease. It is also the reference to determine sequence-based genomic, epigenomic, and transcriptomic variation and identify variation associated with phenotypes and disease. As such, the utility of fragmented reference genomes containing non-chromosomally assigned contigs or scaffolds are limited. Here we combined PacBio long-read and high-fidelity circular consensus sequencing, Hi-C chromatin conformation capture, optical genome mapping (OGM), and manual curation to generate a chromosome-level alpaca reference *VicPac4*. Assignment of sequence scaffolds and super-scaffolds was supported by the alpaca cytogenetic map and the recently available alpaca 76K SNP chip data. The current assembly is 2,572 Mb, with 1200 contigs (N50 = 40.28 Mb) and 783 scaffolds (N50 = 67.53 Mb). Of the initial 790 scaffolds retrieved from OGM, 44 were manually assigned into 37 scaffolds that represent 36 autosomes pairs and the X chromosome, with lengths from 6.7 to 127.7 Mb; 746 scaffolds with a median size of 579 kb remained unassigned. We also corrected several chromosomal mis-assignments in the previous alpaca reference *VicPac3.1* and evaluated the utility of the 76K SNP chip in the context of our highly accurate assembly. Likewise, we were able to locate complex repetitive sequences, such as telomeric TTAGGG repeats in the ends of at least 36 chromosome arms. Furthermore, the conserved sequences of 18S-5.8S-28S rDNA, also known as Nucleolar Organizer Regions (NOR), were found in several unassigned scaffolds, facilitating their assignment to the respective NOR chromosomes. The improved chromosome-level alpaca reference *VicPac4* has considerably enhanced accuracy and contiguity and will be the basis for the first population-level alpaca genome variant database.

Key Words: alpaca, reference genome, NOR, telomeres

P355 Genomic selection for milk fatty acids from Canadian Holstein cows. S. Peters*[1], K. Kadir[1], E. Ibeagha-Awemu[1], and X. Zhao[1], [1]*Department of Animal Science, Berry College, Mount Berry, GA,* [2]*Department of Animal Science, Faculty of Agriculture, Aydin Adnan Menderes University, Aydin, Turkey,* [3]*Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, JIM 0C8 Canada,* [4]*Department of Animal Science, McGill University, Anne de Bellavue, QC H9X 3V9, Canada.*

Milk fat is an important source of fatty acids in human nutrition all over the world. Fatty acids are classified based on the degree of unsaturation (monounsaturated (MUFA), polyunsaturated (PUFA) and saturated fatty acids (SFA)) and the length of the carbon chains in MUFA, PUFA and SFA. Fatty acids have been associated with cardiovascular disease risks and beneficial (anticancer) effects to human health; therefore, producers in dairy industry focus on the optimization of fatty acids in the milk production for the human health. Over the past 2 decades, advances in high-throughput sequencing technologies provide SNP markers which can be used in genomic selection (GS) of complex (fatty acids) traits in dairy cows. The objective of this study is to compare the predictive ability of GBLUP and Bayesian (BayesA, BayesB and BayesC) methods for GS of MUFA, PUFA and SFA traits by using 76,299 genotyping-by-sequencing-generated SNP markers

from 695 Canadian Holstein dairy. The estimates of heritabilities for MUFA, PUFA and SFA traits were obtained from GBLUP and Bayesian methods within random clusters of cross-validations and they indicated that Bayesian methods resulted in the similar heritability estimates that ranged from 0.02 to 0.49; however, the heritability estimates from BayesA (between 0.02 and 0.31) and BayesC (between 0.02 and 0.34) methods were lower than those from GBLUP (between 0.05 and 0.44). On the other hand, BayesB method (0.19, 0.14, 0.28, 0.24, 0.26, 0.41) resulted in higher heritability estimates than GBLUP (0.15, 0.12, 0.16, 0.19, 0.10, 0.23) for Eicosapentanoic PUFA, butyric, caproic, caprylic, tridecylic and lignoceric SFA, respectively. The prediction ability of GBLUP (0.384–0.878, 0.306–0.898, 0.225–0.786) and Bayesian methods (0.385–0.879, 0.306–0.898, 0.223–0.784 from BayesA; 0.395–0.878, 0.302–0.898, 0.221–0.783) from BayesB; 0.392–0.878, 0.304–0.898, 0.225–0.787 from BayesC) was quite similar for MUFA, PUFA and SFA traits, respectively. However, the comparison of the computing time in GS indicated that GBLUP had significantly less computing effort than Bayesian methods.

Key Words: fatty acids, MUFA, PUFA, SFA, genomic selection

P356 Withdrawn

P357 ISAG Bursary Award: Model comparison of genomic prediction for commercial population in Hanwoo (Korean cattle). S. Lee*[1], D. Lee[1], Y. Kim[2], J. Kang[1], D. Lee[1], H. Lee[1], and S. Lee[1], [1]*Chugnam National University, Yuseong-gu, Daejeon, South Korea,* [2]*Quantomic Research & Solution, Yuseong-gu, Daejeon, South Korea.*

Hanwoo (Korean cattle) is an indigenous and this breed has been subject to bull selection through national evaluation since the 1980s. Genetic improvement of the commercial population (CP) has gradually increased from the Hanwoo breeding program. CP is valuable data to re-evaluate bull and cow genetic potential using genetic evaluation (BLUP). However, CP has various systematic effects and other environmental effects. Those systematic and environmental effects should be properly treated to avoid bias of breeding value in the evaluation model. Besides, the amount of commercial data is much larger than the national evaluation data set, and age of slaughter also varies (CP approx. 30, national evaluation at 24) so it is expected to lead to meaningful outcomes. In this study, we aimed to develop an optimal evaluation model for Hanwoo CP that contains 2,130,926 phenotypes (carcass weight), 2,210 individual genotypes, and 6,742,146 pedigree records. We used phenotype records with many variables and examined a total of 12 models by varying combinations of variables, including the farm and location as potential sources that can be derived from residual variance when we applied it to evaluation. This study also conducted the days variable (days of birth to the slaughter) as a fixed effect (month) or covariate (days) to determine the most appropriate type of days variable. Results showed that using birth and slaughter contemporary groups as a combination of variables resulted in a low AIC compared with separated fixed effects. The use of slaughter days as a covariate was also found to be a better fit than using slaughter months as a fixed effect. Incorporating both farm and location random effects in the model was a better fit compared with using them separately. Overall, while a random model may have an advantage due to its complexity resulting in a lower AIC, the model that included birth and slaughters contemporary group, slaughter days as a covariate, and both farm and location random effect was found to have the best fit for this CP reference. (Ongoing: genomic prediction with farm effect will be added.)

Key Words: Hanwoo cattle, commercial population, evaluation model, genomic prediction

P358 Preliminary results: identification of genomic regions associating with wet carcass syndrome in sheep. B. Bhika Kooverjee*[1,2], P. Soma[1], M. van der Nest[3], F. W. C. Neser[2], and M. M. Scholtz[1,2], [1]*Agricultural Research Council, Irene, Pretoria, South Africa,* [2]*University of the Free State, Bloemfontein, South Africa,* [3]*University of Pretoria, Pretoria, South Africa.*

Wet carcass syndrome (WCS) is a condition found in sheep that negatively affects the quality of their carcasses. Before slaughter, an affected animal appears to be physically normal, showing no symptoms of an abnormality. However, after the removal of the skin during the slaughter process, after a 24-h cooling period affected animals show an accumulation of watery fluid on the dorsal parts of the carcass as well as on the hind legs and flanks. The carcass appears to be "wet" and it does not dry off with overnight cooling. Affected carcasses are unacceptable to the consumer from both an aesthetic point of view and due to reduced shelf life. Therefore, the aim of this study was to identify the DNA markers that are associated with Wet Carcass Syndrome in Sheep. For this purpose, eat samples were collected from affected (n = 75) and unaffected (n = 28) animals and DNA was extracted and samples were genotyped using the Ovine 50K SNP BeadChip. Quality control was performed using PLINK v1.9. SNP markers were excluded if minor allele frequency (MAF) was less than 0.05, missing rate per SNP (–geno) was greater than 0.05, and if they failed Hardy-Weinberg equilibrium (–hwe) at 0.01 threshold resulting in 46 289 autosomal SNPs. A genome-wide association analysis (GWAS) was performed using PLINK (–assoc), where the final step included Fisher's exact test (–fisher). Preliminary results revealed one locus positioned on chromosome 1:160339673 that is significantly associated with WCS. Using a GWAS approach allowed us to increase our knowledge about the underlying mechanisms of WCS.

Key Words: case-control GWAS, ovine genomics, ovine 50K beadchip

P359 Genetic analysis of milking temperament and its association with daily milk yield and composition in South African Holstein cattle. T. T. Siwele[1,2], B. J. Mtileni[1], K. A. Nephawe[1], M. A. Madilindi[2], B. Dube[2], and C. B. Banga*[3,4], [1]*Department of Animal Science, Tshwane University of Technology, Pretoria, South Africa,* [2]*Agricultural Research Council, Animal Production, Irene, South Africa,* [3]*Department of Agriculture and Animal Health, University of South Africa, Florida, South Africa,* [4]*Department of Animal Sciences,*

Faculty of Animal and Veterinary Sciences, Botswana University of Agriculture and Natural Resources, Gaborone, Botswana.

Milking temperament (MT) is an important functional trait in dairy cattle, due to its effects on welfare and profitability. This study was carried out to estimate heritabilities and genetic correlations among MT and daily milk yield and composition, in South African Holstein cattle, so as to explore the feasibility of including MT in the breeding objective. Data consisted of MT records of 2 844 cows from 16 herds participating in the National Milk Recording and Improvement Scheme, recorded between 2020 and 2021. Corresponding test-day records of milk yield and composition, and pedigree information, were obtained from the Integrated Registration and Genetic Information System of South Africa. Parameter estimates were obtained by linear animal models, using the ASReml package. The heritability estimate for MT was low (0.05 ± 0.04), while estimates for milk production traits were low to moderate, ranging from 0.11 ± 0.05 for milk yield to 0.24 ± 0.06 for protein percent. Milking temperament had a moderate and favorable genetic correlation of 0.60 ± 0.35 with milk yield. On the other hand, low and unfavorable correlations were observed for MT with fat percent (−0.12 ± 0.24) and protein percent (−0.30 ± 0.32). These results suggest that there is scope to improve MT through selection in South African Holstein cattle, although the accuracy of such selection would be low. Application of multi-trait models including genetically correlated traits such as milk yield, and large-scale recording of MT, may improve the accuracy of selection.

Key Words: genetic improvement, heritability, genetic correlation, milk production traits

P360 ISAG Bursary Award: Population fine structure analyses of the indigenous Croatian cattle populations. I. Drzaic*[1], I. Curik[1], V. Brajkovic[1], D. Novosel[1,2], and V. Cubric-Curik[1], [1]*University of Zagreb Faculty of Agriculture, Svetošimunska cesta 25, 10040 Zagreb, Croatia,* [2]*Croatian Veterinary Institute, Savska cesta 143, 10000 Zagreb, Croatia.*

It is very important to preserve the genetic biodiversity of locally adapted breeds. Besides some highly productive cosmopolitan breeds, there are over 1,000 locally adapted cattle breeds. The indigenous Croatian cattle breeds are late-maturing, long-lived, low-productivity breeds bred in extensive systems without much selection. The objective of this study was to analyze the population structure and genomic position of 3 indigenous Croatian cattle breeds: Croatian Busha (CCB), Istrian Cattle (CCIG) and Slavonian Syrmian Podolian Cattle (CCSSP) using a high-density SNP chip (777K). A total of 112 Croatian cattle belonging to 3 breeds (CCB = 40, CCIG = 40, CCSSP = 32) were collected from different farms. DNA was extracted using the Qiagen Blood and Tissue Kit (Qiagen, Germany) and genotyped using Illumina BovineHD Genotyping BeadChip. After quality control, SNPs with GT score <0.4, GC score <0.8, a call rate <90%, and an individual call rate <95% were removed, leaving 696.419 SNPs for further analysis. Our analyses also included information on 143 individuals from 39 breeds obtained from public open sources. We used a graphical approach to illustrate the relationship between the analyzed breeds in terms of their population structure and admixture. Supervised and unsupervised cluster analyses revealed a fine population structure of Croatian cattle breeds and their close relationship with several regional breeds. Principal component analysis showed clear cluster assignment for 3 Croatian cattle breeds. Population structure analysis showed clear segregation of Croatian cattle breeds at low clusters. This study is the first genome-wide analysis of population structure of 3 indigenous Croatian cattle populations and will help to develop a better breeding and genome diversity conservation strategy for Croatian indigenous cattle breeds.

Key Words: cattle, population genomics, high SNP density, admixture, breed diversity

P361 Multibreed genomic prediction and detection of QTL for fertility traits of tropical bulls. L. R. Porto-Neto*[1], P. A. Alexandre[1], J. Dorji[1], M. R. Fortes[2], and A. Reverter[1], [1]*CSIRO Agriculture and Food, Brisbane, QLD, Australia,* [2]*The University of Queensland, School of Chemistry and Molecular Bioscience, Brisbane, QLD, Australia.*

The genetic improvement of fertility traits in beef cattle lags behind other production traits, especially in tropical cattle in Northern Australia. In bulls, the standardized bull breeding soundness examination performed before young bulls are commercialized opens an opportunity to explore fertility-related traits collected using a detailed protocol. The observed traits include body conformation traits, e.g., sheath score and scrotal circumference, and analyses of semen samples crush-side and laboratory-based, e.g., the percentage of normal sperm. Here, we describe a resource population of more than 8,000 bulls of 6 tropical breeds with fertility traits recorded and SNP genotypes imputed up to ~670,000 markers. Heritability estimates varied from low (0.17, percentage of proximal cytoplasmic droplets in sperm) to high (0.55, sheath score). Genomically enhanced breeding values were largely unbiased. Their accuracies, although varied between breeds and populations, were moderate (0.32, proximal cytoplasmic droplet) to high (0.55, scrotal circumference). Using the estimated SNP effects, we detected QTL in several bovine chromosomes, with highly significant SNP in chromosomes 5, 17, 21, and X. These results confirmed some previously identified QTLs and pointed to new ones. In summary, this research has demonstrated the usefulness of genomic approaches in multibreed scenarios focusing on fertility-related traits of tropical bulls. Genomic breeding values were estimated with useful accuracies, and QTLs were detected. Further work toward the identification of functional variants within those QTL is warranted.

Key Words: fertility, genomic prediction, QTL

P362 The genetic structure and differentiation within and between smallholder and commercial beef cattle of South Africa. M. Ramoroka*[1,2], F. Neser[1], R. Grobler[2], S. F. Lashmar[2], and M. Makgahlela[1,2], [1]*Department of Animal Science, University of the Free State, Bloemfontein, Free State, South Africa,* [2]*Agricultural Research Council-Irene, Animal Production, Pretoria, Gauteng, South Africa.*

The majority of smallholder cattle keepers in South Africa are uncertain of the breed or even the genetic composition of their animals. Smallholder beef cattle populations are adapted to diverse climatic conditions and survive for long in harsh South African (SA) environments. Genomic resources and tools are facilitating elucidation of breeds of origin for smallholder farming systems toward more targeted breeding programs. The aim of this study was to genetically characterize non-descript SA smallholder beef cattle using SNP genotypes generated with the GGP 150K Bovine bead chip, containing 141 716 SNPs. The study included 116 non-descript smallholder (SHD) beef cattle animals from 7 SA provinces (i.e., the Eastern Cape (EC), Free State (FS), Gauteng (GP), Kwa Zulu Natal (KZN), Limpopo (LP), North West (NW) and Northern Cape (NC)) and 366 animals from 8 commercial beef cattle breeds that were used as reference populations. Quality control was done using Plink v1.9, while Arlequin v3.5, ADMIXTURE v2.0 and GENESIS were used for downstream analysis. Principal component analysis, Admixture and genetic diversity measures revealed high heterogeneity in SHD cattle and indicated high gene flow from commercial beef cattle. The results showed a weak sub-population structure in the SHD populations, due to admixture with commercial cattle, especially indigenous breeds. The observed homozygosity ranged between 0.328 ± 0.219 and 0.395 ± 0.139, while the expected heterozygosity ranged from 0.326 ± 0.163 to 0.389 ± 0.112. Inbreeding was low and ranged from −0.023 ± 0.047 to 0.133 ± 0.067. The AMOVA revealed a moderate genetic variation of 5% among populations, 1% among individuals within populations and 94% within individuals. The F_{ST} was low between SHD animals from LP and GP (0.008) and higher between NW and KZN (0.106). The patterns of genetic diversity and population structure showed that effective management and utilization of SA smallholder beef cattle is important. The obtained results can be used as baseline information for the development and implementation of SHD breeding programs.

