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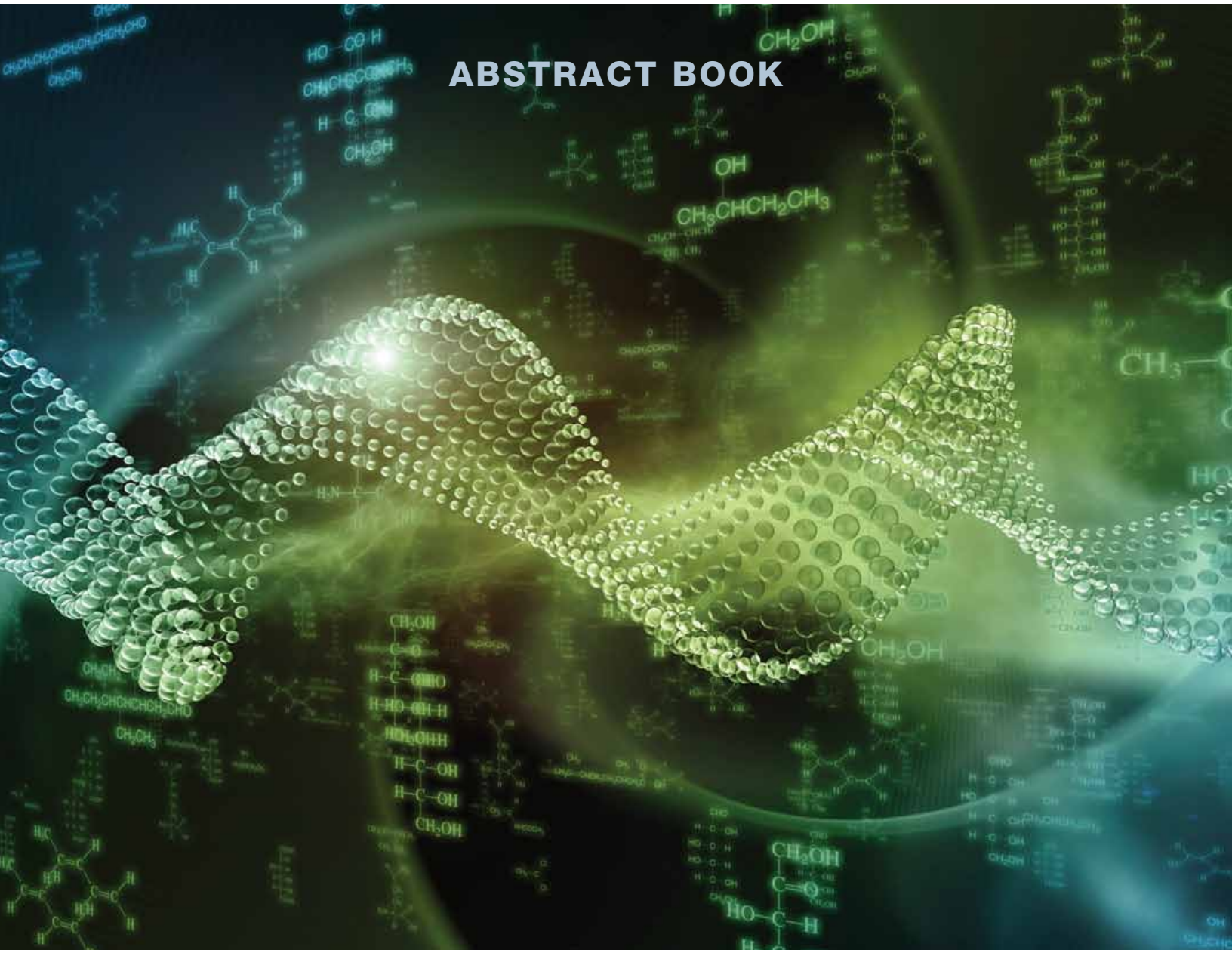

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INVITED SPEAKERS
S0100 – S0124

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INVITED SPEAKERS: FUNCTIONAL ANNOTATION OF ANIMAL GENOMES (FAANG) ASAS-ISAG JOINT SYMPOSIUM

S0100 Important lessons from complex genomes. T. R. Gingeras* (Cold Spring Harbor Laboratory, Functional Genomics, Cold Spring Harbor, NY)

The ~3 billion base pairs of the human DNA represent a storage devise encoding information for hundreds of thousands of processes that can go on within and outside a human cell. This information is revealed in the RNAs that are composed of 12 billion nucleotides, considering the strandedness and allelic content of each of the diploid copies of the genome. Results stemming from the efforts to catalog and analyze the RNA products made by cells in the human (ENCODE), fly-worm (modENCODE), and mouse ENCODE projects have shed light on both the functional content and how this information is organized by various genomes. In human cells, a total of ~161,000 transcripts present within ~50,000 genic regions represent our previously best manually curated annotation (based on v7 Gencode) of the transcriptome. The results from the ENCODE project point to considerable supplementation of these data. Analyses of these transcriptome data sets have resulted in important and underappreciated lessons, such as pervasive, genome-wide transcription prompts a need to redefine the definition of a gene; expression ranges follow transcript types and subcellular localization; expression of isoforms of a gene by a cell do not follow a minimalistic strategy; and genomic characteristics of potential trans-acting enhancer regions are distinguishable from other types of cis-acting regulatory regions. These and other lessons drawn from the landscape of both coding and non-coding RNAs present in eukaryotic cells have been used to assist in understanding and organizing what is often seen as dauntingly complex genomes.

Key Words: annotation, ENCODE, transcriptome

S0101 Causal inference of molecular networks integrating multi-omics data. F. Peñagaricano* (University of Florida, Gainesville, FL)

Recent developments of massively parallel technologies allow assaying different biological molecules at very high throughput rates, including sequencing and genotyping of DNA, quantifying whole-genome gene expression, including measuring mRNA and microRNA abundance, identifying genome-wide epigenetic modifications, such as DNA methylation, and measuring different proteins and cellular metabolites. These advancements provide unprecedented opportunities to uncover the genetic architecture underlying phenotypic variation. In this context, the main challenge is to decipher the flow of biological information that lies between the genotypes and phenotypes under study. In other words, the new challenge is to integrate multiple sources of molecular information (i.e., multiple layers of omics data to reveal the causal biological networks that underlie complex traits). It is important to note that knowledge regarding causal relationships among genes and phenotypes can be used to predict the behavior of complex systems, as well as optimize management practices and selection strategies. Here, we describe a multi-step procedure for inferring causal gene-phenotype networks underlying complex phenotypes integrating multi-omics data. We initially assess marginal associations among genotypes and either intermediate phenotypes (such as gene expression) and endpoint phenotypes (such as carcass fat deposition and muscularity), and then, in those genomic regions where multiple, significant hits co-localize, we attempt to reconstruct molecular networks using causal structural learning algorithms. These algorithms attempt to infer networks, assuming that the pattern of conditional independencies observed in the joint probability distribution of these sets of correlated variables are compatible with the unknown causal model. As a proof of principle of the significance of this integrative approach, we show the construction of causal molecular networks underlying economically relevant meat quality traits in pigs, using multi-omics data obtained from an F2 Duroc × Pietrain resource population. Interestingly, our findings shed light on the mechanisms underlying some known antagonist relationships between important phenotypes, for instance, carcass fat deposition and meat lean content. Generally, the proposed methodology allows further learning regarding phenotypic and molecular causal structures underlying complex traits in farm species.

Key Words: causal inference, graphical models, systems biology

S0102 Genotypes to phenotypes: Lessons from functional variation in the human genome and transcriptome. B. E. Stranger* (Section of Genetic Medicine, Department of Medicine, Institute of Genomics and Systems Biology, Center for Data Intensive Sciences, University of Chicago, Chicago, IL)

Complex trait association mapping in humans has successfully identified genetic loci influencing trait

variation for hundreds of different phenotypes, including disease. The vast majority of associated loci localize to non-coding regions of the genome, suggesting possible effects on gene regulatory mechanisms. Without a clear understanding of the regulatory code of the human genome, deep characterization of the molecular function(s) of genetic variants in the human genome has become increasingly important for defining that code and understanding genetic associations to complex traits. Studies of the human transcriptome, its complexity, and its relation to genetic variation in a variety of contexts have proven highly informative for understanding genome function and for suggesting testable hypotheses involving candidate genes for complex traits and the functional mechanisms though which they may act. These approaches are increasingly leading to successful functional characterization of trait-associated variants, in some cases, suggesting possible targets for trait manipulation. Finally, these characterizations are being used to build models predicting variant function, further extending possible applications.

Key Words: genome function, non-coding variants, regulatory mechanisms

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S0103 Recurrent chimeric transcripts in human and mouse. S. Djebali[*1,2,3], B. Rodríguez Martín[2,3], E. Palumbo[2,3], D. D. Pervouchine[2,3], A. Breschi[2,3], C. Davis[4], A. Dobin[4], G. Alonso[5], A. Rastrojo[5], B. Aguado[5], T. R. Gingeras[4], R. Guigó[2,3] ([1]GenPhySE, INRA, Castanet-Tolosan, France, [2]Universitat Pompeu Fabra (UPF), Barcelona, Spain, [3]Bioinformatics and Genomics Program, Centre for Genomic Regulation (CRG), Barcelona, Spain, [4]Cold Spring Harbor Laboratory, Functional Genomics, Cold Spring Harbor, NY, [5]Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain)

The formation of chimeric transcripts (chimeras) has been widely reported [1,2,3]. Some of them reflect underlying chromosomal rearrangements [4] or are the results of the propensity of reverse transcriptase to engage in template switching [5]. However, a proportion of cases genuinely appear to correspond to trans-splicing of RNAs, as has previously been described [6,7]. Here, we use ENCODE and mouse ENCODE deeply sequenced and bio-replicated RNaseq data from 18 human and 30 mouse samples, and the ChimPipe program to identify chimeras occurring in multiple biological samples (recurrent) and between the same pairs of genes in human and mouse, since they are more likely to be transcriptionally induced and functional. Recurrent common chimeras tend to connect gene pairs located on the same chromosome and relatively near each other (<100 kb), therefore pointing to polymerase read-through. However, interchromosomal chimeras are also observed, pointing to trans-splicing. Importantly, these recurrent chimeras tend to maintain an open reading frame and could therefore generate chimeric proteins. We also observed that not only the gene-to-gene connection is conserved, but strikingly so are specific junction sites. The genes connected in common chimeras tend to be involved in morphogenesis and body plan formation, and consistently tend to be detected in cell lines of embryonic origin. Validation of human chimeras by RT-PCR yielded a success rate of 50% and subsequent cloning and sequencing revealed novel transcript structures, of which some preserve the domains from the 2 parents' genes. Applying this method to multiple animal species and breeds will help us understand chimera evolution, as well as reveal some links between genotype and phenotype.

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Key Words: chimeras, transcripts, trans-splicing

S0104 Improving genomic selection across breeds and across generations with functional annotation. B. Hayes[*1], A. J. Chamberlain[2], H. Daetwyler[3], C. J. Vander Jagt[2], M. E. Goddard[4] ([1]Department of Economic Development, Melbourne, Australia, [2]Dairy Futures Cooperative Research Centre, Bundoora, Australia, [3]Department of Economic Development, Jobs, Transport, and Resources, Bundoora, Australia, [4]Department of Primary Industries, Melbourne, Australia)

Identification of causal mutations that affect complex traits in livestock (including production, health, and fertility) could accelerate genetic gains for these traits by improving the accuracy of genomic estimated breeding values, particularly across breeds and with greater persistency of accuracy across time. Identification of these causal mutations could also reveal facets of the biology underlying such traits. A significant proportion of genomic variation in cattle, for *Bos taurus* breeds at

least, has been identified. The 1,000 bull genomes project now includes whole genome sequences from 1,682 cattle of 55 breeds, from which 67.3 million variants (64.8 million SNP, 2.5 million indel) have been identified. The challenge now is to determine which subset of these variants affects complex traits. This challenge is magnified by the fact that the size of effects of the causal mutations are likely to be small, given the large number of mutations typically affecting complex traits. We propose that an approach that includes a multi -breed reference population (necessary to break down the extensive linkage disequilibrium that exists within many livestock breeds); intermediate phenotypes, such as gene expression and protein abundance, where mutation effect is much larger than on the complex trait phenotype; genome annotation information to identify which classes of variants are more likely to affect complex traits; and a genomic prediction algorithm that uses all this information simultaneously will lead to identification of causal mutations on a genome-wide scale. Several examples identifying potential causal mutations affecting milk composition from dairy cattle are given. The results highlight the need for better annotation of the bovine genome. Many of the most significant mutations are in poorly annotated genomic regions, likely regions regulating gene expression. The functional annotation of animal genomes (FAANG) consortium will greatly improve this situation.

Key Words: genomic selection, functional annotation

S0105 Integrating dynamic omics responses for universal personalized medicine. G. I. Mias* (Michigan State University, East Lansing, MI)

The advent of readily available omics technologies and the recent Precision Medicine Initiative announced by the White House and National Institutes of Health are guiding our efforts to make advances in the implementation of personalized medicine. High quality genomes are now complemented with other dynamic omics data (e.g., transcriptomes, proteomes, metabolomes) that may be used to profile temporal patterns of thousands of molecular components in individuals. We are pursuing the profiling of multiple such omics in parallel $n = 1$ studies that extend the pilot integrative Personal Omics Profiling (iPOP) approach to diseases affecting the immune system. In particular, we will describe our investigations that follow longitudinally healthy and asthmatic individuals, and the integration of multiple omics obtained from peripheral blood cells that we believe may provide novel medical insights. Concurrently, we are developing the necessary statistical and computational methodology for integrating the different omics platforms toward a medical interpretation, including our MathIOmica framework. Our approach enables us to query RNA sequencing, mass spectrometry (proteomics/metabolomics), and any longitudinal omics data, starting from lab samples to raw data, and including downstream quantitation methods for each analysis. We will present a clinically relevant classification scheme of longitudinal patterns, integration that accounts for missing data and uneven time sampling, and ultimately a biological interpretation and dynamic visualization of an integrated profile. Additionally, we are developing the necessary experiments and data sets for future iPOP investigations, with dense profiling of cell-drug treatment responses using Rituximab and other interventions. Our combined transcriptome-proteome profiles enable us to reconstruct dynamic pathways of Rituximab's action on B-cells on a global scale. In summary, our clinical, laboratory, and computational investigations are providing the next steps in the development of omics data generation and integration, toward a universal personalized medicine implementation. G.I.M. and research reported in this presentation are supported by grants from MSU and the National Human Genome Research Institute of the National Institutes of Health under Award Number 4R00HG007065. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Key Words: disease, personal omics profiling, transcriptome-proteome profiles

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S0106 A review of sequencing and assembly methods that enhance computational use. W. C. Warren* (McDonnell Genome Institute, Washington University School of Medicine, St Louis, MO)

In essence, high quality genome references are proven to be a necessity to enable research on so many levels of biological investigation, including disease etiology, small molecule drug screening and interactions, and canonical disease pathway manifestation. To date, very few genomes can be classified as near finished, defined as only missing small regions that are recalcitrant to known molecular biology methods. Ultimately, our goal is to produce contiguous chromosomes for genomes de novo at the lowest cost. So far, most published de novo genome assemblies are derived from deep coverage, Illumina-only sequencing, most often using 2 popular but independent assembly algorithms, yet all are documented to be inadequate for numerous types of genetic investigation. During this surge of short reads genome assembly, new long read sequencing technology arrived, albeit at considerable

cost, about six fold higher than pure Illumina de novo assembly approaches. However, long reads, now averaging ~14 kb in length, have transformed our ability to capture most chromosomes that compel us to fund these approaches to obtain higher quality. Our lab and others now routinely assemble human genomes with N50 contig lengths of 10 Mb and up to 53 Mb size contigs, with contigs defined as uninterrupted consensus sequence. In our studies, we saw how an incomplete genome sequence hindered studies designed to detect signatures of selection in the poultry industry, such as missing microchromosome sequence assignments and partial or completely missing gene models in the chicken. In the chicken, despite the use of older, long read sequencing technology (average read length of 8 kb), we observed an increase of ~180 Mb in assembled size, added 1,920 new gene models, and reduced gaps by sevenfold among ordered chromosomes. Given the intense interest in better genome reference models, I will review the generally compartmentalized phases for producing high quality genome references and provide examples of analysis outcome.

Key Words: assembly, genome reference, long reads

INVITED SPEAKERS: PLENARY SESSION: EPI/GENOTYPE TO PHENOTYPE

S0107 Environmentally induced epigenetic transgenerational inheritance of disease: Ancestral ghosts in your genome. M. K. Skinner* (Washington State University, Pullman, WA)

Transgenerational effects of environmental toxicants significantly amplify the impact and health hazards of these compounds. One of the most sensitive periods to exposure is during embryonic gonadal sex determination when the germ line is undergoing epigenetic programming and DNA re-methylation. Previous studies have shown that endocrine disruptors can cause an increase in adult onset disease, such as infertility, prostate, ovary and kidney disease, cancers, and obesity. Interestingly, this effect is transgenerational (F1, F2, F3, and F4 generations) and hypothesized to be due to a permanent (imprinted) altered DNA methylation of the germ line. The transgenerational epigenetic mechanism appears to involve the actions of an environmental compound at the time of sex determination to permanently alter the epigenetic (i.e., DNA methylation) programming of the germ line that then alters the transcriptomes of developing organs to induce disease susceptibility and development transgenerationally. A variety of environmental compounds have been shown to induce this epigenetic transgenerational inheritance of disease, including fungicide vinclozolin, plastics BPA and phthalates, pesticides, DDT, dioxin, and hydrocarbons. The suggestion that environmental factors can reprogram the germ line to induce epigenetic transgenerational inheritance of disease and phenotypic variation is a new paradigm in disease etiology that is also relevant to other areas of biology, such as evolution.

Key Words: environmental toxicants, epigenetics, transgenerational inheritance

S0108 Genetic background-dependent effects of diet on health and production traits. D. Threadgill* (Texas A&M University, College Station, TX)

Studies looking to optimize diet for health (humans and companion animals) or feed efficiency (production animals) have historically relied on population averages and have not taken into account individual genetic variability controlling dietary responses. In humans and animals, dietary patterns have repeatedly been shown to have profound impacts when studied at the population level. In humans, for example, Japanese and Mediterranean diets are associated with longevity and low rates of chronic disease. Western diets are associated with increased risk of heart disease and specific types of cancer. However, studies evaluating dietary interventions in individuals find large interpersonal variations in diet response. Much of this variation is likely due to underlying genetic differences among individuals. To determine how the genetic background of an individual impacts their response to diet, we exploited inbred strains of mice to examine health- and production-related effects of 4 diets commonly studied in human populations (Western diet, Mediterranean diet, Japanese diet, ketogenic diet, and standard mouse chow as control) in each gender. Mice were fed diets for 6 mo while undergoing a variety of clinical and feed efficiency analyses. We found that most dietary responses showed a strong genetic interaction, which included adiposity, glucose tolerance, blood chemistry profiles, liver triglyceride storage, liver mitochondrial function, metabolic rate, and feed efficiency. The severity and directionality of many of these effects varied, depending on the genetic background, and in some cases, the individual's gender. Mouse strains differed in which diets were optimal or suboptimal, indicating that individuals with unique genetic compositions likely have a specific diet for optimal health or growth, based on specific genetic factors. The results call into question the categorization of diets as "good" or "bad,"

and emphasize the need to evaluate dietary efficacy at a genetic level. Current studies are exploiting an innovative "population-level" model to investigate how individuals can be optimized to specific diets.

Key Words: diet, genetics, production, health

INVITED SPEAKERS: PLENARY SESSION: GENOME EDITING

S0109 Engineering the genome to investigate disease mechanisms. R. J. Platt* (Broad Institute, Cambridge, MA)

The ability to sequence and edit DNA is fundamental to understanding the role of genetic elements in biological and disease processes. The RNA-guided endonuclease, CRISPR-Cas9, is widely being developed to not only simplify and expand genome editing applications but also reduce the cost and speed at which model organisms can be generated. This opens up exciting new avenues of research for a broad range of disease modeling applications.

Key Words: CRISPR-Cas9, disease, genome editing

S0110 Gene editing in livestock. S. C. Fahrenkrug* (Recombinetics, Inc, St Paul, MN)

Natural and artificial selection have resulted in a tremendous variety of adaptive and purpose-bred traits, which have fed humanity since before the Neolithic Revolution. Underpinning this phenotypic diversity lies a genetic inheritance of tremendous value for meeting contemporary societal needs in food and fiber production. Although rich genetic value is segregating in contemporary livestock herds, bottlenecks imposed by geography, climate, and culture have resulted in the loss of valuable genetics, as well as the phenotypic and genotypic stratification of breeds. Increasing the frequency of preferred alleles within a breed by selective inbreeding can also inadvertently increase the frequency of deleterious alleles by so-called "hitchhiking." Crossbreeding can be used to decrease the impact of deleterious alleles via heterosis but also represents a way to introgress desirable genetic variants across breeds and populations. Despite the value that heterosis and introgression provide, crossbreeding dilutes purpose-bred genetics that have taken centuries to assemble. We are using gene editing technologies to eliminate deleterious alleles accumulated via inbreeding and introgress beneficial alleles into naive breeds, bypassing expensive, multigenerational breeding programs. Precision crossbreeding by non-meiotic allele introgression will accelerate efforts to enhance genetic merit and rapidly incorporate traits focused on animal well-being and agricultural sustainability in the face of global climate change and a rapidly increasing world population.

Key Words: gene editing, introgression, precision crossbreeding

INVITED SPEAKERS: PLENARY SESSION: GENETIC DIVERSITY AND ADAPTATION

S0111 Genomics of South American wild cats: Insights into evolutionary history and adaptation. E. Eizirik* (Laboratory of Genomics and Molecular Biology, Faculdade de Biociências, PUCRS, Porto Alegre, Brazil)

In spite of considerable progress over the last century, our understanding of the evolutionary history and intricate ecological interactions that characterize life on Earth is still largely in its infancy. Advancing such understanding at the fastest possible pace is not only a worthwhile academic endeavor but also a critically relevant component of the global effort to halt the ongoing loss of biodiversity driven by human activities. In this context, genomic approaches have revolutionized biodiversity sciences, opening up new research avenues that range from large-scale DNA sequencing of microbial communities to the in-depth characterization of complete eukaryotic genomes. When applied to threatened organisms, such as wild cats, genomic approaches hold great potential to illuminate aspects, such as phylogenetic relationships among species, demographic history of particular populations, patterns of phylogeographic differentiation, and gene flow at multiple spatial scales, as well as signatures of adaptation to distinct environments and the molecular basis of naturally occurring phenotypic variation. In this talk, I will describe and discuss ongoing efforts to apply genomic approaches to address these questions in wild cats, mostly focusing on our current studies targeting South American species. This region of the world is particularly interesting with respect to the evolution of wild cats, as it harbors at least 11 felid species that exhibit distinct and complex histories. I will mostly focus on the Jaguar Genome Project, including an overview of the genomic resources already generated for this species, and analyses targeting its evolutionary history and patterns of local adaptation. In addition, I will describe ongoing efforts to apply genome-wide approaches to investigate other South American cats, prospects for future advances in

this field, and implications of emerging results for the conservation of threatened wild felid populations.

Key Words: adaptation, evolution, wild cats

S0112 Applications of genomics to address adaptation of livestock to stressful environments to prevent food insecurity in the developing world. M. F. Rothschild* (Department of Animal Science, Iowa State University, Ames, IA)

Expectations are that the human population will increase rapidly from 7.3 billion to 9.6 billion by 2050 and that food production must double, despite limitations due to climate change and limited land and water resources. Increased food insecurity and the worldwide food production crises loom in the future as the most significant scientific challenge facing us in the next 30 yr. Improving local production everywhere in the world will be a priority for achieving "zero hunger." A better understanding of the underlying genomic control of adaptation in native and exotic livestock will be required to meet these needs, especially in the developing world. The advent of cheaper, faster sequencing technologies and the realization of good draft sequences and development of SNP chip technologies for livestock have benefitted livestock producers and consumers in the developed world. However, these tools now need to deliver new opportunities for the developing world. Identifying signatures of selection associated with adaptation to heat stress, drought resistance, and susceptibility to major disease agents are first steps to reducing yield gaps. Direction and focus of research, funding issues, and human capacity training will also be required for success. Genomic discoveries will need to be embedded within sustainable programs that address implementation from the outset and benefits to smallholder production will be crucial to meeting this challenge.

Key Words: adaptation, genomics, livestock

INVITED SPEAKERS: PLENARY SESSION: STEVE BISHOP MEMORIAL SESSION ON ANIMAL DISEASE GENETICS

S0113 Unraveling the contribution of host genetics to infectious disease. A. B. Doeschl-Wilson* (The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom)

Host genetic diversity can hugely affect the spread of infectious disease and its impact on fitness and performance. Genetic analyses of infectious disease data usually focus on disease resistance. However, increasing evidence shows that the risk and severity of disease outbreaks also depend on host infectivity (ability to transmit infections) and tolerance to infection (ability to maintain high fitness despite infection). Estimating genetic parameters for these traits has proven difficult, because current quantitative genetics methods fail to account for infection dynamics and dependence among traits. Our studies aim to develop novel statistical methods to estimate additive genetic risks and SNP effects associated with resistance, tolerance, and infectivity. When applied to simulated and real data, these tools provide new insights into the host genetic contribution to infectious disease spread and impact on livestock and human populations. Our dynamic social effects models, when applied to simulated epidemiological data, produce reliable predictions for additive genetic risks and SNP effects for both host susceptibility and infectivity, even when information on time-to-infection is imprecise. Informed by these simulations, large-scale transmission experiments in turbot and chicken were designed, which indicate that all 3 host traits are heritable and likely genetically correlated. Furthermore, recent analyses of infection paths in model and outbred animal populations reveal that contrary to common understanding, resistance and tolerance are not alternative host response strategies. Instead, infection outcome (e.g., death or survival) is determined by carefully timed interactions among genetically controlled resistance and tolerance mechanisms. Our results imply that future genetic studies of infectious diseases should consider the combined contributions of host resistance, tolerance, and infectivity. Opportunities and challenges for implementing these novel disease traits into genetic analyses of infectious disease data and for genetic disease control strategies will be discussed.

Key Words: infectious disease, host genetics, resistance, infectivity, tolerance, mathematical model

S0114 Genetic basis of resistance to infectious disease in aquaculture species. R. D. Houston* (The Roslin Institute and R[D]SVS, University of Edinburgh, Midlothian, United Kingdom)

Study of the genetic basis of host resistance to infectious disease in aquaculture species has been transformed by genomics tools. Genome-wide marker data can now readily be generated in finfish and shellfish populations, using genotyping by sequencing or SNP arrays. Combined with the typically high fecundity of these species and the feasibility of large scale disease

challenge studies, these genomic tools enable high resolution mapping of underlying genes. Such studies can also form the basis of selective breeding for improved resistance using marker-assisted or genomic selection. We have developed and applied genomic tools for several aquaculture species, and found that while disease resistance is consistently heritable, its underlying genetic architecture can vary dramatically. For example, resistance to the infectious pancreatic necrosis virus in Atlantic salmon is largely explained by a single major QTL and this has been widely applied via marker-assisted selection in breeding programs. Using whole genome resequencing and gene expression profiling of resistant and susceptible fish, we gained insight into the causative mechanisms underpinning this QTL. In contrast, resistance to the ectoparasitic salmon louse has a polygenic basis and we investigated the utility of genomic prediction of breeding values for this trait. While salmonid selective breeding schemes are currently the most advanced, breeding for disease resistance is increasingly important in a wide range of aquaculture species. We recently used a large-scale application of RAD genotyping by sequencing to study genetic resistance to a viral disease in European sea bass and developed a high density SNP array to study resistance to herpes virus in farmed Pacific oysters. Research into the genetic basis of resistance to infectious disease in aquaculture provides opportunities to learn about the biology underpinning variation in host response and enables production of more resistant stocks via selective breeding.

Key Words: aquaculture, disease resistance, genomic selection, genotyping by sequencing, SNP array

INVITED SPEAKERS: APPLIED SHEEP AND GOAT GENETICS

S0115 SNP parentage testing in sheep—a comparison of technologies. S. Clarke*, K. Dodds, R. Brauning, T. van Stijn, R. Anderson, J. McEwan (AgResearch, Mosgiel, New Zealand)

Over the past decade, advances in DNA sequencing technology have led to SNP discovery and tools to implement SNP based methods for parentage assignment, a critical component of animal production systems to construct accurate pedigrees. Assigning paternity is particularly important in a multi-sire mating system. There have been several SNP parentage panels developed by a number of countries, ranging from ~80 to ~300 SNPs tailored to their breed(s) of economic importance, however, the International Sheep Genomic Consortium (ISGC) have also established a core panel that is of broad utility. To date these SNP parentage panels are at the low-plex level to provide a low cost genotyping assay for the industry. Recently, the costs of not only DNA sequencing, but also of array based technology have dropped dramatically, allowing parentage panels in the 1000's of markers. Furthermore, developments to genotyping by sequencing (GBS) analysis methods now allow the possibility of delivering genomic selection at a similar cost to that of current parentage tests. A recently developed generic algorithm now allows estimation of genomic relationship matrices based on allele read depths that can be interrogated for: breed composition, pedigree, traceability, inbreeding and co-ancestry as well as be included directly in existing mixed models to estimate breeding values via GBLUP. A comparison of array and GBS technologies will be presented comparing cost, utility and benefits for both parentage and genomic selection.

Key Words: parentage, genotyping by sequencing, Illumina SNP array

INVITED SPEAKERS: CATTLE MOLECULAR MARKERS AND PARENTAGE TESTING

S0116 Genomic evaluations in dairy cattle, beef cattle, and sheep in Ireland. D. P. Berry*[1], F. Kearney[2], R. Evans[2], E. Wall[3], A. Cromie[4] ([1]Teagasc, Moorepark, Fermoy, Co. Cork, Ireland, [2]Irish Cattle Breeding Federation, Bandon, Co. Cork, Ireland, [3]Sheep Ireland, Bandon, Ireland, [4]Irish Cattle Breeding Federation, Bandon, Ireland)

Ireland was the second country to officially implement genomic evaluations in Holstein-Friesian dairy cattle nationally. The vast majority of genotypes in the reference population were obtained from bilateral sharing. Retrospective analysis of the 2-step genomic predictions reveals an average 23% improvement in the accuracy of evaluations across traits. Ireland has now embarked on a national initiative to genotype a large proportion of the national beef herd. Genomic evaluations were undertaken using a 1-step, multi-breed genomic evaluation with the Irish custom genotype panel with >50,000 segregating SNPs on more than 150,000 animals. The cost per genotype, including DNA sampling, postage, DNA extraction, genotyping, genomic evaluations, and tax, is €22. Parentage errors are 17%. The H-inverse matrix was generated with 20% emphasis on the A-matrix; the

G-matrix was constructed using the Euclidian distance among animals. A correlation of 0.813 existed between the off-diagonals of the genomic relationship matrix and the respective off-diagonal of the numerator relationship matrix. The validation data set was entire contemporary groups of recently born animals with phenotypes and contained up to 20,000 animals from multiple breeds and crossbreds. The accuracy of genomic predictions of cow-related traits across breeds was 16% greater than predictions based on parental average. Genotypes on almost 10,000 purebred sheep from 5 major breeds are being used to develop genomic predictions. Genotypes available originate from a combination of medium density (c.a. 50,000 SNPs) panels and a custom low density (c.a. 16,000 SNPs) panel. Mean genotype concordance rate per breed imputing from the low to medium density genotypes was 0.984, 0.972, 0.982, 0.969, and 0.989 for Belclare, Charollais, Suffolk, Texel, and Vendeen, respectively. Genotypes from 89 animals were generated using both an Illumina and Affymetrix platform. The mean genotype (allele) concordance rate per SNP for the 48,326 SNPs on both panels was 0.982 (0.991).

Key Words: genomic prediction, sheep, cattle

INVITED SPEAKERS: COMPARATIVE MHC

S0117 Development of the IPD-MHC Database.

J. Robinson[1], G. Maccari[2], R. E. Bontrop[3], S. Ho[4], U. Grimholt[5], J. Kaufman[6], L. Guethlein[7], K. Ballingall[8], S. G. Marsh[*1], J. A. Hammond[2] ([1]Anthony Nolan Research Institute and UCL Cancer Institute, London, United Kingdom, [2]The Pirbright Institute, Guildford, United Kingdom, [3]Biomedical Primate Research Centre, Rijswijk, Netherlands, [4]Gift of Life Michigan, Ann Arbor, MI, [5]Norwegian Veterinary Institute, Oslo, Norway, [6]University of Cambridge, Cambridge, United Kingdom, [7]Stanford University, Stanford, CA, [8]Moredun Research Institute, Edinburgh, United Kingdom)

The IPD-MHC Database was released in 2003 to provide a curated database of MHC sequences from several non-human species. The system was modeled on the IPD-IMGT/HLA database but expanded to cover multiple species and nomenclature systems. The initial release contained data on non-human primates, felines, and canines. Since this time, further data on horse, sheep, swine, cattle, fish, and rats have been added. Since its release, the database has grown rapidly and now contains nearly 7.000 alleles covering more than 70 non-human species. The site averages ~1,500 unique visitors a month, viewing ~5,000 pages. This growth has led to several challenges in maintaining and developing the site. The bioinformatics challenges of running a locus-specific database across multiple nomenclature systems and locations have impacted the performance and sustainability of the current IPD-MHC model. In 2015, a BBSRC Bioinformatics and Biological Resources (BBR) grant (UK) was awarded to fund both essential upgrades and future expansion of IPD-MHC. Work is currently focused on the underlying database, public website, and submission procedures. The key aims of this first phase are development of a universal cross-species data submission and display tool, and streamlining and standardizing work of the nomenclature committees in curating the data. This will allow for simpler and more frequent species updates. Future developments aim to incorporate cross-species alignment, primer design tools, and incorporation of NGS data. Ultimately, this will create an improved system capable of coping with large numbers of sequence variants, genes, and species within the data set. The project is overseen by a steering committee representing key stakeholders and non-human MHC nomenclature committees. This group is also helping to develop new MHC nomenclature standards and guidelines.

Key Words: database, MHC alleles, nomenclature

INVITED SPEAKERS: DOMESTIC ANIMAL EPIGENETICS

S0118 A hierarchy of epigenetic changes in the developmental transition from brown to white perirenal adipose tissue.

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The developmental transition of the ovine perirenal adipose tissue depot from late gestation to a few weeks after birth shows a rapid change from brown to white adipose tissue. These tissues are functionally and morphologically distinct. The former protects the newborn from hypothermia, whereas the latter is involved in energy homeostasis during a period of rapid growth and

nutritional change. The epigenetic landscape underpinning this transition was investigated using genome-wide analyses of chromatin modifications (ChIP-Seq), DNA methylation (WGBS), and gene transcription (RNA-Seq). Within each depot, there were strong positive and negative relationships between specific chromatin marks and gene expression, although greater complexity and functional stratification arises from chromatin mark subtypes and their combinations. DNA hypomethylation features within each tissue strikingly interfaced with specific chromatin marks and gene features (e.g., promoters, enhancers, and large repressed domains). The developmental transition was associated with large transcriptional and chromatin mark changes, but surprisingly few differentially methylated regions (DMR), despite the intimate relationship between chromatin modifications and DNA hypomethylation features. Few DMRs flanked the edges of DNA hypomethylation features and were enriched near transcription factors, particularly nuclear receptors, several of which showed marked changes in gene expression. It is concluded that the strong interface between DNA hypomethylation features and chromatin modifications, and the dominance of chromatin modification changes acting on a permissive hypomethylation background orchestrate gene expression reprograming and are general characteristics of mammalian developmental programs. The analyses provide rich insight into the hierarchy of epigenetic regulation of mammalian gene expression.

Key Words: epigenetics, development, adipose

INVITED SPEAKERS: GENETICALLY ENGINEERED LIVESTOCK

S0119 CD163 A gatekeeper for susceptibility to porcine reproductive and respiratory syndrome virus. R. S. Prather* (University of Missouri, Columbia, MO)

It is estimated that in North America and Europe, porcine reproductive and respiratory syndrome virus (PRRSV) results in combined losses of $6 million per day. The virus replicates in macrophages, induces prolonged viremia, and animals become persistently infected. Infection by PRRSV predisposes animals to other bacterial and viral pathogens. Young and growing pigs exhibit pneumonia, diarrhea, and mortality of 12 to 15%. Sows and gilts have reproductive failure, late abortion, early farrowing, increased number of mummies, and decreased litter size. Boars have low libido, fever, and low sperm count. Vaccines have not effectively controlled the virus. As with many pathogens, numerous candidates have been proposed to be the receptor for viral entry into the host cell. The most popular receptor candidates for PRRSV have included heparin sulfate, SIGLEC1, VIM, CD151, CD209, and CD163. To determine if these candidate receptors were indeed involved, we first created SIGLEC1−/− animals by homologous recombination and somatic cell nuclear transfer, and challenged them with a Type II (North American) PRRSV. The SIGLEC1−/− animals were not resistant to infection. Next, we created CD163−/− pigs by CRISPR/Cas9 gene editing and challenged these pigs with a different Type II PRRSV strain. In contrast to SIGLEC1−/− animals, the CD163−/− pigs were resistant to the challenge. When the CD163−/− animals were created, other edits in additional animals were introduced that were predicted to modify the structure of CD163. We are working toward in vitro and in vivo challenge experiments with these additional genotypes, with both Type I (European) and Type II strains of PRRSV. This line of research has identified a gatekeeper for PRRSV infection (CD163) and possibly provided an on-farm solution to an otherwise incurable and economically devastating disease.

Key Words: gene editing, PRRSV, disease resistance, pig

INVITED SPEAKERS: GENETICS AND GENOMICS OF AQUACULTURE SPECIES

S0120 Understanding the biology behind selective improvement of rainbow trout for commercially important traits. K. Overturf* (USDA-ARS, Hagerman, ID)

Replacement of fishmeal in the feed of carnivorous aquaculture species is known to reduce growth rates and lead to the development of enteritis. Enteritis is a condition whereby the distal intestine presents with cellular damage, evidenced by increased vaculorization and mucosal folds, and changes to the lamina propria. This condition is also characterized by an increase of inflammatory cells within the tissue. Through genetic selection, our laboratory developed a strain of rainbow trout that does not develop enteritis when reared on plant-based feeds when compared with a control strain. The selected strain of fish also displays improvements in several secondary characteristics important to commercial production, including improved non-specific pathogen resistance. In our interest to understand the

physiological mechanisms behind the changes in identified performance traits, we are evaluating and looking to correlate growth parameters with transcriptomic and proteomic data from multiple tissues, along with histology and microbiota information obtained from the distal intestine. Our findings show that microbiota differs by diet and strain, and that genetic selection is linked not only with the lack of enteritis and enhanced utilization of a plant-based feed but also to clear changes in gut microbiota. We also determined that differences seen in the transcriptome and proteome of specific tissues correlates with growth and histological changes found between these 2 strains.

Key Words: rainbow trout, selection, transcriptome, proteome

INVITED SPEAKERS: GENETICS OF IMMUNE RESPONSE AND DISEASE RESISTANCE

S0121 Are we loosing our sixth sense?

D. Werling* (Royal Veterinary College, Hatfield, United Kingdom)

One of the truly remarkable discoveries in modern biology is the finding that the nervous, endocrine, and immune systems use a common chemical language for intra- and inter-system communication. Specifically, the endocrine and immune systems produce a common set of molecules that act on a common repertoire of receptors in the 2 systems. Here, I will also review more recent studies that have delineated hardwired and humoral pathways for such bidirectional communication. This is discussed in the context of the idea that the sharing of ligands and receptors allows the immune system to serve as the sixth sense that notifies the endocrine system of the presence of entities, such as viruses and bacteria, that are imperceptible to the classic senses. Based on this, attempts are made to explain the increasing strong evidence that high yielding dairy cows are extremely susceptible to infectious diseases and that this has severe economic consequences for the dairy industry and welfare implications. Preliminary functional evidence will be presented showing that the innate immune response differs among cow breeds and that these differences are potentially linked to selective pressure on the endocrine system in the widest sense (meaning selection for higher production), which resulted in a potential appropriate "loss of function" with respect to immune parameters. It will be shown that the ability of macrophages (MØ) to kill pathogens using oxygen-dependent and independent mechanisms differ among cow breeds. The oxygen-dependent mechanisms rely on the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS production has been shown to activate the inflammasome complex in MØ, leading to increased production of the pro-inflammatory cytokine Interleukin-1β (IL-1β). Conversely, RNS inhibits inflammasome mediated IL-1β activation, indicating a division between inflammasome activation and RNS production. These differences are linked to the ability of cows to deal with invading pathogens. Collectively, these preliminary data suggest that the dichotomy of inflammasome activation and RNS production exists in cattle and differs between these 2 breeds. As pattern recognition receptors and signaling pathways are involved in the assessed functional differences presented herein, the data potentially aid the identification of in vitro predictors of appropriate innate immune response. Finally, these predictors may assist in discovering candidate genes conferring increased disease resistance for future use in combination with known production traits.