Key Words: genetic characterization, genetic diversity, population differentiation

P363 Genome-wide association study of footrot in Portuguese native Merino. D. Gaspar*[1,2], C. Ginja[2], C. Leão[1,3], H. Monteiro[4], L. Tábuas[4], S. Branco[3], L. Padre[3], P. Caetano[3], N. Carolino[5], C. Matos[4], A. Ramos[1,3], E. Bettencourt[3], and A. Usié[1,3], [1]*Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/ Instituto Politécnico de Beja (IPBeja), Beja, Portugal,* [2]*BIOPOLIS/CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Porto, Portugal,* [3]*MED - Mediterranean Institute for Agriculture, Environment and Development, University of Évora, Polo da Mitra, Évora, Portugal,* [4]*ACOS – Agricultores do Sul, Beja, Portugal,* [5]*Instituto Nacional de Investigação Agrária e Veterinária, I.P. (INIAV, I.P.), Quinta do Marquês, Oeiras, Portugal.*

Ovine footrot is a necrotic and highly contagious disease caused by a gram-negative anaerobic bacterium, *Dichelobacter nodosus* (*D. nodosus*), and a complex mixture of bacteria in which *Fusobacterium necrophorum* acts as an important primary agent. Footrot affects the epidermal tissues of the interdigital skin and horn of the hooves, being one of the main causes of lameness in sheep, with negative impact on the economy of sheep industry worldwide. Footrot incidence and severity are modulated by 3 key factors: i) environmental conditions; ii) virulence of *D. nodosus* strains; and iii) host genetics. Understanding the genetic basis for footrot would allow breeders to select more resilient animals. We used a genome-wide approach to identify single nucleotide polymorphisms (SNPs) and candidate genes associated with footrot in Portuguese Merino and crossbred sheep. For this, 1,466 animals were clinically evaluated for footrot lesions based on the modified Egerton system (scores from 0 to 5), according to the following: Merino Branco (n = 366), Merino Preto (n = 144), and crossbreds (n = 956). Genomic DNA was extracted from whole-blood collected in these animals and used for genotyping with a set of 47,779 SNPs (29,716 SNPs after quality control). This SNP panel was specifically designed from 39 genomes of these populations. We identified one genome-wide significantly (adjusted Bonferroni threshold of 2.52×10^{-6}) associated SNP on chromosome 24 at 16.68 Mb, and 5 genome-wide suggestive (threshold of 5.05×10^{-5}) SNPs on chromosomes 2 (26.04 Mb), 9 (10.04 Mb), 14 (20.52 Mb) and 24 (16.66 and 16.69 Mb). The gene regions containing these variants have been described to be associated with different immune responses against parasite infections and wound healing. In conclusion, these preliminary data provide new insights into the genomic regions that are involved in the different clinical signs of footrot in Portuguese Merino and crossbreed sheep. In the future, marked assisted selection schemes could be implemented in ovine-breeding programs.

Key Words: *Ovis aries*, Portuguese Merino, footrot, genome-wide association study

P364 ISAG Bursary Award: Genome-wide association studies reveal candidate genes associated with plasma and wool metabolites indicators of water deprivation tolerance in Rasa aragonesa sheep. S. Pérez-Redondo*[1], C. Calvete[1,2], M. Joy[1,2], A. Domínguez[1], S. Lobón[1,2], M. Serrano[3], and J. Calvo[1,4], [1]*Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Zaragoza, Spain,* [2]*Instituto Agroalimentario de Aragón (IA2), Zaragoza, Spain,* [3]*Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC), Madrid, Spain,* [4]*ARAID, Zaragoza, Spain.*

The aim of this study is to identify candidate genes or genomic regions associated with ovine water stress biomarkers through genome-wide association analysis (GWAS). Two hundred one ewes from the Rasa Aragonesa breed were challenged with total water restriction for 5 d. Temperature and percentage of humidity was also measured, as well as intake, live weight, and body condition score daily during the 5-d restriction. Blood samples were collected just before the challenge (0d) and at the end of the challenge (5d), obtaining hematological (white, red blood cells and platelet traits) and metabolite (total protein, glucose, nonesterified fatty acids (NEFA), cortisol, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) concentration phenotypes) measurements. Wool samples were also collected at d 0 and 4 weeks later (28d), and cortisol, DHEA and DHEAS were also measured. In addition to these measurable traits for each blood/wool sample, variation of traits between blood or wool samples collected at different time points were also calculated for each animal. Genotyping was performed using the Ovine680kBeadChip array. AIREMLF90 package from the BLUPF90 family programs was used to estimate variance components for each trait. GCTA software was used for GWAS analysis. Mixed model statistical analysis indicated that all hematological and metabolite levels showed consistent changes throughout water restriction period with the exception of plasma cortisol and mean corpuscular hemoglobin (MCH). It is outstanding that the neutrophils to lymphocyte ratio in close relationship with stress hormones was also altered. We found 56 SNPs associated with different biomarkers at genome-wide significant level (p_Bonferroni <0.05), which were annotated against the ovine reference genome (Oar_rambouillet_v1.0). We performed GO and KEGG pathway enrichment analyses using DAVID through the Functional Annotation Cluster (FAC) tool, finding one cluster of enrichment related to the hypothalamic-pituitary-adrenal axis in wool DHEA phenotype.

Key Words: sheep, water stress, metabolites, GWAS, SNP.

P365 Meta-analysis of physiological responses to heat stress in Dorper and Ile de France sheep populations. P. Soma*[1], B. Kooverjee[1], and M. van der Nest[2], [1]*Agricultural Research Council, Irene, Gauteng, South Africa,* [2]*University of Pretoria, Pretoria, South Africa.*

A major challenge of the 21st century is the risk of climate change to food security. In South Africa, climate change affecting rainfall, temperature patterns and evaporation levels, affect availability of water for agricultural purposes. Sheep breeds differ in their tolerance and susceptibility to heat stress. Dorper is a hardy South African composite meat breed, derived from a cross between the Black-headed Persian and the Dorset Horn. Ile de France sheep is one of the top meat breeds in South Africa that originated from France. Currently, the physiological mechanisms required for thermoregulation and thermo-tolerance in South African sheep breeds is limited. Hence, the aim of this study was to determine the physiological responses, such as ocular and rectal temperature, as well as respiration rate of these 2 sheep breeds in response to heat stress. A meta-analysis was performed on 93 individuals. A one-way ANOVA was performed in R using the 'aov' function within the R stats package v 4.0.3. The ocular temperature and respiratory rate differed significantly ($P < 0.001$) between the 2 breeds. Although, within population, ocular and rectal temperatures differed significantly ($P < 0.001$). The extent of the differences was defined by the breed and the physiological state of the animal. Results of this study demonstrated that heat stress disturbs rectal temperature and respiration rate of sheep and that the South African Dorper breed is better adapted to heat stress than Ile de France breed. Heat stress associated with climate change may increase maintenance requirements, as well as influence production.

Key Words: South African sheep, stress response, climate change, physiological

P366 Genetic analysis of hypospadias in Coburg fox sheep. G. Rudd Garces*[1], A. Letko[2], I. M. Häfliger[2], C. Drögemüller[2], and G. Lühken[1], [1]*Institute of Animal Breeding and Genetics, Giessen, Hessen, Germany,* [2]*Institute of Genetics, Bern, Bern, Switzerland.*

Hypospadias is a common developmental malformation of the penis characterized by the abnormal urethral opening on the underside of the penis or on the perineum. In humans, variants in more than 7 genes related to syndromic and non-syndromic forms of hypospadias have been described. In domestic animals, hypospadias has been reported in dog, cattle, horse, goat and sheep. However, the underlying genetic defect remains largely unknown. In this study, we investigated a family of Coburg fox sheep, a local breed of Germany, in which 2 lambs of consanguineous mating were affected by hypospadias. Using the GGP Ovine50K array, we obtained genotype data of the family and the 2 cases. Assuming monogenic autosomal recessive inheritance, combined linkage and homozygosity mapping revealed 14 critical genome regions on 9 chromosomes totaling 162 Mb. Whole genomes of both affected lambs were sequenced and shared homozygous variants were

compared with 108 control genomes of different breeds. This search yielded 176 private protein-changing variants affecting 58 genes, mostly representing variants of uncertain significance, but 5 were located in likely functional candidate genes of male reproduction. These represent missense variants in *EIF4G3*, *LGR4*, *KIAA1210*, and in-frame deletions in *RSPH6A*, *BCORL1,* that are currently be genotyped in the index family and further sheep of the same breed. The p.(Ser525Leu) missense variant in *LGR4* was the only one that co-localize to a critical region. A possible identification of the genetic cause of this ovine form of hypospadias will enable genetic testing to avoid the unintentional breeding of further affected lambs and provide a first spontaneous large animal model to understand the mechanisms of hypospadias in humans

Key Words: sheep and related species, computational biology, genome sequencing, genetic disorder, animal health

P367 Transcriptome profiling and functional enrichment analysis of abscessed liver tissue in beef cattle. Y. Wang[1,2], J. Wang[3], Z. Pan[1], R. J. Gruninger[4], R. Zaheer[4], T. A. McAllister[4], and L. L. Guan*[1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada,* [2]*Institute of Animal Genetics and Breeding, College of Animal Science and Technology, Sichuan Agricultural University, Chengdu, Sichuan, China,* [3]*State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Animal Science and Technology, Guangxi University, Nanning, Guangxi, China,* [4]*Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada.*

Liver abscesses are lesions found within the liver of cattle that are caused by Gram-negative anaerobes, with a prevalence that often exceeds 25%. However, the etiology of this disease is poorly characterized. Thus, to obtain a more comprehensive understanding of the molecular factors that contribute to liver abscesses, RNA sequencing was performed on 48 liver samples collected from 32 feedlot cattle, 16 from healthy individuals (HH), 16 from the abscesses region of cattle with abscessed livers (AA) and 16 from the non-abscessed regions in those livers that possessed abscessed (AH). After quality trimming, about 35 million clean reads per sample were used for alignment, mRNA classification and quantification. A total of 12,666 of 21,861 mRNA genes with transcripts per million (TPM) > 1 were detected in over 80% of samples and defined as expressed genes. The hierarchical cluster analysis showed that the transcriptome profiles of AH and HH liver samples clustered together, whereas those of AA liver samples separated from these clusters. Differentially expressed genes (DE-genes) were selected based on a strict threshold (The expressed genes with False Discovery Rate (FDR) < 0.01 and log2 fold change > |2|). Compared with HH, 1,630 DE-genes were identified, with 1,151 DE-genes upregulated and 479 DE-genes downregulated in AA. Compared with AH, 1,442 DE-genes were identified, with 1,103 DE-genes upregulated and 339 DE-genes downregulated in AA. However, no DE gene was identified between AH and HH. Functional enrichment analysis showed that compared with HH, the upregulated DE-genes in AA were involved in immune response and the extracellular matrix, while downregulated DE-genes in AA were involved in massive metabolism function, such as glucuronosyltransferase activity and retinol metabolism. The enrichment function of DE-genes between AA and AH was similar to those between AA and HH. These results indicate that inflammation in abscessed liver tissue alters gene expression in a localized manner, while non-abscessed regions retain a mRNAs profile that aligns with healthy liver tissue.

Key Words: liver abscess, RNA-seq, cattle mRNAs, differential expression analysis

P368 Scalepopgen: a bioinformatics workflow resources for population genomic analyses. M. Upadhyay* and I. Medugorac, *LMU Munich, Population Genomics Group, Department of Veterinary Sciences, Lena-Christ-Str. 48, 82152 Martinsried, Germany.*

Population genomics analyses such as the inference of population structure, introgression, and signatures of selection usually involve the application of a plethora of programs, scripts, and procedures. As a result, it is frequently necessary to install numerous tools and dependencies and to perform series of data transformation or pre-processing procedures that make population genomics data analyses challenging. While the usage of container-based technologies has significantly ameliorated the problems associated with installation of tools and its dependencies, population genomics analyses requiring multi-step pipelines or complex data transformations can greatly be facilitated by the application of workflow management systems (WMSs), such as *Nextflow* and S*nakemake*. The usage of WMSs in population genomics can also contribute to the scalability and reproducibility of analyses. Here, we describe *scalepopgen*, a fully automated workflow that can apply widely used population genomics analyses that start from vcf files. The workflow is developed in *Nextflow* and can be run locally or in high performance computing systems using either *conda, ingularity* or *Docker*. The automated workflow procedures include: (1) filtering of individuals and genotypes; (2) running PCA, and admixture analysis, including the identification of optimal K-values and plotting; (3) running *Treemix* with or without migration edges and bootstrapping, including the identification of the optimal number of migration events and results plotting; and (4) implementing single- and multi-population comparison-based procedures to identify signatures of selection. The workflow uses various open-source tools complemented with several in-house developed scripts in python, R, and C++. Some implemented tools are not available elsewhere such as e.g., the genetic diversity index H of Fay and Wu. We validated our pipeline for scalability and reproducibility using vcf files generated from more than 250 publicly available cattle whole genome sequences.

Key Words: nextflow, signature of selection, admixture, treemix

P369 Assessment of different enrichment methods to characterize bovine circRNAs. Y. Wang[1,2], J. Wang[3], R. J. Gruninger[4], T. A. McAllister[4], and L. L. Guan*[1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada,* [2]*Institute of Animal Genetics and Breeding, College of Animal Science and Technology, Sichuan Agricultural University, Chengdu, Sichuan, China,* [3]*State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Animal Science and Technology, Guangxi University, Nanning, Guangxi, China,* [4]*Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada.*

Circular RNAs (circRNAs) characterization using sequencing data is more challenging than linear RNAs because the back-splicing junction reads are the only feature for circRNAs identification. To date, the knowledge of bovine circRNAs is limited. In this study, the total RNA of liver and rumen epithelium from 3 15-mo-old Kinsella Composite beef were extracted, and the circRNAs were enriched using 6 approaches before RNA-sequencing, namely, ribosomal RNAs removal using Ribo-zero (Ribo); linear RNAs degradation using Ribonuclease R (R); linear RNAs and RNAs with structured 3′ ends degradation using Ribonuclease R then tailing and poly $(A)^+$ RNA depletion (RTP); ribosomal RNAs coupled with linear RNAs elimination using Ribo-zero and Ribonuclease R (Ribo-R); ribosomal RNA, linear RNAs and RNAs with poly (A) tailing elimination using Ribo-zero, Ribonuclease R and poly $(A)^+$ RNA depletion (Ribo-RP); and ribosomal RNA, linear RNAs and RNAs with structured 3′ ends elimination using Ribo-zero, Ribonuclease R, tailing and poly $(A)^+$ RNA depletion (Ribo-RTP), respectively. RNA-sequencing analysis showed that different approaches resulted in varied number of circRNAs per million clean reads ($P_{adj} < 0.05$) and false positive rate of identifying circRNAs. Totally, 4,051 of 50,837 circRNAs were defined as high confident circRNAs (Back-splicing junction reads ≥2 and detected in at least all replicates of one method in one tissue), and most of them could be identified with Ribo-RTP method. Besides, 308 of 2,285 and 260 of 2,939 high confident circRNAs were commonly identified from 5 methods in liver and rumen tissues, respectively. Conservation analysis revealed that 507 bovine high confident circRNAs had shared splicing sites with human circRNAs. The present study provides valuable information for researchers to choose the best

circRNAs enrichment methods when studying circRNAs from tissue samples which will enable researchers to study their regulatory function in cattle and other livestock animals.

Key Words: bovine circRNAs, enrichment approaches, RNA-seq, circRNAs conservation

P371 Maternal demographic history of cattle and domestication. V. Cubric-Curik*[1], D. Novosel[1], V. Brajkovic[1], J. Soelkner[2], C. Vernesi[3], P. T. Miracle[4], I. Medugorac[5], and I. Curik[1], [1]*University of Zagreb Faculty of Agriculture, Department of Animal Science, Svetosimunska cesta 25, 10000 Zagreb, Croatia,* [2]*Division of Livestock Sciences, Department of Sustainable Agricultural Systems, BOKU-University of Natural Resources and Life Sciences Vienna, Vienna, Austria,* [3]*Department of Sustainable Agro-Ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, S. Michele all' Adige, Italy,* [4]*Department of Archaeology, University of Cambridge, Cambridge, United Kingdom,* [5]*Population Genomics Group, Faculty of Veterinary Medicine, Department of Veterinary Sciences, LMU Munich, Munich, Germany.*

Due of their great benefits to human cultures, domestic cattle are currently the most significant domestic animal in terms of economic importance. We conducted a thorough full mitogenome analysis of the species to add to the body of knowledge already available on the domestication of cattle and the variety of its mitogenome. A sizable sample from 114 breeds was gathered in South-East Europe, which served as a key agricultural crossing point into Europe during the Neolithic era and is known for its diversity in cattle. We gave Bayesian phylogenetic inference an improved estimate of divergence time than was previously available. Cattle with haplogroup T2 and Q saw the earliest population expansion before domestication, according to Bayesian skyplot estimates (median), but cattle with T3 and T1 experienced later population growth (7.5 kyBP and 3.0–2.5 kyBP, respectively). The growth of N_{ef} in T2 started before domestication and accelerates from the temperature changes in Bølling–Allerød (warm and humid interstadial period occurring in the last stages of the last ice age, from 14.7 to 12.7 kyBP). However, the steepest growth of T2 (10.0 to 7.5 kyBP) and the sudden growth of Q (from 10.0 to 8.0 kyBP), almost overlap with the timing of archeological evidence pointing to the beginnings of cattle domestication. In Africa-specific haplogroup T1, the increase in Nef growth from ~3.0 to 1.0kyBP overlaps with the migration routes of Bantu-speaking farmers and Cushitic- and Nilotic-speaking pastoralists, while it slows down with the assumed arrival date of the Zebu in Africa. Overall, our findings add to the knowledge of cattle during domestication and preservation of maternal cattle variety.

Key Words: cattle, mitogenomes, haplogroups, domestication

P372 ISAG Bursary Award: Ubiquitous impact of sex on gene expression across cattle tissues. M. Bhati*, J. Prendergast, and A. Tenesa, *The Roslin Institute, University of Edinburgh, Midlothian, Scotland, United Kingdom.*

Phenotypes ranging from monogenic traits to polygenic traits/diseases, display variation based on the sex of the organism. The importance of alleles/genes present on the sex chromosomes is widely known in X and Y linked inheritance. However, the mechanism and extent to which genes across the genome are driving sex-specific differences in phenotypes have largely been uncharacterized in livestock. In this study, we leveraged the cattle GTEx data (https://cgtex.roslin.ed.ac.uk/) to characterize sex-specific differences in the cattle transcriptome. We analyzed gene expression data of 2,822 *Bos taurus* samples (1,081 females and 1,741 males) from 10 tissues, with the number of samples per tissue ranging from 114 to 583. We considered 21,307 genes across the autosomes and chromosome X after filtering for low expression. We discovered a total of 11,435 genes differentially expressed (FDR <0.05) between the sexes in at least one tissue (sex-biased genes), with 131 (adipose) to 5,151 (blood) genes discovered per tissue, representing 0.61% to 24.17% of all tested genes, respectively. Of 11,435 sex-biased genes, 6,728 (58.83%) were expressed only in one tissue depicting tissue specificity. Among sex-biased genes, 411 (53% of X genes) were located on the X chromosome. Only 3 sex-biased genes (TXLNG, KDM6A, and ZFX) were discovered in all tissues which were X-linked and exhibited female biased expression. Homogenous tissues, like adipose and liver generally showed fewer sex-biased genes compared with heterogeneous tissue like blood. Additionally, we were able to predict the sex of 1,685 sample with no recorded sex information with an accuracy >0.87 via training a model on known sex-specific gene expression data. Thus, enabling the inclusion of all publicly available data irrespective of whether sex information is available. Overall, our study provides substantial evidence of sex-biased gene expression which requires further evaluation of its importance for understanding economically important livestock phenotypes.

Key Words: cattle and related species, transcriptome, genome regulation

P373 Genome-wide association studies for resumption of postpartum ovarian cyclicity trait during seasonal anestrus in Spanish Merino sheep breed. J. Calvo*[1,2], J. Martí[3], K. Lakhssassi[1,4], M. García-Méndez[1], M. Sarto[1], B. Lahoz[1], J. Bravo[5], A. Domingo[5], and J. Alabart[1], [1]*Centro de Investigación y Tecnología agroalimentaria de Aragón (CITA)-IA2, Zaragoza, Spain,* [2]*ARAID, Zaragoza, Spain,* [3]*UNIZAR-IA2, Zaragoza, Spain,* [4]*INRA, Rabat, Morocco,* [5]*CENSYRA (Extremadura), Badajoz, Spain.*

To elucidate the genetic basis of reproductive seasonality in Spanish Merino sheep breed, a genome-wide association study (GWAS) to detect single nucleotide polymorphisms (SNPs) or regions associated with the resumption of ovarian cyclicity during the postpartum period in conjunction with the seasonal anestrus was performed. In total, 169 multiparous Merino ewes which lambed in February were genotyped using the 50k Illumina Ovine Beadchip. After of lambing, blood samples were obtained weekly for measurement of peripheral progesterone concentrations from d 43 to 120. At 72 d of age, lambs were weaned. Live weight, and body condition score of the ewes were also recorded. The number of days from lambing to ovarian activation trait was used for GWAS. An ewe was considered cyclic when the progesterone concentration was higher than or equal to 0.5 ng/mL. GCTA software was used for GWAS analysis. Only one SNP (rs161969506) on chromosome 3 overcame the genome-wide significance level (p_Bonferroni <0.05). Six potential SNPs overcame the chromosome-wise significance level (p_FDR <0.05). The SNP rs161969506 was located in intron 7 of the *KDM3A* candidate gene in Oar_Rambouillet 1.0. KDM3A is a demethylase enzyme that has been identified as key regulator of many transcription factors, including androgen receptors in the ovary. This gene was partially isolated and Sanger-sequenced, and 5 SNPs were found: 1 SNP in promoter region and 4 nonsynonymous polymorphisms in exons 10 and 17. One and 2 SNPs in promoter and exon 10, respectively, were associated with the studied phenotype in the GWAS population. Haplotype association analysis confirmed the implication of this gene in the studied trait.

Key Words: GWAS, Merino, resumption of ovarian activity, seasonality

P375 Investigating neutral and functional genetic diversity in South African Bontebok (*Damaliscus pygargus pygargus*). M. Mogakala[1], R. Smith*[2,3], C. Mavimbela[1], and D. Dalton[4,2], [1]*Sefako Makgatho Health Sciences Univerty, Pretoria, Gauteng, South Africa,* [2]*South African National Biodiversity Institute, Pretoria, Gauteng, South Africa,* [3]*University of South Africa, Johannesburg, Gauteng, South Africa,* [4]*Teesside University, Middlesbrough, North Yorkshire, England, United Kingdom.*

Bontebok (*Damaliscus pygargus pygargus*) and blesbok (*D. p. phillipsi*) are classified as separate sub-species of *D. pygargus,* which have distinct morphology. Injudicious translocations have resulted in the threat of hybridization between the near-threatened Bontebok, which is endemic to the Western Cape, with the more common blesbok, resulting in fertile offspring. This study investigated the genetic diver-

sity between bontebok and blesbok, and their hybrid and compared the genetic variation between 13 neutral markers (microsatellites) and single nucleotide polymorphisms in the toll-like receptor 2 (TLR2) gene which recognizes molecules for multiple pathogens, including bacteria, viruses, fungi, and parasites. Phylogenetic relationships between the 2 subspecies and their hybrid were also compared. The results showed that bontebok has lower genetic diversity than blesbok and that hybrids have the highest levels of genetic diversity. Five mutations were identified in TLR2 in different individuals and sub-species of *D. pygargus*. This comprised 3 synonymous mutations and 2 non-synonymous mutations. The 2 mutations that resulted in amino acid substitutions were predicted to not affect protein function. Two of the 5 mutations were not present in bontebok, one of which resulted in an amino acid substitution. The other 3 mutations were present in the 3 groups to varying degrees. These mutations provide insights into the genetic diversity and relationships among the 2 subspecies of *D. pygargus* and may have implications for their conservation and management. The study warrants further investigation into adaptive traits necessary for conservation using next-generation sequencing, which assesses the implications of hybridization between the 2 subspecies.

Key Words: bontebok, blesbok, genetic diversity, toll-like receptor 2, microsatellites

P376 Detection of QTL for global recombination rate in Fleckvieh cattle. N. Kadri* and H. Pausch, *ETH Zurich, Zurich, Switzerland.*

Recombination between homologous chromosomes during meiosis plays a key role in gametogenesis and thus fertility. It also contributes to increasing genetic diversity by creating new allelic combinations. By examining phased genotypes for more than 38,000 genome wide SNPs in 429,265 Fleckvieh cattle (using LINKPHASE3), we identified 10,181,122 cross overs in 392,753 male and 2,362,152 cross overs in 102,266 female gametes. Consistent with previous reports in Bovidae, the recombination rate was higher in males than females (25.92 v/s 23.10), mainly due to an increased crossing over in the telomeric region of autosomes in males. The number of cross overs in the autosomes (global recombination rate; GRR) was repeatable and moderately heritable in both sexes with males exhibiting higher h^2 than females (0.15 ± 0.008 v/s 0.08 ± 0.005). A haplotype-based association study identified previously reported QTL on BTA1, 3, 6, and 10 affecting GRR in at least one sex. In addition to these, we identified a novel, highly significant ($P < 1e\text{-}40$) QTL on BTA19 affecting GRR in both sexes. The positions of > 12.5 million cross overs identified in Fleckvieh cattle, the largest catalog of cross over positions in a single cattle breed, is a valuable resource for further studies on genetics of cross over placement.

Key Words: genome biology, recombination rate, meiosis, cattle, fertility

P377 The genetic mechanisms of resistance to heartwater in goats from endemic and non-endemic regions of South Africa investigated using Illumina Goat SNP65K genotypes. X. Nuse*[1,2], M. A. Van Der Nest[3], E. F. Dzomba[1], F. C. Muchadeyi[2], and H. C. Steyn[4], [1]*Discipline of Genetics, School of Life Sciences, University of KwaZulu-Natal, Scotsville, KwaZulu-Natal, South Africa,* [2]*Agricultural Research Council - Biotechnology Platform, Onderstepoort, Pretoria, Gauteng, South Africa,* [3]*Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, Gauteng, South Africa,* [4]*Molecular Biology Department, Agricultural Research Council - OVI, Onderstepoort, Gauteng, South Africa.*

Tick-borne diseases such as heartwater are a constraint to ruminant production in many developing countries. They are responsible for high morbidity and mortality, as well as decreased productivity. This disease poses an economic threat to goat production in endemic areas infested by Amblyomma hebraeum ticks that transmit the causative agent Ehrlichia ruminantium. Previous studies have observed that commercial goat breeds at the age of 2–6 mo are more susceptible to the peracute form of heartwater. It has also been observed that different goat breeds differ in their resistance to the disease. The aim of the study was to investigate the genetic mechanisms underlying resistance to heartwater disease in South African goats. For this purpose, different goat breeds of Tankwa (n = 21), Boer (n = 81), Kalahari Red (n = 31), Savanna (n = 11) and village goat breeds (n = 110) sampled from endemic (n = 174) and non-endemic (n = 80) regions in South Africa were genotyped using Illumina High Density Goat65K SNP BeadChip array. A total of 59 727 SNPs were filtered to estimate genetic diversity and selection signatures. Principal component analysis (PCA) revealed the first principal component (PC1) explaining 13.92% of the variation while the second principal component (PC2) explained 12.59%. The Tankwa goats located in the Northern Cape (non-endemic) region, differ genetically (FST = 0.43) from the other farmed goat populations. SNPs identified by the 2 PCs showed high FST values in Tankwa goats and allowed the identification of candidate genes associated with disease resistance. Overall, preliminary results demonstrated an association between breed/population, geographic location and resistance to heartwater. Selection of resistant individuals in endemic regions is evident. Discovering disease-causing genes could increase our knowledge of the genetic contribution to the animal diseases, leading to new genetic screens, and underpinning research into new remedies and improved breeding programs.

Key Words: goats, genetic variation, SNPs, heartwater-disease resistance, endemnicity

P378 Mapping moose short-read sequences on the bovine genome—a tool to investigate the white moose. D. Paul[1], T. Kalbfleisch[2], M. Baghdy Sar[1], B. Herlemont[1], T. Bergström[1], I. Shutava[1], and S. Mikko*[1], [1]*Swedish University of Agricultural Sciences, Uppsala, Sweden,* [2]*University of Kentucky, Lexington, KY.*

The Scandinavian Moose (*Alces alces alces*) is well camouflaged with a wildtype agouti coat color, but white individuals are frequently seen in a few areas of Scandinavia and Canada. Its unique white coat has intrigued scientists and nature enthusiasts, but the genetic basis is not yet understood. There are 2 moose genomes publicly available on scaffold level, none of them yet annotated. One of them is derived from a Scandinavian moose, and one is a compilation of 3 North American sub-species (*A.a.shirasi*, *A.a.americana* and *A.a.gigas*). We have now sequenced the genome of a white male moose and mapped this to the Bovine genome. The well-annotated Bovine genome have previously shown to be a useful resource in moose genomics. Genes involved in melanin production are highly conserved across different animal species. Finding such variations could serve as a starting point for a functional annotation of the moose genome, as well as advancing our understanding of the genetic mechanisms underlying melanin production and transport in the white moose. In this study, we developed a pipeline to combine genomic data from wild type, and white moose, with the annotated bovine genome to identify genetic variations associated with white coat color. Raw data from were mapped at ~82% to the Bovine genome. We used GATK, to identify SNVs, and indels, possibly associated with white coat color in the moose. Filtration was performed as for an autosomal recessive inheritance, and variants were annotated using the bovine genome as a reference. In this way, 2,914 missense, 100 nonsense, and 313 frame-shift putative variants were detected. Further filtering detected 39 missense variants located within genes known to be involved in pigmentation in other species. Five candidate variants were Sanger sequenced in 12 wildtype moose, and 11 white moose, but none of them was confirmed. Further analyses involve other functional variants, as well as large structural variation. Identifying genetic variations in white moose coat color could provide useful information in the biodiversity, and management of the moose population.

Key Words: wild species, genome sequencing, coat color, biodiversity

P379 ISAG Bursary Award: Tail morphology and environmental adaptations of Ethiopian indigenous sheep: an ecological niche modelling and genomic approaches. A. Amane*[1,2], G. Belay[2], T. Dessie[3], A. M. Ahbara[4], E. Vila[5], and O. Hanotte[3,6], [1]*Amhara Regional Agricultural Research Institute, Bahir Dar, Ethiopia,* [2]*Microbial,*

Cellular and Molecular Biology, Addis Ababa University, Addis Ababa, Ethiopia, [3]LiveGene, International Livestock Research Institute (ILRI), P.O. 5689, Addis Ababa, Ethiopia, [4]Department of Zoology, Misurata University, Misurata, Libya, [5]CNRS/Univ. Lyon 2, UMR 5133 Archéorient, Maison de l'Orient et de la Méditerranée, Lyon, France, [6]School of Life Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom, [7]Centre for Tropical Livestock Genetics and Health, The Roslin Institute, Edinburgh EH25 9RG, United Kingdom.

Different environmental challenges have shaped sheep adaptive diversity, including their tail phenotypes. Ethiopian sheep phenotypic diversity includes 4 tail morphotypes (short fat-tail, long fat-tail, fat-rump, and thin-tail). We previously reported the phenotypic diversity of Ethiopian sheep tails from morphological and osteological perspectives. Here, we aim to identify candidate genome regions and genes associated with tail morphology diversity and environmental challenges. Fourteen Ethiopian sheep populations were studied; it includes sheep with short fat-tail, long fat-tail, and fat-rump tail morphotypes. Based on the genomic, environmental principal component and admixture analyses, the sheep populations were initially clustered in 3 groups: eastern fat-rump, western long fat-tail and southern long fat-tail sheep with short-tail sheep found in the first 2 groups. Following the identification of 7 main drivers of environmental selection (mean temperature of the wettest quarter, variation in precipitation across the year, precipitation during the wettest and driest quarter, proportion of grazing and cultivable land areas, and soil organic carbon content) by ecological niche modeling, these populations were further classified into environmental contrasting groups (Low and High). Candidate regions and genes associated with tail morphology and environmental challenges were identified with 3 selection scan indexes (*ZHp, ZFST* and *XP-EHH*). These include candidate genes associated with fat tail deposition (*NF1, EVI2A, EVI2B, OMG, CALCOCO2, TTLL6, DMXL2*), tail length (*HOXB13*), tail formation (*HOXB13, BMP4*), control of lipogenesis and lipolysis (*PLPPR, CYP2E1*, *ECHS1*, *FOUM*), and adaptations to hypoxic conditions and thermoregulation (*VEGFA, TF, PLCG1*, *KIT, DSC1*, *GNPAT, EXOC8, SPRTN, CHRNA4, EEF1A2, ADSL, FGF2, MSRB3*). The results provide novel insights into sheep genomic adaptations to extreme environments, and they illustrate the impact that environmental challenges may have had on the tail morphology of Ethiopian sheep

Key Words: ecological niche modelling, Ethiopia, genome-wide signature of selection, sheep

P380 ISAG Bursary Award: Integration of reduced representation bisulphite sequencing with RNA sequencing data provides further insights in claw horn disruption lesions susceptibility in dairy cattle. E. Attree*[1], X. Dai[1], D. Xia[1], M. Barden[2], B. Griffiths[2], A. Anagnostopoulos[2], D. Werling[3], G. Oikonomou[2], G. Banos[4], and A. Psifidi[1], [1]*The Royal Veterinary College, Department of Clinical Science and Services, The Royal Veterinary College, Hatfield, United Kingdom,* [2]*Scotland's Rural College, Department of Animal and Veterinary Sciences, Scotland's Rural College, Midlothian, Scotland, United Kingdom,* [3]*University of Liverpool, Department of Livestock and One Health, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Neston, United Kingdom,* [4]*The Royal Veterinary College, Department of Pathobiology and Population Sciences, Royal Veterinary College, Hatfield, United Kingdom.*

Lameness in dairy cows has significant welfare and economic effects through reduced reproduction and milk production, and increased culling rates. Claw horn disruption lesions (CHDL) are non-infectious foot lesions that can cause lameness and were reported to affect over 40% of dairy cattle globally. There is genetic variation related with host resistance to CHDL development in dairy cows, therefore, genetic improvement, along with farm management interventions, could reduce the incidence of CHDL. In this project we have combined meticulous animal phenotyping with multi-omics to improve our understanding of the genetic architecture of CHDL susceptibility. In 24 phenotypically extreme animals (cases and healthy controls) for CHDL lesions, diagnosed by veterinary surgeons during farm visits, RNA-Seq was performed to access the transcriptomic signature of the disease in isolated PBLs from whole blood and on biopsied foot tissue. Identified differentially expressed genes were enriched for functions in immune regulation, ossification and keratinization. In 4 of these animals reduced representation bisulfite sequencing (RRBS) was also performed. Epigenetic differences in the form of differential DNA methylation profiles between cows diagnosed with CHDL and control animals were identified using RRBS. These genes were significantly enriched for pathways including the innate immune and inflammatory responses, regulation of the immune system and hormone activity. Combining results from differential expression and differential methylation has highlighted 5 overlapping genes, both significantly differentially expressed and methylated. Differential methylation within a promoter region has been identified for 2 of these 5 genes, importantly one in the complement cascade of the immune system and one involved in the coagulation pathway. Overall these 5 genes are of great interest and the dissection of their role in genetic susceptibility to CHDL could greatly improve our understanding of this complex disease and inform breeding strategies.

Key Words: bovine, lameness, RNA-seq, RRBS

P381 Genomic breed composition information can optimize stratified randomization strategies in beef cattle experiments. O. Durunna*[1,2] and C. Ekine-Dzivenu[3], [1]*Lakeland College, Vermilion, Alberta, Canada,* [3]*University of Saskatchewan, Saskatoon, Saskatchewan, Canada,* [2]*International Livestock Research Institute, Nairobi, Kenya.*

Beef cattle experiments focusing on feed or growth efficiency and other body performance traits assessments require animals of similar class, age and genetic background. Although crossbred individuals are mostly available for such studies in production settings, the unequal breed compositions in these crossbreds influence their growing patterns. For example, crossbreds with greater proportions of large-framed breeds may be associated with a faster growth rate and higher end-weights than those with small or medium-sized major breeds. Experiments with unbalanced distribution of major breeds may generate inaccurate or misleading results. Genomic breed composition (GBC) profiles provide approximate proportions of each breed in each crossbred cattle. The objective of this study was to determine whether GBC information complements stratified randomization of subjects to experimental groups based on their bodyweight and coat color. Data used for this trial included a total of 248 red-hide steers recruited for a supplement trial over 3 years (n: Yr1 = 104; Yr2 = 80; Yr3 = 64). The GBC of all steers was obtained using the GGP Bovine 100K SNP panel. Within each year, the subjects were randomly assigned to one of 4 groups by either also by balancing for bodyweight information only (RBW) using RandoMice software or by balancing the groups for bodyweight and breed composition (RBWBC) in SAS software. The RandoMice software allocated the subjects to different groups, where 40 groups (balanced for bodyweight) were retained from 100,000 iterations. The major breeds included Angus, Hereford, Simmental, Limousin and Charolais. The relationships between RBWBC and RBW for the differences in proportions of dominant breeds were analyzed with a chi-squared test. Breed frequencies were different ($P < 0.05$) between extreme proportions from RBW, indicating that observed research results from experiments using this strategy may reflect breed-related outcomes rather than treatment effects. However, the breed occurrences were not different ($P > 0.39$) between RBWBC and both extreme proportions from RBW. Using the GBC information to balance for major breeds in crossbred animals will optimize livestock experimental designs and reduce errors.