Key Words: innate immunity, inflammasome, autophagy

INVITED SPEAKERS: HORSE GENETICS AND GENOMICS

S0122 Improving the structural and functional annotation of the equine reference genome.

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The current reference genome for the domestic horse, EquCab2, was released in 2007. It was 1 of the last mammalian genomes assembled from only Sanger sequencing data and has greatly facilitated hundreds of equine studies in such diverse areas as genetic determinants of breed differences, diseases, horse domestication, non-disease traits of interest, and gene expression patterns analyzed on a transcriptome level. Substantial improvements to the equine reference genome, however, are now logistically feasible due to new and complementary high throughput sequencing technologies. We report an improved genomic DNA assembly of Twilight, the same Thoroughbred mare used to generate EquCab2. In addition to the original 6.8X coverage of Sanger reads, new data used includes 40X coverage of paired-end and PCR-free

Illumina reads, 16X coverage of long-read single molecule sequences (PacBio), and 69X coverage from a chromatin, cross-linking library (Dovetail Genomics). Many sequencing errors, mis-assemblies, and missing segments in EquCab2 have been resolved with concurrent substantial increases to the contig N50 and scaffold lengths. To date, functional annotation improvements have centered on greater accuracy in the nucleotide coordinates that define gene loci and individual exons, but efforts are now extending in association with the FAANG initiative to include transcriptional regulatory binding sites and epigenomic parameters. Advancing the structural and functional annotation of the equine reference genome will further enhance genome to phenome studies in the horse.

Key Words: horse, FAANG, gene

INVITED SPEAKERS: ISAG-FAO GENETIC DIVERSITY

S0123 The adaptation of farm animals to northern and arctic environments. J. Kantanen* (Natural Resources Institute Finland (Luke), Jokioinen, Finland)

Natural and human-made selection enables animals to adapt, survive, be productive and reproduce in challenging environments. In the Arctic, traditional animal husbandry is based almost exclusively on reindeer (*Rangifer tarandus*), but in Fennoscandian Lapland, northern Russia and Siberia, other locally adapted animals, namely cattle (*Bos taurus*) and horse (*Equus caballus*) also are used for food production and other societal and cultural needs (for example, Northern Finncattle, Yakutian cattle, Mezen horse and Yakutian horse). These animal breeds represent a valuable genetic resource for northern agriculture and pastoralism.

From the animal science point of view, the Arctic environment guided toward selection of animals with specific metabolic, morphological and reproductive adjustments. From the animal genomics point of view, adaptations to extreme environments or diets are typically associated with structural and functional genomic variations. "Adaptation traits" are complex and often polygenic by nature, but positive selection footprints can be studied through next-generation sequencing (NGS) applications, such as whole genome and mRNA sequencing, analysis of regulatory (miRNAs) elements and DNA methylation profiles. Reindeer, cattle and horse may have different biological capacities to adapt to extremes in temperature, daylight and feed availability. It is suggested that reindeer descended from a large Eurasian glacial reindeer population have the longest adaptation history (but the shortest domestication history) among the three species and can be considered as native to the Arctic. Cattle and horses, on the other hand, have longer domestication histories but have shorter adaptation histories and are regarded as having been "imported" into the Arctic. The recent study on complete genomes of modern Yakutian horses and ancient horses that lived in Sakha (Yakutia) around 5200 yr ago provided evidence that the native Yakutian horse descends from domestic livestock and not from extinct wild horse populations that once existed in Sakha. Genomics of Arctic and northern domestic animals — cattle, horse and reindeer — are studied and compared with Mediterranean cattle and horse breeds native to Portugal in a multidisciplinary study, "Arctic Ark. Human–Animal Adaptations to the Arctic Environment: Natural and Folk Selection Practices (Arc-Ark)." The project is a consortium work between colleagues from genetics and animal science at the Finnish Natural Resources Institute (Luke), Yakutian Research Institute of Agriculture (FGBNU Yakutskij NIISH) and University of Porto and the anthropology team of the Arctic Centre of University of Lapland. The project belongs to the Arctic Research Program "Arktiko" of the Academy of Finland.

Key Words: Arctic, genomics, livestock

INVITED SPEAKERS: LIVESTOCK GENOMICS FOR DEVELOPING COUNTRIES

S0124 Indigenous stocks as treasure troves for sustainable livestock production in the 21st century: Insights from small ruminant genomics. J. M. Mwacharo*[1], A. R. Elbeltagy[2], E. S. Kim[3], A. Haile[4], B. Rischkowsky[5], M. F. Rothschild[3] ([1]International Centre for Agricultural Research in the Dry Areas, Addis Ababa, Ethiopia, [2]Department of Animal Biotech. Animal Production Research Institute, Cairo, Egypt, [3]Department of Animal Science, Iowa State University, Ames, [4]International Centre for Agricultural Research in the Dry areas, Addis Ababa, Ethiopia, [5]International Center for Agricultural Research in the Dry Areas, Addis Ababa, Ethiopia)

The versatility of indigenous sheep and goats to adapt to diverse environmental conditions and production systems positions them as significant animal genetic resources for sustaining livelihoods of

agro-pastoralists, pastoralists and small holder farmers in many developing countries. In most of these countries, recording phenotypic and pedigree information has remained a challenge, cohorts of contemporaries are often of inadequate size, breeding infrastructures are non-existent and where they exist are rudimentary, and advanced reproductive technologies are difficult to implement. These drawbacks can however, be circumvented through community–based breeding programmes (CBBP) which can provide a framework to design and implement basic recording and mating schemes. Variants of the CBBP have been implemented successfully in some countries in Africa and Latin America. The integration of case-control protocols for use in genome-wide association analyses with CBBP offer great opportunities to identify genomic regions with major gene effects that can be used for genomic selection and/or introgression. Furthermore, the availability of genomic data can facilitate the determination of breed composition and admixture in the absence of pedigree records, assessment of genetic diversity and structure to harness biodiversity, and the identification of genome-wide footprints of positive selection. Such data can provide a platform to design mating schemes that optimize productivity and adaptability in a diverse genepool of indigenous sheep and goats and the development of suitable breeds. In the context of the CBBP, the propagation of alleles, underlying production and adaptation traits, via gene/genome/haplotype block editing could be combined with genomic selection in the process of developing synthetic breeds that optimize productivity and adaptability across diverse production and agro-ecological systems. In this regard, genomic data generated recently using various indigenous African sheep and goats and revealing levels of admixture and genome-level multiple breed combinations, genome-wide signatures of positive selection for adaptation to marginal environments and fecundity traits and their potential applications will be discussed.

Key Words: developing countries, genome-wide, goats, selection signatures, sheep

INVITED SPEAKERS: RUMINANT GENETICS AND GENOMICS

S0125 Changing patterns of genomic variability following domestication of sheep. M. Naval Sanchez*[1], R. Brauning[2], S. M. Clarke[2], Q. Nguyen[1], A. McCulloch[3], N. E. Cockett[4], W. Zamani[5], F. Pompanon[6], P. Taberlet[6], S. McWilliam[1], H. Daetwyler[7], J. Kijas[1] ([1]CSIRO Agriculture, Brisbane, Australia, [2]AgResearch, Mosgiel, New Zealand, [3]AgResearch Limited, Mosgiel, New Zealand, [4]Utah State University, Logan, UT, [5]Department of Environmental Sciences, Tarbiat Modares University, Noor, Iran, Islamic Republic of, [6]Laboratoire d'Ecologie Alpine, Universite Grenoble Alpes, Grenoble, France, [7]Department of Economic Development, Jobs, Transport and Resources, Bundoora, Australia)

Patterns of genome variation are highly informative for understanding the diversity and evolutionary history of domestic animal species. We analyzed sheep genomes from a broad collection of domestic breeds, along with their wild ancestor (*O. orientalis*) and other wild sheep species (*O. canadensis* and *O. dalli*). Following variant calling to identify ~30 million high confidence SNP, we calculated nucleotide diversity to assess genome-wide differences in variability and applied homozygosity -based metrics to search for specific genomic regions that have undergone selection sweeps. As might be expected for a wild species, mouflon genomes had generally higher nucleotide diversity compared with domestic sheep. Furthermore, bighorn and thin-horn genomes showed depressed diversity, likely reflecting a strong founder effect and the impact of low effective population size. To approach a deeper understanding of changing patterns of diversity following domestication, we used available gene models and comparative information from human enhancer databases (ENCODE, Epigenetic Roadmap) to partition genomic sequence into a collection of features. These included exons, introns, UTRs, intergenic regions, and the components of gene regulatory machinery, such as promoters. For each genome feature, we compared the distribution in nucleotide diversity between wild and domestic sheep. We found clear evidence that exons have undergone a marked decrease in nucleotide diversity, when comparing wild to domestic sheep, in contrast to other genome features tested. We will also report on the collection of chromosomal regions that have undergone sweeps to build a deeper understanding of the impact of domestication in this important farmyard species.

Key Words: sheep, domestication, polymorphisms, genetic diversity

POSTERS
S1000 – S8008

https://www.asas.org/meetings/isag2016

POSTERS: BIOINFORMATICS, STATISTICAL GENETICS, AND GENOMIC TECHNOLOGIES

P1000 Genomic imputation of a multigenerational Nellore-Angus mapping population. C. A. Gill* (Texas A&M University, College Station, TX)

Genome sequencing is still expensive for populations, so there has been considerable activity in the development of methods to impute single nucleotide polymorphisms (SNP) genotypes up to whole-genome density. Genomic imputation is very tractable in family-based designs and is maximally useful in samples that include large numbers of related individuals. The objective of this study was to cost effectively increase the density of genotype data in our research population to aid discovery of genetic variants associated with production efficiency. We sequenced 7 Nellore bulls and 6 Angus cows that each contributed to more than 10 s generation Nellore-Angus crossbred cattle. Those bulls, cows and the first-generation parents were genotyped with a high density SNP assay, and second-, third-, and fourth-generation crossbred cattle were genotyped at lower density. Depth of coverage of the sequences ranged from 33 to 88X, with 97 to 99% of reads mapped to the UMD3.1 bovine reference genome, and 93.8 to 97.8% of reads properly paired. After removing PCR duplicates and performing local realignment, SNP and indels were called. The SNP quality was recalibrated by applying BovineHD SNP that passed QC and bovine SNP from dbSNP138 as truth sets. We used FImpute to phase and impute SNP haplotypes to sequence-scale for 1,108 cattle across the 4 generations. After imputation, there were 14 million SNP retained for genome-wide association studies. Examples of the impact of increasing SNP density on the power to discover associations for production efficiency traits will be presented.

Key Words: beef cattle, imputation, production efficiency

P1001 SNP calling in transcriptome of Holstein cows and their contribution in genetic variance of residual feed intake. M. H. Banabazi*[1], A. Nejati Javaremi[2], I. G. Imumorin[3], M. Ghaderi-Zefrehei[4], S. R. Miraei Ashtani[2] ([1]University of Tehran, Karaj, Iran, [2]University of Tehran, Karaj, Iran, [3]Animal Genetics and Genomics Laboratory, Cornell University, Ithaca, NY, [4]University of Yasouj, Yasouj, Iran)

In the recent years, single nucleotide polymorphisms (SNP) have been the most important and efficient tool in animal breeding. Genome-wide association studies (GWAS) and genome-enabled predictions (genomic selection) are 2 major applications of SNPs in animal genetics and breeding that both rely on genotyping a lot of SNPs. Some high density (HD) SNP arrays have been applied, particularly in dairy cattle, for this purpose. These chips have still some restrictions, such as computational complexity, high cost and genotyping the previously known SNPs. Therefore, finding small subsets with the same efficiency or better than current SNP arrays is a hot research topic. Our aim was to introduce an SNP panel in transcriptome of a small population of U.S. Holstein cows using RNA-Seq data and then estimate their contribution to genetic variance of the residual feed intake (RFI) trait. We found about 53,478 SNPs by analyzing the transcriptome of 40 U.S. Holstein cows. There are 6,336 common SNPs between our panel and Illumina Bovine HD Chip. The number of common SNPs was very low and therefore could not improve the prediction accuracy compared with common Bovine HD Chip. However, in this study, we found many novel SNPs that are exclusively located on genomic coding regions. In conclusion, the SNPs in transcritome can be identified in RNA-Seq data. However, not as many SNPs can be identified in transcritome compared with the whole genome, which limits this application in improving genomic prediction accuracies.

Key Words: single nucleotide polymorphisms (SNP), RNA-Seq, Holstein cow

P1002 Evolution of hypothalamus-pituitary growth axis among fish, amphibian, birds, and mammals. M. Moaeen-ud-Din* (PMAS-Arid Agriculture University, Rawalpindi, Pakistan)

Hypothalamus-pituitary growth axis (HP growth axis) regulates animal growth and development in pre-natal and post-natal life, governed by many factors. However, until recently, the evolutionary history of this axis among lineages was not understood. The aim of this study was to understand the major events in evolution and evolutionary history, and trend of HP growth axis. The diversity among *Homo sapience, Mus musculus*, *Rattus norvegicus*, *Gallus gallus, Danio rerio,* and *Xenopus laevis* was determined for genes involved in HP growth axis in current study. Sequences of HP growth axis genes were retrieved from NCBI (http://www.ncbi.nlm.nih.gov). Nucleotide diversity using Kimura's 2-parameter method, codon-based test of positive selection, using the Nei-Gojobori, equality of evolutionary rate with Tajima's relative rate test, and

phylogenetic history using the RelTime method were estimated in MEGA6. Estimates of the coefficients of evolutionary differentiation, based on nucleotides and amino acids substitution patterns of HP growth axis genes, showed contrasting evolutionary patterns among the lineages. The results demonstrated that although these genes might have crucial functional roles in each of the species, their sequence divergence did not necessarily reflect similar molecular evolution among the species. Codon-based test of positive selection revealed that human versus mouse, chicken versus rat, human versus rat, and mouse versus rat had similar and higher non-synonymous substitutions ($P > 0.05$). Higher rate of non-synonymous substitutions at similar orthologs level among species indicated a similar positive selection pressure in these species. Results for relative rate test assessed with the chi-squared test showed differences on unique mutations among lineages at synonymous and non-synonymous sites, except chicken versus mouse, human versus mouse, chicken versus rat, human versus rat, and mouse versus rat. This indicated that the mutagenic process that generates substitutional mutation is taking place at approximately the same rate at synonymous and non-synonymous sites. Moreover, despite common ancestry, results indicate a different divergent time among genes of these species. This is the first demonstration that variable rates of molecular evolution may be present within HP growth axis genes among different species. This difference could be of interest for comparative genomics analysis and physiological genes functions identification among those species whose HP growth axis is not explored.

Key Words: HP growth axis, evolution rate, positive selection, comparative genomics

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P1003 MiRNAs expression profiling of myostatin transgenic and wild-type littermate mice by Solexa deep sequencing. R. Javed* (Huazhong Agricultural University, Wuhan, China)

miRNAs are a class of short, non-coding RNA molecules that reportedly play a central role in regulating post-transcriptional gene expression during embryonic stem cell development, myogenesis, adipogenesis, fat metabolism, and glucose homeostasis. For assessment of the effect of loss of myostatin signaling on gene expression in skeletal muscle, RNA from post-developmental myostatin transgenic and wild-type littermate mice were analyzed with Solexa deep sequencing. Sequencing data were analyzed using miRDeep software V. 2.1.2. Four hundred sixty-one mature known miRNAs were identified, out of which 57 miRNAs were found to be differentially expressed. Expression pattern demonstrated that Mmu-miR-22 was most abundant; miRNA, mmu-miR-133a, and mmu-miR-378a were abundant and significantly differentially expressed miRNAs. Sixty-nine novel miRNAs were also identified, out of which 3 NMu-1, NMu-14, and NMu-36 accounted higher read count. For these 3 novel miRNAs and 5 known miRNAs, the expression profiling was done for using Q-PCR analysis. Out of 57 differentially expressed miRNAs, the 20 most abundant miRNAs were selected for target prediction and pathway analysis. Four thousand five hundred eighty-three targets were identified, out of which FST, SMAD3, TGFBR1, ACVR1a, and MEF2c genes, which plays vital role in MSTN signaling, were found to be targeted by miR-101, miR-425, miR-199a, and miR-582 in TGFb-signaling pathway, which activates MSTN signaling. Hence, these miRNAs could prove crucial candidate miRNAs in skeletal muscle development. In conclusion, the present study proffers an initial miRNA transcriptome profile in skeletal muscle development of transgenic and control mice. Findings aided identification of miRNA and their targets, which can possibly contribute to skeletal muscle development. Information generated in this study can be further used to investigate the role of identified miRNAs and their targets in regulation of skeletal muscle development.

Key Words: miRNA, transcriptome analysis, differentially expressed miRNAs, novel miRNA

P1004 Genotyping in thousands by sequencing (GT-seq): A low cost, high-throughput, targeted SNP genotyping method. N. Campbell*, S. Harmon, S. R. Narum (Columbia River Inter-Tribal Fish Commission, Hagerman, ID)

GT-seq is a genotyping method that leverages large read numbers from Illumina sequencers to genotype hundreds of single nucleotide polymorphisms within pools of multiplex PCR amplicons generated from thousands of individual samples (Campbell et al., 2014). This method produces genotypes that are 99.9% concordant to those produced using TaqMan assays at approximately one-fourth the cost. Since its development, GT-seq panels have been created for several species (Chinook salmon, Coho salmon, sockeye salmon, rainbow trout, and pacific lamprey) and have become the preferred SNP genotyping method in our laboratory. New genotyping software allows genotypes and summary figures to be produced from a lane of raw sequencing data in less than an hour, using a desktop Linux computer.

Key Words: multiplex PCR, GT-seq, genotyping by sequencing, SNP genotyping

P1005 Genome-wide association study identifies a QTL for fat percentage in ribeye area on BTA10 in Japanese Black cattle. A. Inoue*[1], T. Nakajima[1], A. Nakajima[1], Y. Uemoto[2], M. Fukushima[3], E. Yoshida[4], E. Iwamoto[4], T. Akiyama[3], N. Kohama[3], E. Kobayashi[5], K. Oyama[6], T. Honda[6], H. Mannen[1], S. Sasazaki[1] ([1]Graduate School of Agricultural Science, Kobe University, Kobe, Japan, [2]National Livestock Breeding Center, Nishigo, Japan, [3]Northern Center of Agricultural Technology, General Technological Center of Hyogo Prefecture for Agriculture, Forest and Fishery, Asago, Japan, [4]Hyogo Prefectural Technology Center of Agriculture, Forestry and Fisheries, Kasai, Japan, [5]Animal Breeding and Reproduction Research Division, NARO Institute of Livestock and Grassland Science, Tsukuba, Japan, [6]Food Resources Education and Research Center, Kobe University, Kasai, Hyogo, Japan)

Beef marbling is an important trait that determines the grade of meat quality. Recent studies suggested that fat percentage in ribeye area, which is measured by image analysis, would be an attractive alternate for evaluation of beef marbling. The objective of this study was to identify genomic regions associated with fat percentage in ribeye area using a DNA pool-based genome-wide association study in Japanese Black cattle. One hundred animals with the highest and 100 animals with the lowest values were selected from 1,836 animals, based on corrected phenotype, and then pooled as the high and low groups, respectively. We performed a DNA pool-based genome-wide association study, using Illumina BovineSNP50 BeadChip v2 with 3 replicate assays for each pooled sample. Genome-wide association study (GWAS) revealed that 6 SNPs on BTA10 were found to be associated with fat percentage at 5% chromosome-wide significance level (P = 4.31E-5). The most significant SNP (BTB-01047707) were further evaluated by individual genotyping to validate the pooling method. We genotyped the SNP by PCR-RFLP method, using 567 animals randomly selected from 1,836 animals and investigated the allelic effect on fat percentage. Tukey–Kramer method using a least mean square value of each genotype revealed that there were significant differences among TT (n = 46), TC (n = 234), and CC (n = 287) genotypes ($p < 0.0001$). The animals with TT type had 5.5% higher values than CC type. These results confirmed the significant SNP detected by pooling GWAS and this SNP can be used in marker-assisted selection. This information will also be useful to select candidate genes for beef marbling in future studies.

Key Words: GWAS, beef marbling, Japanese Black cattle

P1006 Identification of polymorphisms associated with oleic acid percentage by pool-based genome-wide association study in Japanese Black cattle. F. Kawaguchi*[1], A. Nakajima[1], Y. Matsumoto[1], Y. Uemoto[2], M. Fukushima[3], E. Yoshida[4], E. Iwamoto[4], T. Akiyama[3], N. Kohama[3], E. Kobayashi[5], T. Honda[6], K. Oyama[6], H. Mannen[1], S. Sasazaki[1] ([1]Graduate School of Agricultural Science, Kobe University, Kobe, Japan, [2]National Livestock Breeding Center, Nishigo, Japan, [3]Northern Center of Agricultural Technology, General Technological Center of Hyogo Prefecture for Agriculture, Forest and Fishery, Asago, Japan, [4]Hyogo Prefectural Technology Center of Agriculture, Forestry and Fisheries, Kasai, Japan, [5]Animal Breeding and Reproduction Research Division, NARO Institute of Livestock and Grassland Science, Tsukuba, Japan, [6]Food Resources Education and Research Center, Kobe University, Kasai, Hyogo, Japan)

The monounsaturated fatty acid percentage, especially oleic acid, is 1 of the important traits for beef quality because it has positive effects on beef palatability and human health. In our previous study, we selected 100 animals with the highest and 100 animals with the lowest oleic acid percentage from 1,836 animals, and performed pool-based, genome-wide association study (GWAS), using Illumina BovineSNP50 BeadChip v2 to identify genomic regions associated with oleic acid percentage (ISAG conference 2014). GWAS analysis revealed 2 novel candidate regions for oleic acid percentage; 3 and 1 significant SNP on BTA9 and BTA14, respectively, were detected at 5% genome-wide significance level. The objective of this study was to confirm these significant SNPs detected by pooling method and to validate the candidate regions for fatty acid composition. We genotyped the most significant SNPs in each region (Hapmap60557-rs29018515 on BTA9 and BTB-00554873 on BTA14), using 444 animals selected randomly from 1,836 animals. Association analysis showed that the both SNPs on BTA9 and BTA14 were significantly associated with oleic acid percentage (P = 0.0004 and $p < 0.0001$, respectively). In addition, Tukey-Kramer's honestly significant difference test revealed that T/T genotype in Hapmap60557-rs29018515 indicated 1.31 higher percentage of oleic acid than C/C genotype; A/A genotype in BTB-00554873 indicated 1.23 higher percentage of oleic acid than C/C ($p < 0.05$). Our results suggested that these SNPs would be the effective DNA markers for oleic acid percentage in Japanese Black cattle. Further investigation on gene polymorphisms in these candidate regions may lead to identification of the

causative mutation for oleic acid percentage.

Key Words: GWAS, fatty acid composition, Japanese Black cattle

P1007 Genetic and protein study of alpaca fiber.

Y. T. Wong* (Deakin University, Geelong, Australia)

The Australian Alpaca industry is currently the world's second largest commercial herd after Peru, with more than 130,000 alpacas in Australia. It is a growing industry as the Australian Alpaca Association 2020 Vision Report has predicted that as many as 4,000 tonnes of alpaca fiber will be produced in year 2020. Huacaya is the common type of alpaca in Australia, with dense fleece that is highly crimped. Suri is the rare and more precious type, with silky locks of lustrous fleece. The other important and crucial trait for alpaca commercial market price is fiber diameter. Therefore, the focus of this research was to narrow down the genetic region containing genes responsible for the Suri trait and investigate the alpaca genome to determine the order of alpaca's scaffolds using genetic markers. Genetic and protein approaches were used to identify the causative mutation of Suri trait. SNP and microsatellite primers were used for analysis of genetic association. Six scaffolds were mapped in the region identified as containing the genes causing the Suri trait. The location of the causative mutations was narrowed to 2 regions, located on scaffold B at position 11834 and on scaffold A at position 2734593. These 2 regions are approximately 7 million base pairs (bp) apart, based on cow. One of the candidate regions scaffold B is within a keratin-associated protein (KAP) region. Therefore, the analyzed data suggest that there might be 2 genomic regions associated with fleece variation in alpaca. These results are different to the previous generally accepted single-locus genetic model, where the Suri trait has been proposed to be dominant. The results agree with Presciuttini et al. (2010), where they found a 2-gene model better explained their data and the estimated recombination fraction was about 10% (~10 million bp between the genes). To further investigate the regions, protein approach was used to help identify the difference between Suri and Huacaya fiber protein. The protein analyses identified 2 keratin protein candidates that showed high association with the Suri trait and were located in the causative mutation region on scaffold B near marker 11834. This result further confirms that the causative mutation of Suri trait is located in scaffold B region. Identifying the genes and mutations responsible for the highly valued Suri trait will have economic implications for alpaca breeders not only in Australia, but also across the globe.

Key Words: Alpaca, fiber, keratin

P1008 Genetic diversity and origin of mtDNA haplogroup P observed in Japanese Shorthorn.

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Japanese Shorthorn is a native beef cattle breed in the northern region of Japan. To estimate the maternal origin and genetic diversity, we sequenced the whole mtDNA D-loop region of 105 Japanese Shorthorn (59 from Iwate and 46 from Aomori prefectures). We observed 36 variants, including 1 transversion and 35 transitions. On the basis of these variants, Japanese Shorthorn had 20 mtDNA haplotypes (12 and 15 haplotypes in Iwate and Aomori populations, respectively). The NJ phylogenetic tree classified these haplotypes into 3 mtDNA haplogroups, T3, T4, and P, in both populations. The haplogroup P was found in ancient DNA samples from wild aurochs and also observed in a few modern animals from China and Korea. The frequencies of each haplogroup in Japanese Shorthorn were 0.371 in P (n = 39), 0.086 in T3 (n = 9), and 0.543 in T4 (n = 57). The mean sequence divergence values were 0.0011, 0.0029, and 0.0007, within P, T3, and T4 haplogroups, respectively. Network tree showed 8 haplotypes belonging to haplogroup P (JSH1-JSH8). We observed JSH1 haplotype in 16 samples, JSH2 in 11 samples, JSH3 and JSH4 in 3 samples, JSH5 and JSH6 in 2 samples, and JSH7 and JSH8 in 1 sample. Each of JSH2-JSH8 haplotypes differed by 1 variant from major JSH1 haplotype. Japanese Shorthorn was developed by introducing American Shorthorn into indigenous cattle in the northern region of Japan. Interestingly, Japanese Shorthorn had haplogroup P with high frequency, whereas the haplogroup P has not been detected in any other Japanese breeds in previous studies. This result suggests that the maternal origin of Japanese Shorthorn is different from the other Japanese native cattle. In addition, haplogroup P has not been reported in any Shorthorn populations yet. Therefore, it suggests that the haplogroup P of Japanese Shorthorn may be descended from the Japanese indigenous cattle with different maternal origin.

Key Words: mtDNA, haplotype, cattle

P1009 Sheep reference genome sequence updates: Texel improvements and Rambouillet progress.

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The quality of draft genome assemblies is improving beyond that of early Sanger sequencing as a result of the latest methods for producing and analyzing long reads. We have applied these methods to improve the existing genome of the Texel sheep and are in the process of producing a de novo assembly from a single Rambouillet ewe. The Texel ram Pacific Biosciences data were used with the PBJelly software[1] to produce an improved assembly, Oar_v4.0 (GCA_000298735.2). This new assembly, available in GenBank, has improved contiguity with a contig N50 of 150kb and genomic representation with about 2% more genes represented in the RefSeq annotation. This highly contiguous genome has increased the contig N50 more than threefold (from 40 kb), reduced the number of gaps and contigs by two-thirds, and merged 232 scaffolds (4%). Further efforts to produce a very high quality reference genome have shifted focus to the Rambouillet breed where all of the genomic data are being collected from a single ewe. We have completed production of 200 Gb of sequence using the Pacific Biosciences technology. The data have a subread length of 12.6 kb N50 length and 8.9 kb mean length. Error correction of the reads, followed by assembly using Falcon, produced an assembly with a contig N50 of 3.6 Mb with a total length of 2.65 Gb. Two hundred twenty-seven contigs contain 50% of the sequence. Further efforts to scaffold the genome using Hi-C data are planned. Sample collection from the Rambouillet donor animal is planned for further assays, including Pacific Biosciences IsoSeq for RNA sequencing to use as evidence for gene annotation and Hi-C proximity ligation sequencing for genome scaffolding. We will discuss progress of this research to produce high quality genomes without using traditional expensive and manually intensive finishing efforts.

Key Words: sheep genome, reference assembly, Pacific Biosciences long read assembly, Rambouillet reference genome

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P1010 Genotype by environment interaction and genetic heterogeneity of environmental variance of body weight at harvest in genetically improved farmed tilapia (*Oreochromis niloticus*) reared in 3 different countries. S. Agha*[1,2], W. Mekkawy[2,3], N. Ibanez-Escriche[1], J. Kumar[4], A. Mandal[4], C. E. Lind[3], J. Benzie[3], A. B. Doeschl-Wilson[1] ([1]The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom, [2]Animal Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, [3]WorldFish, Penang, Malaysia, [4]Rajiv Gandhi Center for Aquaculture, Tamil Nadu, India)

Establishing breeding programs that improve farmed fish performance across multiple environments is crucial in a globalized aquaculture market. The main objectives of the current study were to assess the growth performance of genetically improved farmed tilapia (GIFT) under different environments, across 3 different countries (Malaysia, India, and China), termed genotype by environment interaction (G × E) or macro-environmental sensitivity, and to quantify the genetic heterogeneity of environmental variance for body weight at harvest (BW) in GIFT, as a measure of micro-environmental sensitivity or robustness. The data consist of 94,351 individuals representing 1,568 full-sibs families. Selection for BW was performed over 13 generations in Malaysia. Subsets of the families from Malaysia were sent to India and China, and reared for 4 and 3 generations, respectively. First, a multi-trait animal model was used to analyze the BW in different countries as different traits. Genetic correlations (r_g) between BW in Malaysia and in India and China were 0.25 and 0.48, respectively, whereas r_g between BW in India and China was 0.13. These low to moderate genetic correlations between BW in different environments indicate a strong G × E interaction and re-ranking of genotypes across environments in regard to the genetic merit for growth. Second, a genetically structured environmental variance model, implemented using Bayesian inference, was used to analyze micro-environmental sensitivity of BW in each country. The results revealed the presence of genetic heterogeneity in both BW and its environmental variance in all environments. The posterior mean of the additive genetic correlation between BW and its environmental variance in Malaysia and China (95% highest posterior interval) were −0.53 (−0.47, -0.59) and −0.66 (−0.80, −0.60), respectively. High negative genetic correlations indicate associations between genes controlling the mean and those affecting its environmental variance. Thus, selection for higher BW would be expected to decrease environmental variation. In contrast, a weak association between additive genes affecting the mean and the environmental variance was found for BW in India, with a posterior mean of the additive genetic correlation −0.03 (−0.17, 0.11). The study indicates a strong G × E interaction in BW for GIFT strain and that the environmental variance of BW is partly genetically determined. Incorporation of macro- and micro-environmental sensitivity information in the selection index of multi-environment aquaculture breeding

programs enables simultaneous improvement of weight gain and increasing homogeneity/robustness.

Key Words: aquaculture, environmental sensitivity, robustness

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P1011 Temporal changes for genomic diversity for poultry conservation population based on genome-wide SNP data. W. Li[*1], M. Zhang[1], W. Han[2], K. Wu[1] ([1]China Agricultural University, Beijing, China, [2]National Chickens Genetic Resources, Yangzhou, China)

The traditional method is limited to measure genetic diversity due to the lack of recording in conservation populations. With the coming explosion of whole-genome information, it is crucial to explore the information for characterizing the population genetic parameters for breed conservation. DNA from 3 poultry conservation populations with 3 successive generations, including Beijing You Chicken, Baier Yellow chicken, and Langshan chicken, were genotyped by sequencing after restriction enzyme digesting. After reads filtering, alignment, and SNPs calling, we obtained 1,286,815 high quality SNPs and estimated the allele frequencies. The nucleotide diversity parameters, such as $\theta\pi$, θw, and Fst, for each breed and each generation, were calculated. With the generation increasing, $\theta\pi$ of each generation from 1 breed showed changing within a narrow range, which is less than 10%. Taking Beijing You Chicken as an example, the $\theta\pi$ of G3 population rose 0.144% relative to the G0, and G8 fell 8.08% compared with G3. As a whole, nucleotide diversity ($\theta\pi$) of Beijing You Chicken remained 92.35% of the initial population after 8 generation conservation program. Same treatment as Beijing You Chicken, Baier Yellow Chicken remained 92.36% after 8 generation and Langshan Chicken remained 96.53% after 5 generations. Moreover, the Fst of each breed ranged from 0.003 to 0.021. Based on the analysis, we found that conservation populations from 3 breeds could maintain the genetic diversity for more than 90% under the conservation management program. Additionally, Fst results showed that no obvious population differentiation was observed in any breed. Overall, the current management program of poultry conservation population, mating at random within family and then selecting sire at random from each male progeny and dam from each female progeny to mate within family, achieved good effects on breed conservation. This study was supported by the program of 973 project (2014CB138501), National Transgenic Creature Breeding Grand Project (2014ZX08006–005), and program for Changjiang Scholar and Innovation Research Team in University (IRT1191).

Key Words: chicken, conservation, genotyping by sequencing

P1012 Liver transcriptome from pre versus post-pubertal Brahman heifers. L. T. Nguyen[*1,2], A. Reverter-Gomez[3], A. Canovas[4], B. Venus[5], A. Islas-Trejo[6], S. A. Lehnert[7], J. F. Medrano[6], S. S. Moore[5], M. R. Fortes[1] ([1]The University of Queensland, School of Chemistry and Molecular Biosciences, St Lucia, Australia, [2]Vietnam National University of Agriculture, Hanoi, Viet Nam, [3]CSIRO Food Futures Flagship, Brisbane, Australia, [4]University of California, Davis, Davis, CA, [5]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, St Lucia, Australia, [6]University of California, Davis, CA, [7]CSIRO Agriculture, Brisbane, Australia)

Heifer age at puberty impacts profitability and reproduction rates. The onset of puberty is triggered by GnRH release, which then stimulates the secretion of LH and FSH. Although these events are similar for *Bos indicus* and *Bos taurus* breeds of cattle, they occur later in life for *Bos indicus* heifers. Apart from genetic drivers, the onset of puberty is also influenced by environmental, nutritional, and metabolic factors. Important metabolic processes take place in the liver, suggesting that gene expression in liver could play a significant role in the onset of puberty. To understand factors involved in the initiation of puberty in *Bos indicus*, we characterized the liver transcriptome from pre- and post-pubertal Brahman heifers. RNaseq was used to study differences in expression profiles of pre- versus post-pubertal heifers, and identify puberty-related genes and their associated pathways. The bovine transcription factors (TF) database and regulatory impact factor metrics were used to identify key regulators of gene expression. A gene co-expression network was predicted from RNaseq data and visualized with Cytoscape. We identified 16,978 genes expressed in liver. Of these, 452 were found to be differentially expressed (DE) ($p < 0.05$) in the comparison between pre- and post-pubertal Brahman heifers; 253 genes were up-regulated and 199 were down-regulated. Among these DE genes, brain-derived neurotrophic factor (BDNF) was observed as the most down-regulated gene ($p < 0.001$) in post-pubertal heifers. In the liver, BDNF plays a role in PPARα and FGF21 mediated regulation of liver glucose and lipid metabolism. Regulation of the PPARα and FGF21 pathways via BDNF expression in the liver can now be proposed to influence pubertal development in Brahman heifers. Furthermore, 82 significant top ranking TF and 1,408 co-expressed genes related to puberty were identified from this study.

A sub-network created by highly ranked TF that co-expressed with highly DE genes ($P < 0.05$) revealed key transcriptional regulators, including BHLHE22, SOX13, ESR2, MESP2, ZNF76, MXD4, and HMG20B. Results from these analyses enhance our knowledge about genes and their interaction in the context of puberty in *Bos indicus* cattle.

Key Words: liver transcriptome, Brahman heifers, puberty, BDNF

P1013 Identification of SNP associated with fertility trait using pool-based genome-wide association study in Japanese Black cattle.

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Female fertility, a fundamental trait required for animal reproduction, has gradually declined in Japanese Black cattle. The objective of this study was to identify genomic regions associated with female fertility traits in Japanese Black cattle. We performed a DNA pool-based genome-wide association study (GWAS) to estimate the associations between genotypes and 3 fertility traits (age at first calving, calving interval, number of calves produced at 4 yr of age), using Illumina BovineSNP50 Beadchip v2. In the GWAS analyses, 100 animals with the highest and 100 animals with the lowest values in each trait were selected from 3,973 animals, based on pedigree and phenotypic information, and then pooled as the high and low groups, respectively. The GWAS revealed that 1 SNP on BTA 5 was associated with age at first calving and 2 SNPs on BTA 12 and 20 were associated with calving interval at 5% significance level. To confirm the significant differences of allele frequencies between high and low groups in each SNP, we individually genotyped the SNPs for high and low groups. The statistical analysis revealed that there were significant differences ($P < 0.05$) in each SNP. In the present study, we performed further analysis, using 1 of 3 SNPs (Hapmap53957-rs29016480 on BTA12). To evaluate the effect of the SNP, we genotyped the SNP using 516 animals randomly selected from 3,596 animals and investigated the association with calving interval by analysis of variance. As a result, this SNP showed significant effects on calving interval ($P = 0.0118$). In addition, Tukey-Kramer HSD test revealed significant differences between AA ($n = 212$) and GG ($n = 52$) genotypes ($p < 0.05$), and the animals with GG type had 24.07 d shorter calving interval than the animals with AA type. Thus, the result suggests that this SNP could be used as a marker to select Japanese Black cattle with superior calving interval.

Key Words: GWAS, fertility traits, Japanese Black cattle

P1014 Genetic association between a missense mutation in the positional candidate gene GRIP1 and backfat thickness traits in pigs.

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Previously, we reported quantitative trait loci (QTL) affecting backfat thickness (BFT) traits on pig chromosome 5 (SW1482–SW963) in an F_2 intercross between Landrace and Korean native pigs. The aim of this study was to analyze glutamate receptor-interacting protein 1 (GRIP1) as a positional candidate gene underlying the QTL affecting BFT traits. Genotype and phenotype analyses were performed, using an F_2 population of 1,105 individuals. Significant association of 2 informative SNPs [c.3163 C > G (G1055R), c.2289 C > T] in GRIP1 with BFT traits were detected. In addition, the 2 SNPs were used to construct haplotypes that were associated with the BFT traits. The SNPs and haplotypes of the GRIP1 gene determined in this study may aid in understanding the genetic structure of BFT traits.

Key Words: GRIP1, backfat, QTL, Landrace, Korean native pigs

P1015 The Caprinae Genome Database: Multispecies goats/sheep genome and incorporation of RNA-Seq data, and re-sequencing data to study comparative genomics and genome assistant breeding.

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Goats are 1 of several economically important animals, especially cashmere goats, which are well known for their high quality Cashmere. In this study, we selected 3 lines of Inner Mongolia and Liaoning Cashmere goats, which are characterized by the quality and quantity of Cashmere. The average depth of 73 goats was about 2.79X. To further study the impact of important genes related to hair follicle, we also selected 12 non-cashmere goat breeds for further comprehensive analysis, with average coverage reaching to about 10X per individual. In Cashmere goat, we detected 2479,437 single-nucleotide polymorphisms and obtained 194,273 indels. The average SNP density in autosome is about 0.95. Genetic variant revealed genetic diversity of Cashmere goats on genome-wide level, providing a genetic basis for the study of economically important traits and guidance for animal breeding and genetic improvement. Genetic structure analyses uncovered that Liaoning Cashmere goats play an important role in crossbreeding program practices. Combined with the unreleased sequencing data of the common goat, we identified several genes that may relate to adaption and important economic traits in Cashmere goat.

Key Words: Cashmere goat, resequencing, comparative genomics, breeding

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P1016 The pig's other genome: A reference gene catalog of the gut microbiome as a new resource for deep studies of the interplay between the host and its microbiome.

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The pig is a major species for livestock production and is also extensively used for biomedical research. A reference catalog of gut microbial genes would clearly complement the recently established pig genome sequence. We established a comprehensive catalog of gut microbial genes from 287 pigs from France, Denmark, and China. Deep sequencing of fecal DNA samples generated 1,758 Gigabases (Gb) of high quality data with an average of 6.125 Gb per sample. The data set allowed us to identify 7685,872 non-redundant (NR) genes with an average contigN50 length of 1.89 Kilobases, together with 719 metagenomic species (MGS). Fifty percent of the NR genes could be taxonomically classified, and of these more than 98% could be assigned to the bacteria super kingdom. At the phylum level, firmicutes was the most abundant, followed by bacteroidetes. At the genus level, *Prevotella* was the most abundant, followed by *Bacteroides, Clostridium, Ruminococcus*, and *Eubacterium*. We identified a common set of 4,430 NR genes, 36 MGS and 3,463, related annotated functions shared by 100% of the 287 pig samples, suggesting the existence of a core of genes, species, and functions in the gut microbiome in pigs. The pig and human catalogs share 12.6% and 9.3% of their genes, respectively, a higher proportion than that shared between the mouse and human catalogs. Importantly, 78% and 96% of the functional pathways are shared, underscoring the potential use of pigs for biomedical research. The pig metagenome exhibited a higher α diversity than both the human and mouse microbiomes, and lower β diversity than the human metagenome at the gene, genus, and KEGG levels. We showed that gender, age, and host genetics markedly influence the pig gut microbiome. We report a common set of antibiotic resistance genes (ARG) found in all pigs, regardless of the country of origin or the supplementation with antibiotics, but show a significantly greater ARG load in animals continuously fed antibiotics. Thus, our data confirmed the efficiency of eliminating antibiotics from animal diet to reduce the risk of dissemination associated with farming systems. The pig microbiome gene catalog reported here provides a straightforward new resource for metagenomics-based research in biomedicine and for sustainable knowledge-based pig farming.