Key Words: crossbred beef cattle, animal breeding, genomic breed composition, experimental designs

P382 DNA variant related to congenital adrenal hyperplasia in cattle. R. Hofmeyer, T. Chen, L. Hampton, W. Y. Low, W. S. Pitch-

ford, K. Petrovski, and C. D. Rottema*, *Davies Livestock Research Centre, School of Animal and Veterinary Sciences, Roseworthy Campus, University of Adelaide, Roseworthy, Australia.*

A female sub-fertility disorder was observed in a stud cattle herd. The phenotypes associated with the disorder included increased muscularity, a shorter vulva, enlarged clitoris and fewer calves than expected for a given age. Subsequent differential diagnoses and pedigree analysis ruled out all potential causes of the sub-fertility disorder other than a recessive genetic mutation. Additional data from body measurements, fertility traits and hormone levels indicated that the disorder resembles congenital adrenal hyperplasia (CAH) in humans and the mutation is most likely to involve an enzyme in the steroidogenesis pathway which affects sex hormone synthesis. Three affected females and their normal dams were whole genome sequenced and 88 genes were screened for DNA variants, including 36 genes in the steroidogenesis pathway and 52 genes from the literature describing other disorders of sexual development with similar phenotypes. There were 87 variants identified and investigated which appeared to be homozygous and/or heterozygous in the affected females and their carrier dams. Of these, 10 variants were selected, based on being homozygous in the affected females and heterozygous in their dams, for further sequencing in additional affected females and their dams. Only one variant, a 4-base deletion in intron 1 of the estrogen sulfotransferase (*SULT1E1*) gene, was homozygous in all affected females and heterozygous in their carrier dams. SULT1E1 sulfonates estrone and estradiol, inactivating these hormones, and thereby, regulating estrogen homeostasis. The 4-base deletion greatly affects the secondary structure of the *SULT1E1* intron. Therefore, it is hypothesized that this deletion affects splicing of the mRNA, which in turn decreases the gene expression of *SULT1E1* and ultimately, the level of SULT1E1 protein. Thus, the deletion in *SULT1E1* could be the cause of CAH in the stud cattle herd, but this must be validated before a DNA test can be provided.

Key Words: bovine, genetic disorder, reproduction, fertility, candidate gene

P383 Insights into the genetic variation, gene-flow and demographic history of African cattle breeds. M. Malima[1,2], K. Nxumalo[1], A. Tijjani[3,4], M. Makgahlela*[1], F. Joubert[2], and A. Zwane[1], [1]*Department of Animal Breeding and Genetics, Agricultural Research Council-Animal Production Irene, Pretoria, South Africa,* [2]*Centre for Bioinformatics and Computational Biology, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa,* [3]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [4]*The Centre for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, The University of Edinburgh, Midlothian, United Kingdom.*

The genomes of many livestock species, including African cattle, have been shaped by domestication and selection pressures. Africa is home to various cattle breeds that are adapted to different environments and used for different purposes. However, little is known about their genomic relationships, history, and gene flow. This study aimed to examine these factors among South, East, and West African cattle breeds using whole-genome sequencing data and bioinformatics techniques. The study analyzed a total of 32 whole genome sequences, including data from Ankole, Kenana, and N'Dama, as well as sequenced data of Nguni, Afrikaner, Bonsmara, and Holstein. The data were analyzed using various methods, including phylogenetic trees, admixture and principal component analyses, D-statistics, Treemix and the Pairwise Sequentially Markovian Coalescent model. The genomic relationships analysis revealed genetic exchange between African breeds and differentiated Bos taurus, Bos indicus, and Bos taurus x Bos Indicus ancestries. The admixture/gene-flow analysis also showed evidence of several genetic exchange events between breeds from different geographical locations followed by unveiling of possible periods where African cattle populations experienced declines and expansions. These findings coincide with events such as domestication and migrations, shedding light on possible migration patterns and co-migration of cattle and humans. Overall, this study highlights the unique genetic diversity and complex genetic changes that African indigenous cattle have undergone, resulting in adaptive traits and unique features important for conservation and breeding programs.

Key Words: indigenous cattle, genomic diversity, population genomics, geneflow, demographic history

P384 A continent-wide genomic resource for African buffalo (*Syncerus caffer*). L. Morrison*[1,2], [1]*Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom,* [2]*Centre for Tropical Livestock Genetics and Health, University of Edinburgh, Edinburgh, United Kingdom.*

The African buffalo (*Syncerus caffer*) is a wild bovid with a historical distribution across much of sub-Saharan Africa. Genomic analysis enables insights into the evolutionary history of the species, and potentially the key selective pressures shaping the current populations, including an assessment of population level differentiation, population fragmentation, and population genetic structure. In this study we generated the highest quality de novo genome assembly (2.65 Gb, with a scaffold N50 of 69.17 Mb) of African buffalo to date, and sequenced a further 195 genomes from populations representing the distribution of all subspecies. Principal component and admixture analyses provided surprisingly little support for the currently described 4 subspecies, but indicated 2 main lineages, located in Western/Central and Eastern/Southern Africa, respectively, with secondary differentiation between Eastern and Southern African populations. Estimating Effective Migration Surfaces analysis suggested that geographical barriers across the continent have played a significant role in shaping gene flow and the population structure. Estimated effective population sizes (N_e) indicated a substantial drop in N_e occurring in all populations 5–10,000 years ago, coinciding with the rapid increase in human populations on the continent. Finally, signatures of selection were enriched for key genes associated with the immune response, suggesting infectious disease exert a substantial selective pressure in shaping the genetics of African buffalo. The data are suggestive of protozoan parasites (the African buffalo is the primary host for the tick-borne *Theileria parva,* an important pathogen of cattle) perhaps exerting particularly strong selection. These findings have important implications for understanding bovid evolution, buffalo conservation and population management, and the pathways involved in bovid tolerance to pathogens.

Key Words: African buffalo, reference genome, population genomics, conservation genomics

P385 Convergent selection of structural variations reveals genes associated with domestication and production traits in sheep and goats. J. Yang*[1], D.-F. Wang[1,2], Q.-H. Zhu[1,2], J.-H. Huang[1], L.-Y. Luo[1], R. Lu[1], X.-L. Xie[2,3], H. Salehian-Dehkordi[2,3], A. Esmailizadeh[4], G. E. Liu[5], and M.-H. Li[1], [1]*College of Animal Science and Technology, China Agricultural University, Beijing, China,* [2]*CAS Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences (CAS), Beijing, China,* [3]*College of Life Sciences, University of Chinese Academy of Sciences (UCAS), Beijing, China,* [4]*Department of Animal Science, Shahid Bahonar University of Kerman, Kerman, Iran,* [5]*Animal Genomics and Improvement Laboratory, BARC, USDA-ARS, Beltsville, MD.*

Small ruminant livestock like sheep and goat have undergone similar domestication process and come in a multitude of analogous traits. Structural variant (SV) is a major source of genomic variation that has been largely ignored in spite of its potential to dramatically affect phenotype. Here, leveraging on the whole genome sequences available for 532 domestic and wild sheep and 442 domestic and wild goats from a broad geographic distribution, we resolved the functional impact of SVs on the domestication and production traits of sheep and goats. Specifically, we estimated the differences in SV allele frequencies between different pairs of sheep or goat populations, e.g., wild progenitor vs. indigenous populations, populations with superior trait vs. populations with common trait, and identified several commonly selected genes between sheep and goats during domestication and for

main production traits such as prolificacy, meat, milk, and wool related traits. Notably, we found that deletions in the bone morphogenetic protein receptor genes *BMPR2* and *BMPR1B*, which have been reported to play an essential role in regulating ovarian function and fecundity of farm animals, were under convergent selection during domestication and improvement of sheep and goats. Collectively, our study offers novel insights into the unique role of SVs in convergent genome evolution of sheep and goats, with widespread importance and utility for future molecular breeding of concerned traits in small ruminant species.

Key Words: structural variant, production traits, domestication, sheep, goat

P386 ISAG Bursary Award: Pangenomes of haplotype-resolved assemblies enable population-scale genotyping of cattle structural variation for eQTL mapping. A. Leonard*, X. Mapel, and H. Pausch, *ETH Zurich, Zurich, Switzerland.*

Genome-wide association studies relate sequence variation to phenotype variation, and so completeness of the marker panel impacts the power to reveal trait-associated loci. Conventional short read sequencing approaches mainly capture single nucleotide polymorphisms and small insertions/deletions but largely neglect structural variation (SV). Long reads, particularly when assembled into haplotype-resolved genomes, produce a much more complete catalog of variation for any type of polymorphism. While generating sufficient samples for a statistically significant association study is still cost prohibitive, imputation of variation discovered from a representative set of haplotypes into large mapping cohorts is feasible. Here we build a Braunvieh cattle pangenome using 16 assemblies generated from PacBio HiFi reads and then use PanGenie to impute pangenome variation into a much larger short read data set of 307 Braunvieh samples. This approach enabled us to genotype 15 million variants in the 307 samples, including approximately 50k SVs of which nearly 10k exceeded 1000 bp in size. This comprehensive set of variation was then tested for association with gene expression in 117 deeply sequenced testis total RNA samples. We identified 3947 genes that were strongly affected by structural variants (approximately 35% of all significant genes). A structural variant was the most strongly associated variant for 45 genes. We were also able to collect PacBio HiFi reads at moderate coverage on 24 of the 117 eQTL samples. We find that the 16 haplotypes in the pangenome do not reach saturation for all SVs present in the Braunvieh population but capture nearly 70% of the SVs discovered directly from the unrelated 48 haplotypes. Efforts like the Bovine Pangenome Consortium or Cattle Long Read Consortium will eventually provide sufficient resources to assess all types of variation, but for the intermediate future, we demonstrate that SV genotyping through a cattle pangenome can reveal eQTL that are missed or incorrectly associated when using only short read variants.

Key Words: cattle, genome assembly, pangenome, structural variants, eQTL

P387 Withdrawn

P388 Withdrawn

P389 Analysis of differential isoform usage in production relevant tissues across pre- and post-natal development in sheep. S. A. Woolley[1], J. G. D. Prendergast[1], M. Salavati[1,2], and E. L. Clark*[1], [1]*The Roslin Institute, Edinburgh, Midlothian, United Kingdom,* [2]*SRUC, Edinburgh, Midlothian, United Kingdom.*

Understanding transcription during early development in sheep can help to inform breeding programs by identifying the genomic drivers of healthy growth in production relevant tissues. This study aims to identify genes that are differentially expressed and exhibit differential isoform usage across pre- and post-natal developmental stages. RNA-sequencing was generated for whole embryo, placenta, liver and

skeletal muscle bicep tissue from 6 different developmental stages from 48 Texel x Scottish Blackface sheep in total. Gene expression levels were estimated across tissues and developmental stages using Kallisto and compared using DESeq2. Tissue- and developmental stage-specific differences in gene expression were observed, especially between d 100 of gestation and one week of age when genes related to muscle growth and development were upregulated (e.g., *MYH3*, *GDF5* and *COL9A2*) in skeletal bicep muscle. Analysis of allele-specific expression also revealed imbalances in expression from either parent in gene families related to growth. In addition, long read Iso-Seq data for 12 of the 48 Texel x Scottish Blackface sheep was generated to investigate differential isoform usage through development. Using the Iso-Seq data we identified 16 isoforms per gene on average across 4 tissue types and 6 time points. The transcription start sites for these transcripts were validated using CAGE-Sequencing data from the same set of samples. The isoform models predominantly originated form 1–2 TSS sites already annotated in the reference gene models for the sheep reference genome ARS-UI_Ramb_v2.0 (NCBI v108). Analysis of the Iso-Seq, RNA-Seq and CAGE data, using the FLAIR analysis pipeline, revealed dominant isoforms across developmental stages, along with functionally important transcript isoforms linked to growth traits in sheep. Integration of these results with GWAS data will assist the identification of expressed genomic variation associated with growth traits in sheep. This information can then be used to inform genomics enabled breeding programs and provide breed-specific annotation information for sheep.

Key Words: sheep, transcriptome, muscle, growth, Iso-Seq

P390 Withdrawn

P391 Genetic diversity and population structure among Central European native sheep breeds using microsatellite markers. Z. Sztankoova, M. Milerski, M. Brzáková, J. Rychtárová, and J. Kyselova*, *Institute of Animal Science, Praha-Uhrineves, Czech Republic.*

Analysis of microsatellite loci is highly informative in reconstructing the historical processes underlying the evolution and differentiation of animal populations. This study used 13 polymorphic microsatellite markers recommended by FAO and ISAG to analyze the genetic diversity, genetic structure, variation, and phylogenetic relationship of 6 Central European sheep breeds (Czech Wallachian, CWA, n = 36, Sumava, S = 46, Slovak Wallachian, SWA, n = 59, Improved Wallachian, IPW, n = 59, Swiniarka, SWI, n = 35, and Uhruska sheep UHR, n = 19). The 172 alleles were observed in 254 animals. The number of observed alleles per locus varied from 7 to 17 per locus (average of 13,23). The mean number of effective alleles per locus was 5.77, with PIC ranging from 0.613 – 0.907 (equal to 0,77). Fst within, subpopulations showed a low level of inbreeding. Nei's genetic distances between breeds were calculated, and results showed that the smallest distance was recorded between CWA and SWA (0.108). The largest was between the polish SWI and UHR sheep breeds (0,283). Principal component analysis showed that Czech and Slovak sheep breeds are closely related compared with Polish sheep breeds, specially SWI. Analysis of molecular variance showed a 6% variance among breeds and a 94% variance within populations. The ΔK value indicated that the most suitable group number was (K = 4). These results showed genetic diversity, which is essential for future selection, animal breeding, and keeping the genetic diversity of native breeds. On the other hand, these results could help preserve genes in these breeds, thereby ensuring their preservation in the Czech and Slovak Republic and Poland. Therefore, future study is recommended to screen other middle European sheep breeds for comparison purposes.

Key Words: native sheep, gene resources, gene diversity, population structure, microsatellite

P392 A time-resolved multi-omics atlas of transcriptional regulation in response to high-altitude hypoxia across the whole-body tissues. Z. Yan* and M. Li, [1]*China Agricultural University, Beijing, China,* Hypoxia serve as the most representative environmental stressors in high-altitude area and profoundly challenge life survive. Previous studies identified a suit of traits favoring local adaptation from indigenous high-altitude population, and indicated the polygenicity of hypoxia adaptation, suggesting that downstream of transcription and participate more directly in crucial cellular activities. Here, we conduct environmental shift experiment (e.g., plain and plateau) and generate time-resolved (i.e., 0 d, 6 d, 13 d, 20 d, and 8 mo) phenotypic and RNA-Seq, single-cell RNA-Seq, ATAC-Seq data from 19 diverse tissues. Our phenotypic changes reveal that short-term hypoxia acclimatization is a 2-stage process. We systematically depict multi-tissue temporal dy-

namic of transcriptome and interplay network under hypoxia. We illustrate that genomic variants associated with high-altitude evolution play a crucial role in cerebellum and exhibit distinct expression pattern. We identify TAD-constrained *cis*-regulatory elements (CREs) and found hypoxia suppress the transcriptional activity. Moreover, we capture phenotypic (e.g., oxygen saturation) and transcriptional (e.g., *UCP3*, *CAT*, *SIK1*) evidences indicate that antenatal hypoxia may increase hypoxia tolerance for offspring. Taken together, our study provides a comprehensive view of hypoxia acclimatization and new insight for human disease research.

Key Words: hypoxia acclimatization, multi-omics, dynamic regulatory, sheep

P393 CNV mapping and CNV contribution to genetic variance of complex traits in dairy cattle. G. Ladeira[1], P. Pinedo[2], J. Santos[1], W. Thatcher[1], and F. Rezende*[1], [1]*University of Florida, University of Florida, Gainesville, FL,* [2]*Colorado State University, Colorado State University, Fort Collins, CO.*

Copy number variation (CNV) are structural genomic variants that can play active role in gene dosage and expression. Hence, CNV can provide substantial insights into the genetic architecture of complex traits and account for some of the additive genetic variance which cannot be accounted for by SNPs. First, CNVs were mapped from 3,601 Holsteins genotyped with the Illumina BovineHD BeadChip. The Log R ratio and B Allele Frequency of 720,732 autosomal markers were used for CNV calling. After quality control, 3,952 non-redundant CNV events were identified spanning the autosomal genome in 2,422 Holsteins. At the population level, individual CNVs were compiled into 943 CNV regions (CNVR). Of those, 927 CNVRs overlapped 12,258 QTLs, and 698 CNVRs overlapped 1,762 genes, most of them (85.81%) classified as protein-coding genes. CNV information was then included into a SNP-based model to assess its contribution in estimating genetic parameters for health traits in 2,900 cows. The variance components were estimated by fitting either only a SNP-derived genomic relationship matrix (SNP_GMR) or both SNP_GMR and CNV_GMR matrices in threshold models implemented in a Bayesian framework using THRGIBBS1F90. The additive genetic variances captured by the pure SNP_GMR model and heritability estimates were, respectively, 0.18 ± 0.09 and 0.14 ± 0.06 for mastitis and 0.16 ± 0.06 and 0.13 ± 0.04 for metritis. When SNP_GMR and CNV_GMR were considered jointly, CNVs accounted for additional genetic variances (0.02 ± 0.02 for mastitis, and 0.03 ± 0.02 for metritis), resulting in greater heritability estimates for mastitis (0.17 ± 0.09) and metritis (0.16 ± 0.06). Therefore, the SNPs and CNVs in the present study were not redundant forms of genomic data, and CNVs accounted for part of the heritability which was not account for by SNPs alone as genetic markers (so called, "missing heritability"). These findings will provide opportunities for a better understanding of the genetic architecture of complex traits and may contribute to the development of more accurate genomic selection methods in Holstein dairy cattle.

Key Words: copy number variation, variance component, health trait

P394 Investigating the role of β-globin in the response to mycotoxin exposure in sheep. K. McRae[1], E. Willems[2], A. Thomas[2], R. Clarke[1], J. Plowman[2], E. Maes[2], S. Clarke*[1], and P. Johnson[1], [1]*AgResearch Ltd., Mosgiel, New Zealand,* [2]*AgResearch Ltd., Lincoln, New Zealand.*

Facial eczema (FE) is an animal health challenge of great importance in ruminants in New Zealand. Ingestion of the mycotoxin sporidesmin leads to liver and bile duct damage, which can result in photosensitization and reduced production. In sheep, there is considerable genetic variation in tolerance to facial eczema, and a quantitative trait locus (QTL) in the β-globin locus has been reported to explain 5% of the phenotypic variance in the response to FE. Mass spectrometry of hemoglobin from animals with differing genotypes at this locus indicated that the QTL is associated with different forms of adult β-globin, with haplotype A animals more tolerant to FE. Adult haplotype A sheep can switch from the synthesis of hemoglobin A (Hb-A; $\alpha_2\beta_2^A$) to the juvenile hemoglobin C (Hb-C; $\alpha_2\beta_2^C$) in response to hypoxia and anemia. To test the hypothesis that animals that are homozygous for haplotype A at the β-globin locus undergo a switch in hemoglobin type from Hb-A to Hb-C in response to exposure to the toxin sporidesmin, 10 animals homozygous for haplotype A and 10 animals homozygous for haplotype B were monitored through a controlled sporidesmin challenge. Blood samples were taken at d 0, 2, 7, 14 and 21 post-challenge for liver enzyme analysis, complete blood counts, and proteomic analyses to determine which forms of hemoglobin were present. In parallel, a DNA sample was taken from each animal at d 0, and the β-globin locus was sequenced using the Oxford Nanopore Technologies adaptive sampling method, which through targeted long-read sequencing of a region enables simultaneous capture of multiple sources of information including methylation and structural variants in a single run.

Key Words: sheep, animal health, disease resilience, proteomics, DNA sequencing

P395 Studying cattle structural variation and pangenome using whole genome sequencing. G. Liu*, *Animal Genomics and Improvement Laboratory, Henry A. Wallace Beltsville Agricultural Research Center, Agricultural Research Service, USDA, Beltsville, MD.*

A cattle pangenome representation was created based on the genome sequences of 898 cattle representing 57 breeds. The pangenome identified 83 Mb of sequence not found in the cattle reference genome, representing 3.1% novel sequence compared with the 2.71-Gb reference. A catalog of structural variants developed from this cattle population identified 3.3 million deletions, 0.12 million inversions, and 0.18 million duplications. Estimates of breed ancestry and hybridization between cattle breeds using insertion/deletions as markers were similar to those produced by single nucleotide polymorphism–based analysis. Hundreds of deletions were observed to have stratification based on subspecies and breed. For example, an insertion of a Bov-tA1 repeat element was identified in the first intron of the *APPL2* gene and correlated with cattle breed geographic distribution. This insertion falls within a segment overlapping predicted enhancer and promoter regions of the gene, and could affect important traits such as immune response, olfactory functions, cell proliferation, and glucose metabolism in muscle. The results indicate that pangenomes are a valuable resource for studying diversity and evolutionary history, and help to delineate how domestication, trait-based breeding, and adaptive introgression have shaped the cattle genome.

Key Words: cattle, structural variation, pangenome, whole genome sequencing

Small Ruminant Genetics and Genomics

P396 Investigating the association of the goat *CSN1S1* polymorphism with milk traits in Murciano-Granadina goats through the use of a KASP assay. A. Castello[1,2], T. F. Cardoso[1], M. Luigi[1], A. Martínez[3], J. V. Delgado[3], J. Jordana[2], G. Cosenza[4], and M. Amills*[1,2], [1]*Centre of Research in Agricultural Genomics, Bellaterra, Barcelona, Spain,* [2]*Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain,* [3]*Universidad de Cordoba, Cordoba, Spain,* [4]*University of Naples, Naples, Italy.*

Alleles at the goat α_{S1}-casein encoding gene (*CSN1S1*) have been associated with high (A, B, C, H, L and M), medium (E, I), low (F, G) and null (N,01, 02) milk α_{S1}-casein content. Several of these alleles

have been also associated with milk and cheese traits in French Alpine and Saanen breeds, while such associations have not been consistently replicated in the Spanish Murciano-Granadina breed. One difficulty in carrying out such studies at a large scale is that the current genotyping techniques rely on methods, such as PCR-RFLP, that cannot be easily automated and require time-consuming agarose gel electrophoresis steps. The main aim of the current work was to implement 5 KASP assays to discriminate the most frequent *CSN1S1* alleles, i.e., A, B, E, F, and the null alleles N and 01. Two allele-specific forward competing primers and one common reverse primer were designed for each KASP assay. The analysis of control individuals with known genotypes was successful. Subsequently, this KASP-based protocol was used to genotype 693 Murciano-Granadina goats with records for milk traits (protein, fat, lactose, dry matter, somatic cell count, and milk yield at 240 d). The E (frequency = 0.46) and B (0.34) alleles were the most common ones, followed by the A allele (0.16). In stark contrast, the F and N alleles were rare (<0.01), and the 01 allele was completely absent. An association analysis between *CSN1S1* genotypes and milk traits was carried out with a general linear mixed model in R software. We observed that the *CSN1S1* genotype is significantly associated with milk dry matter (AA-EF contrast, *q*-value = 9.26E-03), fat (AA-EF, *q*-value = 9.19E-03) and protein (AA-BB, *q*-value = 1.31E-02; AA-FB = 2.48E-02) contents. Unexpectedly, we did not see any significant difference for the milk traits under study between the BB and EE genotypes.

Key Words: casein, goat, milk yield and composition

P397 ISAG Bursary Award: Identification of long non-coding RNAs differentially expressed in the mammary gland of lactating and dry goats. M. Wang*[1], E. Varela-Martínez[1], M. Luigi-Sierra[1], A. Noce[1], A. Martínez[2], J. Delgado[2], A. Salama[3], X. Such[3], J. Jordana[3], and M. Amills[1,3], [1]*Centre de Recerca Agrigenòmica (CRAG), Campus Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain,* [2]*Departamento de Genética, Universidad de Córdoba, Córdoba, Spain,* [3]*Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

Long non-coding RNAs (lncRNAs) have recently attracted attention due to their role as potential regulators of gene expression. In this work, we aimed to characterize the catalog of caprine mammary lncRNAs in lactating and dry individuals and to compare their levels of expression. To achieve this goal, we have used one data set including 7 Murciano-Granadina goats sampled at 3 time points (early lactation, late lactation, and dry period) that was reported in a previous study. Moreover, we have generated a second independent data set consisting of 5 lactating and 5 dry goats to identify lncRNAs with consistent differential expression in both data sets. Total RNA preparations have been sequenced with an Illumina Hiseq 4000 machine. After quality control using Trimmomatic, reads aligned to SILVA rRNA database (release 138.1) were removed with the bbduk software. Then, clean reads were aligned to the goat ARS1 reference genome with the STAR software, and the transcriptome was assembled with Stringtie. We obtained an average of 65 and 78 million reads for the first and second data sets, respectively. After classifying transcripts with the gffcompare software, lncRNAs were screened according to class code ('u', 'i', 'x', 'o') and length. Then, CPC2, CPAT, LGC software and PFAM database were used to detect the coding potential of transcripts as well as the presence of protein domains. Subsequently, candidate lncRNAs were quantified with Kallisto to calculate the gene level of gene expression, and the edgeR software was used to perform differential expression analysis. By doing so, we detected 3,974 lncRNAs transcripts expressed in the goat mammary gland. Moreover, 950 and 546 lncRNA genes were differentially expressed between the lactation and dry stages in the first and second data sets, respectively. Noteworthy, 412 lncRNA genes were consistently identified as differentially expressed in both data sets.

Key Words: LncRNA, RNA-seq, goat, lactation

P398 Genomic improvement in dairy goats using DNA sequencing. A. Caulton*[1], M. Wheeler[2], S. Clarke[1], R. Brauning[1], T. Van Stijn[1], H. Baird[1], R. Anderson[1], B. Foote[3], J. Foote[3], S. Cameron[4], T. Blichfeldt[5], J. Jakobsen[5], K. Dodds[1], and J. McEwan[1], [1]*AgResearch, Mosgiel, Otago, New Zealand,* [2]*AgResearch, Hamilton, Waikato, New Zealand,* [3]*Footes, Hikurangi, Northland, New Zealand,* [4]*Meredith Dairy, Meredith, Victoria, Australia,* [5]*NSG, As, Norway.*

In dairy goats, industry uptake of genomic technologies has been slow due to the small size of the industries coupled with the limited market for SNP array-based technologies. However, the potential benefits of genomic selection in dairy goats are large, because key traits are sex limited, recorded post selection and pedigree recording in large dairy goat herds is problematic. This has led our laboratory to utilize 2 low-cost genotyping strategies based on sequencing: RE-RRS and GT-seq. Historically, these approaches have suffered, in part because they are subject to missing or probabilistic genotyping calls. This made them difficult to integrate with existing genetic evaluation software. The sequencing-based technologies described above are currently used for separate genetic evaluations in Australia, New Zealand and Norway, with more than 87,000 samples genotyped to date using RE-RRS with more than 56K SNPs reported. Evaluation methodology and results for an example herd genetic trend will be presented, which for a 290 lactation length lactation show improvements of 26 L/doe/year and 4.51kg cheese/doe/year over the last 5 year period.

Key Words: goat, genomic selection, sequencing, dairy, genotyping-by-sequencing

P399 Ascertaining the variability and demographic history of the Canarian goat breeds through the use of genome-wide SNPs data. G. Senczuk*[1], M. Macrì[2,3], S. Mastrangelo[4], M. Di Civita[1], M. del Rosario Fresno[5], J. Capote[5], F. Pilla[1], J. V. Delgado[3], M. Amills[6], and A. Martínez[3], [1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy,* [2]*Animal Breeding Consulting S.L, Córdoba, Spain,* [3]*Universidad de Córdoba, Córdoba, Spain,* [4]*Department of Agricultural, Food and Forest Sciences, University of Palermo, Palermo, Italy,* [5]*Instituto Canario de Investigaciones Científicas, Tenerife, Spain,* [6]*CRAG, CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, Bellaterra, Spain.*

The Canary Islands are home to more than 320,000 goats representing one of the most important economic resources, in terms of milk production and cheese industry. The presence of goats in the Canary Islands might trace back to the early 1st millennium BC, when settlers of Berber origin colonized the Archipelago. Considering the relevance of goat genetic resources for the economy of the Canary Islands and the susceptibility of local insular populations to the loss of genetic diversity, we aimed to assess the genetic variability and origins of Canarian local breeds. To do so, we have genotyped, with the Goat SNP50 BeadChip (Illumina), 224 individuals belonging to 4 Canarian breeds (Palmera, Mejorera, South Tinerfeña and North Tinerfeña). Moreover, we have retrieved SNP data from 1,007 individuals from Africa and Southern Europe that were genotyped in the AdaptMap project. After filtering for missing call rate and minor allele frequency, we obtained a final data set of 45,149 SNPs. Diversity indices of the Majorera (Ho = 0.38, He = 0.385, F_{ROH} = 0.03), North Tinerfeña (Ho = 0.363, He = 0.364, F_{ROH} = 0.052) and South Tinerfeña (Ho = 0.36, He = 0.364, F_{ROH} = 0.034) breeds showed values in line with those of other breeds, while the Palmera breed displayed lower levels of genetic variation (Ho = 0.307, He = 0.309, F_{ROH} = 0.103). The strong genetic differentiation of the Canarian breeds resulted evident in all the analyses we performed, confirming a relationship with Northern African breeds. The ADMIXTURE and the TreeMix analyses did not suggest the existence of gene flow between Canarian goats and other continental breeds. This result is fairly unexpected, especially when considering that during the Age of Exploration the Canary Islands were an important maritime port of

call, a circumstance that might have favored the exchanges of livestock genetic resources. This work represents a first step toward the genetic characterization of the Canarian goat breeds, paving the way for conservation of these invaluable insular genetic resources.

Key Words: goats and related species, conservation genomics, population genomics, biodiversity, breed diversity

P400 Combining ATAC-Seq and RNA-Seq data to investigate the molecular basis of lactation in goats. A. Noce*[1], M. Luigi-Sierra[1], A. Martínez[2], M. Wang[1], M. Macri[2], J. Delgado[2], A. Salama[3], X. Such[3], J. Jordana[3], and M. Amills[1,3], [1]*Centre de Recerca Agrigenòmica (CRAG), Campus Universitat Autònoma de Barcelona, Bellaterra 08193, Spain,* [2]*Departamento de Genética, Universidad de Córdoba, Córdoba 14071, Spain,* [3]*Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain.*

The goal of this study is to investigate the molecular mechanisms involved in milk synthesis in goats. Total RNA has been extracted from the mammary gland of lactating (n = 3) and dry (n = 3) goats and sequenced in a NovaSeq6000 (Illumina) platform. Sequencing quality was evaluated with the FastQC software v0.11.7. Raw reads were trimmed to remove adaptors and low-quality reads, using TrimGalore 0.5.0 tool. Trimmed reads were then aligned with the goat ARS1 reference genome using HISAT2 software and the total count of mapped reads was obtained using the featureCounts tool. In parallel, we obtained mammary gland tissue from lactating (n = 3) and dry (n = 3) goats, and it was submitted to the Active Motif company (https://www.activemotif.com/) to carry out ATAC-seq. ATAC-Seq reads quality was also evaluated with FASTQC, aligned to the ARS1 goat genome using BWA-MEM tool, and post-alignment quality control was performed with the ATAQseqQC software. Peak calling was performed using the MACS2 software. Consensus peaks between replicates were obtained with DIffbind. To increase statistical power and provide a more reliable Differential accessibility regions (DARs) analysis, only the peaks overlapping in at least 2 of the samples (consensus = 58,826) have been used. Both differential gene expression (DGE) and DARs analyses have been performed with DESeq2. Differential expression analysis revealed 1,342 downregulated and 1,034 upregulated genes (|FoldChange| > 1.5 and q-value < 0.05) in the milking condition. On the other hand, DAR analysis showed 3,867 significant DAR. Among these, 2,392 regions were enriched in milking goats while 1,475 were enriched in the dry condition (|FoldChange| > 1.0, q-value < 0.05). The integration of ATAC-Seq and RNA-Seq is currently underway. Genes that are DE and map close to DARs will be functionally characterized. Additionally, a genome annotation of the DARs will be carried out to identify which regulatory elements correspond to accessible regions co-localizing with DE genes. The results will shed light on the role of epigenomics in the regulation of gene expression in the mammary gland of lactating goats.

Key Words: ATAC-seq, RNA-seq, goats and related species

P401 Heritability estimates of hematological, serological, morphological and productive traits in Murciano-Granadina goats, using a univariate animal model. M. Macrì[1,2], M. Amills[3,4], J. León Jurado[5], L. Gama[6], M. Luigi-Sierra[3], J. Delgado[2], J. Fernández[7], and A. Martínez Martínez*[2], [1]*Animal Breeding Consulting, 14014-Córdoba, Spain,* [2]*Universidad de Córdoba, 14071-Córdoba, Spain,* [3]*CRAG, CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, 08193-Bellaterra, Spain,* [4]*Universitat Autònoma de Barcelona, 08193-Bellaterra, Spain,* [5]*Diputación Provincial de Córdoba, 14071 Córdoba, Spain,* [6]*Universidad de Lisboa, 1649-004 Lisboa, Portugal,* [7]*Asociación Nacional de Criadores de Caprino de Raza Murciano-Granadina (CAPRIGRAN), 18340-Granada, Spain.*

Eighteen variables corresponding to hematological, serological, morphological and productive traits from 3254 Murciano-Granadina goats, collected during 2016–2018, were analyzed to estimate variance components and heritability using a Restricted Maximum Likelihood (REML) approach. The MTDFREML set of programs was used to obtain restricted maximum likelihood estimations of genetic parameters, with a relationship matrix including 64424 animals. A single trait animal model was used to carry out the above task, using either single-records animal models (hematology, serological and morphology traits) or animal models with repeated measures (milk yield and components). Heritability estimates for hematological traits were 0.23 ± 0.08, 0.17 ± 0.07, 0.22 ± 0.08 and 0.25 ± 0.09 for red blood cells, hemoglobin, hematocrit and leucocytes, respectively. Heritability estimates for the various morphological traits were 0.18 ± 0.09, 0.20 ± 0.07, 0.12 ± 0.07, 0.08 ± 0.08 and 0.07 ± 0.07 for total classification, structure, dairyness, mammary system and feet and legs scores, respectively. For milk production and composition traits, the heritability estimates were 0.19 ± 0.05 for milk yield; 0.15 ± 0.05 and 0.27 ± 0.06 for fat yield and percentage; 0.21 ± 0.06 and 0.41 ± 0.08 for protein yield and percentage; and 0.17 ± 0.05 and 0.31 ± 0.06 for dry matter yield and percentage, respectively. Finally, the heritability estimates for serological traits were 0.02 ± 0.13 for agalactia and 0.05 ± 0.10 for CAEV (Caprine Arthritis Encephalitis Virus). The results of this study show that in Murciano Granadina goats heritability estimates for dairy, morphology and hematology traits are moderate, while those for serology phenotypes appear to be really low.