Key Words: pig, gut microbiome, gene catalog, metagenome sequencing

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P1017 Genome-wide association study using F2 population to reanalyze white feather gene.

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Chicken (*Gallus gallus domestica*) plumage is regulated by multiple genes. Chicken feather color is related to pigment distribution, content, and ratio. Chicken feather color common traits and gene mapping information have made progress in recent studies. As we all know, dominant white feather is regulated by PMEL17 gene and recessive white feather is regulated by TYR gene. We constructed an F2 resource population by crossing of the White Leghorn chicken and recessive White Plymouth chicken, and recorded feather color of the F2 chickens at birth and adult stage. Then, we genotyped the F2 chicken by using the chicken 600K SNP chip from Affymetrix company. We also genotyped the known PMEL17 gene and TYR gene mutations associated with dominant or recessive white. Genome-wide association study was performed with the software Plink1.9 and visualized the associated results with R. According to a chick's phenotype, we found a significant SNP site, which was located in Linkage Group: LGE22C19W28_E50C23. Through adult chicken feather color, it associated with chromosome 1 zone and linkage with TYR gene. In this study, chicken plumage phenotypic changed at different growth stages. It may be the dominant white gene PMEL17 in the chick stage and may be the recessive white gene TYR in the adult stage. The reason for the result may be the phenotype varied in different life stages so that we cannot get the accurate phenotype. In addition, when we genotyped PMEL17 and TYR genes, we found some white feather chickens with genotype CCii or Ccii, so we thought it might have a new white gene. After the correction of PMEL17 and TYR genes, we found no significant SNP loci on the genomic level, but there was a significant SNP site on chromosome 9 (5% significance level), indicating that there may be a new white gene or other mechanism. However, the effect was small. It needs further analysis and experiment verification.

Key Words: white feather, PMEL17, TYR, genome-wide association study

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P1018 Comparison of high-density SNP chip versus Rad sequencing in cattle and related species. L. Pérez-Pardal[1], I. K. Saglam[2], V. Costa[*1], S. Chen[3], M. R. Miller[4], A. Beja-Pereira[1] ([1]Cibio, Porto, Portugal, [2]University of California-Davis, Davis, CA, [3]School of Life Sciences, Yunnan University, Kunming, China, [4]University of California, Davis, CA)

Interest in high throughput SNP genotyping assays in livestock species has increased in recent years. High-density SNP panels allow more accurate calculations of summary statistics and genetic distances between individuals, meaningful estimations of coalescence times, and the option to use haplotype sharing for quantifying breed relationships. Commercial SNP were designed to detect commercial traits from commercial breeds and therefor these SNP chips could underestimate local breed variability. Next-generation sequencing technologies are making a substantial impact on many areas of biology, including the analysis of genetic diversity in populations. Restriction-site associated DNA sequencing (RADSeq), a method that samples at reduced complexity across target genomes, promises to deliver high resolution population genomic data. RADSeq is an important new method for the discovery of thousands of sequenced markers in any organism of choice. It makes possible population genetics studies of unprecedented depth and complexity. In this work, we compared Illumina's CattleSNP 800K Chip versus RADSeq in samples from cattle, zebu, yak, and gaur. Our goal was to study the applicability of these 2 methods in non-model breeds and non-model species.

Key Words: RAD Seq, SNP CHIP, cattle

P1019 Genetic introgression through selection in domestic chickens: Insight from whole genome sequence analysis. R. A. Lawal[*1], D. Wragg[2], P. Silva[3], K. Vanmechelen[4], A. Vereijken[5], D. D. Wu[6], R. M. Al-Atiyat[7], O. Hanotte[8] ([1]School of Life Sciences, University of Nottingham, University Park, Nottingham, United Kingdom, [2]Institut National de la Recherche Agronomique (INRA), UMR 1338 GenPhySE, 31326, Castanet Tolosan, France, [3]Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka, [4]Open Univeristy of Diversity, Hasselt, Belgium, [5]Hendrix Genetics, Boxmeer, Netherlands, [6]State Key Laboratory of Genetic Resources and Evolution, Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Science, Kunming, China, [7]King Saud University, Riyadh, Saudi Arabia, [8]School of Life Sciences, University of Nottingham, Nottingham, United Kingdom)

The evolutionary history of domestic chicken has been subjected to debate since Charles Darwin first proposed the red junglefowl *Gallus gallus spp* as its sole ancestor. However, molecular evidence of introgression from the gray junglefowl *G. sonneratii* in the form of the yellow skin locus (Eriksson et al., 2008) and success in producing fertile offspring from *Gallus*

spp hybrids (Danforth,1958; Morejohn, 1968) has challenged the single species origin for the domestic chicken. In this project, we analyzed the full genomes of 50 birds, including: 4 junglefowl species, indigenous chickens from Ethiopia, Sri Lanka, and Saudi Arabia, as well as European fancy chicken for evidence of introgression from *G. sonneratii*, *G. lafayetii*, and/or *G. varius*. Using the pooled heterozygosity (Rubin et al., 2010), ABBA-BABA (Martin et al., 2013) and *Fst* (Weir and Cockerham, 1984) statistics, we identified several candidate regions of introgression from *G. sonneratii* and *G. lafayetii* into domestic chicken and vice versa. These regions represent new genomic landmarks of the selection pressures that have shaped the genome of domestic chicken and may provide us with new insights on the history of the geographical dispersion of domestic chicken populations.

Key Words: Gallus, introgression, genome

P1020 Identification of polymorphisms modifying gene expression regulation in cattle.

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Thanks to the advent of novel sequencing technologies, an increasing number of polymorphisms have been identified in genic regions. These polymorphisms can play an important role in gene expression regulation. Allele-specific expression (ASE) analysis is a robust approach to detect *cis*-regulatory variations of gene expression. Because of its economic importance, cattle were 1 of the first mammals to have its genome sequenced. During this sequencing, more than 2.2 million putative single nucleotide polymorphisms (SNP) have been detected. Many bovine genomes have been sequenced and there are currently more than 99 Million SNPs. Polymorphisms showing allele-specific expression could be linked to economic important traits and therefore could help improve genetic selection. The aim of our project was to develop a pipeline to predict polymorphisms that modify the regulation of gene expression. Association studies between these predicted polymorphisms and important phenotypes will later be performed. We used RNA-seq data from muscle samples of 19 Limousin bull calves (77 Million reads in average per samples) and from 8 different tissue samples (heart, kidney, liver, lung, muscle, ovary, spleen, and uterus) of 6 Holstein cows (65 Million reads in average by samples). We also had the whole-genome DNA sequences for these 25 animals (an average coverage of 15 Limousin samples and 5 Holstein samples). The RNA-seq data were aligned with STAR, an ultrafast RNA-seq aligner, and we predicted polymorphisms (SNPs and small insertions/deletions) with GATK for DNA and RNA sequence data. The ASE detection was performed using ASEReadCounter. We detected more than 150,000 SNPs showing ASE in all samples with this method. Currently, our pipeline can detect SNPs in genes with an allelic imbalance for species with a reference genome sequence available. This pipeline will be improved by analyzing indels and adding the detection of regulatory SNPs. The most interesting polymorphisms will be validated experimentally.

Key Words: bioinformatics, polymorphisms, regulatory genomics, sequencing, transcriptomics

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P1021 A next generation, semiconductor-based target re-sequencing DNA pool-seq approach for the identification of SNPs and association studies: Application to bitter taste receptor genes in different pig populations.

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In general, association studies for any traits rely on previous identification of polymorphisms that are then genotyped in individuals that are also phenotypically evaluated to establish a link between genotypes and phenotypes. In this study, we applied a next generation sequencing approach based on target re-sequencing to identify, at the same time, novel polymorphisms and to define associations between alleles and production traits. The experimental design was based on a selective DNA pool-seq approach, in which 2 DNA pools, containing equimolar DNA from extreme and divergent pigs for back fat thickness (BFT), 50 pigs with positive estimated breeding values (EBV), and 50 pigs with negative EBVs for this trait, were amplified for several genes. Estimated allele frequencies, obtained by counting generated reads carrying alternative alleles, were compared between the 2 extreme pools and significant differences were calculated. The same approach was also used to characterize population differences by comparing allele distribution in DNA pools from different pig breeds (Italian Duroc, Italian Landrace, Pietrain, Meishan, and Casertana) and wild boars. The targeted genes were 9 porcine bitter taste receptor genes or TAS2R: TAS2R1, TAS2R3, TAS2R4, TAS2R7, TAS2R9, TAS2R10, TAS2R16, TAS2R38, and TAS2R39. About 1.8 million of reads were obtained by sequencing amplicons generated from 8 DNA pools (2 with extreme and divergent pigs

for BFT-EBV and 6 from different breeds and wild boars), using an Ion Torrent PGM sequencer. One hundred twenty-five SNPs were identified, of which 37 were missense mutations, a few of them with important effects on the function of these bitter taste receptors, as inferred by in silico analysis. Variability in wild boars was lower than that in domestic breeds, as a potential result of selective pressure in the wild toward defensive bitter taste perception. Three SNPs were significantly associated with the investigated production trait. Our approach can be further implemented by increasing the number of targeted genes for a whole genome candidate gene association analysis, using this selective DNA pool-seq experimental design

Key Words: nutrigenomics, Ion Torrent, selective DNA pooling

P1022 Bridging SNP genotyping platforms to high-throughput computing for agricultural genomic applications: A perspective from a commercial service provider. X. L. Wu*, J. Qiu, J. Walker, B. Simpson, S. Bauck (GeneSeek, a Neogen Company, Lincoln, NE)

As agricultural genomic data are getting drastically larger and larger, these have increasingly brought challenges on a variety of aspects, including data capture, data curation, search, sharing, storage, transfer, visualization, querying, concordance verification, information privacy, and processing. Strategic management and use of these big agricultural genomic data can lead to improved accuracy in decision making, which in turn results in greater efficiency in agricultural operations and production, as well as reductions in cost and risk. From the prospective of a service provider of agricultural genomics, primary priorities are placed on data storage and management, high-throughput data processing, and automation of pipelines that bridge the upstream genotyping to downstream data processing and reporting. As relational database management systems and desktop applications and visualization packages are becoming inadequate for handling big genomic data, cost-effective and state-of-the-art solutions are in need. Automation of pipelining that bridges the upstream sequencing to downstream applications does not only reduce the chance of human errors, it also increases the throughput of data processing and computing. At the core of data processing, however, is the capacity, infrastructures, strategies, and software of parallel computing for agricultural genomic applications. When Moore's Law meets Amdahl's law, what prevents us from moving to "multiple tracks"? There are 2 apparent paradoxes with parallel computing for agricultural genomic applications. The first is computational, which is seen between multi-core architectures of personal computers and workstations currently in use, and sequential application programs that run on them. The second is methodological, which comes with the need for parallel Bayesian model computing via Markov chain Monte Carlo and the latter is intrinsically sequential in its algorithm. Theories and strategies for parallel MCMC are reviewed, which include running multiple chains, parallelizing single MCMC chains, and cascaded parallel computing. Thumb rules of parallel computing for agricultural genomic applications are presented as well. While task parallelization is the main form of parallel computing, we show an application of data-parallel ridge regression BLUP in genomic prediction for a large U.S. Holstein population. If you were plowing a field, which would you rather use: 2 strong oxen or 1,024 chickens? The famous SRC (Seymour Cray) argument is still remembered, yet are we otherwise in the age of "1,024 chickens," which symbolizes massive computing, using graphic processing units (GPU)?

Key Words: high-throughput computing, Holstein, genomic selection, SNP genotyping

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P1023 RNA editing in swine is associated with PRE-1 retrotransposons. S. A. Funkhouser*[1], J. P. Steibel[2,3], R. O. Bates[3], N. E. Raney[3], C. W. Ernst[3] ([1]Genetics Program, Michigan State University, East Lansing, MI, [2]Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, [3]Department of Animal Science, Michigan State University, East Lansing, MI)

Thousands to millions of adenosine to inosine modifications (A-to-I) have been detected throughout human and mouse transcriptomes, using high-throughput sequencing technology. Caused by a form of transcriptional regulation known as RNA editing, these modifications are catalyzed by the enzyme adenosine deaminase acting on RNA (ADAR), which catalyzes A-to-I edits along double-stranded RNA. ADAR editing has been shown to be conserved across Mammalia. Yet, very little work has been done to evaluate genome-wide RNA editing of mammalian transcriptomes in species such as the pig. Our objective is to provide the first description of the swine "editome," by using whole genome sequencing and strand-specific RNA sequencing from liver, subcutaneous fat, and longissimus dorsi muscle tissues. Bioinformatic algorithms for mapping and variant calling were used in conjunction with our in-house R software editTools to identify mismatches between DNA and RNA sequence, indicative of sites that have undergone RNA editing. Whole genome sequencing was

used to identify homozygous sites with a sequencing depth of at least 10 reads and 95% of all reads in agreement. To be considered a candidate RNA editing site, an allele differing from the genotype call must have been present among at least 5 RNA sequencing reads at a homozygous site. Only uniquely mapped reads and bases of high quality (Phred score >25) were used in variant calling. Candidate RNA editing sites were required to have a Phred-scaled strand bias P-value of at least 20. Positional biases of mismatches indicative of artifacts introduced during first-strand cDNA synthesis were addressed by trimming 6bp of the 5' ends of RNaseq reads before alignment. This approach yielded a total of 8,550 DNA-to-RNA mismatches, with 75% exhibiting the canonical A-to-G "ADAR-like" signature. As is found in other species, the majority of A-to-G mismatches were within repetitive sequences (94%). Interestingly, the swine-specific PRE-1 retrotransposon, an element highly analogous to the human Alu, appears to be preferentially targeted by ADAR RNA editing. Like Alu elements, PRE-1 may influence RNA editing through its ability to enhance double-stranded secondary structure within the transcriptome, thereby creating substrates for ADAR. However, several candidate ADAR edits identified in pig protein coding regions have previously been validated in primate studies, suggesting that although species-specific retrotransposons are associated with ADAR activity, certain features of RNA editing may be conserved across species possessing distinct repetitive element repertoires.

Key Words: PRE-1, transcriptomics, R software

P1024 Inferring genotypes of functional variants in crossbred beef cattle. W. M. Snelling*, L. A. Kuehn, A. K. Lindholm-Perry (USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE)

The current cost of sequencing individual genomes can be justified for influential ancestors of livestock populations, but it is prohibitively expensive to use whole-genome sequencing to genotype large populations. Genotypes for variants detected in sequence might be inferred with haplotype-based imputation, but reported accuracies of imputing sequence variant genotypes are lower than accuracies of imputing high density SNP array genotypes. Linear combinations of high density array SNP were almost perfectly correlated with sequence variant genotypes, suggesting that whole genome approaches might leverage long-range and low-level linkage disequilibrium to infer sequence variant genotypes more accurately than imputation based on haplotypes. Genotypes of animals with low (n = 167), moderate (n = 8596), and high density (n = 1,453) SNP array genotypes, as well as genotypes called from the sequence of 270 bulls, were used to compare haplotype imputation and whole genome approaches to predict sequence variant genotypes. All animals were from the multi-breed Germplasm Evaluation population; the sequenced bulls were the most influential purebred and F_1 sires in that population. Genotypes for SNP on a newly available functional SNP assay were predicted with 4 approaches: 1) population imputation, which filled high density and sequence genotypes based on agreement between high and low density haplotypes; 2) pedigree + population imputation, which considers predicted parent and progeny haplotypes to fill high density and sequence genotypes; 3) GBLUP using imputed high density genotypes to infer sequence variant genotypes; and 4) BayesC to infer sequence variant genotypes from high density genotypes. Accuracy of imputed or inferred genotypes was assessed by correlation with genotypes called from exome sequence, available on a set of crossbred sons and grandsons of the sequenced sires (n = 42). Mean (SE) accuracies for imputing 1988 functional SNP on chromosome 1 were 0.74 (0.01) without and 0.78 (0.01) with pedigree information. Accuracies of inferred genotypes were 0.44 (0.01) using GBLUP and 0.74 (0.01) using BayesC. While imputation with pedigree had the highest average accuracy, 1 of the other approaches was more accurate for two-thirds of the variants. Maximum accuracy was most frequently obtained with BayesC (42%), followed by imputation with pedigree (34%), imputation without pedigree (21%), and GBLUP inference (3%). The mean maximum accuracy was 0.88 (0.003), indicating that accuracy of predicting sequence variant genotypes can be improved by applying multiple approaches. Further exploration is needed to characterize conditions contributing to accuracy of each approach. The USDA is an equal opportunity employer.

Key Words: beef cattle, sequence variants, imputation

P1025 A method for the identification of unfavorable haplotypes contained within runs of homozygosity that impact fitness traits and its application to different swine nucleus lines. J. T. Howard*[1], F. Tiezzi[1], Y. Huang[2], K. A. Gray[2], C. Maltecca[1] ([1]North Carolina State University, Raleigh, NC, [2]Smithfield Premium Genetics, Rose Hill, NC)

The advent of genomic information allows for region-specific stretches of homozygosity that cause inbreeding depression to be identified, rather than

relying on the assumption that 2 individuals with the same inbreeding have similar levels of inbreeding depression. These regions are expected to be at a low frequency so traditional association methods geared toward common variants lack power. Methods that exploit the fact that long runs of homozygosity (ROH) are enriched with deleterious variants may have greater power in identifying unfavorable haplotypes linked to inbreeding depression. Here we implemented a multi-step method that identifies unfavorable haplotypes within ROH and used it in genotyped Landrace (LR; $n = 5001$) and Large White (LW; $n = 6081$) lines. The traits analyzed were number of piglets born alive (NBA), proportion of piglets born dead (PD), and pre-weaning mortality (PWM). After quality control, SNPs were 41,489 and 39,671, for LR and LW, respectively. Genotypes were transformed into presence absence within an ROH of at least 5-Mb (ROH5SNP) and into 5-Mb windows, based on whether the window was an ROH (ROH5W). Across traits, phenotypes were adjusted for farm-year-season and parity, and PWM also included number of initial pigs. Step 1 used yield deviations weighted by the information content in a Bayesian Ridge Regression analysis that included the additive and ROH5SNP effect of an SNP and the individual polygenic effect. Regions in the top 10 percentile based on 1-Mb ROH5SNP variance were investigated further. Step 2 identified ROH5W within regions that passed step 1 that had a significant phenotypic effect (P-value < 0.01). For each ROH5W, a model that included the SNP contained within ROH5W, ROH5W and random and permanent effects of the individual were fitted. The final step involved identifying the unfavorable haplotypes of varying size within ROH5W by using a similar model as step 2 with SNP contained within the ROH5W replaced by haplotype category. Haplotype categories included all non-ROH based haplotypes, as well as each unique ROH haplotype. Contrasts between unique ROH haplotypes and non-ROH haplotypes were estimated. If significant ROH haplotypes (P-value < 0.01) contained the same animals within an ROH5W, only the most significant haplotype was kept. Across breeds, haplotypes that result in a 1.11 to 0.18 decrease in NBA, 1 to 3.9% increase in PD, and 1 to 5% increase in PWM were identified. An optimized version that combines the multi-stage analysis into 1 stage is under development.

Key Words: swine, inbreeding depression, fitness

P1026 Additive and heterotic effects estimation from a F2 Duroc × Pietrain crossbreed using 60K realized breed composition and heterozygosis.

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Heterosis is mainly the result of non-additive genetic effects. It is defined as the difference between the average phenotype of the F1 progeny minus the average of the parental lines. If heterosis is caused by dominance, the values of heterosis are proportional to the loss of heterozygosity, which in turn is defined as the average fraction of the genome with heterozygous loci from the parental lines. Assuming a 2-locus model of genetic effects, the expected value of the phenotype in a F2 progeny from 2 breeds (C and T) can be written as follows: $E(CT) = \mu + k_A A + k_D D + k_{AA} AA + k_{DD} DD + k_{AD} AD$. The coefficients k_A and k_D are functions of the genomic proportion of each breed (S), and of the fraction of the genome in heterozygous state (H), respectively. Usually S and H have been "estimated," using individual breed composition such that all animals at the same crossbreeding stage have the same values of S and H. With the development of SNP arrays technology, thousands of positions in the genome could be genotyped and inheritance of genomic segments could be precisely estimated. Thus, S and H can be "observed" in a specific cross with an adequate method of genotyping. The goal of this research was to estimate the additive and dominance effects from a Duroc × Pietrain F2 crossbred population, using phenotypic data from the F2 animals and genomic data from the F0, F1, and F2. A total of 411 pigs from an outbred 3-generation Duroc × Pietrain resource population were genotyped with the PorcineSNP60 Beadchip. Data on growth (15 traits) and carcass (25 traits) were also collected from the F2 generation (336 animals). For any pair of grandparent–grandson, the identity by descent sharing within the known pedigree, across all SNPs, were inferred with the software PEDIBD and realized values of S and H were estimated in the grandsons by using as reference the breeds of each grandparent. Then, the estimated values of S and H were used to calculate k_A and k_D for each F2 animal, values that were later included as covariates in a mixed model for estimating the additive

and heterotic effects. The realized values of k_A and k_D ranged between −0.480 to 0.377, and from −0.716 to 0.360, respectively, and allowed estimating A, D, AA, AD, and DD for all 40 traits.

Key Words: heterotic effect, SNP, realized heterozygosity

P1027 Application of analysis tools from Affymetrix on Eureka Genotyping Solution to provide accurate and automated animal genotypes. S. Nohzadeh-Malakshah[1], V. Joshi[1], A. Pirani[*2] ([1]Affymetrix, Santa Clara, CA, [2]Affymetrix Inc., Santa Clara, CA)

The BRLMM-P 2-dimensional clustering algorithm is used by mid- to high-plex microarrays from Affymetrix for genotyping single nucleotide polymorphisms (SNP) and insertion/deletions (indels). The algorithm has successfully been implemented across arrays designed to study many dozens of organisms. Its Bayesian step gives it the flexibility to automatically adapt to cluster patterns exhibited by diploid and various levels of allopolyploidy. BRLMMP also incorporates methods for accurately genotyping samples originating from normal and inbred populations. SNPolisher is a tool developed by Affymetrix to calculate SNP quality control metrics, such as call rate, cluster separation, cluster variation, and deviation from expected cluster position. These metrics are used to classify the SNPs into performance categories for downstream applications. Eureka Genotyping Solution is an affordable, low- to mid-plex, high-throughput genotyping assay that uses common next-generation sequencing (NGS) platforms for signal readout. It enables the detection of tens to thousands of genetic markers, which are increasingly in demand for routine animal agrigenomics testing. Using the tools developed by Affymetrix for genotyping high-density SNP microarrays to analyze the low-density Eureka genotyping panels will automate the genotyping process, produce highly accurate results, and allow researchers to migrate between Axiom and Eureka platforms seamlessly. The Eureka NGS read counts are appropriately scaled, normalized, and transformed to process genotyping in the BRLMM-P and SNPolisher framework. The BRLMM-P algorithm has been shown to work on both microarray intensity and NGS reads. Various Eureka genotyping panels are tested, including a bovine panel, for which BRLMM-P generates an average sample call rate of 99.9% and an average sample concordance of 99.5%.

Key Words: Affymetrix, Eureka, genotyping

P1028 Comparing 2 strategies for selecting low density SNPs for imputation-mediated, multiple-trait genomic prediction in a U.S. Holstein population. J. He[*1,2], X. L. Wu[3], S. Bauck[3], J. Q. Xu[2], J. Lee[2], G. Morota[2], S. D. Kachman[2], M. L. Spangler[2] ([1]Hunan Agricultural University, Changsha, China, [2]University of Nebraska-Lincoln, Lincoln, NE, [3]GeneSeek, a Neogen Company, Lincoln, NE)

Genomic selection using single nucleotide polymorphism (SNP) chips to genotype breeding candidates has become pervasive in the dairy industry. Though the cost of SNP genotyping has dropped substantially in the past 10 yr, use of high-density (HD) SNP chips is still relatively expensive in practice. However, low-density (LD) SNP chips offer a cost-effective alternative and thus are gaining interest. LD SNPs are either chosen based on their map locations (e.g., evenly spaced) or selected according to their effects on quantitative traits of interest. Genomic prediction using LD SNP genotypes, however, can suffer from loss of genomic information, leading to decreased prediction accuracy. Preferably, moderate (MD) or HD SNP genotypes are imputed from LD genotypes and used in genomic prediction, yet strategies for selecting LD SNPs for imputation-mediated genomic prediction has not been addressed adequately. Consequently, we evaluated 2 fundamental strategies for selecting LD SNPs for imputation-mediated, multiple-trait genomic prediction, using Holstein animals (n = 11,106), each genotyped with 77,326 SNP (GGPHD or 80K chip). The quantitative traits included predicted transmitting abilities for milk yield, fat yield, and daughter pregnancy rate. Briefly, LD SNPs were selected according to their effects estimated using either single-trait Bayesian regression (STBR) models or multiple-trait Bayesian regression (MTBR) models. The STBR method selected 2K SNPs specific to each trait and then pooled them into a common panel. Because there were overlapping SNPs among these 3 traits, it allowed inclusion of an additional subset of map-optimal, informative SNPs, totaling 7K LD SNPs. The MTBR method selected 7K SNPs that were informative for all the traits, leaving no space for map-optimal SNPs. For each of the 2 sets, 7K SNP genotypes were imputed to 80K SNP genotypes and the latter were used to compute genomic-estimated breeding values. There were 2 salient features with the proposed strategies: 1) LD SNPs included those associated with the quantitative traits, and genotypes of these SNPs were not subject to imputation errors; and 2) genomic predictions were made based on imputed 80K genotypes, and loss of genomic information was not relevant.

The results showed that the STBR method, which included a subset of map-optimal SNPs, had slightly better imputation accuracy and gave higher genomic prediction accuracy for milk yield and fat yield than the MTBR method. Nevertheless, both strategies performed well in terms of imputation accuracy (99.64 to 99.87%) and genomic prediction accuracy (97.66 to 98.09%) in this Holstein population.

Key Words: low-density SNP chip, genomic prediction, Holstein

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P1029 Pan-microbial detection using Axiom genotyping solution from Affymetrix. A. Pirani*[1], P. Rack[1], K. Mcloughlin[2], L. Le[1], C. Sheppy[1], T. Slezak[2], M. Shapero[1] ([1]Affymetrix Inc., Santa Clara, CA, [2]LLNL, Livermore, CA)

The dynamic interplay among microbial, plant, and animal populations is an active area of study. Agrigenomics-related microbiotas include species from archaeal, bacterial, viral, and eukaryotic origins, and can span the spectrum from beneficial to pathogenic roles. As the catalog of sequenced microbial genomes expands, high density microarrays are well suited to capitalize on this information content. Here we present an overview of Axiom Genotyping Solution and a description of the design, development, and testing of an array for microbial detection. Axiom Microbial Detection Array (Axiom MDA) contains approximately 1.3 million probe sequences targeted toward more than 12,000 microbial organisms. The array design enables species/strain-level resolution from individual samples through optimization of probe sequence parameters, such as length, Tm, and mismatch tolerance. Microbial Detection Analysis Suite (MiDAS) is used to ascertain the identities of targets in an unknown sample. This is done using an algorithm that models the likelihood of the observed pattern of probe intensities as a function of the set of targets present in the sample, and follows a greedy maximization procedure to identify a locally optimal set of targets that best explains the observed intensities. Array performance, as measured by sensitivity and positive predictive value, was evaluated, using defined samples of increasing biological complexity. Additionally, Axiom MDA was tested for the ability to identify microorganisms from bona fide biological samples and results were compared to orthogonal approaches, such as 16S rDNA sequencing. The array was also evaluated for compatibility with nucleic acid extracted from a variety of commercial kits, using samples that include mouse stool and porcine stool, tonsil, and serum. Axiom MDA offers a solution for the identification and enumeration of microbial entities comprising complex biological samples. The unbiased array content design is sample-type agnostic and thus has utility in the context of both plant and animal health and food safety. Furthermore, scalable samples throughput enabled by 24- and 96-array formats, with the potential to expand to a 384-format array plate, coupled with laboratory automation allows processing of hundreds to thousands of samples per week with minimal manual intervention. This feature makes Axiom MDA a cost-effective means for detection and examination of microbiomes.

Key Words: microarray, microbiome, Axiom

P1030 Identification of copy number variations in fine wool sheep using Ovine SNP600 BeadChip array. Y. Tian*[1], X. Huang[1], K. Tian[2], J. Di[2], Y. Bai[2], X. Xu[2], X. Fu[2], W. Wu[2], X. Shi[2], B. Zhao[1] ([1]College of Animal Science, Xinjiang Agricultural University, Urumqi, China, [2]Xinjiang Academy of Animal Science, Urumqi, China)

Copy number variations (CNV) are increasingly recognized as an important and abundant source of genetic variation and phenotypic diversity. Compared to SNPs, CNVs have an impact on phenotypic variation mainly through gene dosage effects and are often associated with economically important traits and disease susceptibility. In current research, identification of CNVs were performed using the Ovine SNP600 BeadChip array data, including 225 fine wool sheep of 8 strains. A total of 656 CNV regions (CNVR) were identified, including 519 losses, 60 gains, and 77 with both events (losses and gains), which cover 43.9 Mb of the sheep genomic sequence and correspond with 1.79% of the autosomal genome sequence. The length of CNVRs on autosomes ranged from 1.27 kb to 7.03 Mb, with a mean size of 91.90 kb. Additionally, 145 CNVR events had a frequency more than 3%. Among these CNVRs, 556 CNVRs identified by the PennCNV overlapped with the CNV partition. Functional analysis indicated that most genes in CNVRs were significantly enriched for specific biological functions, such as immunity, enzyme activator activity, purine nucleotide binding, and neurological system processes. Fortunately, 13 keratin genes associated with wool fiber were included in the 720 annotated genes detected from the fine wool sheep population. Furthermore, 10 CNVRs were selected for validation and 7 CNVRs were further experimentally confirmed by qPCR. In this study, we constructed a fine wool sheep CNV map, based on the Ovine SNP600 array. Our results demonstrated the differences of 2 detection tools and that integration of multiple algorithms can enhance the detection of sheep genomic structure

variations. Furthermore, our findings would be of help for understanding the sheep genome and provide preliminary foundation for carrying out CNV association studies with economically important phenotypes of fine wool sheep in the future.

Key Words: copy number variations, fine wool sheep, Ovine SNP600 BeadChip

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P1031 Genetic and genomic testing of cattle from tissue sample units under Australian conditions.

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The increasing integration of livestock production systems of Australia and elsewhere, including the implementation of traceability programs such as the National Livestock Identification System (NLIS), means that animals are tagged early in life. These programs must accommodate a range of production systems, including those extensive operations where the animal may be handled only sporadically for weaning or other husbandry. With the increased use of genetic and genomic technologies for research and as decision making tools in selective breeding programs, these occasions are being seen as an opportunity to sample animals for genetic testing, including parentage analysis, disease diagnostics, and more recently genomics for use in estimating genetic estimated breeding values. Sample types commonly include hair, blood, semen, and most recently tissues collected during the tagging process. Tissue sampling units (TSU) are now marketed by a range of companies as a convenient way to collect tissue for DNA in parallel with tagging, providing benefits, which include labor reduction, traceability, and reduced risk of transcription errors. These TSUs commonly come with or without preservative, with the dry type being used to great effect in Europe and New Zealand. However, dry TSUs have proven problematic under Australian conditions. Transit times for postage under hot and often humid conditions often lead to rapid deterioration of the tissue, such that DNA quality is compromised and testing impossible. To optimize the collection of workable samples for industry, heat challenges were performed that confirmed the superiority of preservative-filled TSUs for genetic testing. These preservative-filled TSUs were further evaluated for tissue stability and quality of DNA extracted for a range of genetic and genomic tests performed in the laboratory, including microsatellite- and SNP-based parentage testing, diagnostics, and genomic applications using SNP genotyping microarray technology. An important research question of this study was also how best to maintain (biobank) tissue or DNA in a cost-effective manner for testing in the future. The current study potentially has insights for future application in studies across a range of tropical and sub-tropical climates.

Key Words: diagnostics, genomics, sampling

P1032 Fine mapping of a QTL for number of teats on SSC7.

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A QTL affecting number of teats has been identified on 936 animals from a Large White population on *Sus scrofa* chromosome 7 (SSC7) in a Genome-wide Association Study (GWAS), using a medium density SNP chip (60K, the Illumina PorcineSNP60 BeadChip). The most promising candidate gene was Vertnin (VRTN), which had been proposed as a candidate gene affecting number of vertebrae in several other pig lines. In a follow-up study, more animals from the same Large White population (n = 2,620) were genotyped and the presence of QTL for number of teats on SSC7 was confirmed. In addition, the same QTL for number of teats was shown to segregate also in a Dutch Landrace, Norwegian Landrace, and Duroc populations (n = 2,491, 6,090, and 3,798 animals, respectively). Subsequently, the most influential sires (n = 64) of the Large White populations were genotyped with the 660K SNP Axiom chip. After imputation from the 60K to the 660K SNP chip of the whole Large White population, the GWAS was repeated, aiming to refine the QTL interval. The most significant SNPs from the 660K SNP chip shift the most likely position around 250.000 bp away from the VRTN locus, which may be an indication that the VRTN is not the candidate gene for number of teats. Currently, the most influential sires (~150 sires per population) from the 2 Landrace and Duroc populations are being genotyped with the 660K SNP. As a further step, genotypes of all populations will be imputed from the 60K to the 660K chip. After imputation, GWAS within and across populations will be performed using the 660K data. For further fine mapping, sequence information is available from about 20 animals of each population. All these data will be evaluated with the aim to refine the QTL interval, determine the candidate gene, and point out possible causal variants.

Key Words: pigs, gene, GWAS

P1033 Relaxation of purifying selection is prevalent among domesticated animals. J. Chen[*1], X. Du[1], C. Zhang[2], S. Zhao[1] ([1]Huazhong Agricultural University, Wuhan, China, [2]Kib, Kunming, China)

Although inherited directly from their wild ancestors, domesticated animals had undergone dramatic dynamics in both population size and selective force. However, it is still poorly understood the extent to which these changes may impact the evolution of animal genomes from wild-types to domesticated forms. In this study, we compared ratios of nonsynonymous to synonymous substitutions (Ka/Ks) in 8 domesticated animals (pigs, dogs, goats, sheep, cow, cat, rabbit, and turkey) and their wild relatives on a genome-wide scale. We observed higher Ka/Ks ratios in these domesticated animals. We suggest that the common trend in these 8 domesticates may indicate the relaxation of purifying selection accompanying domestication, which might be attributed to the strong demographic bottlenecks of their founders and subsequent artificial selection processes. The genome-wide accumulation of nonsynonymous changes may serve as an important source of phenotypic diversification in domesticated animals.

Key Words: relaxation of selection, domesticated animals, Ka/Ks ratio

P1034 CRISPR-offinder: A CRISPR guide RNA design and off-target searching tool for user-defined protospacer adjacent motif. S. Xie*
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CRISPR/Cas system undoubtedly holds great potential for genome editing. Target site cleavage by the CRISPR technology requires a PAM immediately downstream or upstream of the protospacer element to which the sgRNA binds. However, Cas9 from different types of bacteria or variant recognizes different PAM sequences. In addition, the recent study reveals the potential of the Cpf1 nuclease to complement and extend the existing CRISPR-Cas9 genome-editing tools. Cpf1 is a single RNA-guided endonuclease that lacks tracrRNA. It uses a T-rich PAM and is on the 5' side of the guide. Another report showed that C2c1 systems can also mediate DNA interference in a 5'-PAM-dependent fashion analogous to Cpf1. These newly found engineered nucleases broaden the range of genome editing experiments. Design efficient and specific CRISPR small guide RNAs (sgRNA) is 1 of the keys for a successful application of CRISPR technology. Importantly, more and more new RNA-guided endonucleases with different protospacer adjacent motif (PAM) have been discovered. Therefore, there is an urgent need to develop a versatile tool to design sgRNA to satisfy the requirement of different RNA-guided DNA endonucleases. To this end, a flexible sgRNA design program named "CRISPR-offinder" was developed. The most important feature of this new program is that it supports all known PAM types, as well as the customer-defined PAM. Besides, CRISPR-offinder can find and rank the candidate sgRNAs in genome by off-target sites number and also can be used to design single or paired-gRNAs. CRISPR-offinder is freely available as a command-line program or accessible via our web site. CRISPR-offinder is freely available at http://crispr-offinder.com or http://crispr.igenetech.com.

Key Words: CRISPR, bioinformatic tool, PAM

P1035 Ensembl: A comprehensive bioinformatics infrastructure for vertebrate genetics. D. R. Zerbino*, B. Aken, L. Clarke, F. Cunningham, A. D. Yates, P. Flicek
(European Molecular Biology Laboratory, European Bioinformatics Institute, Hinxton, Cambridge, United Kingdom)

Ensembl creates and maintains many widely used and authoritative genomic reference data sets, which help drive the use of genomics from basic biology to translational research. We are constantly on the lookout for research collaborators to expand our coverage of species. We provide a curated set of annotations all available in 1 resource and support 89 reference genomes enriched with comprehensive gene and transcriptome annotations, whole genome alignments, gene phylogenies, genetic markers, QTLs, and regulatory elements. All these resources were built in collaboration with specialists of each species who provided us with the primary data underlying our annotations. Ensembl is also a web browser that allows users to explore the data interactively. In particular, it offers a diversity of specialized angles on the data, along and across species: genomic regions, gene trees, genome alignments, details of a gene and its transcripts, variants associated to a phenotype, details of a genetic marker, and details of a transcription factor binding site. Finally, Ensembl is a database that is available freely and directly to all. A creative bioinformatician who invents a new visualization tool, tailored to the needs of a research community, can automatically obtain our annotations and display or analyze them as they please. Ensembl resources are accessed by thousands of researchers, tens of millions of times each year via our genome browser, programmatic interface, and specialized tools, such as the Variant Effect Predictor (VEP). To go beyond our current collection of

species, we are actively seeking research communities that are building resources around a common genome assembly registered at INSDC and need a solid bioinformatics infrastructure to gather their experimental data. For example, we are currently active collaborators in the Functional Annotation of Animal Genomes (FAANG) consortium and are collaborating on various species, such as zebrafish, rat, baboon, vervet monkey, spotted gar, coelacanth, ferret, cat, cavefish, gibbon, horse, cichlid, and zebra finch. We generally offer gene annotation support. However, we can also provide comparative analyses, variant annotation, and define regulatory elements.

Key Words: bioinformatics, database, genomics

P1036 A reduced panel to determine beef cattle breed composition. L. A. Kuehn*, W. M. Snelling, A. K. Lindholm-Perry (USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE)

The advent of high density marker arrays has revolutionized genetic prediction and selection in beef cattle. In addition to genetic prediction, these marker arrays can be useful for deriving breed composition of cattle with unknown origins. Using marker arrays with thousands of markers, we are able to predict breed composition with high (>95%) accuracy, using multiple regression on known breed frequencies for each marker. Knowledge of breed composition can help with traceback and management objectives, but the cost of buying a marker array can be prohibitive, especially for commercial animals. With low cost tools, such as genotyping by sequencing, it may be possible to estimate breed composition at a significantly lower cost using fewer markers. Our objective was to develop a reduced panel of markers (400 or fewer) that could accurately predict breed composition. This panel was a subset of markers from a large commercially available array with more than 770,000 SNP; approximately 500 animals from 9 different breeds (Angus, Hereford, Red Angus, Shorthorn, Charolais, Simmental, Gelbvieh, Limousin, and Brahman) had been genotyped using this array. The markers for the reduced panel were selected as the marker with the highest F_{ST} over 20 Mb intervals. Because Hereford and Brahman were the most genetically distant breeds, they were excluded from F_{ST} calculations. In total, 350 SNP markers were selected for the panel. Efficacy of the marker set was tested on a set of crossbred animals with known pedigrees. The correlation between pedigree and panel-predicted breed composition was 0.96. Performance was similar for a panel of 145 SNP (r = 0.93 to 0.95) selected from these 350. A random set of 120 SNP markers from the 770,000 SNP array achieved a correlation of 0.88 with pedigree breed composition. A reduced panel of markers can effectively predict breed composition with lower overhead costs relative to high-density arrays. The USDA is an equal opportunity employer.

Key Words: beef cattle, breed composition, genetic markers

P1037 Predicting regulatory SNPs within enhancers and promoters in cattle. Q. Nguyen, R. L. Tellam, J. Kijas, W. Barendse, B. P. Dalrymple* (CSIRO Agriculture, Brisbane, Australia)

Causative SNPs are better predictors of phenotype than associated SNPs. However, very few causative SNPs contributing to complex phenotypes have been identified in production animals. Most causative SNPs lie within non-coding regions and alter the expression of genes by changing the activities of promoters and enhancers. In many cases, this is by affecting the binding of transcription factors (TF). The aim of this work was to identify and weight the effects of such regulatory SNPs to improve genome-wide selection models for breeding in cattle. Compared to research in humans and mice, there has been little functional characterization of genomic regulatory regions in domesticated animals. The Functional Annotation of Animal Genomes (FAANG) project is just starting to address this deficiency in a coordinated fashion. To bridge the gap to the availability of large FAANG data sets for cattle, we have developed a computational pipeline to use human promoter and enhancer databases, many built on the outputs of the human ENCODE, ROADMAP, and FANTOM projects, for the prediction and ranking of general and tissue-specific enhancers and promoters in cattle. The promoter predictions confirmed existing and identified many potential new annotations for transcription start sites of sense/antisense RNAs, coding/non-coding RNAs, and alternative promoter usage in cattle. Predicted enhancers were confirmed and ranked by existing cattle-specific coding/noncoding gene annotation, tissue-specific RNA sequencing data, H3K27Ac epigenetic enhancer marks, and DNA sequence features, including putative TF binding sites, dinucleotide patterns, and conservation scores. Level of expression of TFs in tissues also informed the ranking of SNP effects. Based on the cattle genomic sequence, changes in functional activities of putative cattle enhancers for sequences with SNPs compared to corresponding reference sequences were estimated by a machine learning approach, which weighted functional DNA sequence units (10 kmers) by comparing positive and negative enhancer sets from human data sets. The validity of this cross

species approach was supported by applying it in reverse. For a small set of human enhancers, the predicted scores with a cattle model were compared with published experimental investigations of the effects of mutations on the activity of the human enhancers. Furthermore, using human DNA-DNA interaction data (5C and HiC), gene targets of cattle SNPs can be predicted. This work generates a comprehensive database of regulatory regions in cattle and presents a computational pipeline, using rich data resources from humans and mice to predict regulatory regions and SNP effects on phenotypes in production animals.

Key Words: cattle

P1038 BovineMine: A bovine genome data mining warehouse. C. G. Elsik*, D. R. Unni, A. Tayal, C. M. Diesh, D. E. Hagen (University of Missouri, Columbia, MO)

BovineMine (http://BovineGenome.org/bovinemine), a data warehouse developed using the open-source InterMine system, allows users to create custom-integrated bovine genomics data sets. BovineMine includes bovine gene annotations (NCBI, Ensembl, bovine OGS), protein annotations (UniProt), protein families and domains (InterPro), homologs and orthologs (OrthoDB, TreeFam, EnsemblCompara, HomoloGene) pathways (Reactome), gene-gene interactions (BioGRID), Gene Ontology (GO), QTL (AnimalQTLdb), SNP (dbSNP), and tissue-specific gene expression (SRA). BovineMine provides reports for various entities, such as genes, transcripts, proteins, and ontology terms, along with tools that allow users to analyze and download genome-wide data sets. The QueryBuilder tool allows users to construct custom queries that integrate the BovineMine data sets, whereas predefined query templates provide starting points for data exploration. Researchers can load their own lists of identifiers using the List tool. Or, they can load lists of chromosome locations using the Genomic Regions tool to connect their data sets to the BovineMine data. Results are provided as tables that can be filtered, reorganized, and downloaded in various formats. BovineMine allows users to mine tissue-specific gene expression levels together with genomic variation data, a function not previously available to bovine researchers. Users investigating known or novel SNPs can quickly retrieve functional information for genes within specified ranges. Variant effects for known SNPs are pre-computed using the Ensembl Variant Effect Predictor. The integration of orthologs in BovineMine enables researchers to leverage the curated gene pathways of model organisms (e.g., human, mouse, and rat) based on orthology and is especially useful for GO and pathway analyses in conjunction with GWAS and QTL studies. In addition to the bovine genome, BovineMine includes sheep and goat genes so that those research communities can leverage information from ruminant and model organism orthologs.

Key Words: bovine, genome, data mining

P1039 Bioinformatics resources for animal genomics using CyVerse cyberinfrastructure. J. E. Koltes*[1], J. M. Reecy[1], E. Lyons[2], F. McCarthy[2], M. W. Vaughn[3], J. P. Carson[4], E. Fritz-Waters[1], J. Williams[5] ([1]Iowa State University, Ames, IA, [2]University of Arizona, Tucson, AZ, [3]Texas Advance Computing Center, University of Texas, Austin, TX, [4]Texas Advanced Computing Center, University of Texas, Austin, TX, [5]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY)

In 2015, the life science cyberinfrastructure iPlant Collaborative was rebranded to CyVerse (www.cyverse.org) to emphasize an expanded mission to provide computational resources across the life sciences, including animal genomics. Currently, CyVerse is a community of more than 30,000 users at every level of bioinformatics experience (from little-to-no bioinformatics training to computationally savvy experts). In the area of genomics, we support more than a dozen core workflows (e.g., genome/transcriptome assembly, RNA-Seq, variant calling, and Methylation analysis) and hundreds of bioinformatics applications and services. Community input and needs shape the tools we provide, such as the pipelines and infrastructure developed by research groups in the animal genetics community. A community of animal genomics researchers has developed genotyping pipelines using next generation sequencing (NGS) data, as well as data visualization and processing tools to connect NGS data sets to annotation, data processing, and other existing tools at CyVerse. These resources are tailored for the animal genomics community with a goal of providing computational resources needed to facilitate genotype to phenotype analysis, using large data sets. To date, genotyping has been conducted with NGS data from several genome projects. In addition, users from the aquaculture, cattle, chicken, horse, pig, and sheep genomics research communities have contributed data for the development of CoGe iAnimal data visualization and processing resources. Users of these resources can provide feedback to add additional functionality or they can add additional tools using the existing CyVerse infrastructure. CyVerse supports the life cycle of data with tools for easy data upload, sharing, and even publication directly to the NCBI Short Read Archive. Users within CyVerse can customize

their experience by bringing their own bioinformatics tools and pipelines (via Docker, virtual machines, or deployment via our APIs). Finally, CyVerse tutorials, in-person and virtual training, and community support (e.g., user forum, campus helpers) help users get started analyzing and managing their data. Researchers within the animal genomics community have made extensive use of CyVerse computing and continued collaboration and development of community-driven tools is desired. All tools and services are freely available to the community and CyVerse is funded entirely by the National Science Foundation (Award Numbers DBI-0735191 and DBI-1265383). All tools developed specifically for researchers in animal genomics were supported by Agricultural and Food Research Initiative Competitive Grant award numbers 2013–67015–21231, 2013–67015–21210 from USDA-NIFA.

Key Words: bioinformatics, cyberinfrastructure, next-generation sequencing analysis

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P1040 Diet analysis of grasscutter using next generation sequencing. C. Adenyo*[1,2], H. Ando[3], B. B. Kayang[1], E. Inoue[4], M. Inoue-Murayama[2] ([1]University of Ghana, Accra, Ghana, [2]Kyoto University, Kyoto, Japan, [3]National Institute for Environmental Studies, Tsukuba, Japan, [4]Toho University, Funabashi, Japan)

Grasscutter (*Thryonomys swinderianus*) is a rodent that is endemic to Sub-Saharan Africa, where it is hunted for its meat, especially in Western and Central Africa. In Ghana, the meat of the grasscutter is a delicacy and therefore commands a premium price. There are ongoing efforts to domesticate this species to make its meat readily available to reduce the devastating impact of its hunting on the environment. To fully domesticate the grasscutter, however, understanding its feeding ecology is necessary. In this study, we investigated the diet of the wild grasscutter to identify plant species that constitute their diet. Digesta samples were taken from the cecum of 8 wild grasscutters (4 samples each in the rainy and dry seasons). A plant barcoding marker, trnL, was amplified and sequenced, using the next generation sequencing technology (Roche 454 GS Junior). A total of 20,777 reads were obtained. Homology search of the reads with registered sequences and family-level analysis revealed 54 different families of plants in the grasscutter digesta, with the major component being *Poaceae* (grasses). However, other dominant families, including *Fabaceae* (legumes), *Euphorbiaceae* (spurges), *Cyperaceae* (sedges), *Rutaceae* (Citrus), *Iridaceae* (iris), *Gesneriaceae* (gesneriads), *Fagaceae* (beeches), *Asteraceae* (daisies) and *Arecaceae* (palms), were also found. In terms of seasonal effect, no significant difference was found in the families between the dry and rainy seasons, except *Asteraceae*, which was marginally significant (Mann–Whitney U test, $p < 0.05$). These results shed light on the wild grasscutters' diet and this information could be used to improve the feeding of domesticated grasscutters.

Key Words: digesta, grasscutter, next generation sequencing, domestication, trnL marker, DNA barcoding

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P1041 Application of artificial neural networks to genome-enabled prediction of growth traits in Brangus heifers. S. O. Peters*[1], M. Sinecen[2], K. Kizilkaya[2], M. Thomas[3] ([1]Department of Animal Science, Berry College, Mount Berry, GA, [2]Adnan Menderes University, Aydin, Turkey, [3]Colorado State University, Fort Collins, CO)

Recently, artificial neural networks (ANN) have been proposed as promising methodology for marker-based genomic predictions of complex traits in animal and plant breeding. ANN provides nonlinear relationships between inputs and outputs with the interplay among variables learned adaptively. ANNs are interesting candidates for analysis of traits affected by cryptic forms of gene action. However, there have been only a few empirical applications of ANN to genome-enabled predictions in animal breeding. In this study, Feed Forward Multi-Layer Perceptron ANN model was used for predicting growth traits: birth weights and 205th and 365th day weights in Brangus heifers with first chromosome marker data set. For the Brangus heifers, input variables (first chromosome, 3,552 markers) were derived from 53,695 single nucleotide polymorphisms (SNP) marker information on 743 individuals. MLP-ANN model, which is 2-layer (single hidden layer) feed forward neural network model, has 3,552 inputs, 10-node single-hidden layer, and 1 output. For the MLP-ANN model, 70% of the animals were randomly allocated to a training set, 15% to a validation set, and 15% to a testing set. MLP-ANN model achieved predictive correlations of r = 0.53 for birth weight, r = 0.65 for 205-d weight, and r = 0.63 for 365-d weight. Results suggest that neural networks may be useful for predicting complex traits using high-dimensional genomic information.

Key Words: Brangus, growth, neural network

P1042 Combining RNA sequencing technologies to annotate the bovine genome. D. E. Hagen*, D. R. Unni, C. G. Elsik (University of Missouri, Columbia, MO)

Bovine genome annotation with RNaseq has been challenging due to the complexity of alternative splicing, potential for read mapping errors, high repeat content of the genome and expressed sequences, and the need to assemble full-length transcripts from short reads. We have re-annotated the bovine genome using multiple RNA sequencing technologies. We used RNA sequencing data generated from 96 tissues, or subsets of those tissues, from L1 Dominette 01449, using single-end unstranded Illumina, paired-end stranded Illumina, and PacBio Iso-Seq technologies. After combining these data, we report improvements to the gene set due to adding new isoforms, extending coding regions, extending UTR, and correcting predicted genes that needed to be merged. In addition to updating existing protein-coding gene sets, we identified and characterized novel gene loci, most of which were long non-coding RNA (lncRNA). The lncRNA lack the ability to code for protein but serve as key regulators of diverse biological processes. Criteria for identifying lncRNA sequences include lack of coding potential, lack of similarity to other noncoding RNA classes, and at least 200 nucleotides in length. One challenge in lncRNA identification is that they often lack evolutionary sequence conservation. Furthermore, single exon lncRNA have been difficult to predict due to a lack of splice sites to infer strandedness. The combination of stranded and unstranded libraries allowed us to predict single exon lncRNA. After identifying novel bovine lncRNA, we determined their expression in the 96 tissues. Following gene annotation, we identified sequence differences between RNaseq data and genomic sequence. These differences represent RNA editing events. RNA editing is a post-transcriptional process that results in altered transcript sequences due to the modification of nucleotides. Of the known types of RNA editing, the most common is the deamination of adenosine to inosine (A-to-I), catalyzed by adenosine deaminase acting on RNA (ADAR). These nucleotide substitutions occur in a site-specific and tissue-dependent manner, and can lead to increased protein sequence diversity and altered UTR binding sites. A-to-I editing can be observed as an adenosine to guanine (A-to-G) substitution in RNA with respect to the DNA. Our analysis revealed hundreds of A-to-I RNA editing sites in the bovine transcriptome. We predicted the effects of edits within protein coding regions and whether editing in UTR impact miRNA binding sites.

Key Words: RNaseq, genome annotation, lncRNA

P1043 Identification of regulatory elements in 3 domesticated species. H. Zhou*[1], M. E. Delany[2], H. Cheng[3], P. J. Ross[4], I. Korf[1], C. Kern[1], P. Saelao[1], Y. Wang[1], T. Kim[1], J. Chitwood[1], M. Halstead[1], J. F. Medrano[4], A. L. Van Eenennaam[4], C. K. Tuggle[5], C. W. Ernst[6] ([1]University of California, Davis, Davis, CA, [2]University of California-Davis, Davis, CA, [3]USDA-ARS Avian Disease and Oncology Laboratory, East Lansing, MI, [4]University of California, Davis, CA, [5]Iowa State University, Ames, IA, [6], Michigan State University, East Lansing, MI)

The technologies and assays developed in the human and mouse ENCODE projects provide a solid foundation to functionally annotate domesticated animal genomes. Chicken, pig, and cattle are major farm animals for providing global food production. Robust functional annotations of their genomes could be leveraged to improve their production efficiency and animal and human health. A recent international FAANG (Functional Annotation of Animal Genomes) initiative has stimulated new efforts in functional annotation in these species. The overall objective of this study is to functionally annotate regulatory elements in 3 livestock species. The first key step is to identify regulatory elements in the genomes by integrating RNA-seq, DNase-seq, and ChIP-seq data from important tissues. We will present current progress in generating and analyzing data, including analysis of 48 RNA-seq libraries (16 per species) collected from 2 biological replicates across 8 tissues: adipose, cerebellum, cortex, hypothalamus, liver, lung, muscle, and spleen. For chicken, an analysis of 15 DNase-seq data from all issues with 2 replicates, except hypothalamus (one replicate), show that identified tissue-specific DNase I hypersensitivity (DHS) sites are associated with genes that relate to the unique biological functions of the organs or tissues. Using the 2 replicates to construct a set of DHS sites present in each replicate, we find 29,190 sites in cerebellum, 43,672 in cortex, 52,337 in liver, 64,149 in lung, 27,433 in muscle, and 63,605 in spleen. In addition, 24 ChIP-seq data sets from all tissues (2 replicates), except adipose and muscle (H3K4me3 and H3K27me3 histone modification marks), were generated. For H3K4me3, 19,940 peaks were shared among replicates in cerebellum, 9,979 in cortex, 6,104 in hypothalamus, 5,872 in liver, 29,891 in lung, and 20,323 in spleen. For H3K27me3, 14,000 peaks were shared in cerebellum, 7,525 in cortex, 16 in hypothalamus, 7,642 in liver, 5,807 in lung, and 4,326 in spleen. Integrative analysis of these data sets should allow the identification of genome-wide active and inactive promoter regions, enabling an in-depth comparison of the regulatory landscapes of multiple tissues within chicken and ultimately across the 3 livestock species. This work will lead to a better understanding of the role regulatory elements play on how

the genotype of organisms determines the phenotype.

Key Words: FAANG, domestic species, DNase-seq, RNA-seq, ChIP-seq, regulatory elements

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P1044 Whole transcriptome termini site sequencing: A next generation sequencing method to accurately profile gene expression and alternative polyadenylation with 1 pipeline.

X. Zhou*[1], R. Li[1], J. J. Michal[1], X. L. Wu[1], Z.Liu[2], H. Zhao[2], Y. Xia[2], R. M. Harland[3], Z. Jiang[1] ([1]Washington State University, Pullman, WA, [2]The Chinese University of Hong Kong, Hong Kong, China, [3]University of California Berkeley, Berkeley, CA)

Transcriptome analysis is a powerful tool used to understand the genetic complexity of complex phenotypes that affect animal performance, health, and diseases/defects. Alternative polyadenylation is an essential factor that contributes to transcriptome diversity. We have developed a whole transcriptome termini site sequencing (WTTS-seq) method that can be used to capture 3' ends of transcripts. Our WTTS-seq approach starts with total RNA, followed by chemical fragmentation and enrichment of both polyA+ RNA and polyA+ cDNA. During assay development, we tested 3 types of primers used in PCR for synthesis of second-strand cDNA to complete construction of next-generation sequencing libraries. We found that primer design is a very important factor for accurate coverage of the entire transcriptome. Less than optimal primer design affected product amplification and shifted library composition toward either recessive or dominant noise/bias. By using polyA-anchored primers, we reduced noisy data to less than 0.1%. We also discovered that reduced PCR cycle numbers and lower primer concentrations improved transcriptome coverage. Moreover, we analyzed the same samples using traditional RNA-seq methods and examined WTTS-seq data of biological and technical replicates to reveal both strengths and weaknesses of our WTTS-seq method. Overall, our WTTS-seq method successfully collected polyA sites/regions as signatures for global profiling of gene expression and examination of alternative polyadenylation with 1 pipeline.

Key Words: WTTS-seq, transcriptome coverage, alternative polyadenylation

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P1045 Determination of genome-wide linkage disequilibrium in the South African Bonsmara reference population.

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The largest beef breed in South Africa is the Bonsmara, a locally developed composite breed, consisting of approximately 81,000 registered cows. The Bonsmara was developed specifically for adaptability to sub-tropical conditions, using objective measurements between 1937 and 1963, and as a breed is subjected to mandatory performance recording. Biological samples have also been stored for the last 10 yr. The Bonsmara is therefore well placed to engage in genomic selection and is in the process of compiling a genomic reference population. To date, 583 Bonsmara cattle (388 bulls and 195 cows) were genotyped with the GeneSeek Genomic Profiler Bovine HD Chip (GGP-HD) 80K chip (Neogen, Lincoln, NE, USA) and the results of another approximately 990 Bonsmara cattle are pending, using the GeneSeek GGP-HD 150K chip. In this preliminary study, the genotypes of the initial 583 Bonsmara cattle were characterized for genome-wide linkage disequilibrium, as this may affect the accuracy of genomic estimated breeding values (GEBV). We used 56,248 autosomal SNPs to determine extent of LD in the current reference population. The software package PLINK was used in the analysis and the squared correlation of the alleles at 2 loci (r^2) was used as a measure of LD. A 50 SNP sliding window was used with a 5 SNP increment between windows. Analysis of the marker pairs found that the level of LD in the current reference population decreased with physical distance between SNPs. Overall, the mean r^2 was 0.405. Genomic regions smaller than 420 kb and 815 kb displayed strong LD ($r^2 > 0.8$) and useful LD ($r^2 > 0.2$), respectively. These preliminary results indicate that some population stratification may be present in the reference population and should be taken into consideration during the estimation of GEBVs.

Key Words: genomic selection

P1046 Deciphering chicken fatness trait with integrative genetic and genomic approaches.

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Understanding the functional mechanism of genetic variants that underlie complex traits remains a formidable endeavor, albeit significant efforts have been made toward providing the tools for dissecting

the genotype-phenotype interaction. The limited efficiency and accuracy of QTL mapping often results in large intervals harboring many candidate genes, thereby limiting the ability to identify the causative variants. Nevertheless, the advances in genome sequence information and bioinformatics have enabled molecular phenotyping of organisms quickly at an affordable cost. Through integration of genetic and genomic approaches, we dissected the chromosomal regions involved in regulating fatness traits in broiler chickens. Using 2 broiler lines, divergently selected for plasma very low density lipoprotein (VLDL), we attempted to identify and characterize the genomic regions and genetic variants responsible for regulating this fatness trait. We identified 3 significant QTLs for plasma VLDL and 9 significant QTLs for abdominal fat pad weight, which are both important metrics of fatness. Further incorporation of signatures of selection, using millions of SNPs generated from whole genome resequencing, narrowed the QTL intervals, reflecting the potential of integrative approaches to complement conventional QTL mapping. We found that non-coding regions were enriched in selection signatures, suggesting the underlying variants could be regulatory in nature and further strengthening the regulatory relevance of non-coding loci. Incorporating evolutionary constraint elements previously identified from the multiple genome alignment of 49 sauropods and PacBio transcriptome analysis, this study provided further information for non-coding regions. Adipogenesis pathway and acetate conversion to acetyl-CoA pathway, which are both associated with lipid metabolism, were among the canonical pathways enriched. Our study demonstrated that the integration of analysis of selection signatures with functional annotation of variants enabled refinement and further characterization of the QTL and selection signature regions. Combining quantitative and population genetics with the knowledge of selection history, our integrated approach identified plausible candidate genes and nucleotides, and further elucidated the potential interplay among genes and biological pathways involved in regulating fatness traits in selection lines of broiler chickens.

Key Words: fatness, genetic variants, VLDL

POSTERS: EPIGENETICS AND EPIGENOMICS

P2000 DNA methylation and hydroxymethylation in early rabbit embryo: Consequence of in vitro culture. M. N. Bedhane*[1], J. Salvaing[2] ([1]Jigjiga University, Jigjiga, Ethiopia, [2]INRA, Paris, France)

During the first developmental stages, the embryo's genome is transcriptionally silent and developmental changes are under control of maternally inherited factors (RNA and proteins). Embryonic genome activation (EGA) takes place at later stages (8-16 cell stage in rabbit) and involves epigenetic modifications. CpG methylation is depleted at the early stages and reinstated at the blastocyst stage. DNA methylation at CpG dinucleotides is an important epigenetic mark for embryonic development. Recent findings have shown that demethylation is achieved by oxidation of the methylated DNA into hydroxymethylated DNA. However, the role of hydroxymethylation can probably not be restricted to an intermediate product in DNA demethylation. Indeed, hydroxymethylation seems involved in gene activation and maintenance of pluripotency, and could therefore be important for EGA. Several studies have suggested that in vitro conditions can have a negative impact on epigenetic reprogramming. Thus, 5-methylcytosine (5MeC) and 5-hydroxymethylcytosine (5hMeC) appeared as interesting candidates to investigate the impact of culture media on methylation and hydroxymethylation in rabbit embryos. We used rabbit as a model because the metabolism and timing of EGA in this species is closer to human embryos. The 2 chosen culture media that are commonly used for artificial reproduction technologies (ART) are 1 single-step medium (global), which allows development from zygote to blastocyst, and 1 sequential medium (G1+/G2+), which needs to be changed at the time of EGA. Embryos were fixed at different developmental cell stages: 2-, 4-, 8-, and 16-cell stages. To quantify the level of methylated and hydroxymethylated DNA in the nuclei, we implemented an immunofluorescence-based detection protocol. Finally, the methylated and hydroxymethylated DNA were quantified using an appropriate procedure developed in the host laboratory. Our result shows that the dynamics of 5MeC and 5hMeC are different between the 2 culture media. In the sequential 1, methylation increases between 4-cell and 8-cell stages, while there is no significant change in hydroxymethylation between 2-cell and 16-cell stages. In the single-step 1, hydroxymethylation decreases until the 8-cell stage and increases afterward, while no change

is observed in methylation between 4-cell and 8-cell stages. To draw solid conclusions, it is advisable to reproduce the experiment with other applicable species, such as bovine embryos, ahead of further steps to demonstrate on human embryos. Our results will be helpful for the advancement of ART, which is challenged by abnormal embryonic development and unsuccessful pregnancy.

Key Words: DNA methylation and hydroxymethylation, epigenetics, in vitro culture, embryo

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P2001 CD4 promoter hyper methylation is associated with lower gene expression in clinical mastitis cows and vice versa in the healthy controls. T. Usman[*1,2], Y. Yu[3], Y. Wang[3] ([1]Abdul Wali Khan University Mardan, Mardan, Pakistan, [2]Institute for Farm Animal Biology, Dummerstorf, Germany, [3]College of Animal Science and Technology, China Agricultural University, Beijing, China)

Mastitis is the most common and costly disease of economic and animal welfare concern in dairy cattle. Cluster of Differentiation 4 (CD4) is known for its role in a variety of inflammatory conditions in different species. This study was designed with the objectives to assess methylation levels of CD4 promoter CpG island in peripheral blood cells using pyrosequencing assay, and investigate gene expression by quantitative real time PCR for clinical mastitis in Chinese Holsteins and healthy controls. A total of 120 samples of Chinese Holstein cattle, including clinical mastitis (n = 60) and healthy controls (n = 60), were analyzed in this study. Student t test was used to examine the differential methylation levels and mRNA expression of CD4 gene between clinical mastitis cows and healthy controls. The results showed that all of 5 CpG sites in the CD4 promoter were highly significant hyper methylated in clinical mastitis cows compared with the healthy controls ($P < 0.0001$). The gene expression analysis showed that the mRNA expression was significantly lower in the clinical mastitis cows compared with the healthy controls ($P < 0.01$). Four active transcription factors (cap, Sp1, GATA-1, and GATA-2) were predicted to be present on the CpG sites of the bovine CD4 gene. Moreover, all of the CpG sites were highly significant correlated with each other, except for the correlation between CpG site 1 and CpG site 5. The results showed that hyper methylation of the CD4 promoter was associated with depressed gene expression in clinical mastitis cows and vice versa in healthy controls. The negative correlation between methylation and expression in the CD4 implies that DNA methylation in CD4 could play an essential role in mastitis susceptibility in dairy cattle and thus can be considered a useful epigenetic marker in mastitis resistance studies.

Key Words: CD4, DNA methylation, gene expression, clinical mastitis

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P2002 The conserved functional role of non-CpG methylation in mammalian and avian brain. K. M. Schachtschneider[*1,2], M. F. Derks[1,3,4], O. Madsen[1], V. N. Laine[3], L. B. Schook[2], M. A. Groenen[1], K. J. Verhoeven[5], K. van Oers[3] ([1]Animal Breeding and Genomics Centre, Wageningen University, Wageningen, Netherlands, [2]Department of Animal Sciences, University of Illinois, Urbana, IL, [3]Department of Animal Ecology, Netherlands Institute of Ecology, Wageningen, Netherlands, [4]Bioinformatics Group, Wageningen University, Wageningen, Netherlands, [5]Department of Terrestrial Ecology, Netherlands Institute of Ecology, Wageningen, Netherlands)

DNA methylation is an epigenetic regulator of gene expression that plays a role in many cellular processes affecting a variety of traits. In this study, DNA methylation was assessed in neuronal tissue from 3 pigs (frontal lobe) and 1 great tit (whole brain), using reduced representation and whole genome bisulfite sequencing, respectively. In addition, gene transcription patterns were profiled using RNA-seq. In total, more than 1.5 and 10.2 million CpG, and 5.5 and 167.4 million non-CpG sites were covered in the porcine and great tit samples, respectively. The observed genome-wide DNA methylation patterns in both species were consistent with previous mammalian findings, including low but significant non-CpG methylation that occurred predominantly at CpA dinucleotides. Both great tit brain and pig frontal lobe CpG methylation were negatively correlated with gene expression at transcription start sites (Spearman's rho = -0.30 and -0.16, respectively, $P < 1 \times 10^{-15}$) and gene bodies (Spearman's rho = -0.32 and -0.08, respectively, $P < 1 \times 10^{-15}$). In addition, both great tit brain and pig frontal lobe non-CpG methylation were negatively correlated with gene expression at transcription start sites (Spearman's rho = -0.24 and -0.05, respectively, $P < 1 \times 10^{-14}$) and gene bodies (Spearman's rho = -0.46 and -0.11, respectively, $P < 1 \times 10^{-15}$). Increased CpG and decreased non-CpG methylation were also found within transposable elements (TE) compared with surrounding regions in the great tit brain. TE activity was negatively correlated with non-CpG methylation, both within TEs (Spearman's rho -0.11, $P < 1 \times 10^{-15}$) and the surrounding upstream and downstream 2 kb regions (Spearman's

rho = −0.20 and −0.19, respectively, $P < 1 \times 10^{-15}$). These findings provide the first evidence for conservation of non-CpG methylation in mammalian and avian neuronal tissue, and suggest a functional role for non-CpG methylation in avian neuronal tissue. These results raise interesting questions regarding the universal role of non-CpG methylation in neuronal epigenetic regulation and its potential role in learning and memory.

Key Words: epigenetics, non-CpG methylation, brain

P2003 Altered hippocampal DNA methylation, gene transcription, and RNA editing in response to early life environmental insults in 2 independent studies of cognitive development.

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DNA methylation is an epigenetic mark that occurs at cytosines throughout the genome, is involved in regulating gene expression, and can be altered in response to environmental signals. This study investigated DNA methylation and gene expression patterns in hippocampus samples from 2 studies observing reduced hippocampal-based spatial learning and memory in response to early life environmental insults (iron deficiency and PRRSv infection) in porcine biomedical models of cognitive development (Rytych et al., 2012; Elmore et al., 2014). Reduced representation bisulfite sequencing and RNA-seq were performed on 16 hippocampus samples (iron deficiency, with 3 deficient and 4 control; PRRSv infection, with 4 infected and 5 control). In total, 192 and 455 differentially expressed genes (DEG) were detected in the iron-deficient and PRRSv-infected groups, respectively. Of these, 53 were differentially expressed in both studies, including genes involved in neurodevelopment and function, such as CARTPT, NTNG1, PRSS12, GABRE, and HTR2C. Differential DNA methylation was assessed at more than 600,000 CpG and 2.4 million non-CpG sites in both studies, identifying 853 differentially methylated (DM) CpG and 99 DM non-CpG sites in the iron-deficient group, including 12 sites associated with 9 DEGs. In total, 1,857 DM CpG and 153 DM non-CpG sites were identified in the PRRSv-infected group, including 26 sites associated with 19 DEGs. Increased expression of VWF (log2 fold change >1.8) and HTR2C (log2 fold change >1.0) was associated with hypomethylation of the same genomic regions in the iron-deficient and PRRSv-infected groups. In addition, as HTR2C undergoes adenosine-to-inosine (A-to-I) RNA editing at 5 sites affecting human HTR2C receptor activity and brain function, HTR2C editing frequencies were determined. Increased editing was detected at the first site (site A) in both groups, although the difference was only significant for the iron-deficient group ($P = 0.019$). In addition, 1 RNA isoform (IAAAI) and 1 protein isoform (V-S-I) were expressed exclusively in both the iron-deficient and PRRSv-infected groups. Together, these results provide evidence for altered hippocampal DNA methylation, gene expression, and HTR2C RNA editing in response to early life environmental insults in 2 independent studies of cognitive development.

Key Words: DNA methylation, porcine biomedical model, cognitive development

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P2004 Association of bta-miR-24-3p with serum antibody response to mycoplasma spp. in beef cattle.

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The objective of this study was to identify microRNAs associated with a serum antibody response to *Mycoplasma spp.* in beef cattle. Serum from 16 beef calves was collected at 3 points: summer of 2013, after calves were born; fall of the same year at weaning; and spring, 2014. All sera collected in summer were negative for IgG reactive with *Mycoplasma spp.* By the fall, 8 animals were seropositive (positive group), whereas 8 remained negative (negative group). By spring, all animals in both groups were seropositive. MicroRNAs were extracted and sequenced on the Illumina HiSeq next-generation sequencer. A total of 967,152 normalized sequences were identified as bta-miR-24-3p. There was a significant interaction between groups and season ($P = 0.0268$). During summer, both groups had similar number of sequences for the bta-miR-24-3p ($P = 0.773$). In the fall, the positive group had an increased number of sequences when compared with animals in the negative group ($P = 0.021$). The difference in the number of sequences for the bta-miR-24-3p increased in the positive group, when compared to the negative group during the following spring ($P = 0.0001$). Differential expression of miR-24-3p has been observed in human liver cell lines infected with hepatitis B virus. Production of bta-miR-24-3p may be associated with a host defense mechanism triggered by infection or it may provide some advantage to a pathogen during infection of a

host. Further studies are needed to establish if bta-miR-24-3p could be used as a diagnostic indicator of exposure or whether intervention strategies could be developed to be used as an alternative to antibiotics for controlling disease due to *Mycoplasma spp.*

Key Words: cattle, disease, microRNA, mycoplasma, respiratory disease

P2005 Identifying DNA methylation differences that contribute to an age-dependent increase in bovine innate immunity using reduced representation bisulfite sequencing and the dermal fibroblast model. F. Korkmaz*, D. E. Kerr (University of Vermont, Burlington, VT)

Reduced representation bisulfite sequencing (RRBS) was used to identify DNA methylation differences in paired dermal fibroblast cultures taken from 6 heifers when they were 5 and 16 mo of age. Before RRBS, we determined that the older fibroblast cultures responded with greater ($P < 0.05$) interleukin-8 (IL8) and interleukin-6 (IL6) protein secretion and gene expression, following a 36-h LPS treatment. From the same fibroblast cultures, DNA was analyzed for CpG methylation by RRBS (Methyl-MiniSeq, Zymo Research). A paired t test comparing methylation ratios revealed 14,094 differentially methylated CpGs ($P < 0.05$) with >5X coverage. Clustering analysis of the top 100 differentially methylated sites was performed using a complete linkage method and Euclidean distance metric, which revealed 2 separate methylation clusters representing cultures from young and older animals, respectively. To identify sites with greatest biological relevance, we refined our analysis to include only CpGs with >25% methylation ratio difference and >7X coverage, leaving 1,063 sites. The majority (833, 80%) of these sites were hypermethylated in young cultures, including 77 out of the 90 sites found within gene promoter regions (−2.5 kb to +1.0 kb from the transcription start site). Transcription factor analysis was performed (TRANSFAC v8.2) on genes containing differentially methylated sites ($P < 0.05$, >25% meth diff, >7X coverage) between young and old cultures. In agreement with their lower LPS response, genes hypermethylated in cultures from young animals showed an association ($P < 0.05$) with NF-κB-regulated genes and genes controlled by MAZR, which regulates expression of the acute phase proteins serum amyloid A (SAA1–3). Genes hypermethylated in old cultures showed enrichment ($P < 0.05$) of genes controlled by IRF6 and STAT1, which both regulate interferon production. To determine if differential methylation was associated with gene expression, a panel of 6 differentially methylated ($P < 0.05$, >25% meth diff, >7X coverage) immune-related genes (TNFSF13, FES, PIK3R1, RORA, NFATC1, and TCF7) were selected for analysis by RT-qPCR on RNA obtained 0, 2, and 8 h post-LPS treatment. With the exception of TCF7, young cultures with greater methylation in all 6 genes also had lower levels ($P < 0.05$) of gene expression. This indicates that methylation may have affected expression of these genes. The current study shows that DNA methylation may contribute to age-dependent differences in the dermal fibroblast response to LPS. Identifying epigenetic mechanisms that affect the innate response may be informative to the potential causes of inter-animal variation in susceptibility to economically important diseases, such as bovine mastitis.

Key Words: epigenetics, immunity

P2006 Transgenerational effects of modifications of the embryonic environment in quail. T. Zerjal*[1], S. Leroux[2], D. Gourichon[3], C. Leterrier[4], Y. Labrune[2], V. Coustham[5], J. L. Coville[1], M. Morisson[2], F. Minvielle[1], F. Pitel[2] ([1]INRA, AgroParisTech, Université Paris-Saclay, GABI, Jouy en Josas, France, [2]UMR INRA/INPT ENSAT/INPT ENVT-GenPhySE, Castanet Tolosan, France, [3]UE1295 Pôle d'Expérimentation Avicole de Tours, Nouzilly, France, [4]INRA, UMR85 Physiologie de la Reproduction et des Comportements, Nouzilly, France, [5]INRA-URA, Nouzilly, France)

Epigenetic phenomena, such as DNA methylation, which participate in the regulation of gene expression, can influence phenotypes. The influence of the embryonic environment on the adult phenotype, through epigenetic marks, has been observed in numerous cases. Recent studies show that epigenetic information may be transmitted across generations. Our aim was to observe if a modification of the quail embryonic environment would have transgenerational effects. We observed phenotypic differences in the third generation between 2 lines, obtained after treating (Epi+) or not treating (Epi-) eggs from a common set of founders G0, by injection of genistein. We used genistein as it is known to interfere with the epigenome, especially with DNA methylation. A "mirror" device was set, with parallel genealogies in each line, to minimize the putative influence of genetics on trait variability. After 3 generations without any further treatment, a significant difference in sexual maturity was observed between the lines, with the Epi+ G3 birds starting egg laying later. A significant interaction between line and sex was observed for 3-wk body weight and for eye temperature. Two behavioral traits in the G3 were also significantly affected by the initial treatment. Global methylation analyses are ongoing. These observations

demonstrate the impact of a modification of the founders' embryonic environment on the phenotype of quails, 3 generations later. While genetic variability cannot definitely be ruled out, the mirror animal device should have minimized its effects, and observed differences in the G3 may be attributed, at least partly, to transgenerational epigenetic phenomena.

Key Words: quail, epigenetic, transgenerational effects

P2007 Sulforaphane enhances proliferation of porcine satellite cells through suppression of TGF-β signaling pathway. R. Zhang[1], C. Neuhoff[¶], H. Fan[2], J. Welzenbach[*1], Q. Yang[1], M. J. Uddin[3], M. U. Cinar[4], D. Tesfaye[1], E. Tholen[5], C. Looft[5], K. Schellander[5] ([1]Institute of Animal Science, University of Bonn, Bonn, Germany, [2]Department of Basic Medical Science and Center for Cancer Research, Purdue University, West Lafayette, West Lafayette, IN, [3]School of Veterinary Science, The University of Queensland, Gatton, Australia, [4]Faculty of Agriculture, Department of Animal Science, Erciyes University, Kayseri, Turkey, [5]Institute of Animal Science, University of Bonn, Bonn, Germany)

Satellite cells, the muscle stem cells, play a critical role in muscle growth, maintenance, and regeneration. A lot of muscle diseases result from defective function of satellite cells. Porcine satellite cells are a good model for studying the role of satellite cells in muscle development. Sulforaphane (SFN), a natural molecule rich in cruciferous vegetables, is a potent inducer for the NF-E2-related factor 2 (Nrf2) signaling and also inhibits the activity of histone deacetylases (HDAC). Our previous study found that SFN epigenetically suppressed the transcription of myostatin in porcine satellite cells. However, the effects of SFN on the proliferation of porcine satellite cells and the related mechanisms are far from understood. In the present study, we report that SFN enhanced the proliferation of the porcine satellite cells and modified the expression myogenic regulatory factors. SFN altered the expression of HDACs and inhibited the activity of HDACs. The activity of TGF-β signaling was suppressed by SFN treatment, which was accompanied with up-regulated Smad7, an endogenous suppressor of TGF-β signaling. Furthermore, we found that SFN increased the mRNA expression of Smad7's transcription factors and decreased the expression of miRNAs targeting Smad7. The DNA methylation of a studied fragment in Smad7 promoter was not influenced by SFN treatment. SFN has received substantial attention because of its potential application in cancer therapy. The present study, for the first time, investigated the effects of SFN on the proliferation of porcine satellite cells and the underlying mechanism. We found that both mRNA and protein level of Smad7 were greatly increased by SFN. Thus, besides reducing TGF-β1 protein abundance, SFN also inhibits the activity of TGF-β signaling by increasing expression of Smad7. It has been shown that overexpression of Smad7 led to enhanced skeletal muscle differentiation and cellular hypertrophy. In summary, our studies state that SFN enhances the proliferation of porcine satellite cells by suppressing TGF-β signaling through activation of Smad7.