Key Words: goats and related species, animal breeding, bioinformatics tools, heritability, milk production

P402 Withdrawn

P405 Genetic analysis of body weight of Mecheri sheep using robust model. A. K. Thiruvenkadan[1], K. Kizilkaya[2], S. O. Peters*[3], J. Muralidharan[4], and C. Bandeswaran[5], [1]*Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Salem, Tamil Nadu, India,* [2]*Aydin Adnan Menderes University, Faculty of Agriculture, Department of Animal Science, Biometry and Genetics Unit, Aydin, Turkey,* [3]*Department of Animal Science, Berry College, Mount*

Berry, GA, [4]Mecheri Sheep Research Station, Pottaneri, Tamil Nadu, India, [5]Department of Animal Nutrition, Madras Veterinary College, Chennai, Tamil Nadu, India.

The Student's-t distribution is a heavy-tailed distribution and a viable alternative to normal distribution for the robust analysis of the data including unusual or outlying observations. We compared estimates of genetic parameters by fitting univariate Normal and Student's-t distributions for residuals in the analysis of 5080 birth and weaning weights records from Mecheri sheep between 2010 and 2021. Model comparisons using deviance information criteria (DIC) did not favor the Student's-t residual specification models for birth and weaning weights. The posterior mean estimates of degrees of freedoms in the Student's-t error model for birth and weaning weights were higher than 100. Higher value of posterior density of degrees of freedom for the univariate Student's-t models confirms that the assumption of normally distributed residuals is adequate for the analysis of the birth and weaning weights. Posterior mean estimates of direct and maternal genetic variances and error variance from univariate Normal and Student's-t models were similar for birth weight. However, posterior mean estimates of direct and maternal genetic variances from Student's-t model were lower than from univariate Normal model. The additive and maternal heritabilities for birth weight under normal error and student's error model were 0.3419 and 0.205 and 0.3292 and 0.225 respectively. The additive and maternal heritabilities for weaning weight under normal error and student's error model were 0.2841 and 0.105 and 0.2438 and 0.083 respectively. The posterior inference on additive and maternal heritabilities and genetic correlations between additive and maternal genetic effects for birth and weaning weights using univariate Normal and Student's-t models indicated moderate heritability. Also, posterior means of additive and maternal genetic correlations for birth and weaning weights were variable and negative which showed an antagonistic relationship.

Key Words: Mecheri sheep, robust model, heritability, genetic selection

P406 Phenotypic and genomic variation in gastrointestinal nematode (GIN) infection in Tunisian indigenous sheep. J. Mwacharo*[1,2], M. Rouatbi[3], A. Ahbara[2], M. Gharbi[3], M. Rekik[1], A. Haile[1], and B. Rischkowsky[1], [1]*Small Ruminant Genomics, International Centre for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia,* [2]*Animal and Veterinary Sciences, Scotland's Rural College (SRUC) and Centre for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, Midlothian, Scotland,* [3]*Laboratoire de Parasitologie, Université de la Manouba,École Nationale de Médecine Vétérinaire de Sidi Thabet, Sidi Thabet Tunisia.*

GINs are the most significant parasites to grazing ruminants from an animal, human and environmental health, welfare and economic standpoint. The long-term use of chemoprophylaxis has seen the emergence of drug-resistant GINs and presence of drug residues in animal products widely consumed by humans, thus calling for alternative control strategies. We investigated individual phenotypic variability and genome-wide genetic divergence to GIN infection in indigenous Tunisian sheep that are traditionally managed on communal pastures. Feacal, blood parameters and abomasum contents of 310 sheep from 8 abattoirs across North Tunisia were assessed. The proportion of fecal samples that were positive for GIN's eggs was 30.82% while, the recovery of worms from abomasum contents showed an infection rate of 75.90%. Principal component analysis identified 2 clusters of animals. One had individuals with high incidence of abomasum nematodes, low total feacal egg worm counts (<500), and normal range in the values of albumin, red blood cell count, hemoglobin and packed cell volume, suggesting possible GIN resistance. The other cluster comprised all the other observations in which subgroups of animals could be distinguished based on their potential resistance to abomasum nematodes. Using ROH, LR-GWAS, F_{ST} and XP-EHH tests, we analyzed 600K SNP genotype data from the 2 groups. It revealed 35 genomic selection candidate regions that were identified by at least 2 tests. Nineteen regions overlaid QTLs for parasite resistance, immunity, and disease susceptibility and, 10 had QTLs for production (growth) and meat and carcass (fatness and anatomy) traits. Selection sweeps spanning genes enhancing innate immune defenses (SLC22A4, SLC22A5, IL-4, IL-13), intestinal wound healing/repair (IL-4, VIL1, CXCR1, CXCR2) and GIN expulsion (IL-4, IL-13) were also revealed. Our findings suggest that indigenous sheep are an alternative and sustainable genetic treasure-trove to chemoprophylaxis. They have naturally evolved genetic strategies that invoke and enhance GIN resistance while ensuring growth and developmental stability under traditional management.

Key Words: Africa, endo-parasites, SNP

P407 Withdrawn

P409 Withdrawn

P410 Whole-genome diversity across Nubian, Old English, and Anglo-Nubian goat breeds. S. A. Rahmatalla*[1,2], D. Arends[3], G. B. Neumann[1], H. Abdel-Shafy[4], J. Conington[5], M. Reissmann[1], M. K. Nassar[1,4], and G. A. Brockmann[1], [1]*Albrecht Daniel Thaer-Institute for Agricultural and Horticultural Sciences, Humboldt-Universität zu Berlin, Berlin, Germany,* [2]*Faculty of Animal Production, University of Khartoum, Khartoum, Sudan,* [3]*Department of Applied Sciences, Northumbria University, Newcastle upon Tyne, UK,* [4]*Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt,* [5]*SRUC, W Mains, Rd, Edinburgh, Scotland, United Kingdom.*

Monitoring and maintaining genetic diversity is essential to ensure the long-term viability of goat breeding programs. Nubian goats are found widespread in Northeastern African countries such as Sudan and Egypt and show an important contribution to food security as a source of milk and meat. During the latter half of the 19th century, Anglo-Nubian goats were developed by crossing Nubian goats from Africa with native Old English goats from Britain. To evaluate the genetic diversity within and between Nubian goats from Northeast Africa, and their relationship with Old English, and Anglo-Nubian breeds, 188 goats were genotyped using the Axiom Caprine 60K SNP chip. Genetic diversity was estimated using nucleotide diversity, observed and expected heterozygosity, while inbreeding was estimated as excess of homozygosity. Relationship between breed was assessed using the fixation index (F_{ST}), hierarchical clustering, and principal component analysis (PCA). Anglo-Nubian breed had the lowest nucleotide diversity compared with other breeds. Nubian goats from Sudan showed the highest observed heterozygosity (35.97), while Anglo-Nubian goats had the lowest heterozygosity (24.62). As expected, the pattern was reversed for the excess of homozygosity. Anglo-Nubian goats exhibited the highest excess of homozygosity, while Nubian goats from Sudan had the lowest. The estimated pairwise F_{ST} values ranged between 0.06 (Nubian from Sudan vs. Nubian from Egypt) to 0.26 (Anglo-Nubian vs. Old English). The PCA revealed the presence of 4 clearly defined clusters, namely Nubian from Sudan, Old English, Anglo-Nubian, and Nubian from Sudan and Egypt, which were consistent with the findings of the hierarchical clustering. The first 2 principal components accounted for 14.3% and 6.7% of the genetic diversity between breeds, respectively. SNPs that contributed most to the first principal component allowed us to identify genomic regions, particularly on chromosome 25, that differentiate Nubian from Old English goats. These regions contain genes for adaptation, immune response, reproduction, pigmentation, and metabolic process. Further research will focus onidentified regions and subsequent gene discovery.

P411 ISAG Bursary Award: Investigation on already known variants and markers for horn phenotypes in Icelandic sheep. R. Simon*[1], K. Elísabetardóttir[2], and G. Lühken[1], [1]*Institute of Animal Breeding and Genetics, Justus Liebig University, 35390 Giessen, Germany,* [2]*Hvammshlíð, Iceland.*

The Icelandic sheep population as such has not been considered much in the context of animal genetics. The long isolation of the entire population, including strict import restrictions, and the fact that all sheep belong to a single diverse breed, turns the Icelandic sheep into an interesting research population. The basis of the genetic pool were sheep imported from Northern Europe around 900 BC. One of the heterogeneous characteristics is the horn status. It is variable in both sexes, knobs are described, and different horn shapes can be observed. Particularly striking is the occurrence of polyceraty. Overall, samples from 93 Icelandic sheep with different horn phenotypes were analyzed. Depending on the horn phenotype, the samples were genotyped for variants already published to be involved in i) polledness (1.78-kb insertion in *RXFP2* (Oar_v4.0: chr10 g.29,433,060–29,434,923) and a SNP (Oar_v4.0: chr10 g.29458450 GG)), ii) horn shape and size (OviAri3: chr10 g.29,461,968 C/T + g.29,462,010 C/T), and iii) polyceraty (*HOXD1* (OAR_4.0: chr4 g.132,832,249_132,832,252del)). As in other breeds with variable horn status, the *RXFP2* insertion did not segregate with polledness in Icelandic sheep, supporting the hypothesis that at least one additional variant must be present in such breeds. Similarly, no association was found between horn status and the SNP on chr10 predicting polledness in Merino, as no variation from wild type was seen. The region on sheep chr10 associated with horn shape and size in Chinese breeds did not show any polymorphism regarding one of the 2 SNPs included, but showing a slight association with horn shape in the second SNP, like described previously. However, the sample set was too small to confirm the association completely, even though considered promising. Interestingly, the 4-bp sized deletion in *HOXD1* segregated with multihorndness in the tested Icelandic sheep and thereby confirms former results. These preliminary results represent the base for further studies, on the molecular basis of external and functional traits plus the diversity of the Icelandic sheep population.

Key Words: sheep and related species, animal breeding, genotyping, breed diversity

P412 Assessing runs of homozygosity Kazakh Edilbay sheep breed. A. Khamzina*[1], S. Darkhan[1], A. Shamshidin[1], and K. Khamzin[2], [1]*Zhangir Khan University, Uralsk, Kazakhstan,* [2]*Kazakh National Agrarian Research University, Almaty, Kazakhstan.*

Sheep breeding in Kazakhstan has one of the main roles in animal husbandry locally. The indigenous Edilbay breed was created at the end of the 19th century in the Ural region as a result of natural and artificial selection. No documented data are available on the appropriation of this sheep breed. Edilbay is well adapted to the harsh conditions of dry steppes and deserts, with almost year-round grazing. Ear tissue samples were collected from Edilbay breed (n-500) in West Kazakhstan (Birlik farm). Samples were stored in 2 mL Eppendorf tubes containing 70% ethanol. Genomic DNA was extracted from ear tissue samples by applying the protocol from the MasterPure Complete DNA kit. The concentration of DNA samples was measured by spectrophotometer and diluted to 50 ng/µL for genotyping. 500 animals genotyped using the Illumina Ovine SNP600K, which contains oligo probes for 685,734 SNPs. After Quality Control, 385 individuals and 385,786 autosomal SNPs were subjected to the subsequent ROH detection and analysis. The X and Y chromosomes were excluded. Among the Edilbay breed studied the individual ROH length is 789.9 Mb. The individual ROH numbers was displayed is 138. The mean genomic inbreeding coefficient values is FROH = 0.039 QTL analysis were observed within the recorded ROH islands using the Sheep QTL database. The *MSTN* gene is, which is associated with meat production and weight gain is also observed in the recorded ROH islands, which makes sense given that this breed has a good meat constitution. In this study observed lipid metabolic process and galactose metabolic process. The productive traits of the Edilbay breed were identified using ROH analysis, since this breed is an indigenous breed and has a national meaning for Kazakhstan.

Key Words: sheep, Edilbay breed, ROH analysis, MSTN gene, inbreeding coefficient

P414 Monitoring of genetic polymorphism at *CSN2* and *CSN3* loci in Czech goat population using primer extension analysis (PEA). Z. Sztankoova, L. Tichý, K. Novák, and J. Kyselová*, *Institute of Animal Science, Praha-Uhrineves, 104 00 Czech Republic.*

The main objective of the current study was to investigate the genetic polymorphism at exons 7 and 9 of the β-casein (*CSN2*) and exon 4 of the kappa-casein (*CSN3*) locus in the population of historical

Czech goat breeds comprising White and Brown Short-Haired goats. The reliable, sensitive, and reproducible assay PEA in the commercial version of SNaPshot was applied for efficient milk protein genotyping. The genetic polymorphism in the *CSN2* locus was determined at 3 years sampled (2008–2022), and in the *CSN3* locus at 5 years (2011–2022). We analyzed 543 animals from the genetic resources program for the variants of *CSN2* (*A*, *C*, *C1*, and null alleles). The development of allelic frequencies showed that the *A* allele increased from 35% to 37%. On the contrary, there was a significant decrease in the occurrence of the *C* allele group (*C* + *C1*) from 68% to 14.51% +48.39%, respectively. However, we noted an increase in the *C1* allele to 48%, an increase in the frequency of the heterozygote AC1 to 37%, and the homozygote C1C1 to 22% in the monitored goat population. The positive result is that we did not detect any null allele in the observed population. This allele reduces the casein content in milk, with consequences for goat milk production and economic parameters. In addition, 989 animals were genotyped for *CSN3* (*A*, *B*, *C*, *D*, *F*, and *G*). The results demonstrated that the predominant variant was the *B* allele (66%) compared with the *A* allele (27%) in the population. The frequency of the BB genotype increased from 31% to 47% during the following period. Conversely, the AA genotype was significantly reduced from 20% to 7%. Other identified variants, alleles *C* and *G*, had a minor occurrence 1.8% and 5%, respectively. Alleles *D* and *F* were not detected in the monitored population. Changes in the occurrence of genetic variants might be influenced by unintentional selection, inbreeding, or climate changes. The PEA method identified these variants accurately, efficiently, and reliably. Consequently, it can be used in population studies as a routine genotyping method for the *CSN3* and *CSN2* loci, including the null allele.

Key Words: goat, *CSN2*, *CSN3*, SNP, PEA

P415 ISAG Bursary Award: Genetic diversity among Swakara sub-populations and their founders. A. Njilo*[1,2], F. Muchadeyi[1], and E. Dzomba[1], [1]*Agricultural Research Council, Pretoria, Gauteng, South Africa,* [2]*University of KwaZulu-Natal, Pietermaritzburg, KwaZulu-Natal, South Africa.*

Originally derived from the Karakul breed of Uzbekistan, the Swakara is a fat-tailed breed known for its high-quality pelts. Introduced into Southern Africa in the 1900s, the Karakul was subjected to intense crossbreeding with indigenous Namaqua Afrikaner and Blackhead Persian breeds to produce the Swakara, whose pelt is superior with unique features. Selection and crossing of Karakul to indigenous white-wooled breeds resulted in a sub-vital factor in the pure white sub-population that causes the lamb to die within 48 h of birth. With a complex ancestral background, little is known about the genomic architecture of the sub-populations, making it difficult to understand the factors contributing to the occurrence of genetic disorders. A total of 244 sheep from 8 sub-populations sampled in Namibia, South Africa and Germany were genotyped using the OvineSNP50 beadchip. We report the genetic diversity and structure of Swakara sub-populations and their founding breeds. Genetic diversity ranged from $H_O = 0.29 \pm 0.15$ for the White Sub-vital to $H_O = 0.41 \pm 0.22$ for the Swakara, and the highest inbreeding was observed in the Namaqua Afrikaner ($F_{IS} = 0.13 \pm 0.10$) and White Vital Swakara ($F_{IS} = 0.08 \pm 0.09$). The principal component analysis produced 5 clusters with PC1 explaining 27.35% of the total variation separating cluster 2, which contained the Namaqua Afrikaner only, from clusters 1 and 3–5, to which the Black Vital, Brown Vital, Grey Vital, White Sub-vital and Swakara all belonged. PC2 accounted for 19.25% of the total variation separating cluster 1 from all the other clusters. The PCA demonstrated the effects of geographic origin with sheep breeds sampled from Germany clustering together, separating from the Southern African sheep. Per marker F_{ST} showed SNPs within QTLs associated with milk production, wool quality, and coat color. The detection of coat color genes provided evidence to selection targets due to a human-led selection. This study gave insight into the genomic architecture of Swakara sheep and its founding breeds toward a better understanding of the underlying causes of the prevalent genetic disorders.

Key Words: sheep, genome-wide association, genotyping, breed diversity, genetic improvement

P416 Genetic diversity of United States Rambouillet, Dorper, and Katahdin sheep. G. Becker*[1], J. Thorne[1,2], J. Burke[3], R. Lewis[4], D. Notter[5], J. Morgan[6], C. Schauer[7], W. Stewart[8], R. Redden[2], and B. Murdoch[1], [1]*Department of Animal, Veterinary and Food Science, University of Idaho, Moscow, ID,* [2]*Texas A&M AgriLife Extension, Texas A&M University, San Angelo, TX,* [3]*United States Department of Agriculture, Agricultural Research Service, Dale Bumpers Small Farms Research Center, Booneville, AR,* [4]*Department of Animal Science, University of Nebraska–Lincoln, Lincoln, NE,* [5]*Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA,* [6]*Round Mountain Consulting, Fayetteville, AR,* [7]*Hettinger Research Extension Center, North Dakota State University, Hettinger, ND,* [8]*Department of Animal Science, University of Wyoming, Laramie, WY.*

Analyses of genetic diversity can facilitate identification of signatures of selection which may contribute to specific health, production and visual characteristics of breeds or populations. Breeds with well-characterized traits such as fine wool production (Rambouillet, n = 745), environmental hardiness (Dorper, n = 265) and parasite resistance (Katahdin, n = 581) were evaluated for measures of genetic diversity at 36,113 autosomal SNPs. Statistics assessed included runs of homozygosity (ROH), inbreeding (F_{ROH}), Wright's fixation index (F_{ST}) and effective population size (Ne). The Rambouillet breed had the most highly conserved ROH region, with a region on chromosome 6 containing 202 SNPs called in an ROH in 50 to 94.23% of individuals. Rambouillet had the lowest average F_{ROH} (0.169), while the F_{ROH} for Katahdin and Dorper were similar (0.187; 0.188) and were above the overall average (0.178). Pairwise F_{ST} between breeds were 0.161 for Dorper-Katahdin, 0.156 for Rambouillet-Dorper, and 0.140 for Katahdin-Rambouillet. The Rambouillet animals had the largest Ne (n = 268 at 13 generations ago) and the lowest average linkage disequilibrium (r^2 of 0.024). To better understand the potential biological importance of signatures of selection, genes present within ROH peaks and regions of high breed divergence in F_{ST} were analyzed through pathway enrichment by gene ontology or KEGG Mapper tools. Signatures of selection in all breeds contained genes of known economic importance, including *RXFP2* related to horn growth, *LCORL* related to body size and *IRF2BP2* related to wool type. Signatures in Dorper were found to be significantly enriched for pathways related to epidermis development and olfactory receptor activity. Pairwise F_{ST} from the Dorper-Katahdin analysis revealed enrichment of immune-related pathways (chemokine signaling pathways and cytokine-cytokine receptor interaction) which may provide insight into the genetic basis for divergent immune responses in these breeds. This study identified signatures of selection within 3 diverse and economically important US sheep breeds and suggested candidate genes and pathways for further understanding of breed diversity.