Key Words: sulforaphane, satellite cells, epigenetics

P2008 Lipopolysaccharide-induced gene expression of CD14 in TRIF pathway is epigenetically regulated by sulforaphane in porcine pulmonary alveolar macrophages. Q. Yang[1], M. J. Pröll[*1], D. S. Wondim[1], R. Zhang[1], D. Tesfaye[1], H. Fan[2], M. U. Cinar[3], C. Grosse-Brinkhaus[1], E. Tholen[4], C. Looft[4], A. Islam[5], M. Hölker[1], K. Schellander[4], M. J. Uddin[6], C. Neuhoff[¶] ([1]Institute of Animal Science, University of Bonn, Bonn, Germany, [2]Department of Basic Medical Science and Center for Cancer Research, Purdue University, West Lafayette, IN, [3]Faculty of Agriculture, Melikgazi Kayseri, Turkey, [4]Institute of Animal Science, University of Bonn, Bonn, Germany, [5]Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh, [6]School of Veterinary Science, The University of Queensland, Gatton Campus, Gatton, Gatton, Australia)

Cluster of differentiation 14 (CD14) is the pattern recognition receptor (PRR) involved in the recognition of bacterial component lipopolysaccharide (LPS) through the MyD88-dependent and TRIF pathway of innate immunity. It may be a modulator to prevent and mitigate the LPS-induced lung inflammation in pigs. However, reports on CD14 activation induced by LPS in TRIF pathway are controversial. Furthermore, the gene expression regulation of CD14 by the epigenetic factor sulforaphane (SFN) is still poorly understood. To identify the epigenetic changes of CD14 mediated with SFN in LPS-induced TRIF pathway, the PAMs model in vitro was investigated. For this, the mRNA expression of CD14 and downstream genes of TRIF pathway were quantified using qPCR. The cytokine levels of tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β) were measured by enzyme-linked immunosorbent assay (ELISA). The gene expression of the epigenetic enzymes DNA methyltransferase-1

(DNMT1) and DNMT3a were quantified. The protein level of NF-κB was analyzed by western blot. Furthermore, the DNA methylation alterations of CD14 at promotor and gene-body (CDS region) were analyzed using bisulfite sequencing in SFN- and LPS-treated PAMs. It was shown that CD14 gene expression was induced by 5 μg/ml LPS in time-dependent manner. At time point 12 h, the gene expression of CD14 and downstream genes in TRIF pathway, including TRIF, TRAF6, $NF_{\gamma}B$, TRAF3, IRF7, and cytokines, such as TNF-α, IL-1β, IL-6, and IFN-β, were significantly induced by LPS. The LPS-induced gene expression was suppressed by SFN in a dose-dependent manner. The LPS-induced cytokine levels, including TNFα, IL-1β, and NF-κB levels, were also inhibited by SFN. Similarly, the DNMT3a mRNA expression was increased by LPS and downregulated by SFN at a dose of 5 μM. Furthermore, the bisulfite sequencing results showed that gene body methylation of CD14 was positively associated with gene expression of LPS-treated PAMs and this methylation status was inhibited by SFN in a dose-dependent manner. This in vitro study suggests that CD14 is involved in TRIF pathway, including TRIF-TRAF6 and TRIF-TRAF3 pathway, by LPS induction. Furthermore, this LPS-CD14 activation was suppressed by SFN via the epigenetic regulation of CD14 gene body methylation associated with DNMT3a. This study provided novel insights into SFN-mediated epigenetic downregulation of CD14 gene in LPS-induced TRIF pathway inflammation and may open new avenues for approaches to prevent and mitigate LPS-induced inflammation in pigs.

Key Words: CD14, LPS, sulforaphane

P2009 Initial analysis of sperm DNA methylome in Holstein bulls using whole genome bisulfite sequencing. G. E. Liu* (Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD)

Aberrant DNA methylation patterns have been associated with abnormal semen parameters, idiopathic male infertility, and early embryonic loss in mammals. Using Holstein bulls with high (Bull1) or low (Bull2) fertility rates, we created 2 representative sperm DNA methylomes at a single-base resolution using the whole genome bisulfite sequencing (WGBS). Totally, 442 million reads (~44 Gb, 15X coverage) and 612 million reads (~61 Gb, 23X coverage) were obtained for Bull1 and Bull2, respectively. We anchored these reads onto the cattle reference genome by filtering monoclonal reads and allowing multiple mapping reads, and then determined the methylation status of each cytosine site. While these 2 sperm genomes have high coverage of methylation, we observed that the methylation status of individual CpG sites can vary, even when in very close proximity to apparently invariable methylated cytosines. We also performed differentially methylated region (DMR) analyses and identified 392 DMRs, ranging from 123 bp to 3973 bp (mean 436 bp, total 268,582 bp) in Bull1, and 690 DMRs, ranging from 148 bp to 6639 bp (mean 620 bp, total 722,822 bp), in Bull2. We evaluated the functional impacts of these DMRs on genomic features, including functional genes, regulatory elements, and common repeats. We are processing 20 more similar genome-wide DNA methylation maps and designing high-throughput DNA methylation assays for semen quality control, which will facilitate a full assessment of the impact of sperm DNA methylation on male fertility in dairy cattle.

Key Words: cattle, DNA methylation, whole genome bisulfite sequencing

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P2010 Discovery of tissue-specific and gene-expression associated CpG methylations in the swine genome. M. K. Choi*, J. Lee, J. Lee, M. T. Le, C. Park (Konkuk University, Seoul, Korea, The Republic of)

DNA methylation is an important component of the epigenetic regulation of gene expression in the mammalian genome. Although several genome-wide DNA methylation profiling studies have been reported in pigs, the genome coverage of data is still limited, especially for tissue diversity and analysis at the single nucleotide level. We experimentally generated the DNA methylation profiles of the neocortex, olfactory epithelium, spleen, liver, and muscle tissues, and a pulmonary alveolar macrophage (PAM; 3D4/2) cell line, using reduced representation bisulfite sequencing (RRBS). On average, 3.92 Gb of clean reads were analyzed from each of the 6 samples. The results showed that 42.82 to 45.71% and 4.85 to 5.07% of detected cytosine and guanine dinucleotides (CpG) were located in CpG islands (CGI) and 2 kb upstream region of transcription start sites (TSS), respectively. We observed a low rate (average of 1.67%) of non -CpG methylation in the 6 samples, except for the neocortex (2.3%). The observed global CpG methylation patterns of pigs indicated high similarity to other mammals, including humans. The general characteristics of the methylation pattern of the pig genome by RRBS analysis in this study were consistent to the results of another recent pig genome RRBS study. Observed patterns of differentially methylated cytosines (DMC) were compared among different tissues. However, DNA methylation pattern of PAM cells was quite different with those of other tissues. In addition,

we analyzed the correlation between the levels of DNA methylation and gene expression among neocortex, liver, muscle, spleen, and a macrophage cell line. We identified 21 neocortex-, 42 liver-, 20 muscle-, 28 spleen-, and 652 macrophage-specific differentially expressed genes (DEG) harboring tissue specific DMCs in upstream 10 kb region from TSS and gene bodies, including exons and introns. These results confirm and support that changes in DNA methylation in the swine genome are associated with alteration in gene expression and phenotypic differences.

Key Words: pig, DNA methylation, gene expression, RRBS, RNA-seq, epigenetics

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P2011 Novel analysis of global DNA methylation in the limbic system of the bovine brain.

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There has been limited research focusing on the genetic-environmental interactions in bovine brains. Global DNA methylation has been measured in brains of several species, but has yet to be examined in bovine. The objective of this study was to characterize global DNA methylation in 9 regions of the limbic system in the bovine brain: amygdala, bed nucleus of the stria terminalis, cingulate gyrus, dorsal raphe, hippocampus, hypothalamus, nucleus accumbens, periaqueductal gray, and prefrontal cortex. DNA was extracted from brain and blood samples of 6 Red Angus × Simmental steers (less than 20 mo of age), using the DNA Extraction Kit from Agilent Technologies (Santa Clara, CA) and a phenol chloroform extraction. Percent of global DNA methylation was determined using the MethylFlash Methylated DNA Quantification Kit (Colormetric) from Epigentek (Farmingdale, NY). Varying amounts of global DNA methylation were observed among the 9 functionally distinct regions of the bovine limbic system. Amygdala, bed nucleus of the stria terminalis, cingulate gyrus, dorsal raphe, periaqueductal gray, prefrontal cortex, and nucleus accumbens are all significantly different ($P < 0.05$) from 1 or more brain tissue type, using a paired t test in SPSS (IBM, Armonk, NY). Conversely, global DNA methylation of blood was not significantly different ($P < 0.05$) from any brain tissue type. This study shows significant differences in global DNA methylation among different tissue types in the limbic system of the bovine brain. Understanding the differences in global DNA methylation within different tissues in the brain will facilitate future research involving the effects of differential methylation with regard to economically important traits.

Key Words: bovine, brain, methylation

P2012 Investigation of genomic imprinting in chicken embryonic brain and liver through RNA sequencing.

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Genomic imprinting refers to the epigenetic phenomenon that some autosomal genes are exclusively expressed from either the maternal or paternal allele, whereas, based on Mendelian inheritance, expression of alleles is expected to be in equal amount and independent of their parental origin. DNA methylation in *cis*-acting manner is the major mechanism for genomic imprinting. Imprinted genes have been identified in several animal species and are frequently associated with embryonic growth and survival functions. Yet, whether genomic imprinting exists in chickens is still debatable, as previous studies reported conflicting evidence regarding the topic. Albeit no genomic imprinting has been found in the chicken embryo as a whole, we investigated whether certain embryonic tissues exhibit genomic imprinting. In this study, we interrogated the existence or absence of genomic imprinting in chicken the embryonic brain and liver by examining mRNA expression of parental alleles in an F1 generation. Eggs from 2 highly inbred chicken lines (Fayoumi and Leghorn) and their reciprocal crosses were collected and incubated for 12 d; then, brain and liver were harvested from embryos for cDNA library preparation. To establish the genotypes of the inbred lines and F1 hybrids, and to minimize reference bias of RNA-Seq sequence alignment, genomic DNA from inbred Fayoumi and Leghorn chickens were pooled separately and each pool was sequenced at 20X coverage. The SNP loci identified from DNA-Seq data were masked to create a customized reference genome (based on Ensembl Galgal4) for RNA-Seq reads mapping. Of 65 million RNA-Seq reads per sample generated using the Illumina HiSeq 2000 sequencer, 88% were mapped to the customized reference genome. The genome-wide ratio of mapped reads containing reference allele was reduced by 1.5% when compared with results from the original reference genome. Our analyses indicated that in the F1 crosses, about 9.2% of the heterozygous loci show allele-specific expression (binominal test, p value $\leq$

0.05), but there was no evidence detected of genomic imprinting in chicken 12-d embryonic brain and liver.

Key Words: genomic imprinting, chicken, RNA-Seq, allele specific expression

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P2013 Impact of collection season and storage of semen on methylation activity in swine placental and fetal tissues derived from summer or winter breedings. L. A. Rempel*, J. R. Miles (USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE)

DNA methylation patterns in extra-embryonic tissues have been linked to irregular fetal growth and early pregnancy loss. The objective of this study was to evaluate methylation profiles of placental and fetal tissue collected from pregnancies derived using cooled-extended (ExT) or cryopreserved (FrZ) semen from June (spring), August (summer), or January (winter), and breeding gilts in summer (August) or winter (January). Gilts were artificially inseminated 1 time following synchronization in August or January, with 1 of 3 semen types (August ExT or January ExT, August FrZ, and June FrZ) and a control group of females artificially inseminated following natural heat detection and 2 inseminations with ExT semen. Gilts were harvested at approximately 45 d post-breeding. A portion of fetal liver and companion placenta were collected from the smallest, an average, and largest live piglet from each litter. Methylation (5-methylcytosine; 5mC) and hydroxymethylation (5-hydroxymethylcytosine; 5hmC) activity in fetal liver and placenta was measured to estimate gene transcription activity. Fetal liver 5mC activity was greater ($P = 0.0472$) from pregnancies derived from semen collected during cooler periods in comparison to semen from summer (8.0% ± 0.51 and 6.7% ± 0.56, respectively). No differences ($P \geq 0.1363$) were detected within fetal liver for 5hmC activity. Fetal livers had decreased ($P = 0.0058$) 5mC:5hmC, and therefore increased gene transcription activity, when derived from summer breedings versus winter breedings (8.8 ± 2.54 and 19.9 ± 2.69, respectively). Placental 5mC activity was affected by breeding season with summer breedings less ($P = 0.0383$) than winter breedings (6.3% ± 0.51 and 7.7% ± 0.51, respectively), and semen collection period tended ($P = 0.0762$) to be greater from summer versus cooler collections (6.6% ± 0.66 and 5.0% ± 0.58, respectively). Placental 5hmC activity was also less ($P = 0.0013$) from summer matings in comparison to winter matings (4.3% ± 0.62 and 7.3% ± 0.63, respectively). In contrast to liver ratios, placentas derived from summer breedings had reduced 5mC:5hmC versus winter breedings (4.2 ± 0.67 and 0.9 ± 0.67, respectively). The findings in the current study support the occurrence of male and female factors contributing to seasonal differences in methylation activity. However, breeding season of the gilt, summer or winter, may still harbor the greatest influence on transcriptional activity in fetal liver and placenta, as measured by reduced ratio of 5mC to 5hmC. USDA is an equal opportunity provider and employer.

Key Words: cryopreserved semen, fertility, methylation, season, swine

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P2014 Examining conserved DNA methylation in the bovine 5' AMPK gene family. F. Betancourt, S. Friedman, S. Perlee, H. Lachance, S. D. McKay* (University of Vermont, Burlington, VT)

The 5' AMP-activated protein kinase (AMPK) gene family includes an evolutionary conserved serine/threonine heterotrimeric protein kinase. The primary function of this kinase is regulation of cellular energy and metabolism. The activation of this gene family primarily occurs when there is a depletion of cellular ATP due to environmental and nutritional stressors, which is associated with a rise of AMP levels. Subsequently, the genes initiate energy-conserving measures within the cell to protect it from the decrease in ATP levels. The regulation of these genes has been linked to the epigenetic mechanism of methylation. Methylation is an epigenetic modification of DNA that regulates gene transcription. DNA methylation primarily involves the addition of a methyl group to the 5 position of cytosines that are found in CpG dinucleotides. The location of 5-methylcytosine relative to genic regions can either facilitate or prevent transcriptional machinery from binding to its target site. We investigated the presence of methylation in the bovine AMPK gene family. The bovine AMPK gene family includes 7 genes that are also classified as members of 1 of 3 subunits. In this study, we performed combined bisulfite restriction analysis (COBRA) with 5 AMPK genes, using restriction enzymes that recognize CpG recognition sequences. When performed in the liver and muscle tissue of Angus and Charolais cattle, COBRA results indicated regions of conserved intragenic methylation in PRKAA1 and PRKAB1. Subsequently, DNA methylation patterns were analyzed through bisulfite sequencing of cloned PCR products. This study confirms the presence of DNA methylation in the bovine AMPK gene family and provides evidence of conservation of methylation across breeds of cattle. Furthermore, we believe that conservation of gene body methylation in the bovine AMPK gene family plays an important evolutionary

role in maintaining the function of this kinase.

Key Words: bovine, AMPK, conserved methylation

P2015 Fto and Irx3 transcription and methylation profiles in adipose tissues of rats fed with high-fat and high-protein diets. J. Nowacka-Woszuk*, E. Pruszynska-Oszmalek, M. Szydlowski, I. Szczerbal (Poznan University of Life Sciences, Poznan, Poland)

Obesity is 1 of today's main civilization problems. It was recently found that the identified SNP cluster in intron 1 of the Fto gene is associated with body mass. Thus, Fto was announced as a major gene in obesity pathogenesis. Recent findings showed that in the first intron of Fto, a promoter enhancer for neighboring Irx3 gene exists. This shows that chromatin interaction between both genes is essential for their expression and plays a key role in obesity. It is also known that both genes are expressed in adipose tissue. Thus, the aim of our study was to test how different diets and age of animals influence transcriptional and DNA methylation profiles in rats. The transcript level was analyzed by real-time PCR and the DNA methylation profile was determined by bisulfate sequencing. We performed all analyses in 2 types of adipose tissue: subcutaneous and abdominal fats. Two diet types were tested: high-fat and high-protein versus control. Samples were collected in 3 time points: at 30, 60, and 120 d of age. In terms of transcript level for both genes, we found the interactions between diet type and age of animals in subcutaneous and abdominal adipose tissues. Moreover, the tissue/diet interaction for the Fto promoter methylation profile was noticed. For the Irx3 DNA methylation, the tissue/diet/age interaction was observed. In conclusion, all tested factors, such as tissue type, diet, and age of animal, interact with each other in a complex way, modulating the Fto and Irx3 genes transcript level, as well as DNA methylation profile. The studies were financed from projects: N N303 551639 and 2013/09/D/NZ2/02006.

Key Words: Fto, Irx3, obesity

P2016 Combined analysis of DNA methylome and transcriptome reveal novel candidate genes relevant with susceptibility to bovine *Staphylococcus aureus* subclinical mastitis. M. Song* (China Agricultural University, Beijing, China)

Subclinical mastitis is a widely spread disease of lactating cows and causes tremendous economic losses to the modern dairy industry. *Staphylococcus aureus* (*S. aureus*) is a major subclinical mastitis-causing pathogen in dairy cows. In this study, we performed genome-wide integrative analysis of DNA methylation and transcriptional expression to identify candidate genes and pathways relevant to bovine *S. aureus* subclinical mastitis. The genome-scale DNA methylation profiles of peripheral blood lymphocytes in subclinical mastitis cows infected by *S. aureus* (SA) and healthy controls (CK) were first generated by methylated DNA immunoprecipitation combined with microarrays (MeDIP-chip). A total of 2,881 methylated genes were detected in SA and CK groups, and 1,078 differentially methylated genes were identified in SA cows compared with the controls. By integrating DNA methylation and transcriptome data, 58 differentially methylated genes were shared with differentially expressed genes, in which 20.7% distinctly hypermethylated genes showed down-expressed in SA versus CK, whereas 14.3% considerably hypomethylated genes showed up-expressed in the comparison. Integrated pathways analysis suggested that these genes are related to inflammation and cancer progression, ErbB signaling pathway, and mismatch repair. Further functional analysis revealed that 3 genes, NRG1, MST1, and NAT9, are strongly correlated with the progression of *S. aureus* subclinical mastitis and could be used as powerful biomarkers for the improvement of bovine resistance. Our studies lay the groundwork for epigenetic modification and mechanistic studies of bovine *S. aureus* subclinical mastitis susceptibility and prevention.

Key Words: genome-wide DNA methylation, epigenetic regulation, *S. aureus* subclinical mastitis, novel candidate genes

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P2018 Maternal periconceptional overnutrition alters the adipose tissue epigenome of offspring. T. Vuocolo (CSIRO Agriculture, Brisbane, Australia), D. C. Bauer (CSIRO Data61, Sydney, Australia), S. McWilliam (CSIRO Agriculture, Brisbane, Australia), S. Zhang (The University of South Australia, Adelaide, Australia), M. Buckley (CSIRO Data61, Sydney, Australia), J. L. Morrison (The University of South Australia, Adelaide, Australia), I. C. McMillen (The University of Newcastle, Newcastle, Australia), R. L. Tellam* (CSIRO Agriculture, Brisbane, Australia)

Maternal nutrition during pregnancy is linked with metabolic changes in newborn and adult offspring of a number of mammalian species. These changes can lead to increased adipose tissue deposition and increased risk of metabolic disease in humans and animal models. However, it is unclear whether maternal nutrition at conception can impact offspring metabolism and, if so, how memory of this state is propagated through

development and postnatal life. Using a sheep embryo transfer model pioneered by Caroline McMillen and Janna Morrison from the University of South Australia, we investigated the impacts of maternal periconceptional overnutrition on perirenal adipose tissue deposition, gene expression and the epigenome in late gestation fetal offspring from control recipient ewes. Genome-wide profiling of gene expression (RNA-seq) and three chromatin modifications, H3K4me3, H3K27ac and H3K27me3 (ChIP-Seq) were performed. Within each group there were strong positive and negative relationships between the specific chromatin marks and gene expression. H3K4me3 and H3K27ac were strongly linked to actively transcribed genes, while H3K27me3 identified repressed genes that were often developmental transcription factors or gated ion channels. Periconceptional overnutrition had little impact on gene expression in late gestation fetal perirenal adipose tissue from the embryo transfer offspring, although there were considerable changes in the H3K27ac modification indicating the presence of an altered but latent epigenetic state. We suggest that this epigenetic state directs changes in gene expression that alter adipose tissue metabolism and deposition in later postnatal life.

Key Words: epigenetics, maternal nutrition, adipose

P2019 Adipocyte gene expression and DNA methylation patterns differ significantly between lean and obese pigs. M. J. Jacobsen* (Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark), J. H. Havgaard (Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark), C. M. Junker Mentzel (Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark), P. M. Sørensen (Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark), S. Pundhir (BRIC, University of Copenhagen, Copenhagen, Denmark), C. Anthon (Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark), P. Karlskov-Mortensen (Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark), C. S. Bruun (Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark), S. Cirera (Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark), J. Gorodkin (Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark), C. B. Jørgensen (Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark), R. Barrès (The Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark), M. Fredholm (Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark)

Adipose tissue plays a central role in metabolic diseases, and, in particular, abdominal obesity is considered unfavorable due to its tight relationship with development of diabetes and cardiovascular diseases. As both genetic and environmental factors contribute to the development of obesity and its co-morbidities, we aimed to explore how the DNA methylome contributes to an altered transcriptome profile of adipocytes in obesity. The gene expression profile of mature adipocytes isolated from the retroperitoneal fat pad (abdominal fat) was compared between a group of lean and a group of obese pigs. Simultaneously the epigenetic signature was profiled by analyzing the global methylation of the DNA isolated from the same adipocytes. The pigs used in this study were housed in the same farm under the same environmental conditions with free access to food and water. This very stringent and controlled environment suggests that the differences in epigenetic profile detected in the study primarily reflect variations due to obesity. We found a total of 1155 coding genes and 66 non-coding RNAs that were differentially expressed between the lean and obese animals. The methylation analysis revealed a higher degree of DNA hypomethylation in obese animals, and we identified more than 6000 differentially methylated regions. Combining the transcriptome and the methylation analysis revealed a total of 100 genes, which have expression profiles that are directly (*cis*-acting) influenced by methylation. Gene ontology analyses of these genes show an overrepresentation of genes involved in lipid and fatty acid metabolism and inflammatory response, clearly indicating that the altered methylation profile of the adipocytes is implicated in development of obesity and subsequent inflammation progress in the obese pigs.

We are currently validating the most relevant results.

Key Words: DNA methylation, obesity, pigs, adipocytes

P2020 Genome-wide assessment of inbred chicken lines indicates epigenetics signatures of resistance to Marek's disease. J. Song* (University of Maryland, Animal Science and Avian, College Park, MD)

Marek's disease (MD) is a highly contagious, lymphomatous disease of chickens induced by a herpesvirus, Marek's disease virus (MDV), that is the cause of major annual losses to the poultry industry. MD pathogenesis involves multiple stages, including an early cytolytic phase and latency, and transitions between these stages are governed by several host and environmental factors. In this study, we investigated temporal chromatin signatures induced by MDV by analyzing early cytolytic and latent phases of infection in the bursa of Fabricius of MD-resistant and -susceptible birds. Major global variations in chromatin marks were observed at different stages of MD in the two lines. Differential H3K27me3 marks were associated with immune-related pathways, such as MAP kinase signaling, focal adhesion and neuroactive ligand receptor interaction, and suggested varying degrees of silencing in response to infection. Immune-related microRNAs, e.g., *gga-miR-155* and *gga-miR-10b*, bore chromatin signatures, which suggested their contribution to MD-susceptibility. Finally, several members of the focal adhesion pathway, e.g., *THBS4* and *ITGA1*, showed marked concordance between gene expression and chromatin marks indicating putative epigenetic regulation in response to MDV infection. Our comprehensive analysis of chromatin signatures, therefore, revealed further clues about the epigenetic effects of MDV infection, although further studies are necessary to elucidate the functional implications of the observed variations in histone modifications.

Key Words: epigenetics, histone modifications, chickens, genetics

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P2021 Age-related methylation patterns of equine blood leukocytes. T. Ząbek*, E. Semik, T. Szmatoła, A. Gurgul, A. Fornal, M. Bugno-Poniewierska (National Research Institute of Animal Production, Balice, Poland)

Horses belong to the one of the longest-living species of farm animals. Advanced age in horses is mainly associated with a decrease in body condition and dysfunction of the immune system. Due to this, the search for new solutions in the prevention and treatment of pathological conditions of the advanced age in this farm animal species are desirable. Identification of changes in the genome activity of aged horses is interesting in this respect. The recent research on aging includes the study of age-related epigenetic marks using methylome data. For example, epigenome-wide association studies in humans revealed a number of differentially methylated regions (DMRs) that correspond to the particular phenotypes of aging. To find molecular pathways involved in the horse aging, we have conducted methylome studies of blood leukocytes, including groups of juvenile and aged horses of the primitive Hucul breed. Bisulfitome sequencing of blood leukocytes was performed using Illuminas' sequencing-by-synthesis method. The protocol for RRBS libraries preparation was applied to extract genomic areas enriched in CG sites. Majority of identified DMRs were placed in the intergenic regions or genomic deserts. A significant part of DMRs were located inside the coding loci and also DMRs placed upstream to or at the transcription start sites of the genes. Genes associated with identified DMR sites are the members of pathways involved in nucleic acid binding transcription factor activity (GO:0001071), enzyme regulator activity (GO:0030234), and also catalytic (GO:0003824) and transporter activity (GO:0005215). Age-related methylation state of particular loci were consisted with their transcriptional activity in blood leukocytes derived from investigated horses. Obtained results might facilitate the determination of molecular basis of aging of equine blood system.

Key Words: horses, aging, blood leukocytes, methylome study

P2022 Mining functional genomics and epigenetics data with livestock EpiDB. E. Fritz-Waters (Iowa State University, Ames, IA), M. W. Vaughn (Texas Advance Computing Center, University of Texas, Austin, TX), J. P. Carson (Texas Advanced Computing Center, University of Texas, Austin, TX), J. M. Reecy (Iowa State University, Ames, IA), J. E. Koltes (Iowa State University, Ames, IA; University of Arkansas, Fayetteville, AR)

The livestock EpiDB was developed to facilitate reuse of next-generation sequencing data generated from ChIP-seq, methyl-seq, RNA-seq and small RNA-seq in cattle, chicken, horse, pig and sheep. More than 3300 well-annotated Illumina next-generation sequencing functional genomics data sets have been identified from the NCBI SRAdb or EBI's ENA and processed to date. These datasets represent an untapped resource for researchers working on animal functional genomics. The objective of the livestock

EpiDB is to facilitate reuse of functional genomics data by providing an easily searchable database of metadata sorted by data type, tissue and species and also to provide reference expression data in the form of a gene atlas and easily downloadable tracks of tissue-specific gene expression levels. Data used for gene expression estimation are further filtered based on quality control to remove sequences that do not pass an array of filters from FASTQC software. Data analysis pipelines have been developed to utilize gold standard quality control (FASTQC, Sickle), alignment (Bowtie/Tophat2), variant calling (GATK), and quantification (RSEM, MACS) software wherever possible based on FAANG guidelines. A pipeline was also developed to identify allele-specific expression in RNA-seq datasets that are indicative of monoallelic expression. At present, more than 1200 RNA-seq samples, comprising 98 tissues in cattle, have been processed to identify high-quality gene expression data. A total of 63 tissues met the quality control standards, resulting in reference expression levels for each of these tissues. In addition, ChIP-seq data has been processed for 23 samples in cattle. Pipelines have been developed for all data types and are currently being deployed on TACC's Lonestar 5 petascale system to facilitate automated analysis in all species. We will also implement the Agave REST API to orchestrate data management to allow for easy updates in data processing when new reference genomes become available. These resources will serve as valuable reference resources for GWAS and functional genomics studies. They will also permit large-scale comparative analysis of functional genomics data to identify conservation of gene expression and epigenetic regulation across species. EpiDB is publically available at: http://epidb.animalgenome.org/.

Key Words: gene expression atlas, epigenetics, functional genomics

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P2023 Analysis of methylation patterns in bovine spermatozoa. M. R. Prause (Texas A&M University, College Station, TX), B. M. Murdoch (University of Idaho, Moscow, ID), J. E. Sawyer (Texas AgriLife Research, College Station, TX; Department of Animal Science, Texas A&M University, College Station, TX), J. L. Williams (University of Adelaide, Adelaide, Australia), S. D. McKay (University of Vermont, Burlington, VT), C. A. Gill (Department of Animal Science, Texas A&M University, College Station, TX)

Epigenetic remodeling of chromatin structure is required to compact DNA sufficiently for packaging into spermatozoa. Methylation of the carbon 5 position of cytosine is a common epigenetic mark in vertebrates and has been associated with male infertility. Methylation can be affected by environmental changes in utero, and our long-term goal is to establish whether maternal nutrition affects the bovine sperm methylome and variation in male fertility. As a first step, our objective here was to establish the general pattern of methylation in ejaculated bovine spermatozoa. We performed whole genome bisulfite sequencing for five Nellore-Angus crossbred bulls. Sequences were aligned to a bisulfite-converted version of the UMD3.1 bovine reference assembly, and ~15x genome coverage was obtained per sample. Circos plots of the global methylation pattern in bovine spermatozoa will be presented, and examples of differentially methylated regions will be highlighted.

Key Words: epigenetics, DNA methylation, sperm

P2024 Profiling of open chromatin in chicken tissues using ATAC-seq. M. Halstead*, C. Kern, P. Saelao, Y. Wang, H. Zhou, P. J. Ross (University of California, Davis, CA)

Accessible or "open" chromatin constitutes regions in which nucleosomal structure is less compacted, facilitating DNA–protein interactions important to active transcription and functionality of regulatory elements. To date, genome-wide profiling of open chromatin has been limited by the technical constraints of DNase-seq, a highly specialized and complicated assay. Recently, a novel technique termed Assay for Transposase-Accessible Chromatin (ATAC-seq), was developed as an alternative to DNase-seq; however, ATAC-seq has only been reported to work with cultured cells. Consequently, we aimed to adapt ATAC-seq for tissues, using the chicken as our model, and evaluate the efficacy of our protocol by comparing ATAC-seq data with DNase-seq data generated from the same tissues. Fresh tissues were prepared for ATAC-seq and DNase-seq by gentleMACs homogenization in sucrose buffer, filtration through a 100 μm vacuum filter system, and cryopreservation at -0°C. DNase-seq was performed by the John Stam Lab at the University of Washington according to their established protocols. For ATAC-seq, nuclei were thawed and isolated via centrifugation in a sucrose buffer gradient, then counted and evaluated for quality using a hemocytometer and finally subjected to the standard ATAC-seq protocol. Resulting libraries were submitted for 100 bp paired-end sequencing on the HiSeq3000 platform. Both ATAC-seq and DNase-seq raw reads were analyzed using the same bioinformatics pipeline. In brief, reads were trimmed using Trimmomatic and aligned with BWA to Galgal4, read duplicates were removed using picard-tools and peaks

were called using MACS2. The ATAC-seq and DNase-seq data were compared for signal-to-noise ratio, intersection of peaks and correlation of peak intensities. The fragment length distribution of ATAC-seq reads recapitulated the expected nucleosome spacing pattern, and ATAC-seq peaks generally coincided with DNase-seq peaks. These results suggest that ATAC-seq, preceded by appropriate nuclei preparation, may be used as an alternative to DNase-seq for profiling regions of open chromatin in tissues.

Key Words: ATAC-seq, chicken, open chromatin

P2025 Identification of tissue-specific promoters in chickens. C. Kern* (University of California, Davis, CA), P. Saelao (University of California, Davis, CA), Y. Wang (University of California, Davis, CA), M. Halstead (University of California, Davis, CA), J. Chitwood (University of California, Davis, CA), T. Kim (University of California, Davis, CA), P. J. Ross (University of California, Davis, CA), I. Korf (University of California, Davis, CA), M. E. Delany (University of California, Davis, CA), H. Cheng (USDA-ARS Avian Disease and Oncology Laboratory, East Lansing, MI), H. Zhou (University of California, Davis, CA)

The importance of epigenetics in understanding the link between an organism's genome and resultant phenotypes has become clear in recent years. This is especially significant in the food production industry, where such knowledge can be used to improve production efficiency, animal welfare and food safety. We present our progress toward compiling a catalog of functional genomic elements for the chicken, a species utilized in one of the largest global meat production industries. We have generated genome-wide profiles of DNase I hypersensitivity (DHS) sites as well as H3K4me3 and H3K27me3 histone modifications from cerebellum, cortex, liver, lung and spleen tissues. Two biological replicates were used to permit consistent identification of DHS sites and histone modification peaks, with only those features present in both replicates being used for further analysis. DHS sites and H3K4me3 peaks are associated with enhanced gene expression when found in promoter regions, while H3K27me3 is associated with repressed expression. A total of 25,503 promoter regions (2kb upstream of transcription start site) belonging to annotated transcripts contained a DHS site co-localizing with an H3K4me3 peak that was seen in each of the five tissues. The number of tissue-specific active promoters varied considerably among tissues, with 175 identified in liver, 180 in cerebellum, 458 in cortex, 800 in lung and 2615 in spleen. Combining such data with RNA expression measurements further verified the regulatory role of these features and supports the existence of expressed, but unannotated, transcripts, such as novel isoforms and long noncoding RNA.

Key Words: epigenetics, tissue-specific, promoters

P2026 Polar overdominance and maternal genome effects in placenta drive heterosis in utero. C. A. S. Estrella (Robinson Research Institute, The University of Adelaide, Adelaide, Australia; JS Davies Epigenetics and Genetics Group, School of Animal and Veterinary Sciences, Roseworthy Campus, Adelaide, Australia), K. L. Kind (Robinson Research Institute, The University of Adelaide, Adelaide, Australia; School of Animal and Veterinary Sciences, Roseworthy Campus, Adelaide, Australia), M. Ghanipoor-Samami (Robinson Research Institute, The University of Adelaide, Adelaide, Australia; JS Davies Epigenetics and Genetics Group, School of Animal and Veterinary Sciences, Roseworthy Campus, Adelaide, Australia), A. Javadmanesh (Robinson Research Institute, The University of Adelaide, Adelaide, Australia; JS Davies Epigenetics and Genetics Group, School of Animal and Veterinary Sciences, Roseworthy Campus, Adelaide, Australia), C. T. Roberts (Robinson Research Institute, The University of Adelaide, Adelaide, Australia; Discipline of Obstetrics and Gynecology, School of Medicine, The University of Adelaide, Adelaide, Australia), S. Hiendleder* (Robinson Research Institute, The University of Adelaide, Adelaide, Australia; JS Davies Epigenetics and Genetics Group, School of Animal and Veterinary Sciences, Roseworthy Campus, Adelaide, Australia)

Heterosis, defined as the superior performance of F_1 hybrids over their parents, has been used for centuries to increase yield in plants and animals. However, the biological basis of heterosis is poorly understood, as it does not follow standard genetic models. Based on theoretical prediction that genomic imprinting may mimic overdominance and heterosis, we investigated whether imprinting effects could explain heterosis. We used purebred and reciprocal cross *Bos taurus taurus* (Angus, A) and *Bos taurus indicus* (Brahman, B) cattle that display one of the strongest known heterotic phenotypes in mammals. We intercepted concepti at mid-gestation, when the fetus enters accelerated growth but does not yet display heterosis in weight, to map drivers of heterosis in placenta as the major organ regulating prenatal growth that predicts postnatal performance. Our analyses at the gross morphological, histomorphological and molecular level revealed nine

maternal, three paternal and nine polar over/underdominance patterns consistent with genomic imprinting effects but only two with additive genetic effects. Strikingly, placental polar overdominance patterns at midgestation mirrored polar overdominance in birth weight. We found that increased nutrient supply via maternal A genome effects on placental phenotype, combined with increased nutrient transfer capacity via polar overdominance effects of paternal B genome on umbilical cord phenotype, provide the basis for heterosis in birth weight of B×A hybrids. Polar overdominance in expression of imprinted *IGF2R* in *Placenta fetalis* of B×A hybrids, and correlation of transcript abundance with number of feto-maternal syncytia in placenta, are consistent with an active signaling role of IGF2R in placenta and a further indicator of superior placental performance as the driver of heterosis. In conclusion, we have shown that phenotypic expression patterns consistent with imprinting effects on placental and umbilical cord parameters and in agreement with the conflict of interest theory of genomic imprinting drive mammalian heterosis in utero.

Key Words: heterosis, polar overdominance, maternal and paternal genome effects, placenta, IGF, bovine

POSTERS: FUNCTIONAL GENOMICS

P3000 Variation of goat interferon regulatory factor 3 gene and its implication in goat evolution.

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Members of the interferon regulatory factor (*IRF*) gene family are major regulators of host defense in vertebrates, controlling many different aspects of the innate and adaptive immune responses. Among these, *IRF3* plays important roles in many biological processes. Considering that immune systems are crucially fundamental for the survival and evolution of all species, patterns of selection and polymorphisms in innate immune loci are of significant interest in molecular evolution research. We assembled and evaluated 1353 bases on the encoding regions of the *IRF3* gene in domesticated goats from Nigeria (West Africa), Ethiopia (East Africa), Iran (West Asia) and China (East Asia) and in wild goat (*Capra aegagrus*). The sequence diversity in domesticated goats was quite low but significantly different from that of wild goats. The Fu and Li's tests were significant and positive, while the Tajima's D test was significant but negative, suggesting a deviation from neutrality. Two of the six observed haplotypes across all the sequences were common in Asian goats. Two haplotypes were shared with wild goat, by West African Dwarf (WAD) and African Borena goats. In assessing the mode of evolutionary activity affecting the evolution of the gene, we found that the codon models d_N/d_S ratio for all goats was greater than a unit (1.667; $P = 0.025$). Likelihood ratio test (LRT) to compare the models used was significant (24.56; $P < 0.001$). Positive diversifying selection inferred with recent evolutionary changes in domesticated goat *IRF3* led us to conclude that the gene evolution has been influenced by domestication process in goats.

Key Words: evolution, goat, gene

P3001 Transcriptomic signature of high dietary selenium supplementation in sheep: A nutrigenomic insight using a custom microarray platform and gene set enrichment analysis. R. Elgendy* (University of Padova, Padova, Italy), M. Giantin (University of Padova, Padova, Italy), F. Castellani (University of Teramo, Teramo, Italy), L. Grotta (University of Teramo, Teramo, Italy), F. Palazzo (Parco Tecnologico Padano, Lodi, Italy), M. Dacasto (University of Padova, Padova, Italy), G. Martino (University of Teramo, Teramo, Italy)

The objective of this study was to investigate the effect of a high dietary selenium (Se) supplementation on the whole-transcriptome of sheep. A custom-sheep whole-transcriptome microarray, with more than 23,000 unique transcripts, was designed and then used to profile the global gene expression of sheep after a high dietary supplementation of organic Se. Lactating cross-bred ewes (N = 10, 3 to 4 y of age; 55 to 65 kg BW) at their late lactation [100 ± 8 d in milk (DIM)] were acclimated to indoor individual pen feeding of a basal control diet (0.40 mg Se/d, Na-selenite) for 4 wk. Sheep were then kept on a diet with an extra (high) supplementation of organic Se (1.45 mg Se/d as Sel-Plex, Alltech, Australia) for 40 d. Whole blood (2.5 mL) was collected at two time-points [last day of the acclimatization period (T0), and after 40 d of the high Se supplementation (T40)], then total RNA was isolated and labeled for the subsequent microarray analysis. Significant analysis of microarray (SAM), using a paired t test, of the microarray data (T40 versus T0) evidenced the up- and down-regulation of 942 and 244 transcripts (FDR < 0.05), respectively. Seven genes showed the same trend of expression (up- or down-regulation) when tested by qPCR in a cross-validation step. The microarray evidenced the up-regulation of some selenoproteins at T40, such as the selenium binding protein 1 (SELENBP1), selenoprotein W1 (SEPW1), glutathione peroxidase 3 (GPX3) and septin 8 (SEPT8), where the expression trend for SEPW1 and SEPT8 has been additionally validated using qPCR. Functional annotation of the differentially expressed (DE) genes showed the enrichment of several immune system-related biological processes (lymphocyte activation, cytokine binding, leukocyte activation, T cell differentiation and B cell activation) and pathways (cytokine and interleukin signaling). Moreover, gene set enrichment analysis (GSEA) evidenced the enrichment of B and T cell receptors signaling pathways with an enrichment score (ES) of 0.63 and 0.59, respectively. Overall, these results provide, on a global gene expression (whole-transcriptome) scale, the main genes, biological processes and pathways regulated by a high Se supplementation in sheep, which mainly reflect an immune-system and transcription-modulation-induced transcriptomic signature. Moreover, the study delivers a custom whole transcriptomic microarray platform that can be used in further global gene expression studies in the ovine species.

Key Words: selenium, microarray, gene set enrichment analysis

P3002 Functional annotation of the equine genome. C. J. Finno* (University of California, Davis, CA), J. L. Petersen (University of Nebraska, Lincoln, NE), R. Bellone (University of California, Davis, CA), J. N. MacLeod (University of Kentucky, Lexington, KY)

The reference genome sequence of the domestic horse has enabled unprecedented advances in the field of equine genetics. Of the 32 equine traits for which a commercial genetic test is available, 22 were discovered since this milestone was generated in 2007. Most of these known mutations reside in coding regions of genes, yet the vast majority of all mammalian genomes are composed of non-coding DNA. Many important genetic/genomic discovery opportunities lie in unraveling the location and function of the regulatory elements that reside in the non-coding genome and determining, on a tissue-specific basis, how they regulate gene expression. As members of the FAANG initiative with a strong interest in advancing equine genetics, our long-term objective is to create a tissue-specific functional map of the equine genome, including annotation of gene expression patterns, histone modification marks, DNA methylation sites and transcription factor occupancy. This new functional annotation will become the basis for analyzing and comparing gene function and regulation across cell types, tissues, horse breeds and among species, providing a resource for advancing our understanding of development, normal function and pathology in the horse. Our initial objective is to characterize tissue-restricted patterns of gene expression and regulatory regions across eight tissues (skeletal muscle, laminae, liver, ovary/testis, cerebral cortex, lung, spleen and heart) in two well-phenotyped, healthy, adult thoroughbred horses using RNA-sequencing and ChIP-sequencing, respectively. These data will be associated with the underlying genomic DNA nucleotide sequence of each individual. Subsequently, this annotation will be extended to include the location of additional modifiers of gene expression using CTCF-sequencing and the discovery of additional regulatory regions with DNase-I hypersensitivity assays. All data collected will be made publically accessible

in existing genome browsers. Finally, while the initial analyses will be performed on eight tissues, we will archive > 50 tissues from each horse that will be available for future studies.

Key Words: FAANG, transcriptome, gene regulation

P3003 The extent of *cis*-regulation of gene expression and its influence on complex trait variation in cattle. A. J. Chamberlain* (Department of Economic Development, Jobs, Transport and Resources, Bundoora, Australia; Dairy Futures Cooperative Research Centre, Bundoora, Australia), M. Khansefid (Department of Economic Development, Jobs, Transport and Resources, Bundoora, Australia; Dairy Futures Cooperative Research Centre, Bundoora, Australia; University of Melbourne, Parkville, Australia), C. J. Vander Jagt (Department of Economic Development, Jobs, Transport and Resources, Bundoora, Australia; Dairy Futures Cooperative Research Centre, Bundoora, Australia), B. J. Hayes (Department of Economic Development, Jobs, Transport and Resources, Bundoora, Australia; Dairy Futures Cooperative Research Centre, Bundoora, Australia; La Trobe University, Bundoora, Australia), L. C. Marett (Department of Economic Development, Jobs, Transport and Resources, Ellinbank, Australia), Y. Chen (NSW Department of Primary Industries, Menangle, Australia), S. Bolormaa (Department of Economic Development, Jobs, Transport and Resources, Bundoora, Australia), C. A. Millen (Dairy Futures Cooperative Research Centre, Bundoora, Australia; University of Melbourne, Parkville, Australia), T. T. Nguyen (Department of Economic Development, Jobs, Transport and Resources, Bundoora, Australia), M. E. Goddard (Department of Economic Development, Jobs, Transport and Resources, Bundoora, Australia; University of Melbourne, Parkville, Australia)

Cis-regulatory genetic variation can cause variation in gene expression between individuals and between tissues. Allele-specific expression (ASE) and expression quantitative trait loci (eQTL) mapping have both been used to locate this *cis* regulation. Here we present two studies that utilize RNA sequence (RNaseq) data to gain insight into the genetic variation in complex traits, including gene expression and production traits in cattle, resulting from *cis* regulation of gene expression. The first study tested ASE in 18 tissues taken from a lactating cow and two tissues from 20 lactating cows. 89% of all genes tested (total 7985) in the single cow contained at least one SNP with significant ($p < 0.01$) ASE in at least one tissue. ASE ranged from mono allelic to slight overexpression of the major allele. There were some genes that displayed ASE consistently across tissues however many were tissue specific. A large proportion of ASE genes displayed divergent ASE across tissues. The levels of ASE and tissue specificity were validated in the two tissues from 20 cows. We conclude that ASE is pervasive in cattle, some of which is tissue specific, with divergent ASE across tissues common. The second study compared SNP significantly associated with gene expression (detected with ASE and local eQTL) with those significantly associated with variation in complex traits. ASE was tested and local eQTL mapped in four RNaseq datasets from three different tissues from a total of 102 animals from two breeds, and combined in a meta-analysis. Genome-wide association studies (GWAS) were conducted with 729,068 SNP genotypes in (1) 3296 *Bos taurus* cattle for 20 complex traits, (2) 10,191 *Bos taurus*, *Bos indicus* and their crosses for a multi-trait test and (3) 24,041,262 sequence variants in 5614 *Bos taurus* cattle for residual feed intake (RFI). Results showed that SNP driving ASE were also often local eQTL implying that they were *cis*-eQTL. These SNP often affected gene expression in more than one tissue and the allele increasing expression was usually the same. Also, SNP significantly associated with gene expression were more likely than by chance to influence complex traits, indicating that some mutations influence complex traits by changing the expression level of genes. Identification of *cis*-regulatory variants responsible for phenotypic variation in cattle production traits may lead to rapid identification of causative mutations affecting complex traits and thus more accurate genomic selection.

Key Words: allele-specific expression, expression quantitative trait loci, gene expression

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P3004 Differential expression of micrornas in synovial fluid as biomarkers of osteochondrosis in equine hock joints. E. Barrey* (GABI, INRA, AgroParisTech, Universite Paris-Saclay, Jouy-en-Josas, France J. Rivière (GABI, INRA, AgroParisTech, Universite Paris-Saclay, Jouy-en-Josas, France), C. Morgenthaler (GABI, INRA, AgroParisTech, Universite Paris-Saclay, Jouy-en-Josas, France), F. Rossignol (Veterinary Clinic of Grosbois, Boissy St Léger, France), C. Mespoulhès-Rivière (Université Paris-Est, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France), C. Robert (GABI, INRA, AgroParisTech, Universite Paris-Saclay, Jouy-en-Josas, France)

Osteochondrosis (OC) is a developmental disorder reported in several domestic species (pigs, dogs, chickens, etc.), including horses, with differences in prevalence among breeds. OC is defined as a focal disturbance of endochondral ossification. The exact pathophysiology is not yet understood, but it is generally accepted that both genetic and environmental risk factors influence the development of lesions. The findings of several genetic projects have not been consistent because of the variability of phenotypic criteria for defining OC and the variability between breeds. To better understand the pathophysiology of OC and to better define the OC phenotype, we proposed to analyze the miRNAs expressions in synovial fluids of horses from the same breed sharing a similar OC phenotype. Synovial fluid samples were collected from the tibiotarsal joints in 10 French Trotters, seven diagnosed with fragmentation of the intermediate ridge of the tibia (10 OC affected joints) and three controls (six joints), and were frozen until use. Total RNA was extracted using a specific filtration protocol on column and a quality control of RNA was performed. The miRNA expression screening was performed on each sample using RT-qPCR with a panel of 752 human hsa-miRNAs (miRCURY LNA Universal RT micro RNA, Exiqon). Biostatistics and annotation analysis were used to study the differential expression of the miRNAs. Fifteen microRNAs were expressed in all the samples and their average was used to normalize the raw expressions Cp of all the other miRNAs. An average of 87 different miRNAs per sample was detected and less microRNAs counts were observed in OC joints. Benjamini-Hochberg corrected *t* test revealed a significant differential expression in 10 microRNAs downregulated in OC vs. control joints ($p < 0.05$; Figure: volcano plot). Using the top 25 miRNAs commonly expressed in all samples as input variables, principal component analysis discriminated clearly the two groups on the first axis and the variability among OC on the second axis. Using the 10 significant miRNA as input in miRPath, significant enrichment ($p < 10^{-6}$) was observed in many pathways (number of predicted target genes): endocytosis (56), actin cytoskeleton (59), PI3K-Akt signaling (82), TGF-β signaling (26), focal adhesion (53), ubiquitin proteolysis (39), calcium signaling (47), MAPK signaling (63) and insulin signaling (37). Additional synovial sampling of the same joint in the same breed will be performed to further explore these potential miRNA biomarkers and their putative physiopathologic implications in OC.

Key Words: regulome, transcriptome, bone

P3005 Genome-wide transcriptomic analysis of liver in sex-linked dwarf and wild-type chickens.

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The sex-linked dwarf (SLD) recessive mutation is characterized by a significant reduction of body size in hemizygous females (30%) and homozygous males (40%). The SLD chickens of the Leghorn line are affected by a growth hormone (GH) resistance condition due to a missense mutation at the growth hormone receptor (GHR) gene causing a loss of function of the GHR. In addition to its commercial use in the broiler dam lines to improve production efficiency, the SLD mutation is also an interesting model to unravel regulatory pathways depending on GH and to investigate complex traits such as energy expenditure and heat resistance, for which the SLD chickens differ compared to normal-size ones. In this study we performed a genome-wide transcriptome profiling of liver using a 8x60K custom Agilent microarray. In total we analyzed 24 SLD and 24 wild-type birds of 12 wk of age obtained by crossing a heterozygous "dw" Leghorn cock with 12 females of an inbred Leghorn line to obtain wild-type and dwarf sib and half-sib hens. Differential expression analysis revealed that the sex -linked dwarf mutations produce dramatic changes in liver gene expression. In total 615 differentially expressed (DE) genes were identified, 168 of which had an absolute fold-change > 2. Extreme expression reduction was observed for the insulin-like growth factor-1 (IGF1), the IGF-binding protein 4 (IGFBP-4) and the Type II iodothyronine deiodinase (Dio II), consistent with the significant reduction of plasma IGF-I and triiodothyronine (T3) levels previously observed in SLD chickens. Biological process analyses of sex -linked dwarf-induced DE genes indicate an alteration of lipid and amino acid metabolism, molecular transport and cellular growth and proliferation

Key Words: chicken, dwarf mutation, transcriptomic analysis

P3006 Integrated network multi-omics approach highlights muscle late fetal maturation process.

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While transcriptomic analysis has provided incredible insight into cell operation, an integrated multi-omics approach is crucial to gain further insights into complex biological systems. Here, we chose to develop an integrated network method of proteomic and phenotypic data, with integration of transcriptomic information, to highlight some important proteins during the end of gestation in pig skeletal muscle. Networks are increasingly used to analyze and visualize data in biology and genetics. An integrated network analysis was first developed to explore relationships between co-expression network models, built from proteomic data, and targeted biological phenotypes of interest to identify molecular signatures underlying late fetal muscle development. Second, correlation with muscle transcriptomic data was also investigated to complete and combine different layers of expression. Piglet maturation, which occurs at the end of gestation, leads to a state of full development after birth and is an important determinant of early survival. The objective of our project is an integrated global multi-omics analysis (transcriptome, proteome and targeted biological phenotypes) with a focus on skeletal muscle because of its key role in adaptation to extra-uterine life (locomotion and thermogenesis). Progeny from two extreme purebreds for maturity (Large White and Meishan) were investigated. The Large White (LW) breed is a highly selected breed with a high rate of mortality at birth, whereas the Chinese Meishan (MS) is a more robust breed exhibiting an extremely low neonatal mortality. The late fetal maturation process was analyzed on the progeny from these two breeds (LW, MS and reciprocal F1) at two developmental time points during the end of gestation (90 and 110 d of gestation). First, three targeted biological phenotypes (glycogen, MyHC adult fast and embryonic) were found as good descriptors of muscle maturity. The proteomic approach showed that proteins associated with the cytoskeleton and muscle filaments were overexpressed at 90 dg, whereas proteins involved in muscle energy metabolism were strongly up-regulated at 110 dg. The integrated network analysis revealed a high number of proteins involved in the mitochondrial oxidation/reduction metabolic process that were overexpressed at 110 dg with a higher expression in MS than in LW. In particular, CKMT2 and ATP5A1 were identified as important nodes and possible good biological markers of muscle maturity at 110 dg. Our data also showed that some of these proteins are transcriptionally regulated and that PPARGC1A could be an important transcriptional factor.

Key Words: biological data integration, muscle fetal development, pigs

P3007 Time course of the response to ACTH in pig: biological and transcriptomic study.

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The hypothalamic–pituitary–adrenal (HPA) axis plays a major role in physiological homeostasis. It is also involved in stress and adaptive response to the environment. In farm animals in general and more specifically in pigs, breeding strategies have highly favored production traits such as lean growth rate, feed efficiency and prolificacy at the cost of robustness. On the hypothesis that the HPA axis could contribute to the trade-off between robustness and production traits, we have designed this experiment to explore individual variation in the biological response to the main stress hormone, cortisol, in pigs. We used adrenocorticotropic hormone (ACTH) injections to trigger production of cortisol in 120 juvenile Large White (LW) pigs from 28 litters, and the kinetics of the response was measured with biological variables and whole blood gene expression at four time points. A multilevel statistical analysis was used to take into account the longitudinal aspect of the data.

Cortisol level reached its peak 1 h after ACTH injection. White blood cell composition was modified with a decrease of lymphocytes and monocytes and an increase of granulocytes ($FDR < 0.05$). Basal level of cortisol was correlated with birth and weaning weights. Microarray analysis identified 65 unique genes whose expression responded to the injection of ACTH (adjusted $P\text{-}value < 0.05$). These genes were classified into four clusters with distinctive kinetics in response to ACTH injection. The first cluster identified genes strongly correlated to cortisol and previously reported as being regulated by glucocorticoids. In particular, *DDIT4*, *DUSP1*, *FKBP5*, *IL7R*, *NFKBIA*,

PER1, *RGS2* and *RHOB* were shown to be connected to each other by the glucocorticoid receptor NR3C1. Most of the differentially expressed genes that encode transcription factors have not been described yet as being important in transcription networks involved in stress response. Their co-expression indicates possible co-regulation, and they potentially provide new biomarkers of sensitivity to cortisol.

Key Words: stress, pig, genes

P3008 Transcriptome profiling of reproductive tissues characterizes genetic basis of the prolificacy traits in sheep (*ovis aries*).

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Fertility traits such as high ovulation rate and litter sizes are affected both by the genetic and environmental factors. We have investigated mRNA and microRNA transcriptomes of three tissues important in reproduction — ovary, *corpus luteum*(CL) and endometrium — in Finnsheep (n = 11), Texel (n = 11) and their F1 crossbred ewes (n = 9). Tissue sampling for RNA extraction was done in two different time points during the establishment of pregnancy: follicular growth phase (ovarian tissues) and early pregnancy before implantation (CL and endometrium). We also studied the effect of nutrition on reproduction by keeping half of the individuals in flushing diet (additional energy and protein supply). During the feeding experiment, hormonal and energy measurements were recorded, and during the tissue sampling number of follicles and CL were counted. The mRNA and miRNA sequencing was done using Hiseq2000 (ovarian samples) and Hiseq3000 (CL and endometrium samples) Illumina technology. We found that Texel ewes responded to the flushing diet in terms of number of CLs and gene expression levels, while no clear effect was seen in the highly prolific Finnsheep. Interestingly, F1-ewes behaved more like Finnsheep. The number of CL in Finnsheep was on average twice than that found in Texel. The highest number of genes was expressed in the ovaries (on average 16,402), followed by CL (16,067) and endometrium (15,457); however, there were differences in these numbers between the groups. We observed a recently identified GDF9-gene mutation, a putative causative mutation for prolificacy, in majority of Finnsheep and half of the F1 ewes but none in Texel. Differentially expressed genes in our study were associated with gene ontology (GO) terms and pathways related to reproductive and metabolic processes such as angiogenesis, steroidogenesis, cell migration and VEGF signaling pathway. A number of novel miRNAs were expressed in the data. Only few miRNAs were significantly differentially expressed in the different tissues between breed groups. A high proportion of miRNAs are clustered within 194 kb on Chr 18, which is homologous to human (Chr 14), dog (Chr 8), horse (Chr 24) and other mammals. These results will improve the knowledge of important fertility traits in sheep, providing an invaluable new data for genomic research.

Key Words: mRNA, miRNA, ovary, corpus luteum, endometrium

P3009 Polymorphisms of NELL1 and RNCK1 in relation to porcine growth, carcass and meat quality traits.

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Nel-like molecule-1 (*NELL1*), discovered in prematurely fused cranial sutures, is a secreted osteogenic growth factor and plays an important role in osteoblast differentiation, bone formation, regeneration and adipose differentiation and cell focal adhesion. The RBCC protein interacting with protein kinase C1 (*RBCK1*) is a nuclear-cytoplasmic shuttling protein

and possesses transcriptional and ubiquitin ligase activities. *RBCK1* is involved in immune regulation, antiviral signaling, iron and xenobiotic metabolism and cancer. This study was to genotype single nucleotide polymorphisms (SNPs) of *NELL1* and *RBCK1* with PCR-RFLP in the Pietrain (Pi) and Duroc × Pietrain (DuPi) F2 population. The SNPs of porcine *NELL1* were associated with backfat (middle) in Pi population and with conductivity 45 min and 24 h *post mortem* in ham ($Con24_H$) in DuPi population. The SNP in *RBCK1* was associated with backfat (middle), muscle area in Pi population and with shoulder weight, daily gain (from 30 to 105 kg), meat color, pH 24 h p.m. in loin and ham ($pH24_L$ and $pH24_H$) and thawing loss in DuPi population. Haplotypes were constructed within *NELL1* and *SOX-6,* where two SNPs in each gene showed significant associations. It was found that the haplotypes of *SOX-6* were associated with net daily gain and meat color in Pi population. At the same time, the haplotypes of *NELL1* were associated with backfat (middle) in Pi population. In this study, we found that the polymorphisms of *NELL1* and *RBCK1* were associated with porcine growth, carcass and meat quality traits. The association and explained variance of SNPs into haplotypes generally normalized the association of involved each SNP to the respect traits in Pi population. However, the haplotypes of *SOX-6* were associated with net daily gain, which is not found for either SNPs of *SOX*-6 in our previous work. This may indicate the existence of other valuable SNPs in porcine *SOX-6* loci. This report shows that *NELL1* and *RBCK1* could be potential candidate genes for porcine growth, carcass and meat quality traits.

Key Words: SOX-6, meat quality, single nucleotide polymorphism

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P3010 Effect of rumen content exchange on gene expression in rumen epithelium of lactating cows. J. Vilkki* (Natural Resources Institute Finland, Jokioinen, Finland), D. Fischer (Natural Resources Institute Finland, Jokioinen, Finland), I. Tapio (Natural Resources Institute Finland, Jokioinen, Finland), K. J. Shingfield (Aberystwyth University, Aberystwyth, United Kingdom)

The effect of rumen digesta exchange on gene expression in rumen papillae was analyzed from an experiment involving a total rumen content exchange between three pairs of lactating cows fed the same diet. Papillae samples were sequenced for both mRNA and miRNA at three time points: at the exchange and 1 or 2 wk afterward. Papillae samples were obtained during rumen evacuation from the ventral sites of rumen, immersed in liquid nitrogen and submitted for RNA extraction (AllPrep DNA/RNA/miRNA Universal Kit, Qiagen). Sequencing libraries were prepared according to Illumina TruSeq® Stranded mRNA and TruSeq® Small RNA sample preparation for mRNA and miRNA, respectively. Paired-end sequencing with 2 × 150 bp read length and the Illumina HiSeq 3000 platform was used for mRNA (average 50.6 M reads per sample) and single-read sequencing with 1 × 50 bp read length using Illumina HiSeq 2500 for miRNA (average 2.5 M reads per sample). From mRNA libraries, 67.8% of reads were mapped. About 53.5% of the mapped reads were located to known genes, with the remaining reads mapping to unannotated regions of the bovine genome. Novel gene candidates were analyzed with our in-house R-package hoardeR to identify potential orthologs. From miRNA libraries 36.3% reads were uniquely mapped. From 811 annotated miRNAs, 382 miRNAs were expressed. Differential expression (DE) analysis by edgeR provided a set of genes found to be differentially expressed before and after the rumen exchange, indicating that specific genes respond to changes in the rumen contents and microbial communities. From the DE analysis two groups of cows that responded differently to the rumen exchange were indentified. The biological significance of these two groups was further confirmed by links with metabolic data such as nitrogen metabolism and ruminal VFA concentration. Affected canonical pathways, identified using Ingenuity Pathway Analysis (Qiagen), included pathways putatively involved in epithelial proliferation and Acyl-CoA metabolism. The miRNA expression patterns within the two groups were compared and correlated to the expression of their known target genes and rumen metabolism.

Key Words: rumen, papillae, transcriptome

P3011 Toward robust blood biomarkers for residual feed intake in pigs. M. Schroyen* (Department of Animal Science, Iowa State University, Ames, IA), K. M. Feye (Department of Biomedical Sciences, Iowa State University, Ames, IA), Y. T. Nguyen (Department of Statistics, Iowa State University, Ames, IA), A. Rakhshandeh (Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX), N. K. Gabler (Department of Animal Science, Iowa State University, Ames, IA), D. Nettleton (Department of Statistics, Iowa State University, Ames, IA), J. C. M. Dekkers (Department of Animal Science, Iowa State University, Ames, IA), C. K. Tuggle (Iowa State University, Ames, IA)

To study the genetics and physiology of feed efficiency in pigs, genetic selection based on residual feed

intake (RFI) was used to create two divergent lines: a more efficient low RFI line and a less efficient high RFI line. The objective of this study was to examine the effect of selection for low RFI on response to an immunological stressor, lipopolysaccharide (LPS), since selection toward feed efficiency may negatively impact the immune system. Twenty-eight 4-mo-old Yorkshire gilts, 14 of each line, were intramuscularly injected with either increasing amounts of LPS (immune system stimulated, ISS+; $n = 16$) or saline (control, ISS-; $n = 12$) at 0, 24, 48, 96 and 144 h. Paired-end strand-specific RNA-sequencing was performed on whole blood collected at 0 and 168 h. Forward selection of covariates determined the best statistical model, and the QuasiSeq R package was used to identify differentially expressed (DE) genes between lines or due to LPS treatment. We found 860 genes were DE between ISS+ and ISS- (FDR < 0.05). Cell type enrichment (CTEN) and gene ontology (GO) analyses revealed that the DE genes were expressed by monocytes evoking an "inflammatory response" ($p < 4e\text{-}9$) accompanied with a significant "cytokine" ($p < 3e\text{-}4$) and "chemokine receptor activity response" ($p < 2e\text{-}3$). However, there was no line-by-treatment interaction effect, indicating this response to be similar for both RFI lines. Between lines, 318 and 213 genes were DE (FDR < 0.05) at 0 and 168 h, respectively, of which 116 overlapped between these two time points. These genes also had a similar direction of DE between lines on both days. CTEN analyses indicated that these 116 common DE genes were mainly expressed by B cells, which was supported by GO annotation results such as "B cell receptor signaling pathway" ($p < 6e\text{-}4$) and "lymphocyte activation" ($p < 9e\text{-}4$). Interestingly, 26 of these DE genes were also identified to be significantly DE between these two lines, and in the same direction, in blood collected on a different set of non-LPS-treated pigs (Liu et al., 2016). Enrichment of these 26 DE genes within the Liu et al. study DE list was highly significant (Fisher's exact test $p = 8.3e\text{-}5$). This common DE list includes two out of five genes that Liu et al. found to be correlated with RFI value and proposed as potential blood RFI biomarkers. We therefore propose that this replicated set of 26 RNAs may be predictive blood biomarkers for RFI line under both healthy and immune stimulated conditions. Acknowledgment: USDA-NIFA-AFRI#2011–68004–30336.

Key Words: RFI, pig, biomarker

P3012 Deconstructing the pig genome-metabolome functional interactions. L. Fontanesi* (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), S. Bovo (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), G. Schiavo (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), G. Mazzoni (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), A. Ribani (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), V. J. Utzeri (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), S. Dall'Olio (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), F. Bertolini (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy; Department of Animal Science, Iowa State University, Ames, IA), F. Fanelli (Department of Surgical and Medical Sciences, Endocrinology Unit, University of Bologna, Bologna, Italy), M. Mezzullo (Department of Surgical and Medical Sciences, Endocrinology Unit, University of Bologna, Bologna, Italy), G. Galimberti (Department of Statistical Sciences "Paolo Fortunati," University of Bologna, Bologna, Italy), D. G. Calò (Department of Statistical Sciences "Paolo Fortunati," University of Bologna, Bologna, Italy), P. Trevisi (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), P. L. Martelli (Biocomputing Group, University of Bologna, Bologna, Italy), R. Casadio (Biocomputing Group, University of Bologna, Bologna, Italy), U. Pagotto (Department of Surgical and Medical Sciences, Endocrinology Unit, University of Bologna, Bologna, Italy), P. Bosi (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy)

Metabolomics has been changing the way in which differences among animals can be investigated. Metabolomics can detect and quantify hundreds of metabolites that constitute internal (or molecular) phenotypes, providing information on the metabolic state of the animals that is influenced by genetic and environmental factors. Metabotypes are referred as phenotypes defined by the level of metabolites in a biological fluid. In this study, we determined about 200 plasma metabotypes in two cohorts of Italian Large White (~1000 animals) and Italian Duroc pigs (~400 animals) that have been genotyped with the Illumina PorcineSNP60 BeadChip. Genome-wide association studies identified a large number of significant regions affecting metabotypes in both breeds, partially overlapping comparing the two groups of pigs, suggesting that common genetic determinants and, on the other hand, different genetic factors are acting in Italian Large White and Italian Duroc populations to shape their breed-defined

metabolomic profiles. Metabotypes from metabolite families were influenced by the same genomic regions providing useful information to indirectly disentangle unknown metabolomic pathways. Genome-wide association results were also able to de-orphanize a few genes based on the affected metabolites. Metabolomic-genomic interactions were used to predict production traits opening new opportunities to integrate molecular phenotypes for breeding purposes.

Key Words: GWAS, metabolomics, phenomics

P3013 Characterization of endometrium protein expression during mid-late gestation in Meishan and Duroc sows with iTRAQ analysis. K. Wang,* M. Fang (China Agricultural University, Beijing, China)

More than 30% embryonic mortality was observed during pregnancy in pig and mainly occurred in early and mid-late gestation, which accounts for 10–15% proportion. Meishan breed is one of the most prolific pig breeds known, farrowing between three and five more live piglets per litter than European commercial breeds, including Duroc pig. To illustrate the characterization of endometrium protein expression during mid-late gestation between Meishan and Duroc sows, iTRAQ was selected to detect global protein level. Endometrium tissues at Day 49 and Day 72 of gestation from Meishan and Duroc sows were collected. In total, 4499 proteins were identified, of which 2170 proteins were quantified. In relative to DUD49, 45 up-regulated and 69 down-regulated protein was detected in MSD49. Comparing to DUD72, 56 up-regulated and 42 down-regulated proteins were detected in MSD72. During the pregnancy process, 43 up-regulated and 27 down-regulated proteins were observed in Meishan sows, and 35 up-regulated and 79 down-regulated proteins were identified in Duroc sows. Lots of GO terms were clustered between stages or breeds. Of these, we were interested in GO:0004867 term, which were enriched by SLPI, SERPIN1, UFAP, UFBP, ITIH1 and ITIH2. These genes were partly found involved in matrix degradation required for inplantation. Two GO terms which involved intermediate filament and contractile fiber part were extracted in differentially expressed proteins between MSD72 and MSD49. These genes were demonstrated maybe responsible for the substantial adaptive responses of entire uterine wall to the increasing mechanical forces generated as a consequence of rapid fetal growth and accumulation of fetal fluids. Furthermore, GO term: regulation of angiogenesis was discovered between MSD49 and DUD49 which involved genes also provided a higher level in MSD72 than DUD72. Collectively, this data characterized the protein expression during the pregnancy process between Meishan and Duroc sows that could provide a foundation for uncovering the genetic basis for embryonic mortality.

Key Words: iTRAQ, endometrium, sow

P3014 The study on the genetic mechanism of varied atrogin-1 expression in different chicken lines. J. Li* (Beijing Institute of Genomics, Chinese Academy of Science, Beijing, China; China Agricultural University, Beijing, China), Y. Hu[3], H. Lan (The State Key Laboratory for Agro-Biotechnology, China Agricultural University, Beijing, China), L. Li (The State Key Laboratory for Agro-Biotechnology, China Agricultural University, Beijing, China), X. Hu (China Agricultural University, Beijing, China), N. Li (China Agricultural University, Beijing, China)

Chicken is bred all over the world and has significant economic value as one of the major agricultural commercial animals. The behavior of growth, reproduction and disease resistance varies among different chicken breeds. Deciphering the genetic basis of these differences has great value in both biological science and agricultural economy. In this study, we used next-generation sequencing technique to study the genetic mechanism of *MAFbx* (muscle atrophy F-box) varied expression in different chicken breeds.

Ubiquitin E3 ligase–*MAFbx*/*Atrogin-1* plays an important role in muscle protein degradation. In our previous study, we found that the expression of *MAFbx*/*Atrogin-1* was significantly higher in wild chicken than in broiler, which implied the importance of *MAFbx*/*Atrogin-1* in muscle development. Thus, we sought to unveil the molecular mechanism of how the *MAFbx*/*Atrogin-1* affects the development of muscle. Our study may help to shorten the period of chicken breeding and improve the productivity.

We proved that the expression of *MAFbx*/*Atrogin-1* was higher in Red Jungle Fowl (RJF) and Chahua than in broiler chicken. HE staining of skeleton results demonstrated that the myofiber was thinner in RJF. The *MAFbx*/*Atrogin-1* expression profile in differential development stages showed that the gene expression divergence had emerged at Day 4 after born. The sequencing results of *MAFbx*/*Atrogin-1* gene region in eight different domestic chicken breeds performed that heterogeneity reduced in broiler chicken. We presumed that a causative mutation occurred in *MAFbx*/*Atrogin-1* gene region. In vitro, luciferase and gel shift assay of candidate SNPs confirmed that a single base mutation of gene's intron 4 reduced the enhancer activity and inhibited the binding of transcription regulator in broiler chicken. Our results suggested that

MAFbx/*Atrogin-1* expression variance was probably caused by the mutation.

Key Words: *MAFbx*/*Atrogin-1*, SNP, chicken

P3015 Analysis of G protein-coupled receptor gene expression during bovine intramuscular adipogenesis. T. Kuwabara,* Y. Mizoguchi (School of Agriculture, Meiji University, Kawasaki, Japan)

It has been identified that G protein-coupled receptors (GPCR) 41 and 43 are characterized by binding to the short chain fatty acids (such as acetic acid, propionic acid and butyric acid) (Hong et al., 2005) which were related at adipose differentiation and development in mice (Brown et al., 2003; Nilson et al., 2003). Recently, both GPCR 41 and GPCR 43 have also been identified in bovines (Wang et al., 2009). In this study, we investigated the GPCR 41 gene involvement in bovine intramuscular adipogenesis by analyzing the gene expression level after differentiation in a clonal bovine intramuscular preadipocyte (BIP) cell line (Aso et al., 1995). We harvested the BIP cells 0, 3, 6, 9 and 12 d after adipogenic stimulation with either 4.5 g/L (high) or 1 g/L (low) glucose contents and then measured the GPCR 41 gene expression levels and the accumulation of triglycerides (TG). TG contents in the BIP cells significantly increased between Day 0 and Day 12 after stimulation with both glucose concentrations. Moreover, the GPCR 41 gene expression level increased during the latter half of adipogenesis. Therefore, these data suggest that the GPCR 41 gene might be involved in accumulation of TG in the BIP cells. To understand more details about the function of GPCRs on bovine intramuscular adipogenesis, these gene expression profiles should be conducted by the dose changes in addition of short chain fatty acids into media.

Key Words: bovine, adipogenesis, G protein-coupled receptors

P3016 Breed and feeding factors influencing adipose tissue lipogenic and lipolytic gene expression in growing Iberian and Duroc pigs. R. Benítez* A. Fernandez (INIA, Madrid, Spain), B. Isabel (UCM, Madrid, Spain), Y. Nuñez (INIA, Madrid, Spain), E. Alves (INIA, Madrid, Spain), E. De Mercado (Instituto Tecnológico Agrario, Segovia, Spain), E. Gómez-Izquierdo (Instituto Tecnológico Agrario, Segovia, Spain), J. M. García-Casco (INIA, Zafra, Spain), M. C. Rodríguez (INIA, Madrid, Spain), C. López-Bote (UCM, Madrid, Spain), L. Silió (INIA, Madrid, Spain), C. Ovilo (INIA, Madrid, Spain)

Tissue composition largely determines the quality of meat and meat products and is influenced by factors as diet, genetic type, age or sex. Diet influences animal body and tissue composition due to direct deposition and to the nutrients' effects on metabolism. The influence of specific nutrients on the regulation of lipogenesis may be conditioned by feeding level or genetic background. In this study we evaluated breed, dietary energy source and 24-h fasting effects on gene expression of candidate genes involved in lipogenesis (*SCD, ME1, FASN* and *ACACA)* and lipolysis (*ATGL* and *HSL*) in Duroc and Iberian growing pigs. A total of 30 Iberian and 19 Duroc males started the dietary treatment at 19.3 kg of average weight (LW) and were kept under identical management conditions and fed with two different isocaloric and isoproteic diets (3.3 Kcal of digestible energy and 15.6% of crude protein) provided ad libitum: HO diet enriched with 6% high-oleic sunflower oil and CH standard diet with carbohydrates as energy source. All animals were slaughtered after 47 d of treatment, with 50.7 kg of average LW. Greater feed intake, backfat thickness, percentage of intramuscular fat, size of adipocytes and SFA content of subcutaneous fat were observed in Iberian pigs, whereas a greater ham weight and PUFA content were registered in Duroc. In both breeds, FA composition of subcutaneous fat samples showed significant differences between diets, with higher MUFA and oleic acid and lower SFA and PUFA in the HO treatment. Regarding the diet and breed effects on gene expression in fasting and postprandial status, breed and feeding status had significant effects, with evidence of quantitative interaction between them, while diet showed negligible effects. The expression of lipogenic genes was higher in Iberian pigs and higher in the samples obtained in postprandial status. On the contrary, the expression of lipolytic genes tended to be higher in Duroc and in the samples obtained in fasting status, with complex interaction effects. Results agree with the hypothesis of a fasting inhibition of lipogenesis and stimulus of lipolysis. Quantitative interactions breed x feeding status point to a different response between breeds to nutritional interventions, with a more stable expression of lipogenic genes in Iberian pigs, in agreement with their higher lipogenesis potential.

Key Words: Iberian pig, nutrigenomics, oleic acid, gene expression, lipid metabolism, fasting, breed

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P3017 Functional analysis and association studies of bovine MYOT gene with meat quality. C. M. Adoligbe* (University of Abomey-Calavi, Abomey-Calavi, Benin), L. Zan (Northwest A&F University, Yangling, China), S. Farougou (University of Abomey-Calavi, Abomey-Calavi, Benin)

The main objective of the present work was to study the molecular mechanisms of MYOT gene expression and its association with beef production traits. MYOT gene is a multifunctional gene involved in muscle development, which makes it a candidate gene for the improvement of meat quantity and quality. In the current study, the full length of bovine MYOT gene cDNA spanning 2234 bp was successfully cloned using skeletal muscle tissue. Real-time PCR analysis revealed that the gene is highly expressed in skeletal muscle followed by heart and heart fat tissues. Bioinformatics analysis showed that bovine MYOT protein contain eight different conserved functional domains. Polymorphism analysis of the gene sequence showed correlation of its mutations with loin muscle area, backfat thickness and intramuscular fat ($P < 0.05$) in Chinese Qinchuan cattle breed. We have further investigated the longitudinal expression profile of the MYOT gene and its corresponding cell differentiation process during myotube formation. Expression of the gene was initiated on Day 3 of differentiation and scored a peak on Day 6. This is identical to the expression pattern exhibit by three other myotube markers included in this experiment (MYOG, MYL1 and MYF6). Hence, MYOT gene may play a pivotal role in myoblast fusion and could also be considered as myotube marker. As showed by Western blot analysis, protein expression of the gene started from Day 6 of differentiation and increased slightly with the increase in the number of days. Lentivirus-mediated down-regulation of MYOT has inhibited significantly the expression of type I (slow-oxidative) and type IIA (fast-oxidative) muscle fiber types. However the inhibition of the gene has led to the increase in expression of type IIX (fast glycolytic) muscle fiber. These suggest that MYOT gene could affect muscle fiber characteristics and subsequently sensory quality of cooked meat. In conclusion, our findings showed that MYOT gene is essential for the regulation of muscle traits, and its SNPs can be used as genetic markers to expedite genetic change using marker-assisted selection in cattle breeding.

Key Words: bovine MYOT gene, SNP, meat production traits

P3018 Gene expression analysis in backfat and identification of eQTL regions for fatness and fatty acid composition candidate genes in pigs.

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Fat content and fatty acid (FA) composition determine important sensory and technological aspects of pork and meat products because of their influence on the melting point and oxidative status of porcine tissues. Artificial selection to increase meat production in pigs has caused a reduction of intramuscular fat and changes in meat FA composition in some breeds. Gene expression levels can be analyzed as a quantitative trait to identify genomic regions regulating its expression. These regions are known as expression quantitative trait loci (eQTLs). The detection of eQTLs has been proposed as a good strategy to deepen the study of the genetic architecture of complex traits. The aim of this work was to study the genetic basis of the backfat expression of 43 lipid-related genes associated with meat quality traits in pigs. We performed an expression genome-wide association (eGWAS) with the backfat gene expression measured by real-time PCR and the PorcineSNP60 BeadChip genotype information in 115 Iberian x Landrace backcross (BC1_LD) animals. The eGWAS identified 224 eSNPs located in 21 chromosomal regions on SSC2-SSC4, SSC6, SSC8-SSC10 and SSC13-SSC15 and associated with the *ACSM5*, *ELOVL6*, *FABP4*, *FADS2*, and *FATP4* genes. Three out of 21 eQTLs corresponding to *ACSM5*, *FABP4*, and *FADS2* were classified as *cis*-acting eQTLs, whereas the remaining 18 eQTLs have trans-regulatory effects. In addition, four polymorphisms were identified and genotyped in the BC1_LD animals for the genes with *cis*-acting eQTLs: two SNPs located in the proximal promoter region of *ACSM5* (SSC3) and *FADS2* (SSC2), and one Indel and one SNP located

in the intron 1 and in the 3'UTR region of *FABP4*, respectively. It is noteworthy that the strongest association signal for *ACSM5* gene expression was shown for the polymorphism located in the promoter region of this gene. For *FABP4* gene expression, the SNP in the 3'UTR region showed the lower *P*-value, and the indel was also one of the most significantly associated, as has been previously described. Thus, these SNPs are strong candidate polymorphisms to explain the mRNA variation of the *ACSM5* and *FABP4* genes and may have also a role in the determination of the FA composition in the BC1_LD animals. In concordance with these results, *FABP4:g.2634_2635insC* polymorphism has been associated with the percentage of palmitoleic and eicosatrienoic FA and polyunsaturated FA in muscle. These findings provide resources to decipher the functional regulatory mechanisms implicated in the variation of meat quality traits in pigs.

Key Words: gene-expression, expressionQTL, lipid-metabolism

P3019 Screening and characterization of copy number cariation in South African nguni cattle using next-generation sequencing data. M. D. Wang* (ARC Biotechnology Platform, Pretoria, South Africa; University of Stellenbosch, Stellenbosch, South Africa), K. Dzama (University of Stellenbosch, Stellenbosch, South Africa), J. Rees (Agricultural Research Council-Biotechnology Platform, Pretoria, South Africa), C. Hefer (ARC Biotechnology Platform, Pretoria, South Africa), F. C. Muchadeyi (Agricultural Research Council-Biotechnology Platform, Pretoria, South Africa)

Copy number variations (CNVs) are modifications in DNA structure comprising of deletions, duplications and insertions. Prevalent in bovine genomes, CNVs have been designated as playing a role in adaptation and interindividual and between breed variations in cattle. South African Nguni cattle have undergone years of natural selection in harsh environmental conditions, resulting in a breed that is well adapted to the abrasive conditions of Southern Africa. To date no next-generation sequence data of any cattle breed from Southern Africa has been published. Next-generation sequencing data has been deemed a suitable means of supplementating array based CNV studies, as breakpoints can be more accurately determined, while analyses are not limited to predefined marker regions. It is hypothesized that CNVs are prevalent within the genome, genetic diversity and adaptation of Nguni cattle. Twenty-one South African Nguni cattle were thus sequenced on the Illumina Nextera HiSeq 2500 at an average of 10 × coverage. Paired end reads were trimmed and mapped against the UMD3.1 and Btau 4.6.1 reference genomes using Timmomatic v0.33, Burrows Wheeler Alignment and samtools. The average mapping percentaged was 97.05 and 97.29 for UMD3.1 and Btau4.6.1 references. The recently developed RAPTR-SV software was utilized to identify regions of variable copy number by means of hybrid split-read and paired end method. CNVs were filtered according to the number of reads that support the event with low stringency (F10), medium stringency (F45) and high stringency (F75). Adjacent and overlapping CNVs were merged to from 185, 21 and 10 unique CNVRs of between 1 kb and 1.59 Mb in length at F10, F45 and F75. Comparisons with previously published Bovine 50K BeadChip data from the same breed demonstrate notable discrepancies with considerably more CNVs of smaller size being reported by sequencing data. CNVRs at F10, F45 and F75 covered or lay within 1Mb of 218, 65 and 12 genes, respectively, that represented a number of biological processes, cellular components and molecular functions. Four CNVR genes were shared between F10 CNVRs and those previously reported from array data. The addition of sequence data to array data provides a more comprehensive picture of CNV prevalence within the genome. The occurrence of CNVRs within regions of the Nguni genome involved in processes like biological regulation, metabolic process and response to stimulus designate a possible correspondence of CNVR prevalence with adaptation traits.