Key Words: population genomics

P417 Unravelling of genes associated with coat color and coat color patterns in South African meat-type goats. S. Gcabashe*[1], F. Muchadeyi[2], and E. Dzomba[3], [1]*University of KwaZulu-Natal-School of Life Sciences, University of KwaZulu-Natal-School of Life Sciences, Pietermaritzburg, KwaZulu-Natal, South Africa,* [2]*Agricultural Research Council-Biotechnology Platform, Agricultural Research Council-Biotechnology Platform, Pretoria (Onderstepoort), Gauteng, South Africa,* [3]*University of KwaZulu-Natal-School of Life Sciences, University of KwaZulu-Natal-School of Life Sciences, Pietermaritzburg, KwaZulu-Natal, South Africa.*

Goat genetic improvement in South Africa is lagging due to a lack of genomic studies about goat production traits and breed characteristics. Coat color is of high economic importance in goat production since it forms a part of breed characteristics and usually determines the selling price of goats, particularly in smallholder farming systems. Using within-population iHS analysis and between-population XP-EHH analysis, this study aimed to identify genes underlying coat color and coat color patterns in South African meat-type goats. A total of 311 meat-type goats belonging to Boer (n = 97); Kalahari Red (n = 53) Savanna (n = 40) and village ecotypes (n = 121) was genotyped on the Illumina Goat SNP60K panel. The Boer (white and redhead), Kalahari

Red (red) and Savanna (white) were of uniform breed standard coat color patterns. The village ecotypes were of various coat colors and coat color patterns including white (n = 30), red (n = 17.), black (n = 25), gray (n = 17), belted (n = 17), brown (n = 10) and patched (n = 5). A total of 371 candidate genes were detected by this study. Between the Boer and the Kalahari Red, 65 candidate genes were detected including the *CHST1* gene on chromosome 15, which is involved in hair follicle development in goats. A total of 162 genes were detected between the Boer and the Savanna including 4 genes on chromosome 4 (*SEMA3D*), 10 (*HSPA2*), 11 (*FOX13*), and 22 (*IL17RB*) which are involved in hair follicle growth and development in cashmere goats. A total of 144 genes were detected between the Savanna and the Kalahari Red, including the ASIP, ITCH, and ACHY genes. Overall, this study provides information that can be used to design and implement improved breeding and conservation programs for SA goats, particularly in small-scale farming systems where goats remain poorly characterized.

Key Words: goats, coat color diversity, genetics of coat color, genome-wide SNP data

P418 Withdrawn

P419 Differentiation of Indigenous Veld goats (IVG) breed in South Africa. L. Rashijane*[1,2], T. Tyasi[2], and K. Hadebe[1], [1]*Agricultural Research Council, Pretoria, Gauteng, South Africa,* [2]*University of Limpopo, Polokwane, Limpopo, South Africa.*

Indigenous goat populations have for years supported households in communal areas in the form of food security, income and sacrifices for cultural ceremonies among other roles. This study was conducted to investigate the differentiation of South African Indigenous Veld Goats breed using PCAdapt, where a total of 115 goats viz. Cape Lobear (37), Cape Speckled/Skilder (17), and Mbuzi (61) from Limpopo and Kwa-Zulu-Natal were evaluated using the Illumina 65K Goat Bead chip. A total of 7 differentiation SNPs were observed. The SNPs were detected on the following chromosomes, 1(snp58254-scaffold945–582202, position: 108667531), 3(snp17665-scaffold183–1128427, position: 112306142), 4(Random2.2K-443, position: 56041179), 6(ilmnseq_rs664392780; position: 70316692), 6(snp9844-scaffold1352–23038, position: 97704927), 22(snp15314-scaffold163–1176183, position: 39984346) and 24(GoatD01.017418; position: 41254800). DNA sequence variation plays a crucial role in breeding and genetics, in support of selection. The polymorphisms discovered in this study have the potential of influencing the farmers decisions in selecting the best animals for future breeding, however, more breed representations and number of animals are required for validation of the SNPs for future use.

Key Words: single nucleotide polymorphisms (SNPs), indigenous goat populations, breeding, and genetics

P420 Polymorphism and association of growth hormone gene with growth traits in Dorper sheep. K. Molabe*[1], T. Tyasi[1], V. Mbazima[1], B. Gunya[1], and L. Bila[2], [1]*University of Limpopo, Polokwane, Limpopo, South Africa,* [2]*Potchefstroom College of Agriculture, Potchefstroom, North West, South Africa.*

Dorper sheep are South African breed resulted from crossbreeding Dorset Horn and Blackhead Persian sheep. The *Growth hormone* gene (*GH*) has been employed in many research since it has impact on food partitioning which contributes to the rapid growth of an animal. However, single nucleotide polymorphisms (SNPs) of the *GH* gene and their association with growth traits are not yet known. Hence, the objective of the study was to identify SNPs of the *GH* gene and their association with growth traits. Growth traits such as body weight, withers height, body length, heart girth, sternum height and rump height were collected from 50 Dorper sheep aged between one and 2 years. PCR-FRLP and DNA sequencing were used for SNPs detection. The results showed that the 2 genotypes (AA and AB) were observed. DNA sequencing findings showed a synonymous SNP (T/A) on position 735 of the exon 4 of the *GH* gene. Marker-trait association results showed that there was no significant association between genotypes (AA and AB) and growth traits except for withers height, whereby genotype AA had the highest impact on withers height. In conclusion, the results of the current study suggest that Dorper sheep with genotype AA of *GH* gene might be used when improving withers height. These findings might be used to assist breeders in selecting animals based on molecular genetic markers to improve growth traits. However, more research on *GH* gene polymorphisms and their association with growth traits has to be done with a large sample size and including more growth traits.

Key Words: SNP, growth hormone gene, marker-traits association, withers height

P421 Absolute quantification of growth differentiation factor-9 (GDF9) gene in ovarian tissues of high prolific and low prolific sheep breeds. J. Mamutse*[1], A. Molotsi[1], K. Dzama[1], and C. Urbano-Braz[2], [1]*Stellenbosch University, Capetown, Western Cape, South Africa,* [2]*University of Illinois, Champaign, IL.*

The efficiency of lamb production is influenced by several aspects which include litter size. Selection for litter size, a trait that is directly related to ovulation rate, may be responsible for the productivity and economic efficiency of ewes. Growth differentiation factor-9 (GDF9) is a member of the transforming growth factor β superfamily with crucial roles in folliculogenesis. Therefore, the aim of this study was to examine the expression of GDF9 gene in ovarian tissues of high prolific and low prolific sheep. The sheep breeds that were used were Dohne Merino and Merino. Six ovarian tissues were obtained from low prolific sheep along with 6 tissues from high prolific sheep. RNA was isolated from the tissues and then stored at −20°C until real-time qPCR. Absolute quantification RT-qPCR method was used to quantify the gene expression levels of GDF9 gene in ovarian tissues using 2-fold DNA serial dilutions as standards. The ANOVA was used to determine the significant difference of GDF9 gene expression levels between high and low prolific ewes. Findings of this study showed that GDF9 gene expression levels in the sampled animals ranged from 0.5 to 3.45 copies/µL. Statistical analysis showed that there was no significant difference

($P > 0.05$) of the expression levels of GDF9 gene between high and low prolific ewes in Dohne Merino sheep (2.03 ± 1.19 copies/µL and 0.77 ± 0.29 copies/µL), Merino sheep (1.72 ± 1.31 copies/µL and 1.33 ± 0.35 copies/µL) and all breeds combined (1.82 ± 1.09 copies/µL and 1.05 ± 0.41 copies/µL). Although no statistical difference was reported between the 2 prolific groups, GDF9 gene was expressed in ovarian tissues of all the sampled ewes. Therefore, this study concluded that GDF9 gene plays a major role in sheep prolificacy.

Key Words: gene expression, RT-qPCR, reproduction, sheep

P422 ISAG Bursary Award: Breed composition of South African sheep affected by wet carcass syndrome. R. Grobler*, P. Soma, B. B. Kooverjee, and M. M. Scholtz, *Agricultural Research Council – Animal Production Institute, Irene, Gauteng, South Africa.*

Wet carcass syndrome (WCS) is a condition found in sheep, which negatively affects the quality of their carcasses. Before slaughter, an affected animal appears to be physically normal, showing no symptoms of an abnormality. However, after removal of the skin during the slaughter process, affected animals show an accumulation of a watery fluid on the dorsal parts of the carcass as well as on the hind legs and flanks. These carcasses are condemned at the abattoir and results in high economic losses in the South African (SA) sheep industry. Currently the etiology of WCS and the breeds that are affected by WCS are unknown. The aim of this study was to characterize the breed composition of sheep affected with WCS in regions of South Africa where WCS is prevalent. A total of 75 animals affected with WCS were genotyped with the Ovine 50K SNP array. SNP genotype data for reference populations were included from SA sheep breeds, i.e., SA Merino, SA Mutton Merino, Dorper, Blackhead Persian and Namaqua Afrikaner. Quality control of the genotypic data were performed using Plink v1.9. To identify patterns of admixture and relationships among breeds, data analysis was performed with GCTA and ADMIXTURE 1.23 software. Even though the WCS affected animals were separated into 2 clusters, poor population substructure was observed within this population. Some of the affected animals grouped with the SA Merino, and were closely clustered with the SA Mutton Merino, while absence of clustering with a particular breed were observed for the majority of affected animals. Admixture based clustering indicated that the affected population are most likely from commercial breeds and did not include ancestry from indigenous breeds. Results from this study indicates that the incidence of WCS are not breed specific and that breed most likely does not influence the etiology of this condition. These findings are contradictory to reports that this condition is specific to the Dorper breed.

Key Words: breed specificity, genetic relatedness, principal component analysis

P424 The benefit of genomic information for enhancing genetic prediction of production and reproduction traits in South African Merino sheep. C. Nel*[1,2], P. Gurman[3], A. Swan[3], J. van der Werf[4], M. Snyman[5], K. Dzama[2], W. Olivier[5], A. Scholtz[1], and S. Cloete[2], [1]*Directorate: Animal Sciences, Western Cape Department of Agriculture, Elsenburg, Western Cape, South Africa,* [2]*Department of Animal Sciences, Stellenbosch University, Stellenbosch, Western Cape, South Africa,* [3]*Animal Genetics & Breeding Unit, University of New England, Armidale, New South Wales, Australia,* [4]*School of Environmental and Rural Science, University of New England, Armidale, New South Wales, Australia,* [5]*Grootfontein Agricultural Development Institute, Department of Agriculture, Land Reform and Rural Development, Middelburg, Eastern Cape, South Africa.*

Genomic selection (GS) requires validation in South African (SA) sheep breeding. Testing of GS methods in SA currently depends on a reference population combining both commercial and research data sets, which have not been concatenated in a single analysis. This study compared the accuracy, bias and dispersion of pedigree BLUP (ABLUP) and single-step genomic BLUP (ssGBLUP) for genetic prediction. Animals in this study provided production records for weaning weight (WW), yearling weight (YW), fiber diameter (FD), clean fleece weight (CFW) and staple length (SL). For reproduction traits, the data set included 58 744 repeated records of the number of lambs born (NLB), the number of lambs weaned (NLW) and the total weight weaned (TWW). The single-step relationship matrix, H, was calculated using the PreGS90 software combining 2,811 medium density (~50k) genotypes and the pedigree of 88,600 animals. All flocks had animals genotyped ranging from 79 to 516 individuals per flock. The accuracy of ABLUP and ssGBLUP was compared according to the "LR-method" following single-trait analysis using ASREML V4.2 software. Validation candidates were assigned according to scenario I: born after a certain time point; and scenario II: born in a particular flock. In Scenario I, ssGBLUP increased the accuracy of prediction for all traits except NLB, ranging between + 8% (0.62 to 0.67) for FD and + 44% (0.36 to 0.52) for WW. This showed a promising gain in accuracy despite a modestly sized reference population. In Scenario II, overall accuracy was lower, but with greater differences between ABLUP and ssGBLUP, ranging between 17% (0.12 to 0.14) for TWW and 117% (0.18 to 0.39) for WW. There was little indication of severe bias, but some traits were prone to over dispersion and the use of genomic information did not improve these observations. These results were the first to validate the benefit of genomic information in South African Merinos. However, because production traits are highly heritable and easy to measure at an early age, GS methods are likely to rather focus on sex-limited or lowly heritable traits.

Key Words: marker data, genomic selection, ovine

P425 Rumen microbial composition in sheep supplemented with *Acacia mearnsii* Tannin extract for methane reduction. I. Lawal, E. van Marle-Koster*, and A. Hassen, *University of Pretoria, Pretoria, Gauteng, South Africa.*

The emission of methane from ruminants can be reduced to varying degrees through the manipulation of the rumen microbiome by dietary interventions such as tannin. The aim was to study the rumen microbial composition in Merino sheep on a diet supplemented with crude or encapsulated *Acacia mearnsii* tannin extracts. Twenty-four sheep were divided into 4 groups that were fed a standard diet (negative control), standard diet with 75 mg/kg DM monensin (positive control, ionophore), standard diet with 20 g/kg DM *Acacia mearnsii* crude tannin extract and 29 g/kg DM of *Acacia mearnsii* tannin extract micro encapsulated in Sunflower oil. Rumen samples were collected at slaughter and stored at −21°C. DNA was extracted for metagenomic analyses. Sequence data were analyzed using MG-RAST version 4.0.3 (Meyer et al., 2008). Although inclusion of crude or encapsulated *Acacia mearnsii* tannin extract has in previous studies shown an increase in fecal nitrogen excretion and a reduction in methane emission of 19.1–21.7%; in this study, the encapsulated tannin didn't reduce digestibility and rumen fermentation parameters. *Bacteroidetes* (72%) and *Firmicutes* (21%) were the most prevalent phyla out of the 28 identified. Alpha and β diversity indices showed no significant differences ($P > 0.05$) among the 4 dietary treatment both at the phylum and genus level and was confirmed using Kruskal-Wallis test. Forty-one archaeal genera were identified with *Methanobrevibacter* having the highest abundance. The total bacteria and methanogens did not significantly differ between the tannin and non-tannin treatments suggesting that the tannin might have likely acted as a hydrogen sink.

Key Words: sheep and related species, microbiomics, DNA sequencing, digestive system, environment

P426 DNA-based vaccine design against *Toxoplasma gondii* in ovines using rhoptry protein antigens through immunoinformatics approach. T. Madlala*[1], M. Adeleke[1], M. Okpeku[1], and S. Tshilwane[2], [1]*University of KwaZulu Natal, Durban, KwaZulu Natal, South Africa,* [2]*University of Pretoria, Onderstepoort, Pretoria, South Africa.*

Ovine toxoplasmosis is a zoonotic disease with significant impact on the welfare of livestock and on the economy of the farming industry worldwide. This disease threatens public health due to the high risk of transmission of parasite from livestock to humans through in-

gestion of undercooked meat containing parasite tissue cysts. To alleviate the economic burden imposed by *Toxoplasma gondii* parasite in the farming industry and public health, the development of novel vaccines against *T. gondii* that are safer has become imperative and shows great promise in controlling toxoplasmosis. Treatments currently used to control this disease often present critical throwbacks such as partial protection and short shelf-life, contributing to the parasite's resistance and inefficient elimination of the parasite tissue cysts. This study focused on ovine rhoptry proteins to predict potential antigenic epitope candidates and computationally design multiepitope vaccine effective against *Toxoplasma gondii* through immunoinformatics approach. The *in-silico* technique implemented in this study successfully identified 20 T-cell and 2 (2) B-cell epitopes which were classified as conserved, antigenic, immunogenic, and non-allergen. These epitopes were joined to construct a vaccine adjuvanted with monophosphoryl lipid A and Cholera B subunit to enhance the vaccine's immunogenicity. The protein of the designed vaccine had molecular weight of 75.93 kDA, theoretical pI of 9.78 and was observed to be thermostable (instability index = 36.73) and hydrophilic (GRAVY = −0.235). it was observed as highly soluble with solubility of 0.946847. The structural validation of the refined vaccine revealed a Ramachandran plot with 96.1% residues in the most favored regions and a Z-score of −7.68. The properties observed from our proposed vaccine showed potential of eliciting robust cellular and humoral immunological response against *T. gondii*, crucial baseline information for future laboratory validation and designing a potential vaccine.