Key Words: CNVs, next-generation sequencing, cattle, adaptation

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P3020 The potential relationship between comb color and egg production revealed by GWAS in blue-shelled chicken. X. Dong,* J. Li, Y. Zhang, X. Deng, C. Wu (Key Laboratory of Animal Genetic Improvement, Beijing & Animal Genetic Resources and Molecular Breeding Laboratory, China Agricultural University, Beijing, China)

In the last decade, a lot of genetics of traits in domestic animals were deciphered, they may account for the origin of interesting phenotypes. It was found that the exceedingly fantastic appearance not only represents the physiognomy but relates to development and health, which are treated as complex traits; that is to say, the appearance of animals may associate with growth and development or so-called economic traits, and the monogenic character may engage the formation of complex traits. In Dongxiang Blue-Shelled chicken, two different appearances of comb are segregated, with the color being red and dark. By collecting the egg number from 20 to 60 wk in a homogeneous population in continuous generations, we found the egg production of red

comb subpopulation is significantly higher than the dark comb subpopulation ($p < 0.05$). We inferred that the potential genetic link may exist between comb color and egg production. In this study, we used the 600K Affymetrix Axiom HD genotyping array to conduct a genome-wide association study (GWAS) on comb color to find the related genes and analyzed the relationship between comb color and egg production. We first explored the physiological basis of comb color by tissue section and found melanin is the main cause inducing the difference of comb color. Then the GWAS was performed on comb color. It revealed that 66 SNPs densely distributed on the Chr 20 at a region of ~1.4 Mb were significantly associated with comb color, among which eight significant SNPs were located in four known genes: SLMO2, VAPB, SLC35C2 and SNRPB. The genomic region we identified is consistent with a previous report. The region covers the structure variation of a complex genomic rearrangement involving EDN3 which causes the pigmentation of the dermal layer of skin in chicken. Association study was conducted on egg production on Chr 20, we found three significant SNPs in the identical genomic region as well as comb color, among which one SNP was located at 7 kb downstream of the known hyperpigmentation gene EDN3, suggesting that the existence of pleiotropism of the structure rearrangement which causes the variation of both comb color and egg production, in addition to EDN3, other potential genes including non-coding RNA in the genomic region may play crucial roles in the two different traits.

Key Words: comb color, egg production, GWAS

P3021 Effects of diet on the expression of lipid metabolism signaling genes in the longissimus dorsi muscle of Polish Holstein bulls.

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The aim of the study was to determine the effect of supplementation on gene expression of stearoyl-CoA desaturase (SCD), acetyl-CoA carboxylase α (ACACA), lipoprotein lipase (LPL) and diacylglycerol O-acyltransferase (DGAT) involved in lipid metabolism in longissimus dorsi muscle (LM) of young Holstein-Friesian bulls. The study was conducted on 25 bulls ($n = 25$) from five different nutritional groups: bulls extensively fed on a pasture, bulls fed TMR based on corn silage, bulls intensively feeding TMR with the addition of lineseed and mineral mixture of bioplexes, bulls intensively fed with the addition of selenium yeast and bulls fed meadow grass with supplement of concentrate. Controlled bulls supplementation took around 90 d between 12 and 15 mo of age. Gene expression was analyzed by reverse-transcription polymerase chain reaction (RT-PCR). As the reference gene was used glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The results were expressed as $2\Delta\Delta Ct$: $\Delta\Delta Ct = (Ctij - CtGAPDHj) - (Cti1 - CtGAPDH1)$. Where Ctij and CtGAPDHj are the Ct values for gene i and for GAPDH in a sample (named j); Cti1 and CtGAPDH1 are the Ct values in sample 1. Differences between groups were evaluated with analysis of variance by using One-Way ANOVA. Bonferroni's tests were used for interpretation of the data. All normalized gene expression values are expressed on a value of natural logarithm. The data were expressed as least squares means with standard errors. Significance was declared when $P < 0.05$. The present paper describes differences in expression of genes associated with systems of feeding. In this study, the results of RT-PCR showed that the expression of studied genes was remarkably different in diverse systems of feeding. The expression level of LPL gene was relatively higher in muscle of pasture cattle. All procedures involving cows were approved by Local Ethics Commission I, Agricultural University, Warsaw (permission No. 27/2010).

Key Words: gene expression, supplementation, cattle

P3022 Hepatic genes and pathways related to hematological and biochemical traits promoting resilience.

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Liver is the central metabolic organ and also exhibits fundamental functions of defense as well as in haemopoiesis and synthesis of coagulation proteins. Accordingly, liver expression profiles are indicative for the metabolic status and traits related to response to stimuli. Haematological traits and clinical-chemical, biochemical traits of blood are important biomarkers for immune and metabolic status, perturbation of homeostasis and diseases. To detect genetic variation related to hepatic pathways promoting resilience, i.e., metabolic stability and health, we obtained hepatic expression of 297 German Landrace pigs (180 d of age) using the Affymetrix Snowball microarrays, as well as hematological (12 traits) and biochemical traits (eight traits) from blood samples and genotypes (PorcineSNP60 BeadChip, Illumina) for assessing trait-associated expression and eQTLs. At FDR < 1%, 5387 transcripts showed correlated expression with at least one of 12 hematological traits ($r^2 = \backslash 0.22$–$0.48\backslash$). The sets of genes correlated with traits of the white blood cell counts were related to Acute Phase

Response Signaling and Hepatic Fibrosis/Hepatic Stellate Cell Activation. For erythrocyte related traits transcripts of Oxidative Phosphorylation, Mitochondrial Dysfunction and Ephrin A Signaling were correlated. Coagulation System and Complement System were found correlated with platelets related traits. At FDR < 1%, 6321 transcripts were correlated with one of eight biochemical traits (ALB, NH3, BUN, TCHO, TG, GLU, IP, CREA) (r^2 = \0.22–0.41\). For example, the expression of genes related to PXR/RXR Activation, FXR/RXR Activation and TR/RXR Activation, i.e., endogenous processes of bile acid metabolism, cholesterol homeostasis, lipoprotein, lipid and glucose metabolism, were found to be correlated to TCHO, TG and GLU. Expression-QTL analysis integrates gene-expression levels and genome-wide genotyping information to find genetic variation associated with changes in gene expression. Here 20,517 significant (FDR < 5%; $p < 10^{-7}$) eQTL for 1401 transcripts were found; 6865 eQTL indicated *cis* regulation, and of these, 808 and 1148 were eQTL of transcripts correlated with hematological or clinical-chemical traits, respectively. The analyses of trait-correlated hepatic expression and the eQTL-detection complement genome-wide association studies for hematological and biochemical, clinical-chemical biomarkers of hepatic functions contributing to metabolic homeostasis, innate defense and resilience.

Key Words: eQTL, hepatic genes expression, resilience

P3023 Dietary supplementation with vitamin E or grape pomace influences antioxidant and lipid metabolism candidate gene expression in broiler muscle. Y. Núñez* (INIA, Madrid, Spain), A. Fernández (INIA, Madrid, Spain), R. Benítez (INIA, Madrid, Spain), I. Arija (Facultad de Veterinaria. UCM, Madrid, Spain), A. Viveros (Facultad de Veterinaria, UCM, Madrid, Spain), A. Brenes (Instituto de Ciencia y Tecnología de Alimentos y Nutrición, CSIC, Madrid, Spain), C. Ovilo (INIA, Madrid, Spain)

Feed supplementation with vitamin E (VE) is widely employed in the meat industry as a method to avoid adverse reactions in meat due to its antioxidant properties. VE is the naturally occurring antioxidant that has shown greater potency. Grape pomace (GP) has been proposed to improve the oxidative stability of meat due to its content in polyphenolic compounds. Supplementation with GP in broiler chicks does not affect performance but has a protecting effect similar to VE, reducing susceptibility to lipid oxidation. Also it increases plasma α-tocopherol and meat PUFA content. These effects might be produced by changes in gene expression of key genes. To understand the molecular mechanisms associated to VE and GP supplementation, we have studied their effect on broiler breast muscle gene expression of four candidate genes related to antioxidant activity and lipid metabolism. A total of 36 male broiler chicks were employed and fed a starter diet from 1 to 21 d of age. From Day 21 to 35, chickens received three treatments (12 animals each): control corn-soybean diet, control supplemented with red grape pomace (8%) and control supplemented with VE (200 IU), provided ad libitum. At 35 d of age, birds were slaughtered, and breast muscle was sampled. RNA was obtained, and gene expression was measured by RT-qPCR. Significant gene-expression differences among experimental groups were observed for three out of the four analyzed genes. No difference was observed for δ9-desaturase (SCD) gene expression. The catalase gene (CAT) showed significantly higher expression in both VE- and GP-supplemented diets in comparison with control ($p < 0.0005$), in agreement with its role in antioxidative processes. Nevertheless, the second gene involved in oxidative stability, Heme-oxygenase 2 (HMOX2), showed higher expression in GP than VE groups ($p < 0.01$), without differing from control group. This result might indicate a higher potential of polyphenols for controlling oxidative stress by means of heme-catabolism pathway, in comparison with VE. At last, Sterol Carrier Protein 2 (SCP2) showed the same pattern as CAT gene, with higher expression in supplemented animals ($p < 0.005$). SCP2 protein is a universal fatty acid binding and trafficking protein, whose expression is associated with increasing PUFA contents, according to the effects of the employed diets at muscle composition level. Although a wider study is necessary, the results suggest biological mechanisms for VE and GP actions at both metabolic and antioxidant levels.

Key Words: vitamin E, grape pomace, gene expression, antioxidant, PUFA

P3024 Transcriptome analysis of longissimus thoracis et lumborum from pigs divergent in residual feed intake. J. Horodyska* (Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany; Teagasc Food Research Centre, Dublin, Ireland), M. Oster (Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany), K. Wimmers (Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany), A. M. Mullen (Teagasc Food Research Centre, Dublin, Ireland), P. G. Lawlor (Teagasc Pig

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Residual feed intake (RFI) is described as the difference between an individual's actual feed intake and its predicted feed requirements for maintenance and growth. The objective of this study was to investigate the molecular mechanisms contributing to differences in RFI. cDNA obtained from Longissimus thoracis et lumborum (LTL) muscle of 20 commercial line Maxgro x (Landrace x Large White) gilts from low and high RFI groups was hybridized on Affymetrix Snowball Array. Samples were RMA normalized, and probe sets with a low standard deviation ($s \leq 0.23$) were discarded. A further analysis involved filtering by both control probe sets and means (means ≤ 2.5 were rejected). Mixed-model analysis was implemented, and pathway analysis was conducted. A total of 30,992 probe-sets remained after filtering, and 423 genes were found to be at least 1.5-fold differentially expressed. The most altered genes were AP2M1 (2.37; highRFI < lowRFI) and NCOA2 (3.32; highRFI > lowRFI), respectively. The most significant molecular and cellular functions of differentially expressed (DE) genes in relation to RFI were "accumulation of fatty acid" and "accumulation of lipid." The most significant canonical pathways DE in relation to RFI were "TR/RXR activation" and "PEDF signaling." To validate the microarray, a set of reference genes was selected (B2M, RPL10, RPS11). Out of 10 DE genes, mRNA abundance of nine transcripts (ACACA, ACSL1, BCL2, CAPN6, JMJD1C, NCOA1, RHOA, WIPF1 and PPARG) showed a numerical change in the same direction when compared to the microarray. In conclusion, a number of pathways were altered in relation to RFI groups in LTL muscle, and many of the addressed biological processes could be broadly classified into three categories: accumulation of fatty acids, adhesion of connective tissue and apoptosis. Thus, those transcripts are potential candidate genes for improved efficiency.

Key Words: residual feed intake, gene expression, pig

P3025 RNA depletion for highly abundant transcripts in bovine mammary gland improves the sensitivity of RNaseq analysis. R. Weikard,* C. Kühn (Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany)

Deep RNA sequencing (RNaseq) generates the comprehensive transcriptomic landscape of cells and tissues and has opened a new horizon for understanding global gene expression. In the transcriptome of the mammary gland of lactating dairy cows, genes encoding for milk proteins are highly abundant, which can impair prevent the detection of lowly expressed transcripts at a given level of sequence depth. The aim of this study was to develop a cost-efficient, bovine-specific procedure to decrease the proportion of highly expressed transcripts in mRNA from mammary gland for improving the sensitivity to discover transcripts with marginal expression levels. Therefore, antisense oligonucleotides targeting genes of the casein cluster (CSN1S1, CSN1S2, CSN2 and CSN3), α-lactalbumin (LALBA) and β-lactoglobulin (LGB) near their polyA tail were hybridized to total RNA isolated from mammary gland of lactating dairy cows. Digestion with RNase H followed by poly(A+) mRNA selection leads to a RNA depletion of the targeted genes encoding milk protein genes (deadenylated) in the mRNA pool. The effect of this RNA pre-treatment before RNaseq was monitored by comparative expression analysis of depleted and nondepleted RNA samples using reverse-transcription qPCR (RT-qPCR). The results showed that the mRNA expression level of targeted milk protein genes was reduced by 30% to 90% in the depleted samples depending on the specific gene targeted. Exemplarily, RNaseq libraries were prepared from depleted and nondepleted RNA from the same animals and subjected to paired-end mRNaseq analysis on the HiSeq 2500 Sequencing System (Illumina). The results obtained by RT-qPCR were also reflected by whole transcriptome analysis. In response to RNase H-mediated RNA depletion, the ratio of reads mapping to the targeted milk protein genes relative to the whole number of reads decreased from about 60% in the nondepleted sample to 30% in the depleted sample. Furthermore, the sensitivity for discovering transcripts with low expression levels was improved. To further optimize the efficiency of the RNase H-mediated RNA depletion, experimental conditions could be modified — for instance, the sequences or ratios of oligonucleotides used for depletion of targeted milk protein genes.

Key Words: RNaseq, mammary gland, RNA depletion, cattle

P3026 RNA silencing-targeted transcriptome of porcine alveolar macrophages on infection with porcine respiratory and reproductive syndrome viruses (PRRSV) of different virulence. S. Pollet (GABI, INRA, AgroParisTech, Universite Paris Saclay, 78350 Jouy en Josas, France), P. Renson (ANSES, Unité Virologie Immunologie Porcines, 22440 Ploufragan, France), F. Jaffrezic (GABI, INRA, AgroParisTech, Universite Paris Saclay, 78350 Jouy en Josas, France), G. Marot

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Porcine reproductive and respiratory syndrome (PRRS) is a major swine disease caused by PRRSV, a positive-sense ssRNA virus present worldwide with a wide range of strains with different virulence and pathogenicity. RNA silencing is a crucial cell component of host virus interactions. Some host miRNAs are known to modulate fundamental components of the host immune response to PRRSV and may directly interact with the expressed PRRSV genome and/or be altered by PRRSV for immunosuppression and/or immunoevasion mechanisms. Here we characterized the pool of host genes modulated by RNA silencing following in vitro infection of porcine alveolar macrophages with two European PRRSV strains (Finistere and Lena). We used four biological replicates and multiplicity of infection of 2, and collected cells at 7 h p.i. and 10 h p.i. Total cell and RISC (RNA-Induced Silencing Complex)-bound immunoprecipitated transcripts were profiled using a custom Agilent 8x60K microarray enriched for host immunity related genes and the expressed genome of each PRRSV strain. Analyses of differentially expressed transcripts in total cell RNA were performed using the Limma R package, while the Anota R package was used for the relative enrichment analysis of RISC-bound vs. total cell transcriptome. As expected, Lena was highly virulent compared to Finistere. Major differences were found in virus titers and total cell RNA expression, with principal component analysis clearly grouping each virus/time and controls. The number of differentially expressed transcripts at 7 h p.i. and 10 h p.i. ($p < 0.05$) increased markedly, from 535 to 2530 for Finistere and from 11,850 to 53,400 for Lena. A completely different pattern was found in the RISC. Between 7 h and 10 h p.i., the number of relatively RISC-enriched host transcripts in Finistere-infected cells was very high at 7 h p.i. (2880) and sharply decreased at 10 h p.i. (250), indicating that several host genes were targeted by RNA silencing mechanisms but only at the early stage of PRRSV infection. Conversely, no significant host transcripts were found to be enriched in RISC for the Lena-infected cells either at 7 h or 10 h p.i. This suggested that at 7 h p.i., the infection was already too progressed to detect any effect, but also that the modulatory effects of the RNA silencing pathways were rapidly overpowered by high virulence strains. Finally, no PRRSV transcripts were found enriched in RISC. Analyses are currently in progress to characterize the gene pathway components of RISC-enriched genes and their predicted targeting by host miRNAs.

Key Words: RISC, miRNA

P3027 Bioactivity of colostrum and milk exosomes containing microrna from cows genetically selected as high, average and low immune responders based on their estimated breeding values. M. Ross* (Department of Pathobiology, University of Guelph, Guelph, ON, Canada), H. Atalla (Department of Pathobiology, University of Guelph, Guelph, ON, Canada; Department of Animal Biosciences, Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada), B. Mallard (Department of Pathobiology, University of Guelph, Guelph, ON, Canada; Department of Animal Biosciences, Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada)

Bovine milk contains bioactive components with immune-regulatory potential in humans. Expression of milk bioactive components is often controlled by microRNA (miRNA). Colostrum and milk miRNAs are enclosed in exosomes, conferring their protection from degradation and potentially promoting uptake by recipient cells. While dairy cows classified as high immune responders (HIR) have improved colostrum and milk quality compared with average (A) and low (L) responders, the bioactivity of colostrum and milk exosome-derived miRNA at the human intestinal epithelial barrier remains to be explored. Therefore, the purpose of this study is to evaluate the functional role of milk exosomes at the intestinal epithelial interface using healthy and cancerous human intestinal epithelial cells. Exosomes were isolated by differential ultracentrifugation from the colostrum and milk of cattle genetically selected as L, A or HIRs based on their estimated breeding values. Exosomes were viewed by electron microscopy and confirmed by immunoglod labeling, ELISA ExoEL kit and Western blot analysis for the presence of common exosomal-proteins (CD9, CD63, CD81, and Hsp70). Quantification of exosomal protein was conducted by BCA protein assay. Exosome surface markers are more abundantly expressed in colostrum exosome isolates across all immune response groups compared with milk. Specifically, expression of colostrum exosome markers is higher in A and HIR exosome isolates, compared with L responders. To assess bioactivity, exosomes

are co-cultured with human intestinal epithelial cells (IECs). An MTT assay is conducted to determine if exosomes are cytotoxic or promote the viability of IECs. Uptake of PKH67 labeled exosomes by IECs is conducted to assess the bioavailability of bovine milk exosomes in humans. Finally, transfer of exosomal miRNA to IECs is being validated using qPCR array. This research will help determine the functional importance of bovine milk on gastrointestinal health of humans.

Key Words: high immune response technology, bovine milk exosomes, microRNA

P3028 The suppression of miR-16 maturation induced by 54-bp insertion activates a novel feedback regulatory via the insulin signaling pathway. X. Jia* (South China Agricultural University, Guangzhou, China; Iowa State University, Ames, IA), H. Xu (South China Agricultural University, Guangzhou, China), Q. Nie (South China Agricultural University, Guangzhou, China), X. Zhang (College of Animal Science, South China Agricultural University, Guangzhou, China), S. J. Lamont (Department of Animal Science, Iowa State University, Ames, IA)

Both genetic mutation and miRNA have been implicated in complex genetic regulatory mechanisms, but interactions between these pathways are poorly understood. In this study, we identified that chicken miR-16 is a major candidate for growth; the expression is associated with growth, and the location overlaps a quantitative trait locus region confirmed in our previous genome-wide association study. A 54-bp insertion mutation caused decreased miR-16 expression through affecting alternative splicing, resulting in a series of downstream regulatory changes. In vitro, miR-16 directly downregulated FOXO1 expression. Furthermore, FOXO1 widely participated the early period of myoblast differentiation through increasing MyoD and decreasing slow muscle fiber formation. ChIP-seq and in vitro investigations confirmed that FOXO1 could control MYCN expression by directly binding to its promoter. We also determined that FOXO1 increased most miRNAs encoded by the miR-17–92 cluster, which were shown in a previous study to be positively regulated by MYCN and directly down-regulated PTEN. In summary, our findings identified a new feedback regulation loop induced by abnormal miR-16 expression: miR-16 mediates the insulin pathway by suppressing FOXO1, resulting in decreased miR-17–92 expression through inactivating MYCN, while lower miR-17–92 expression increased PTEN activity, leading to PI3K pathway inhibition and FOXO1 activation. As a result, this novel feedback regulatory can compensate FOXO1 transcriptional activity inhibited by miR-16.

Key Words: miRNA FOXO1 insulin pathway

P3029 Identification of regulatory genes involved in longissimus dorsi transcriptomic differences between pig genotypes. M. Ayuso (UCM, Madrid, Spain), J. Garrayo (UPM, Madrid, Spain), A. Fernández (INIA, Madrid, Spain), Y. Núñez (INIA, Madrid, Spain), R. Benítez (INIA, Madrid, Spain), B. Isabel (UCM, Madrid, Spain), A. I. Fernández (Departamento de Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain), A. I. Rey (UCM, Madrid, Spain), A. Gonzalez-Bulnes (INIA, Madrid, Spain), J. F. Medrano (University of California, Davis, CA), A. Cánovas (University of Guelph, Ontario, ON, Canada), C. López-Bote (UCM, Madrid, Spain), C. Ovilo* (INIA, Madrid, Spain)

Iberian pig production is based on both purebred Iberian (IB) and crossbred Duroc X Iberian (DUxIB) pigs. These two genetic types show important differences in growth, fattening and tissue composition. This study was conducted to assess Longissimus dorsi muscle gene expression profiles and to identify regulatory genes potentially responsible for gene expression and phenotypic differences between pig genotypes. Nine IB and 10 DUxIB piglets were slaughtered at birth, and 7 IB and 10 DUxIB were slaughtered at 4 mo of age (growing stage). Carcass traits were measured and samples from Longissimus dorsi were taken to study intramuscular fat (IMF) content and composition and to analyze the muscle transcriptome with RNA-seq technology. Differences in growth and fatness patterns were observed between genotypes. Genetic type significantly affected expression of 261 genes ($P < 0.01$ and Fold change > 1.5) at birth and 113 genes at growing stage. To understand the molecular mechanisms underlying gene expression differences between IB and IBxDU, a regulatory gene examination was conducted following three different approaches. Identification of regulators was based on biological data mining (Ingenuity Pathways Analysis software), coexpression data (Regulatory Impact Factor study) and differential expression data (DE regulators). Regulatory genes identified at both ages were deemed to have a deeper impact in the final phenotype. Some of the genes were closely related to muscle development (*MYOD1*, *BHLHE40* and *HDAC2*) and adipogenesis and fat accumulation (*NFKBIA*, *ATF4* or *CEBPA*). Regulators identified in more than one approach were considered

to be the most robust results. In newborns, these regulators were mainly involved in muscle cell differentiation (*MEF2C, MEF2D, MYOG, SOX4*) and protein degradation (*CREB3L1, HSF1* and *CREBBP*), thus playing a role in muscle development. *FOS* and *FOXO* regulatory genes control muscle differentiation but also adipogenic genes expression and IMF accumulation. In growing animals, three regulators were identified by complementary approaches, being involved in the regulation of the cell cycle (*EN1* and *IRF2*) and lipid metabolism (*EN1* and *TCF7L2*). Moreover, a functional analysis was performed combining the DE and the regulators studies information. Several pathways were enriched at both stages. Among them, pathways involved in adipocyte differentiation and protein degradation (the PPAR signaling, the adipogenesis, the Wnt and the unfolded protein response) are of special relevance. The present work identifies regulatory genes potentially involved in differences in metabolism and productive traits between IB and IBxDU pigs.

Key Words: transcriptome, regulatory genes, Iberian pig, RNA-seq

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P3030 Identification of expression quantitative trait loci for longissimus muscle microrna expression profiles in the Michigan State University Duroc × Pietrain pig resource population.

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MicroRNAs (miRNAs) are a class of small, non-coding RNAs shown to post-transcriptionally regulate gene expression through complementary binding with target mRNAs. To date, there has been little exploration of the effects of miRNA regulation in skeletal muscle of market-age pigs. The objective of this study was to conduct an expression Quantitative Trait Loci (eQTL) analysis of miRNAs expressed in the Longissimus dorsi (LD) muscle of 174 F2 pigs from the MSU Duroc x Pietrain Resource Population to identify genomic regions containing variants associated with variation in miRNA expression (miRNA eQTL). Animals selected for this study exhibited extremes for phenotypes of loin muscle area and backfat thickness. These animals were previously genotyped with Illumina PorcineSNP60 BeadChips, and markers were filtered for removal of non-informative markers, markers with extreme allele frequencies ($MAF < 0.10$) and markers located on sex chromosomes. Total RNA extracted from LD tissue for each pig was sequenced on an Illumina HiSeq 2500 sequencing platform in 1x50 bp, single-end format. Raw sequence reads were trimmed for adaptor sequences, size- and quality-filtered, and PCR duplicates were removed. High-quality reads were aligned to the *Sus scrofa* reference genome (10.2.79), and annotated *Sus scrofa* miRNAs (miRBase) were quantified using miRDeep2. After filtering for low abundance across samples, 295 mature miRNA expression profiles were normalized utilizing the *voom* function of the Limma R package, and resulting log-counts per million were treated as response variables in a GBLUP-based genome-wide association (GWA) analysis utilizing the *gwaR* R package developed by our group. The GBLUP model included fixed effects of sex and selection group, random additive genetic effects with variance-covariance proportional to the genomic relationship matrix, and heterogeneous independent Gaussian residuals with variance proportional to inverse weights obtained from the *voom* function in Limma. Results of the GBLUP analysis indicated the average heritability of the 295 miRNAs was 0.118, whereas average heritability of the 47 miRNAs exhibiting significantly heritable expression was 0.329 ($q < 0.05$). Twenty-six significant miRNA eQTL peaks were identified from the GWA analysis, mapping to 11 chromosomes and associated with 18 miRNAs ($q < 0.05$). Seven of the 26 miRNA eQTL map to a region on SSC15 (133.9 Mb- 140.1 Mb). This is the first miRNA eQTL analysis reported in pigs, and future work will assess the biological effects of variation in miRNA expression on the regulation of mRNA associated with economically important pig phenotypes.

Key Words: miRNA, eQTL, skeletal muscle

P3031 Toward resolving long noncoding RNAs in fish: Identification, mapping and association to disease using strand-specific RNA-seq in rainbow trout fed alternative diets.

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Long noncoding RNAs (lncRNAs) are broadly classified as transcripts –200 bp that do not encode proteins.

They can regulate precise physiological processes and be altered during disease-states. We set out to understand their importance in rainbow trout, a carnivorous fish that develops severe enteritis when fishmeal in their diets is replaced with sustainable plant-based proteins. Through years of selective breeding, our group has developed an enteritis-free model rainbow trout strain that thrives on a 100% fishmeal-free diet. As dietary substitution is known to effect hepatic metabolism, commercial (susceptible to enteritis development) and selected (no enteritis) trout strains were fed replacement diets for several months, and then livers (n = 20) were sampled and prepped for strand-specific Illumina HiSeq. Reads (~25M/liver) were clustered with Trinity. Transcripts were subjected to Annocript pipeline using BLASTn, BLASTx and rpsBLAST in searches of gene identification, gene ontology, open reading frame and conserved domain matches. Data was compiled with strand-information, a protein-coding-potential algorithm applied and non-coding probabilities calculated. To date, we identified 911 and 778 putative lncRNAs (> 0.95 Pr) between commercial and selected trout, respectively. Similar to human GENCODE, the majority (> 60%) identified were intergenic. Interestingly, exonic lncRNAs enriched in symptomatic fish include potential regulators of bile acid transporters and apolipoproteins, also observed in mammalian gastrointestinal disorders. With the trout genome in early stages of description, these data will be useful additions as we prepare for functional studies. Ongoing work includes addition of individuals, tissues and treatments to the map while confirmation experiments and full-length assessments are in preparation.

Key Words: noncoding RNA, trout, salmonid, enteritis, nutrition, diet, aquaculture

P3032 Association of skeletal muscle transcripts with fatty acid content in Nellore cattle.

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Fatty acids have been implicated in a variety of different biological processes — for example, activation of transcription factor. In this study, we utilized *Longissimus* muscle (skeletal muscle) with extreme fatty acid (FA) content to evaluate the association of different fatty acids and gene expression. The transcriptome and fatty acid profile of skeletal muscle from 200 Nellore steers was obtained by RNA-seq using Illumina platform (HiSeq 2500) and gas chromatography, respectively. Thirty animals with high (H) and 30 with low (L) skeletal muscle FA content were selected for this study for each fatty acid evaluated. The FAs used herein were oleic acid (OA), palmitic acid (PA), stearic acid (SA), linoleic acid (LA), conjugated linoleic acid *cis*9-tran11 (CLA-c9t11), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which were chosen based on their importance in many biological processes. These animals did not differ in their intramuscular fat content (IMF) or backfat thickness. Tophat2, HTSeq and DESeq2 programs and R packages were utilized to performed differential expression analysis between H and L groups. No differentially expressed genes (DEGs, FDR 10%) were identified for LA or SA; only a few DEGs were identified for EPA (5), DHA (4 DEGs) and PA (123 DEGs); while a large number of DEGs were identified for OA (1134) and CLA-c9t11 (872). Functional annotation and enrichment from OA DEGs identified important genes and canonical pathways such as *SCD*, *PLIN5*, LDL-cholesterol, *CPT1* and *PPAR,* related to oxidative phosphorylation, insulin receptor signaling, docosahexaenoic acid (DHA) signaling and oleate biosynthesis. Enrichment analysis of CLA-c9t11 DEGs identified one KEGG pathway, Ribosome (BH-adj = 1.2e-02), and several molecular functions such as nucleotide binding (BH-adj = 1.7e-03), ATP binding (BH-adj = 2.2e-02) and structural constituent of ribosome (BH-adj = 2.4e-02). In this study, animals of common nutrition, sex and similar age with no statistical difference in IMF and backfat thickness had many DEGs due to variation in OA and CLA-c9t11 content. These results indicate that only a couple of fatty acids appear to have potential biological activity, by either direct or indirect effects, on skeletal muscle and possibly other tissues in Nellore beef cattle.

Key Words: conjugated linoleic acid, oleic acid, RNA-seq, differentially expressed genes

P3033 A comprehensive porcine blood transcriptome. H. Liu (Bioinformatics and Computational Biology Program, Department of Animal Science, Iowa State University, Ames, IA), T. P. L. Smith (USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE), D. J. Nonneman (USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE), J. C. M. Dekkers (Department of Animal Science, Iowa State University, Ames, IA), C. K. Tuggle* (Bioinformatics and Computational Biology Program, Department of Animal Science, Iowa State University, Ames, IA)

Blood sample analyses are extensively used in high throughput assays in biomedicine, as well as animal genetics and physiology research. However, the draft quality of the current pig genome (Sscrofa 10.2) is insufficient for accurate interpretation of many of these assays because of incomplete gene and transcript isoform annotations. In this study, we assembled a comprehensive blood de novo transcriptome by using the Trinity platform on 162,285,683 pairs of paired-end and 183,116,578 single-end, clean and normalized Illumina reads (25 to 100 bases in length) from five independent RNA-seq studies of pig blood. This raw assembly consisted of 490,209 putative transcripts (PTs) from 397,560 genomic loci and includes more than 97% of the normalized reads. To verify the porcine origin of these assembled PTs, we mapped them to a PacBio long read-based USMARC pig genome assembly (T. Smith et al., unpubl.) and to the Sscrofa 10.2 assembly. Overall, 99.4% and 94.2% of PTs could be mapped to the USMARC and Sscrofa 10.2 assemblies, respectively, with more than 97% coverage. Notably, the majority of 3089 PTs that could not be mapped to the USMARC assembly were of bacterial, viral or plant origin. We removed unmapped PTs that did not align to mammalian sequences in the NCBI nt database and filtered the PTs using relaxed criteria based on minimum expression level, length and splicing potential, producing a set of 159,146 unique PTs, 121,057 of which were putative spliced transcripts. We aligned the reduced set of PTs along with a newly available dataset of IsoSeq transcripts from pig liver, spleen and thymus (H. Liu et al., unpubl.) to the current Sscrofa 10.2 genome assembly. Visual inspection of these alignments in IGV showed that a large number of the assembled blood transcripts were structurally more complete/accurate than their counterparts in the Sscrofa 10.2 annotation. We will report further filtering, validation and annotation of these PT, including alignment to IsoSeq-derived transcripts, as well as comparisons to the Ensembl pig transcriptome, the NCBI nucleotide database and the SwissProt Uniprot/UniRef90 and EMBL-EBI Xfam databases to support robust prediction of the coding/non-coding potentials of novel PTs. This assembled and validated transcriptome can be used to improve pig genome annotation and enhance future high throughput studies of blood samples. Acknowledgment: USDA-NIFA-AFRI #2011–68004–30336.

Key Words: porcine blood transcriptome

P3034 Analysis of microRNA of ovine preimplantation embryo developed in vitro. W. Wu*, H. Tulafu, X. Xu, X. Fu, K. Tian (Xinjiang Academy of Animal Science, Urumqi, China)

To improve IVF efficiency, global analysis of miRNA transcriptome profile in ovine embryos during in vitro development were investigated. In this study, we analyzed the sequences and relative expression levels of ovine miRNAs of 20,850 sheep IVF embryos at different developmental stage by Solexa sequencing with computational techniques. The result showed that 900 miRNA differentially expressed between unfertilized egg and two-cell embryo, 613 miRNA differentially expressed between two-cell and four-cell, 521 miRNA differentially expressed between four-cell embryo and eight-cell embryo, 551 miRNA differentially expressed between eight-cell embryo and morula. To verify accuracy by sequencing, real-time quantification of six kinds known miRNAs by stem-loop RT-PCR, most of six kinds miRNAs expression level was consistent with sequence result in different development stages of embryo. Specifically, we found 398, 429, 467, 485 and 242 known miRNA families expressed, respectively. In the meantime, we predicted 1797 candidate miRNAs in which miR-10b expression level is the highest in the five different developmental stages and miR-708–3p, miR-5398–3p, miR-960–3p, miR-99b-3p, miR-874 expression level is the lowest, respectively, in unfertilized-egg, two-cell embyo, four-cell embryo, eight-cell embryo and morula. The above data greatly widened the ovine miRNA information and laid a foundation of further screen differentially expressed miRNA in different development period of embryo.

Furthermore, we performed additional investigation for identifying the potential target mRNAs using TargetScan, 2430 target genes, 3540 target genes, 1577 target genes, 3045 target genes, 2411 target genes and 4181 target genes respectively predicted by mir-28–3p, mir-202, mir-150, let-7b-5p, mir-21–5p and mir-143. Most of target genes have binding and catalytic activity by function annotation and significant enrichment in 13 signal pathway, such as Wnt signal pathway, Autophagy signal pathway and Axon

guidance signal pathway. Our results provide useful information for the investigation into embryonic miRNAs of ovine and provide a valuable resource for investigators interested in the regulation of embryonic development in ovine and other animals.

Key Words: ovine, preimplantation embryo; RNA-seq, miRNA

P3035 Analysis of transcriptome profile of ovine preimplantation embryo developed in vitro.
W. Wu*, H. Tulafu, X. Xu, X. Fu, K. Tian (Xinjiang Academy of Animal Science, Urumqi, China)

To provide a comprehensive view of the transcriptome changes in ovine early stage embryos, the total RNA content of ovine unfertilized-egg, two-cell, four-cell, eight-cell embryos and morula were sequenced by Illumina 2000 Genome Analyzer. The result showed that 15,271 transcripts and 7445 new transcripts in unfertilized-egg, 14,867 transcripts and 8090 new transcripts in 2two-cell embryo, 15,025 transcripts and 8491new transcripts in four-cell embryo, 15,594 transcripts and 8963 new transcripts in eight-cell embryo and 16,260 transcripts and 8177 new transcripts in morula. ES, IR, A3SS and A5SS of alternative splicing major in ovine embryo. We also found 616,234 SNPs and optimized 37,544 genes. We identified 7950 mRNA differentially expressed between unfertilized egg and two-cell embryo, 1169 mRNA differentially expressed between two-cell embryo and four-cell embryo, 3631 mRNA differentially expressed between four-cell embryo and eight-cell embryo and 5851 mRNA differentially expressed between eight-cell embryo and morula. These differentially expressed genes were functionally annotated by related gene ontology terms and KEGG pathway. Functional analysis revealed stage-specific functions of the differentially expressed genes. In conclusion, we have obtained a preliminary landscape of genes differentially expressed in ovine embryo during the transition from unfertilized egg to morula. Our results provide an opportunity to study the functions of these genes in relation to the development and survival of pre-implantation ovine embryos.

Key Words: ovine transcriptome profile preimplantation embryo

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P3036 Differential expression in feed- and energy-absorbing, partitioning, metabolizing and depositing tissues of broilers divergent for feed conversion efficiency.
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Traits related to feed conversion efficiency (FCE) in livestock are of particular interest due to their economic and ecological implications. Improving FCE provides the potential to save both feeding costs and resources while increasing productivity and reducing greenhouse gas emission. The efficient conversion of feed for the formation of animal tissues depends on exogenous factors like diet and feeding regimen and endogenous properties including gut microbiota. Processes driving FCE comprise digestion and absorption of nutrients in the gut, partitioning and primary metabolism in the liver, and superior mechanisms to orchestrate resource allocation for maintenance, growth, physical activity and thermoregulation. Thus, FCE is strongly related to the metabolism of muscle and fat tissues, the major consumers and stores of energy and the main animal products. Holistic analyses of genes that are constitutively active in gut and liver, differentially expressed depending on the organismal phenotype or induced in response to various stimuli reflect genomic contributions to the variation in FCE. In the ECO-FCE project, we applied RNA-seq to generate 64 high-resolution transcriptome profiles of liver, small intestine and muscle tissues of FCE-divergent broilers. In duodenum, jejunum and ileum a number of 172, 120 and 81 differentially expressed genes (DEG) were identified between high and low FCE animals. More than 80% of these DEGs were uniquely observed in a distinct part of the small intestine, arguing for largely independent tissues with specific functions in the context of FCE. In fact, networks of duodenal DEGs were related to weight gain, immune-cell enrichment and cell death, whereas in jejunum a significant number of DEGs belonged to leukocyte migration. In ileum, genes related to glucose uptake, transporters and lipid metabolism were prevalent among the DEGs. For liver, leg and breast muscle the analyses revealed 159, 186 and 54 DEGs, respectively. The overlapping DEGs among these tissues were scarce. In liver, DEGs were enriched in fat and protein metabolic pathways. The holistic transcriptome analyses complement our

earlier genome-wide association studies and provide additional experimental evidence for the role of particular genes and pathways in FCE.

Key Words: feed conversion efficiency, comparative RNA-seq analysis, resource allocation

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P3037 Molecular cloning and characterization of the promoter region of the porcine stearoyl-CoA desaturase gene. S. Gol* (University of Lleida-Agrotenio Center, Lleida, Spain)

In pigs, pork fat content and composition is controlled by nutritional and environmental cues, but it also has a strong genetic component. Recently, our group identified a haplotype of three polymorphisms in the porcine stearoyl-coA desaturase (SCD) promoter that is strongly linked to the desaturation index of intramuscular fat and, therefore, to nutritional, sensory and technological quality of pork (Estany et al., 2014 PlosOne 20;9(1):e86177). In particular, the haplotype H1 (composed by nucleotides C-T-A) is considered beneficial as it promotes deposition of less saturated fat and increases the monounsaturated fatty acids content and unsaturation index of pork. In Duroc pigs, the most common alternative variant is H2 (composed by nucleotides T-C-G). Animals of H1H1 diplotype express higher levels of SCD compared to H2H2 pigs, being the effect clearly additive. We are currently conducting a functional study in cultured cells in vitro to investigate whether the haplotype H1 is responsible for increasing the gene expression and, supposing that, which of the three is the causal mutation. For this proposal, 750 bp of the genomic DNA of the SCD promoter from pigs with diployte H1H1 and H2H2 were amplified by PCR. These fragments were cloned into the pGL3 vector that directs the expression of firefly luciferase reporter gene from heterologous promoters, generating clones PH1 and PH2. In a second step, clones will be transfected into immortalized human liver cells (HepG2 line) with the pRL-TK vector, which promotes the expression of Renilla luciferase. The pGL3-basic vector will be transfected using Turbofect (Thermo Fisher) with pRL-TK in parallel as control standard. After transfection, cells will be incubated for 48 h with basal medium or two reagents that promote or inhibit SCD promoter. Each reagent will be tested at two different concentrations. Past 48 h, cell extracts will be collected and held in triplicate in a dual luciferase Firefly and Renilla assay with the kit Dual-Glo Luciferase Assay System (Promega). The activity of SCD promoters will be expressed as the ratio of activities Firefly/Renilla. This experiment allows us to validate that the nucleotide substitution in the SCD promoter results in a higher transcriptional activity and, second, to identify which signaling pathway is affected by these mutations.