Key Words: infectious disease, immune system, vaccine, sheep, immunoinformatics

P427 Goat milk oligosaccharide composition determined by genes with a large effect. R. Gonzalez-Prendes*[1,2], H. Bovenhuis[2], L. Pellis[1], and R. P. M. A. Crooijmans[2], [1]*Ausnutria BV, Zwolle, the Netherlands,* [2]*Animal Breeding and Genomics, Wageningen University & Research, Animal Breeding and Genomics, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands.*

Milk oligosaccharides (MOS) are complex molecules with direct impact on newborns' health. They promote the growth of beneficial bacteria in the gut, inhibit the adhesion of pathogens, stimulate brain development, and modulate the immune system. Goat milk has higher concentrations of MOS in colostrum and mature milk as compared with cow or sheep milk. As such, goat milk has been suggested as a potential natural source of MOS for infant formula. However, the genetic factors underlying MOS composition remain poorly understood. This study aims to address this knowledge gap by investigating the genetic parameters of the goat milk oligosaccharides (*g*MOS) and conducting a genome-wide association analysis (GWAS) in a population of 996 Dutch dairy goats. Blood and milk samples were collected from goats of 18 farms in the Netherlands. In milk samples 10 *g*MOS were determined by Eurofins using the ultra-high pressure liquid chromatography. Additionally, DNA extracted from the blood was genotyped using the Neogen GGP Goat 70k array. Sialylated oligosaccharides were found to be more abundant than neutral ones, with large differences in *g*MOS composition observed between goats. The estimated heritability varied from 0.22 (LNT) to 0.84 (3-FL) and the values of the genetic correlation (r_g) between the *g*MOS ranged from −0.59 to 0.76. The GWAS identified significant associations (*q-value* < 0.05) at whole genome level between 7 *g*MOS and 176 SNPs, located in 16 genomic regions on 5 goat chromosomes. Most significant associations were detected on ARS15 (3-FL), ARS18 (2'-FL), and ARS23 (3′-SL). Interestingly, the most significant SNPs in 4 genomic regions were associated with more than one sialylated *g*MOS, suggesting that these MOS may be synthesized through a shared biosynthetic pathway. The analysis of positional candidate genes showed that genes related to the metabolism of MOS were significantly enriched, including genes involved in the transfer of L-fucose and sialic acid. Our findings suggest that genetic differences play a significant role in defining the composition of oligosaccharides in goat milk.

Key Words: goat milk oligosaccharides, genomic, infant formula

P428 Identification of genetic regions associated with resistance to gastrointestinal nematodes in Comisana sheep using a genome-wide association study based on EBV ranking. C. Persichilli[1], S. Biffani[2], G. Senczuk[1], M. Di Civita*[1], M. K. Bitew[1], A. Bosco[3], S. Grande[4], and F. Pilla[1], [1]*University of Molise, Department of Agricultural, Environmental and Food Science, Campobasso, CB, Italy,* [2]*National Council of Research, Institute for Agriculture Biology and Biotechnology, Milan, MI, Italy,* [3]*University of Naples Federico II, Department of Veterinary Medicine and Animal Production, CREMOPAR, Naples, NA, Italy,* [4]*National Sheep and Goat Breeders Association, Rome, RM, Italy.*

Gastrointestinal nematodes (GINs) have significant economic, environmental, and animal welfare implications in small ruminant. This study is aimed to identify genetic regions responsible for sheep resistance to GINs. Fecal samples were collected from 642 Comisana sheep over 3 years to assess Fecal Egg Counts (FEC) with the FLOTAC technique. Using pedigree data and logn(FEC+2) as phenotypes, Estimated Breeding Values (EBVs) for GIN resistance were estimated by a BLUP animal model. The EBVs in the 99.95th and the 0.05th percentile were used to identify the most and the least genetically resistant individuals to GINs, later genotyped with the Illumina OvineSNP50 beadchip. Using the software PLINK a case/control GWAS was performed. A threshold for the 0.005% most significant FDR corrected p-values was chosen. Using the R package GALLO, QTLs associated with the significant SNPs were annotated and enriched. With the ToppGenes utility, genes associated with the SNPs have been enriched for KEGG pathways, using a threshold of 0.05 FDR. As a result, 18 significant SNPs involving 13 genes were identified on 12 chromosomes. Among these, many are involved in the physiology or pathology of the gastrointestinal tract (the UGT1A* family, KIF6, LOXL2, CALN1 and TWISTNB). Others play a role in adaptive processes and production traits (LOXL2, GPC6, MYT1 and SS18L1). Among the found QTLs, 6 are in the Health class, 5 of which are associated with traits related to FEC. Enrichment analysis of the found genes highlighted 11 significant pathways classifiable as involved in the regulation of the immune response, involved in drug metabolism and detoxification, and involved in other metabolic processes. Previous research has linked some of the discovered pathways to GIN resistance, such as Ascorbate and aldarate metabolism, Glucuronate pathway (uronate pathway), and Metabolism of xenobiotics by cytochrome P450.

Key Words: sheep and related species, genome-wide association, disease resilience, single-nucleotide polymorphism (SNP), quantitative trait locus (QTL)

P429 ISAG Bursary Award: First look into the genetic architecture influencing liver copper concentration in Merinoland sheep. O. O. Adeniyi* and G. Lühken, *Institute of Animal Breeding and Genetics, Justus Liebig University, Giessen, Hessen, Germany.*

Economic losses due to copper (Cu) intoxication or deficiency is a problem encountered by sheep farmers. The aim of this study was to investigate the ovine genome for regions and candidate genes responsible for variability in liver Cu concentration. For this, a total of 134 liver samples were collected from slaughtered lambs of the Merinoland breed from 2 farms, and used for measurement of Cu concentration and genome-wide association study (GWAS). All samples were genotyped with the Illumina Ovine 50k SNP BeadChip, after which a total of 45,512 SNPs and 130 samples were left for analysis following quality control. For our analysis, single-locus and several multi-locus GWAS (SL-GWAS; ML-GWAS) methods were employed. Gene enrichment analysis was performed for identified candidate genes to detect gene

ontology (GO) terms significantly ($P \leq 0.05$) associated with hepatic Cu levels in Merinoland sheep. The SL-GWAS and a minimum of 2 ML-GWAS identified 2 and 13 significant SNPs, respectively. The identified regions harbor some promising functional candidate genes such as *DYNC1I2*, *VPS35*, *SLC38A9* and *CHMP1A* associated with endosomal cargo sorting and trafficking, as well as lysosomal transport. Additionally, genes such as *SPG7*, *ATP5MF* and *SLC25A12* were identified as functional genes involved in mitochondrial membrane permeability which has been associated with Cu toxicity. Likewise, the genes *SLC9B1* and *SLC9B2* which are involved in luminal and intraluminal pH and multivesicular body fusion to lysosome, were observed as potential candidate genes influencing hepatic Cu levels in Merinoland sheep. These genes need to be further investigated to ascertain their involvement in liver Cu variation in Merinoland sheep in particular, and other sheep breeds in general. In addition, this study provides evidence for a polygenic inheritance of this trait and delivers promising clues for further studies to identify potential causal variants that may be associated with variation in Cu tolerance in sheep and used for practical breeding.

Key Words: sheep and related species, genome-wide association, candidate gene

P430 The extreme genotypes of *CSN1S1* gene have a significant effect on milk composition and cheese yield in Carpathian goat. V. A. Balteanu*[1], R. K. Sigartau[2], D. Nadolu[3], and A. H. Anghel[4], [1]*University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Institute of Life Sciences, Cluj-Napoca, Cluj, Romania,* [2]*Babes-Bolyai University, Faculty of Mathematics and Computer Science, Cluj-Napoca, Cluj, Romania,* [3]*ICDCOC Palas, Constanta, Constanta, Romania,* [4]*Ovidius University, Constanta, Constanta, Romania.*

Goat milk represents in many European countries a valuable raw material for cheese industry due to an increased demand for cheeses, which are produced mainly from specialized breeds *i.e.* Alpine, Saanen or Murciana. Carpathian goat population from Romania is over 1.5 million heads, occupying the third place in Europe. They are raised on extremely diverse natural pastures from Transylvanian hilly areas to southern Danubian plains. This imprints to milk a specific flavor and a high nutritional value. However, a high variability in cheese yield was reported by breeders. In goat *CSN1S1* locus a wide variety of mutational events were characterized, accounting for 20 alleles. They are classified according to the expression levels (3.5 to 0 g/l) in strong (A, B), medium (E), week (F) or null (0) alleles. The defective F allele is characterized by a cytosine deletion at the 9th exon, which causes a drop in α_{S1}-casein synthesis from 3.5 to 0.45 g/l. Although *CSN1S1* gene polymorphism might be a major determinant of goat milk composition variability, this effect might vary depending on the breed. To determine nationwide the frequency of F allele we genotyped over 3000 Carpathian goats. Additionally, we tested in 150 goats, exhibiting extreme *CSN1S1* genotypes (ex. AA, AF and FF), the associations with milk composition (ex. whole protein, casein, fat, nonfat solids, lactose) and cheese yield. We found a high frequency of the defective F allele (0.27). The association tests highlighted significant differences between investigated genotypes, particularly for whole casein (AA: $3.00 \pm 0.10^{a+}$; AF: 2.64 ± 0.13^{b}; FF: $2.58 \pm 0.11^{b+}$) and whole protein (AA: $4.07 \pm 0.13^{a+}$; AF: 3.58 ± 0.17^{b}; FF: $3.50 \pm 0.14^{b+}$) contents and for liters of milk needed / kg of cheese (AA: 6.62 ± 0.47^{a} AF: 7.58 ± 0.47^{a}; FF: 8.11 ± 0.47^{b}). If we extrapolate nationwide these results, we can assume that more than 405000 individuals carry at least one copy of the defective F allele. This could be translated in important economic losses. We concluded that selection for strong alleles could represent a valuable tool to improve these traits in Carpathian goat and to fulfil European market demands for goat cheeses.

Key Words: goat, milk, *CSN1S1*, cheese

P431 Positional candidate genes involved in the response to heat stress in sheep. M. Ramon*[1], C. Diaz[2], M. Serrano[2], and M. J. Carabaño[2], [1]*CERSYRA-IRIAF, Valdepeñas, Ciudad Real, Spain,* [2]*INIA-CSIC, Madrid, Spain.*

Future climate scenarios derived from climate change (CC) point to the Mediterranean basin as one of the world areas that will be most affected by global warming. An important part of the world sheep and goat population is distributed in this region. A great effort is being put into the study of the consequences that CC will have on sheep and goat farming and into the development of mitigation and adaptation strategies. In line with this, this work aims to identify candidate genes involved in the response of sheep to heat stress. For this purpose, historical production data were used for the characterization of the individual response curve in milk, fat and protein yield along the temperature scale using random regression models and from them several thermotolerance indicators were developed. A total of 1,320 ewes with such thermotolerance indicators were genotyped with a 50K SNP chip and a GWAS analysis was carried out using the mixed-model approach in GCTA software and correcting for population structure. Genes were mapped using the ovine *Ovis aries* OAR v3.1 reference assembly within 500Mbp windows flanking the markers with significant association. Potential genes associated with thermotolerance in sheep were interleukins (IL13, IL2, IL21, IL12RB1) and several genes of the SLC39 family, with a significant role in promoting the immune system; genes of the family of chaperones (HSF1, DNAJB1) with specific function related to heat stress events; LEP and LEPR involved in the regulation of fat metabolism, as well as PPARG, involved in the fixation, absorption and storage of fatty acids, and associated with the insulin resistance path that has been described to be affected by heat stress (HS). Genes FGF2 and VEGFA are involved in the activation of angiogenesis; the latter also in the inhibition of apoptosis and the induction of permeabilization of blood vessels. These functions largely related to HS, are also very appealing to explain mechanisms used to protect the normal functioning of cells and mechanisms of heat dissipation in response to HS. Information from this study might be used to enhance selection of HS tolerant sheep.

Key Words: sheep and related species, genome-wide association, adaptation

P432 Selection of an ovine SNP parentage panel for consideration as the ISAG comparison test panel. R. Ferretti*[1], K. Schutt[2], M. Dowling[2], J. Qiu[1], and R. Tait Jr.[1], [1]*Neogen GeneSeek Operations, Lincoln, NE,* [2]*Neogen Australasia, Ipswitch, QLD 4304, Australia.*

Advances in medium- and high-throughput genotyping platforms have allowed for significant reduction in genotyping costs. A decade ago, this was a prohibiting factor for transitioning away from microsatellites over to single nucleotide polymorphism (SNP) technologies. At the 33rd International Society for Animal Genomics (ISAG) conference in 2012, this topic was raised and initial work was done by the International Sheep Genomics Consortium (ISGC) to adopt an official Ovine SNP comparison test (CT) panel using 88 autosomal SNPs and one male specific SNP. Here we propose an expanded panel of 201 SNP markers for consideration as the accepted ISAG Ovine Parentage CT panel. The aim for this parentage panel was to build off the original ISGC 89 SNP panel by incorporating SNP markers from newer iterations of academic and commercial parentage panels. Furthermore, to foster greater adoption we have considered attributes of a SNP panel including: 1) backward compatibility to multiple historic genotyping platforms; 2) global relevance across populations; and 3) the ability to be platform agnostic. To achieve this, we used a 3-step approach for SNP selection. First, the candidate SNPs should be available in the public domain. Second, SNPs should be represented on at minimum 2 genotyping platforms: Agena (Sequenom), KASP, Illumina, Affymetrix, GBS/NGS. Lastly, final SNP selection was made using SNPs displaying high Minor Allele Frequency (MAF) and highest average call rate across data sets and platforms. A total of > 200,000 animals consisting of more than 20 breeds and sample representation from 6 different geo-

graphic regions were evaluated. From this data a subset of 200 highly informative SNPs from a candidate pool of 857 SNPs were selected.

Key Words: sheep and related species, animal breeding, genotyping, parentage

P433 Modulation of innate immune memory and systemic effects of Gum Arabica in goats. Y. Ahmed and M. Worku*, *North Carolina A&T State University, Greensboro, NC.*

Acacia senegal is part of the goat's natural diet. Gum Arabica (GA) is a water-soluble complex polysaccharide antimicrobial, prebiotic derived from *Acacia Senegal*. Antimicrobial functions of GA may involve Toll-like receptors (TLRs). This study evaluated the effect of GA on TLR transcription. Clinically healthy Boer and Spanish goats (n = 20) were randomly assigned to 2 groups of 10. Goats in the treatment group received 10 mL of GA in water daily for 6 weeks, controls received sterile water. Total RNA was isolated from blood using Trizol (Sigma-Aldrich). The RNA concentration (ng/µL) and purity was assessed using a Nanodrop Spectrophotometer (Thermo Scientific Inc.). Whole transcriptome Illumina sequencing via rRNA depletion was conducted on pooled RNA (GENEWIZ, South Plainfield, NJ, USA). Bioinformatics analysis included mapping, differential gene expression, alternative splicing, and gene ontology analysis. Briefly, sequence reads were trimmed using Trimmomatic v.0.36 and mapped to the Capra_hircus reference genome available on ENSEMBL using the STAR aligner v.2.5.2b. Only unique reads that fell within exon regions were counted. Using DESeq2, a comparison of gene expression between wk 1 and wk 6 control and treated group samples was performed. The Wald test was used to generate p-values and log2 fold changes. Genes with a p-value <0.1 and absolute log2 fold change >1 were called as differentially expressed genes for each comparison. Time and GA treatment related differential gene expression was observed in goat blood. All goats expressed TLR 1–10. Only TLR10 was differentially expressed in blood from goats treated with GA (absolute log2 fold change >1). Toll-like receptor 10 (TLR10) is involved in trained memory, inhibits the induction of innate immune responses and inflammation. These studies have implications for application of GA in modulation of TLR 10 mediated innate immune memory. Further analyses are needed to validate individual and co-regulated genes, for applications in goat health

Key Words: goat, blood, prebiotic, RNASeq, TLR

P434 Gene expression profiling of the abomasum, duodenum, jejunum and ileum of resistant and susceptible Dohne Merino sheep naturally infected with *Haemonchus contortus*. T. M. Ramantswana*[1,2], D. P. Malatji[2], R. E. Pierneef[1], P. Soma[3], M. Van Der Nest[4], and F. C. Muchadeyi[1], [1]*Agricultural Research Council, Biotechnology Platform, Biotechnology Platform, Onderstepoort, Pretoria, South Africa,* [2]*University of South Africa, Florida, Gauteng, South Africa,* [3]*Agricultural Research Council, Animal Production Institute, Irene, Pretoria, South Africa,* [4]*University of Pretoria, Hatfield, Pretoria, South Africa.*

Gastrointestinal nematode (GIN) infections are a concern that is affecting sheep production and causes economic loss. The South African Dohne Merino sheep was bred to maximize wool and meat production and represent an important genetic resource that can be harnessed to breed for resistance to GIN as an alternative strategy to chemical control of nematodes. In this study, RNA-Seq and differential gene expression profiling was used to understand the underlying molecular mechanism associated with the infection of *H. contortus* in sheep. Total RNA was extracted from the abomasum, ileum, jejunum and duodenum tissue samples and used to generate an average of 6 691 243 paired-end reads with a length of 125bp. Six adult Dohne Merino sheep were categorized into resistant (n = 3) and susceptible (n = 3) to *Haemonchus contortus* based on Estimated Breeding Values derived from fecal egg count phenotypes. Differential gene expression analysis between resistant and susceptible animals resulted in 34 significantly expressed genes (DEGs) (False discover rate ≤ 0.05, log2fold change values > ± 1). Genes such as *APLF*, *ART1*, *YIPF7*, *SIVA1* and *CDKN1A* were reported. Functional annotation of the DEGs revealed association with immune response process, responses to stimuli and other cellular processes. KEGG pathway analysis identified the Rap1 signaling pathway and PI3K-Akt signaling pathway. The differential gene expression analysis of the specific segments of the gastrointestinal tract resulted in 146 significantly expressed DEGs for abomasum, 302 for ileum, 584 for jejunum and 332 for duodenum. Functional annotation and pathway analysis demonstrated tissue specific response mechanisms to *H. contortus* infection in sheep. Overall, the study provides information that forms the basis for understanding the genetics for resistance to GINs and has potential use in the selection of animals and breeding for resistance to infections by *H. contortus* nematodes.

Key Words: gastrointestinal nematodes, RNA sequencing, South African Dohne Merino sheep, *Haemonchus contortus*, gene expression

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C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

Key Word Index

Numbers following entries refer to abstract numbers. A number preceded by OP indicates an oral presentation, and a number preceded by P indicates a poster. Orals are listed first, followed by posters in session and number order.

D

E

F

G

N

O

P

Q

R

S

T

U

V

W

X

Y

Z