Key Words: cell culture, luciferase, SCD, PIGS

P3038 Chromatin accessibility in the liver and circulating immune cells of pigs, goats and chickens. E. Giuffra* (GABI, INRA, AgroParisTech, Universite Paris Saclay, 78350 Jouy en Josas, France), K. A. Munyard (Curtin University, School of Biomedical Sciences, CHIRI Biosciences, Perth, Australia; GenPhySE, INRA 31320, Castanet-Tolosan, France), A. Goubil (GABI, INRA, AgroParisTech, Universite Paris Saclay, Jouy-en-Josas, France), S. Vincent-Naulleau (GABI, INRA, AgroParisTech, Université Paris Saclay, Jouy-en-Josas, France; SREIT, iRCM, CEA, Université Paris Saclay, Jouy-en-Josas, France), D. Esquerré (INRA, UMR1388 GenPhySe, GeT-PlaGe Genomic Facility, Castanet-Tolosan, France), S. Djebali (GenPhySE, INRA, Castanet-Tolosan, France), S. Foissac (INRA UMR 1388 GenPhySE, Castanet-Tolosan, France)

Functional annotation of the genomes of agriculturally important species is required for research and applications on health, food security and environmental sustainability purposes (Andersson et al., 2015 Gen. Biol. 16:57). One important aspect of functional control of mammalian genomes is chromatin accessibility (Kornberg & Lorch (1992) Ann. Rev. Cell Biol. 8:563–587; Mellor (2005) Molecular Cell 19:147–157). The chromatin accessibility of major agricultural species is being measured as part of the French pilot project (Fr-AgENCODE) within the Functional Analysis of Animal Genomes (FAANG) consortium. Assay for transposase-accessible chromatin using sequencing (ATAC-seq) is an emerging method for measuring chromatin accessibility with advantages over other such methods (e.g., DNase 1 hypersensitive sites sequencing: DNase-seq) because it requires fewer cells and is fast and simple (Buenrostro et al., (2013) Nat. Methods 10(12):1213–1218). We previously presented, for the first time, ATAC-seq data from porcine primary tissues in MeLim pigs and have now extended this to liver, $CD3^+CD4^+$, & $CD3^+CD8^+$ T cells collected from pigs, goats and chickens. Transposition was completed on fresh liver samples at time of sampling and on preserved, thawed $CD3^+CD4^+$ and $CD3^+CD8^+$ cells (Buenrostro et al., 2015, Curr. Prot. Mol. Biol. 109:21.2). Libraries that passed QC were sequenced on an Illumina HiSeq3000 to generate > 100 million PE 150 bp raw reads. An analysis pipeline has been developed using best-practice bioinformatics tools to trim reads (Trimgalore), map reads to the reference (Bowtie2), remove mitochondrial reads

and duplicates (Samtools/Picard-Tools) call peaks (MACS2) and incorporate into this pipeline quality control metrics. We compare and contrast the nucleosome occupancy of avian and mammalian cells/tissues and discuss the implications for chromatin accessibility and functional annotation of genomes.

Key Words: ATAC-seq

P3039 Elucidating the genetic basis of tick resistance in nguni cattle. N. O. Mapholi* (ARC-Animal Production Institute, Irene, South Africa, Pretoria, South Africa), A. A. Maiwashe (ARC-Animal Production Institute, Irene, South Africa), O. Matika (The Roslin Institute and R(D) SVS, University of Edinburgh, Midlothian, United Kingdom), V. Riggio (The Roslin Institute and R(D) SVS, University of Edinburgh, Midlothian, United Kingdom), M. D. MacNeil (Delta G, Miles City, MT), C. B. Banga (Agricultural Research Council, Irene, South Africa), J. F. Taylor (University of Missouri, Columbia, MO), K. Dzama (University of Stellenbosch, Stellenbosch, South Africa)

Ticks are an important constraint to cattle production, particularly in tropical and subtropical regions of the world. Acaricides are the primary method of tick control; however, their cost has a large, negative impact on farm profitability. Hence, there is a need to find alternative tick control methods, such as genetic improvement, that are more affordable and sustainable. Most cattle breeds indigenous to regions with high tick infestations possess some degree of natural resistance to ticks and tick-borne diseases. Objectives of this study were to estimate heritability of tick count and identify genomic regions associated with tick resistance in South African Nguni cattle. Tick-count data were from 586 Nguni cattle exposed to natural infestation from four herds located in different provinces of South Africa. Tick counts by species were collected for eight anatomical locations over a 2-yr period (2013 and 2014) and data for November, December and January were $\log_{10}(x + 1)$ transformed to achieve normality and analyzed separately for each month. DNA was extracted from hair and blood samples and genotyped using the Illumina BovineSNP50 assay. After quality control (call rate > 90%, minor allele frequency > 2%), 40,436 SNPs were retained for analysis. Heritabilities were estimated using ASReml to fit animal models. A genome-wide association analysis for tick count was performed using GenABEL. Heritability estimates for the eight analyzed tick-count traits ranged from 0.04 ± 0.04 to 0.20 ± 0.04. Two genome-wide significant regions on Chr 1 and 19 were identified for total tick count on the perineum and for total body *Amblyomma hebraeum* ticks, respectively, and these have been previously identified. Additional regions significant at the suggestive level were identified on most chromosomes for several of the traits. The regions identified here as harboring QTL underlying variation in tick burden now enables candidate gene analyses to identify polymorphisms related to tick resistance for marker-assisted selection in Nguni cattle. There is significant genetic variation among cattle for tick counts, and this offers the potential to use genetic tick control approaches.

Key Words: indigenous cattle, tick resistance, heritability, SNP markers, genomic analysis

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P3040 Large-scale gene co-expression network as a source of functional annotation for bovine genes. H. Beiki (Department of Animal Science, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran), J. M. Reecy* (Iowa State University, Ames, IA), A. Pakdel (Isfahan University of Technology, Isfahan, Iran), A. Nejati Javaremi (University of Tehran, Karaj, Iran), A. Masoudi Nejad (University of Tehran, Tehran, Iran)

Genome sequencing has led to the discovery of tens of thousands of potential new genes. Seven years after sequencing of bovine genome, there are genes that have limited annotation, and the function of many genes are still not understood or are partly understood at best. Based on the assumption that genes with similar patterns of expression across a vast array of tissues and experimental conditions are likely to encode proteins with related functions or within a given pathway, we constructed a genome-wide bovine gene co-expression network (BGCN) using 72 microarray datasets that contained a total of 1470 Affymetrix Genechip Bovine Genome Arrays that were retrieved from either NCBI GEO or EBI ArrayExpress. The total of 16,607 probe sets, which represented 11,397 genes, with unique Entrez ID were consolidated into 32 co-expression modules that contained between 29 and 2569 probe sets. All of the identified modules showed strong functional enrichment for particular gene ontology terms and REACTOME pathways. Modules with important biological functions such as response to virus, response to bacteria, energy metabolism, cell signaling and cell cycle have been identified. Moreover, the cellular roles of 153 functionally unknown cattle genes are predicted by the gene co-expression networks using "guilt-by-association" principle. Four un-known hub genes were identified in modules highly enriched for GO terms related to leukocyte activation (LOC509513), RNA processing (LOC100848208), nucleic acid metabolic process

(LOC100850151) and organic-acid metabolic process (MGC137211). Such highly connected genes should be investigated more closely, as they likely to have key regulatory roles in the bovine. We have demonstrated that the BGCN and its corresponding regulons provide rich information for experimental biologists to design experiments, interpret experimental results and develop novel hypothesis on gene function in this poorly annotated genome.

Key Words: gene network, functional genomics, data mining, cattle

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P3041 A selective region on OAR17 is associated with blackbone trait in lanping blackbone sheep.

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Lanping Blackbone sheep were found in Lanping, Yunnan province of China, which have dark-colored inner organs and blood. The blackbone trait is thought of as a kind of hyperpigment trait, which is similar to that in silky fowl. The genetic basis of the blackbone trait in sheep remains to be elucidated. Population structure inferred by principle component analysis (PCA) and STRUCTURE showed that blackbone sheep and non -blackbone sheep from Lanping clustered together and separated from other groups. The extend and pattern of decay of LD in Lanping blackbone and non-blackbone population are very similar, inferring that they suffer from an identical evolution history. Even though there are no extensive genetic differences between blackbone and non-blackbone Lanping sheep individuals, there is an extreme divergent blackbone phenotype between them; the candidate loci of the trait were probably fixed in Lanping blackbone group or non-blackbone group. Hundreds of blackbone and common individuals in Lanping district were collected and genotyped by Illumina OvineSNP50 Beadchip. To correct the effect of population structure in GWAS, we used model-based clustering method and PCA method to classify individuals into clusters. Genomic regions with genome-wide significant association were detected on OAR3 and OAR17, respectively. On the other side, eight blackbone individuals and five non-blackbone individuals were collected in Lanping district, and genomic re-sequencing was performed on Illumina HiSeq 2000 platform. Average 10× coverage genomic data per individual were applied to further variants calling and genomic sweep analysis. Smoothed Fst/$\Theta\pi$ value were used to select the putative divergent regions, and 408 genes were screened out. The genomic sweep analysis with re-sequencing data revealed the significant association region on OAR17 may suffer from positive selection. This region is also proved to be in a long-range haplotype in blackbone individuals by nuclear haplotype analysis. Both the SNP chip and genome re-sequencing data support that the selective region on OAR17 with high degree of fixation in blackbone group is an important candidate region for backbone trait in Lanping blackbone sheep.

Key Words: blackbone, re-sequencing, GWAS

P3042 Transcriptome profile of genes differentially expressed in the mesenteric adipose tissue of beef cattle with variation in geed intake and gain[1].

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Mesenteric fat, a depot within the visceral fat, is responsible for hydrolyzing fatty acids and glycerol, and compared to subcutaneous fat, mesenteric fat releases more free fatty acids into the plasma. In humans, visceral fat depots contribute to obesity and obesity-related disorders. The aim of this study was to determine whether the genes expressed in the mesenteric fat of steers were associated with body weight gain and feed intake. Sixteen steers chosen from quadrants for gain and feed intake based on their distance from the bivariate mean were selected for this study. Mesenteric fat was obtained and evaluated for differences in gene expression. A total of 1831 genes were identified as differentially expressed among steers with variation in feed intake and gain. Many of these genes were involved with metabolic processes such as proteolysis, transcription and translation. In addition, the GO annotations including transport and localization were both over-represented among the differentially expressed genes. Pathway analysis was also performed on the differentially expressed genes. The superoxide radical degradation pathway was identified as overrepresented based on the differential expression of the genes *GPX7*, *SOD2*, *TYRP1*, suggesting that some of these animals may be experiencing oxidative stress. *GPX7*, *SOD2* were in lower transcript abundance and *TYRP1* was higher in transcript abundance

among the low gain-high feed intake animals. The retinoate biosynthesis pathway was also enriched due to the differential expression of the genes *AKR1C3*, *ALDH8A1*, *RDH8*, *RDH13* and *SDR9C7*. These genes were all more highly expressed in the low gain-high intake animals. The pathways identified suggest a role for oxidative stress within the mesenteric fat among the low gain-high intake animals. Mesenteric fat is a highly metabolically active tissue, and in this study we identified genes involved in proteolysis, transcription, translation, transport and oxidative stress as differentially expressed among beef steers with variation in gain and feed intake. ([1]Mention of trade name proprietary product or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable. USDA is an equal opportunity provider and employer.)

Key Words: beef cattle, mesenteric fat, transcriptome

P3043 Study of differentially expressed short-RNAs in swine backfat between fat and lean animals and target prediction of genes regulating fat traits.

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Short noncoding RNAs (sRNAs) are one of the major categories of transcripts modulating the expression of messenger RNAs (mRNAs) in almost all tissues. In pigs, the number of papers describing the micro-RNAs (miRNAs) expressed in backfat tissue is limited. and only a few of them are included in the present release of miRBase (Release 21, June 2014). Nevertheless, the identification of the molecular mechanisms regulating the aptitude of fat deposition in pigs can lead to the detection of key genes and markers for the genetic improvement of the fat trait in pigs. We analyzed by RNA-seq the sRNAs differentially expressed (DE) in backfat tissue between two groups of Italian Large White (ILW) pigs divergent for backfat deposition. We detected 31 DE sRNAs, 14 up-regulated and 17 down-regulated in fat pigs compared with lean pigs. MiRanda v.3.3a software was used to predict the putative target mRNA of the DE sRNAs. For the target prediction we used the porcine differentially expressed mRNAs detected on the same samples (Zambonelli et al., 2016, Animal Genetics doi: 10.1111/age.12413) used for the identification of the DE sRNAs. Using this approach we detected on the whole 40 DE transcripts targeted by 30 DE sRNAs with a total of 193 interactions. This result shows a strong relationships between the two categories of transcripts opening new possibilities to understand the patterns of regulation of differentially expressed coding genes in pigs with different aptitude for backfat deposition. The target DE mRNAs and the sRNAs were localized on porcine genome and were used to detect some co-localization with QTL region available in pigQTLdb identified as responsible of fat traits. On the whole, our findings add new data useful to elucidate the complex mechanism regulating fat deposition on pigs that can be used to implement innovative strategies based on genomic markers.

Key Words: porcine backfat, miRNA, genetic improvement

P3044 Characterization of exosomal immune-related micrornas in colostrum and milk from average, low and high immune responder cows.

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The expression of bioactive host defense proteins in bovine colostrum and milk is regulated by miRNAs. MiRNAs are abundant in milk at different lactation stages, often enclosed within exosomes that protect them from degradation and likely allow for cell-to-cell communication. Notably, immune-related miRNA are highly expressed in milk, particularly in colostrum, with potential to regulate gut immunity of the newborn calf and humans. To date, only few studies have evaluated the expression profile of miRNAs in cow's milk. An interesting observation with these studies was the considerable variation in the total and identified individual miRNAs. These discrepancies were explained by differences in the RNA isolation methods and the background of the cows used in the studies. While high immune responder (HIR) cows have superior colostrum quality and concentrations of bioactive proteins

compared with average (A) and low (L) responders, the expression profiles of colostrum and milk miRNAs remain to be elucidated. The central hypothesis of this study is that colostrum and milk-derived exosomes containing immune-related miRNAs are differentially expressed in A and L responders compared with HIR. The objectives of the study are to: (1) establish a reliable protocol for the fractionation of colostrum and milk for further purification and isolation of exosomes-containing miRNAs, and (2) compare and validate the expression profile of colostrum and milk exosomal miRNAs from A, L and HIR. Exosomes were isolated by differential ultracentrifugation and their presence was confirmed by immunogold labeling electron microscopy. Six RNA extraction methods were compared and RNA quantity and quality were assessed by Qubit Fluorometer and bioanalyzer. The total exosomes RNA/protein isolation kit (Invitrogen) had high RNA yield and the least ribosomal contamination. Milk samples from average responders that passed the library generation quality control (four out of 10 samples) were subjected to next-generation sequencing using the Illumina mirRNA-HiSeq2500 and bioinformatics analysis. A set of identified and novel miRNAs is being selected for validation by PCR array. This study provides platform for optimal colostrum and milk exosomal miRNAs identification that is necessary for down-stream applications. These include, but are not limited to, the discovery of novel miRNAs that have the potential to be utilized as biomarkers for milk quality control or for better understanding of the molecular mechanisms that underlie the regulatory role of miRNAs in calves and humans with emphasis on the health benefit of milk.

Key Words: HIR technology, colostrum/milk exosomes, miRNA isolation, next-generation sequencing, PCR

P3045 Identification and expression analysis of bovine X degenerate YcChromosome genes.

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The Y chromosome male-specific region known as the MSY region comprises approximately 95% of the DNA content of the Y chromosome. Based on the few Y chromosomes that have been sequenced, it has been determined that its euchromatic region contains at least four different types of sequences: X-transposed (99% similarity with X), X-degenerate (60–96% to the X), ampliconic and centromere repetitive sequences. X-degenerate Y-chromosome genes, are single copy, have an X chromosome counterpart (sequence homolog) and are largely housekeeping genes with broad expression profiles, or in some cases have acquired more specific functions, such as SRY, which regulates male sex determination. Understanding of sequence differences between X-degenerate Y-chromosome and their corresponding X chromosome homolog genes will allow dissecting their specific spatial and temporal functional gender differences and provide us with a tool to identify whether the male copy or the female copy of the gene, or both, and of their isoforms are being expressed in any particular tissue. Our hypothesis is that for some of these genes there exist Y specific and X specific isoforms versions and that these X degenerate Y-chromosome gene isoforms as well as the X chromosome counterpart genes are expressed differentially across the fifteen different tissues that are being examined. To investigate our hypothesis we have used single molecule real time (SMRT) isoform sequencing (Iso-Seq) of several tissues from a sire-daughter pair of Hereford animals (Domino/Dominette). Iso-seq transcriptome testes and liver libraries were obtained from Domino, and Iso-seq transcriptome liver, lung, adipose, muscle and hypothalamus were obtained for Dominette. These seven Iso-seq libraries were used for bioinformatic analysis and isoform characterization of X-degenerate Y chromosome genes. Sequence comparisons allowed the identification of two isoforms for *UTY* and three for *UTX*; one isoform for *ZFY* and three for *ZFX*; six isoforms for *RPS4Y1* and three for *RPS4X*; three isoforms for *TBL1Y* and two for *TBL1X*. Similarly, development of specific primers targeting gene sequence sites that differ between the Y and the X copy of the gene has allowed the specific amplification of the X-degenerate Y-chromosome copy of *UTY*, *ZFY* and *USP9Y* genes but not of *RPS4Y1* in male tissues.

Key Words: Y-chromosome, X degenerate genes

P3046 Gene expression in developing goat testes: Sequencing, assembly and identification of caprine spermatogenesis transcriptome.

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Understanding gene networks necessary to establish and maintain spermatogenesis will provide insight into the development of molecular markers for potential fertility of individual animals. The objective of the current study was to identify candidate genes and gene networks important for the establishment and maintenance of spermatogenesis in the goat testes. We performed a high-throughput RNA sequencing project of caprine testis using Alpine goats as a model system. Sampling included pre-meiotic and meiotic testis parenchyma from 2-mo- and 4-mo-old Alpine bucks (n = 3 animals/time-point). Total RNA isolations were used to generate six single-stranded cDNA libraries, each treated for poly-A selection. In total, the raw reads for this project represented 436,408,082 sequences reads of an average length of 801 bp. To compare available transcriptomic information of *Capris hircus* (Yunnan goat) with our high-throughput sequencing project data, we mapped raw reads against publically available goat transcriptome data (ftp://ftp.ncbi.nih.gov/genomes/Capra_hircus). A total of 343,704,188 reads mapped against the reference, and 92,703,894 reads were unmapped. Unmapped reads were used to generate a de novo testes transcriptome assembly of Alpine goat. Mapping information provided insights into the top 1000 highly differentially expressed genes. Results showed an age-specific enrichment for the apoptosis pathway (19 genes; KEGG). Apoptosis is important to establish and maintain spermatogenesis. The expression of 19 apoptosis genes decreased between 2-mo and 4-mo testes. To determine the presence of the apoptosis gene network in the de novo testes transcriptome, we generated a custom BLAST database and searched 19 genes and 25 variants involved in the apoptosis pathway. BLAST searches and alignment examinations showed evidence for 17 proteins and 20 variants members of the apoptosis pathway. The protein kinase, cAMP-dependent catalytic β protein (PRKACB) showed the highest number of variants. Interestingly, this protein signals cell survival and integrity and is not involved in the execution of cell apoptosis. We have identified gene networks important during pre-meiotic and meiotic phases of spermatogenesis. Future studies will focus on implementing additional bioinformatics approaches to exhaustively and carefully compare completeness of current de novo transcriptome assembly by analysis of additional de novo transcriptome assemblies generated from each individual sample studied in the project (e.g., tissue, age, individual). In addition research efforts will focus on validating the expression of apoptosis genes and other candidate genes by qPCR and in situ hybridization and characterizing the biological significance of the expression of these pathways during caprine testes development

Key Words: apoptosis, de novo assembly, RNA-sequencing

P3047 Reduced cell cycle gene expression in adipose tissue of chickens during juvenile development. X. Wang* (Tennessee State University, Nashville, TN), A. Ropelewski (Pittsburgh Supercomputing Center, Pittsburgh, PA), N. Cook (Tennessee State University, Nashville, TN), A. Bohannon-Stewart (Tennessee State University, Nashville, TN), S. Nahashon (Tennessee State University, Nashville, TN)

Intensive selection for rapid growth is adversely accompanied by increased adipose tissues in livestock and poultry. Reduction of adipose tissue accretion is of great interest. By genetic selection through application of molecular genetics knowledge, it is possible to reduce fat accretion in farm animals. Despite much effort, much remains unknown regarding the key genes that may be targets for selection for reduced adipose tissue accumulation. Interrogation of adipose transcriptome may lead to deeper understanding of adipose tissue development. We have analyzed the transcriptome of abdominal adipose tissue using massive parallel sequencing. RNA samples from 2 and 8 wk of age were sequenced using Illumina HiSeq2000. We have acquired 320 million paired-end reads. Analysis of these transcripts revealed 172 differentially expressed genes between 2 and 8 wk of age. A significant portion of these genes are involved in cell cycle, DNA replication and repair, and signal transduction.

Key Words: RNASeq, adipose tissue, transcriptome, juvenile development

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P3048 Gene network analysis identifies rumen epithelial processes perturbed by diet and correlated with methane production and yield. R. Xiang (CSIRO Agriculture, Brisbane, Australia), J. McNally (CSIRO Agriculture, Armidale, Australia), S. J. Rowe (AgResearch, Mosgiel, New Zealand), A. Jonker (AgResearch, Palmerston North, New Zealand), C. Pinares-Patino (CSIRO Agriculture, Canberra, Australia), J. Bond (NSW Department of Primary Industries, Armidale, Australia), H. V. Oddyar (NSW Department of Primary Industries,

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Ruminants are major contributors to the greenhouse gas, methane (CH_4), contributing up to 14% of the anthropogenic contribution to global methane. Since CH_4 is produced by the rumen Archaea from H_2 and CO_2 from bacterial fermentation of ingested feed, studies for mitigating CH_4 have focused on suppressing rumen microbial methanogenesis. However, CH_4 yield (CH_4 production per unit of dry matter intake) has a heritable component; one possible mechanism is host-mediated changes in rumen flow rate. To identify signals of the genetic mechanism for variation in CH_4 yield in the rumen wall, we sequenced mRNAs of full-depth rumen wall tissue from 24 female sheep from an experiment conducted at AgResearch, New Zealand. These sheep had high and low genetic potential to produce methane and were offered different amounts and quality of feed. Feed quality and amount and volatile fatty acid (VFA) concentrations are correlated with total CH_4 production, which are all highly correlated with the expression of rumen cell cycle genes. This suggests that the turnover of the rumen epithelium is influenced by the level of energy intake. VFA concentrations and total methane production are also positively correlated with the expression of a gene module with enrichment for metabolism of keto-acids. To increase the signal to noise ratio of the analysis, the NZ gene expression data was combined with an independent experiment undertaken in Australia. The Australian study was on gene expression of full-depth ventral rumen wall tissue of 62 female sheep phenotyped extensively for CH_4 and associated traits. The small set of genes correlated with methane yield (albeit weakly) in both experiments included those encoding key enzymes in the ketone body synthesis pathway: ACADS, HMGCL and BHD1. Ketone body metabolism is a critical process in energy transactions for ruminants, in particular capturing butyrate from the rumen contents. The positive correlation between CH_4 yield and expression of these genes suggests that ketone body synthesis is downstream of the genetic control of methane production. No significant correlation between rumen wall muscle gene expression and CH4 yield was detected. Further analysis of the relationships between gene expression and CH_4 yield are underway to identify drivers of the genetically determined differences in methane production in sheep. These analyses may identify potential pathways for host regulation of methane production and elucidate some of the down-stream effects of rumen derived metabolites in animals inherently different in methane production.

Key Words: methane

P3049 Gene expression profile of satellite cells differentiation from longissimus dorsi and semimembranosus muscle. S. De las Heras-Saldana* (School of Environmental and Rural Science, University of New England, Armidale, Australia), K. Y. Chung (Hanwoo Research Institute, NIAS, RDA, Pyeongchang, Korea), S. H. Lee (Chungnam National University, Daejeon, Korea), C. Gondro (School of Environmental & Rural Science, University of New England, Armidale, Australia)

Hanwoo cattle are known for their high meat quality, particularly their high marbling (intramuscular fat) ability compared with most other breeds. Meat flavor and tenderness are largely determined by intramuscular fat composition, muscle fiber characteristics and connective tissue structures. All of these factors differ largely between muscle types, but it is not well known how this differentiation occurs and what genes and pathways regulate the process. To better understand the myogenic processes involved in differentiation of Hanwoo muscle types, we performed a time-series RNA-seq experiment to measure transcriptome expression levels during the development of muscle satellite cells (MSC) in Longissimus dorsi (LD) and Semimembranosus (SM). MSC differentiation is a good model for muscle development because their nuclei contribute to postnatal muscle growth remodeling of preexisting fibers. RNA-seq was sampled at Days 0, 2, 4, 7 and 14 in both LD and SM. Between 77% and 84% of the reads were mapped (from around 45,594,803 total reads) to the reference genome (*Bos taurus* UMD3.1). In general, there were genes differentially expressed between time points. Transcriptome profiles differed significantly between LD and SM already at Day 0. Myogenic regulatory factors (MRFs) *Myf5* and *MRF4* were expressed during muscle satellite cell differentiation. Other myogenic markers usually expressed in cells undergoing differentiation were MYL2 and MYH3, which we found to be significantly expressed in relation to Day 0 (pre-differentiation) and Day 14 (post-differentiation) in both muscles. Also in this study, IGF-I was differentially expressed. Gene ontology analysis (GO) revealed that the main enriched terms were genes with functions necessary for regulation of cell cycle, mitosis, nuclear division, cell cycle checkpoints and DNA biosynthetic processes. Also, processes related to cell shape were enriched, such as cytoskeleton organization and embryonic morphogenesis. The comparison of LM and SM transcriptome profiles may provide a better

understanding of muscle-specific differentiation signals that relate back to the phenotypic differences that influence the meat quality of Korean Hanwoo cattle.

Key Words: Hanwoo, satellite cell, differentiation

P3050 Functional genomics of high altitude disease in angus cattle: Leveraging-OMICS and systems biology to better understanding of the function and role of key contributing genes. A. Canovas* (University of Guelph, Guelph, ON, Canada), R. Cockrum (Virginia Polytechnic Institute and State University, Blacksburg, VA), D. Brown (University of Colorado, Denver, CO), S. Riddle (University of Colorado, Denver, CO), J. M. Neary (Colorado State University, Fort Collins, CO), T. N. Holt (College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO), J. F. Medrano (University of California, Davis, CA), A. Islas-Trejo (University of California, Davis, CA), R. M. Enns (Department of Animal Sciences, Colorado State University, Fort Collins, CO), S. E. Speidel (Department of Animal Sciences, Colorado State University, Fort Collins, CO), K. Cammack (Colorado State University, Fort Collins, CO), K. R. Stenmark (University of Denver, Denver, CO), M. G. Thomas (Department of Animal Sciences, Colorado State University, Fort Collins, CO)

High-altitude (> 1800 m) disease is a challenging problem for beef cattle. The disease is a consequence of hypoxia-induced right ventricular heart failure as per vascular inflammation of the pulmonary artery and hypertension. An indicator trait of this condition, pulmonary arterial pressure (PAP) has moderate to high heritability (0.2–0.4) when measured in yearling cattle; however, knowledge of candidate genes that can be used in genetic improvement for this trait and physiological-disease condition are minimal. The transcriptomes of left and right ventricle, pulmonary artery, aorta, muscle and lung were examined in samples harvested from fattening-yearling Angus steers phenotyped to be of low or high pulmonary arterial pressures (LPAP and HPAP; n = 10/group). Gene-expression analyses from RNA-seq data revealed the highest number of differentially expressed genes between groups were in right ventricle (n = 1394) and aorta (n = 1173). Also, splice variant analyses revealed the highest differential expression in right ventricle (n = 555), aorta (n = 547) and pulmonary artery (n = 152; $q < 0.05$ and Fold-change > 2) between LPAP and HPAP animals. Pathway analyses of right ventricle differentially expressed genes suggested importance of IL-8/IL-10 signaling, leukocyte extravasation, factors promoting cardiogenesis, coagulation, thrombin and cardiac hypertrophy signaling. Systems biology analyses of right ventricle data suggested that 101 genes were acting as key regulators of 705 genes differentially expressed between LPAP and HPAP steers. Most of the key regulators had roles in angiogenesis and atherosclerosis, cardiomyopathy (NFATC1), movement of leukocytes and neutrophils (OLR1, PLAUR), failure of heart (CTGF), hypertrophy of heart ventricle (TREM1, GATA2, P38-MAPK) and vascularization (SYVN1). These approaches helped identify splice variants corresponding to key regulator genes in a polygenic disease induced by high-altitude in Angus cattle. Also, regulatory impact factor (RIF) and transcription factor binding sites (TFBS) approaches were used to identify regulators with the highest evidence contributing to differentially expressed and/or tissue-specific gene specificity in LPAP and HPAP animals. Using the RIF metrics, which exploits the concept of differential co-expression, 1329 regulators were contrasted against a unique list of genes that were either differentially expressed or tissue-specific, identifying several transcription factors. Several SNP variants segregated specifically in either the LPAP or HPAP animals in key regulator genes. The integration of structural and functional genomic data associated with high-altitude disease will help to develop more robust approaches for genetic selection in beef cattle.

Key Words: cattle, transcriptomics, RNA-seq, gene-networks, functional genomics, systems biology

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P3051 Comparative aspects of functional annotation of genomes in the FAANG project. C. K. Tuggle and the FAANG Consortium

The Functional Annotation of Animal Genomes (FAANG) consortium is a grass-roots organization formed to advance the annotation of newly assembled genomes of domesticated and non-model organisms (www.faang.org). FAANG projects on several major domesticated livestock are developing, including cattle, chicken, goat, horse, pig and sheep. A major requirement for immediate functional annotation is a high-quality draft genome sequence, so that functional assays using sequence can be mapped to specific positions in the genome assembly. Many of these draft assemblies are being greatly improved through the use of PacBio technology to extend contiguity and accuracy. However, scientists with interests in annotating additional species, such as buffalo, salmon, trout and others, without a current public draft assembly are participating in FAANG discussions. Sequencing technology and assembly improvements promise to create useful genome assemblies for many additional

animal species very soon. Overall, nearly 300 scientists are contributing to the FAANG efforts. The first phase of FAANG is predicted to focus on mapping functional data to the genome within each species so that predictive tools assigning functional state to chromatin regions can best be brought to bear. These data include RNA-seq, histone modification, chromatin accessibility and higher-order chromatin interactions. Several groups are developing data and data analysis plans to create such a first-stage annotation using the above assays on two to four individuals from multiple tissues. However, comparative approaches will also play a role in these early analyses, as prior work on predicted function of defined elements through evolutionary constraint of genome sequence, integrated with specific human genome functional annotations (e.g., Lindblad-Toh et al., 2011 Nature. 478:476), can be extended to these species as well. Finally, we anticipate that more detailed comparative functional genomics will be possible in the future, through combining available functional annotation from the human and mouse ENCODE projects with similar new data from domesticated animals. Such analyses will inform and reinforce chromatin state predictions for the domesticated species, as these appear stable within cell types across human and mouse (Mouse ENCODE Consortium, 2014 Nature 515: 355). As well, adding species with deep functional annotations to these two species data sets may also elucidate the mechanisms underlying *cis*-regulatory element divergence in orthologous genes, yet congruent transcription factor networks seen in the human-mouse comparisons.

Key Words: annotation, functional genomics, genome assembly

P3052 Characterization of Circular RNAs in relation to embryonic muscle development in chicken. H. Ouyang (College of Animal Science, South China Agricultural University, Guangzhou, China), Q. Nie* (South China Agricultural University, Guangzhou, China), X. Zhang (College of Animal Science, South China Agricultural University, Guangzhou, China)

Skeletal muscle is the most important component of animal body, and its growth and development is regulated by many signaling pathways, genes, transcription factors and non-coding RNAs (ncRNA). Circular RNAs (circRNAs) is a novel class of functional ncRNA, which have been identified widespread in various cell types or tissues of eukaryote. They are abundantly and widely expressed in eukaryote and often show tissue and developmental stage specific expression patterns. CircRNAs have been identified to play key roles in growth and development of skeletal muscle in mammals. Thus, in this study, we identified circRNAs during chicken embryonic skeletal muscle development by RNA sequencing, so as to explore their functions in relation to skeletal muscle development. Leg muscles of female Xinghua chicken in three different development stages (11 embryo age, 16 embryo age, and 1 d post hatch) were used for RNA sequencing. The sequencing libraries were constructed by $rRNA^-$ and RNase R^+enriched and then performed using Illumina Hiseq3000. Sequencing data were assembled by cufflinks, and circRNAs were identified by find_circ. CircRNAs and its expressions were validated by PCR with convergence and divergence primers, real-time quantitative (q) PCR and RNase R resistance assay. In this study, we identified 24,814 circRNAs in chicken skeletal muscle of three different development stages through deep sequencing. Among them, 3459 circRNAs are expressed abundantly (read counts > 100) in muscle, and most of these circRNAs are from exonic sequences, especially from coding sequences (numbers = 2, 237). There were 5106 genes have circular isoforms, some of them have a considerable number of alternative splicing circularization patterns. A total of 2464 differentially expressed circRNAs were identified in three different stages (fold change > 2; p-value < 0.05). Junction sequences of several abundant circRNAs were confirmed, and differential abundance and RNase R resistance of circRNAs were also verified by qPCR. GO analysis for the parental genes of differentially expressed circRNAs found that there were enrich in several muscle biological process. Chicken circular RNAs harbor many canonical miRNA seed matches, especially within coding regions. The *circRBFOX2* gene was found interacted with gga-miR206. Our study reveals the prevalence of circRNAs in chicken and has identified circRNAs differentially abundant in different stage of embryonic skeletal muscle, suggesting its important functions during poultry muscle development.

Key Words: circular RNA, chicken, skeletal muscle, embryonic development

POSTERS: GENETIC DIVERSITY AND POLYMORPHISMS

P4000 Specific polymorphisms in mitochondrial region D-loop of the Tunisian domestic goat. Y. M. Ressaissi* (ISA-Chott Mariem, Tunis, Tunisia)

The mitochondrial DNA analysis is the most

meaningful approach for evaluating the genetic diversity of populations since it allows determining a population genetic history and its geographical origin. The objective of this study was to examine maternal lineages of the Tunisian native goat population by partially sequencing the mtDNA hypervariable region "D-loop" in 26 local goats. Sequences were aligned with eight Tunisian local individuals, 20 individuals from six identified maternal groups in domestic goat which are geographically distant, 16 individuals of different local African breeds, the wildlife Bezoar of species and sequences from the introduced exotic breeds to Tunisia. Data were analyzed using the neighbor-joining methods and median-joining network. Most of the identified mutations in the 26 sequences were found in the eight local sequences previously published in GenBank. According to genetic distances, the local population belong to the most common A maternal haplo-group while sharing a large genetic heritage with the African and European populations. However, it is distinguishing itself by a remarkable genetic diversity resulting from a narrow genetic variability based on few punctual mutations whose four are specific mutations and were only observed in 12 local sequences. This finding demonstrated a unique gene pool in the local goat population by revealing five new maternal lineages in the Tunisian local population.

Key Words: indigenous goats, maternal lineage, genetic diversity, phylogeny, Tunisia

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P4001 Molecular analysis of genetic variability in Egyptian buffalo using microsatellite DNA markers. S. Abou Bakr* (Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt), M. Attia (Animal Production Department, Faculty of Agriculture Cairo University, Giza, Egypt), A. A. Nigm (Animal Production Department, Faculty of Agriculture Cairo University, Giza, Egypt), S. Abdelghany (Animal Production Department, Faculty of Agriculture Cairo University, Giza, Egypt), N. Abdallah (Genetic Department, Faculty of Agriculture, Cairo University, Giza, Egypt)

A total of nine microsatellite DNA markers — BM1329, BMS483, BM143, AFR227, BMS2460, CSSM38, CSSM70, ETH02 and BM1706 — were tested to assess and analyze the molecular genetic variability among and within six governorate-located groups of Egyptian buffalo. A total of 312 sampled animals were collected from six governorates: Behera, Menoufia, Kaliobia, Giza, Sharkia and Alexandria. All studied microsatellites showed allelic polymorphism. Across all loci, 139 alleles were observed. The average number of observed alleles per polymorphic locus was 15.444 ± 3.206, ranging from 10 (BMS2460) to 20 (BM1706). The effective number of alleles per locus showed lower values varying between 8.988 and 17.176 for BMS2460 and BM1706, respectively, with a mean of 13.233 ± 2.630. The total observed number of alleles per governorate group varied between 110 (Alexandria) and 116 (Kaliobia and Giza) with an overall mean of 113.3 alleles per governorate group. The mean observed number of alleles per locus per group varied between 12.22 (Alexandria) and 12.89 (Kaliobia and Giza), while the mean effective number of alleles per locus per group varied between 10.22 ± 2.08 (Alexandria) and 10.95 ± 2.49 (Kaliobia). The observed heterozygosity (Ho) ranged from 0.792 (CSSM38) to 0.930 (BM143), while the expected heterozygosity (He) ranged from 0.890 (BMS2460) to 0.943 (BM1706). The overall means of Ho and He values were 0.890 ± 0.048 and 0.923 ± 0.017, respectively. Polymorphic information content (PIC) ranged from 0.878 (BMS2460) to 0.939 (BM1706) with a mean of 0.915. At the nine microsatellite loci, the mean of fixation index was 0.024 ± 0.017. These results indicate the presence of a wide genetic variation within the Egyptian buffalo at the molecular level, giving an opportunity to exploit marker-assisted selection (MAS) in improving economic traits of Egyptian buffalo.

Key Words: Egyptian buffalo, genetic variability, microsatellite markers

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P4002 Genetic polymorphisms of caprine stearoyl-coa desaturase (SCD) gene and their relationship with blood cholesterol and triglyceride of goats for meat in southern Thailand. C. Supakorn* (Walailak University, Tha sala, Thailand)

The present study attempts to classify SNPs of stearoyl-coA desaturase (SCD) gene and their relationship with blood cholesterol and triglyceride in goats for meat at a commercial farm in southern Thailand. Genetic variability in caprine SCD was analyzed in 290 animals belonging to several types of Boer (B), Thai native (TN), Anglo-nubian (AN) and Saanen (SA) breed crosses by SSCP technique and DNA sequencing. Four SNPs were identified in exon 3 (A/G), exon 5 (C/T), exon 6 (C/G) and 3'untranslated region (3'UTR) (TGT deletion). Associations between genetic polymorphisms of SCD gene and blood cholesterol and triglyceride were also analyzed. Individuals with genotypes BD and DD in exon 6 had significant low blood plasma cholesterol. Goats with genotype AA in exon 3 and genotype EE in 3'UTR had significant low triglyceride. These results indicated SNPs of SCD gene as a critical player in blood

cholesterol and triglyceride of goats in this herd.

Key Words: stearoyl-coA desaturase (SCD) gene, blood cholesterol, triglyceride, meat goat

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P4003 Variations of adipocyte fatty-acid binding protein (A-FABP) gene in Chinese sheep.

W. Yan,* L. Xu, J. Hu, Y. Luo (Gansu Key Laboratory of Herbivorous Animal Biotechnology, Gansu Agricultural University, Lanzhou, China)

The adipocyte fatty-acid binding protein (A-FABP) belongs to the fatty-acid binding protein family (FABPs), and it participates in fatty acid metabolism by combining and transporting the long-chain fatty acid. Based on ovine A-FABP gene in Ensemble database, the long sequences (6474bp) of A-FABP gene in Chinese sheep were sequenced and analyzed using 20 samples collected from seven breeds (Kazakh, Chinese Merino, Duolang, Tashikuergan, Tibetan, Gansu alpine fine-wool and Qinghai fine-wool). These results showed that four DNA bases (A, T, G and C) occupied about 31.48%, 33.02%, 17.21% and 18.29% of the total bases, respectively. The average content of A+T and G+C occupied about 64.5% and 35.5% of the total content, respectively. Forty-eight base mutation sites and two microsatellite loci for M1((TG)n) and M2((TA)n) were observed, and the single base mutation site, two/three base mutation sites and insert/deletion base mutation sites occupied about 31.25%, 45.83% and 22.92% of the total base mutation sites, respectively. Four mutation types (transition, transversion, insertion and deletion) were observed, which occupied about 45.84%, 31.25%, 2.08% and 20.83% of the total mutation sites, respectively. Nineteen haplotypes (H1-H19) were found in 20 samples in our study; therefore, this observation reflects the rich diversity of haplotype in ovine A-FABP gene. The NJ tree constructed by 19 haplotypes developed two main clades, which also indicates that 19 haplotypes are initially derived from two main haplotypes.

Key Words: A-FABP gene, sheep, variation

P4004 Polymorphism information content as a measure of the usefulness of microsatellites for genetic analysis.

L. H. McClean* (The University of the West Indies, Cave Hill Campus, Bridgetown, Barbados)

The mean statistical values for all of the genetic parameters measured at the 20 microsatellite loci in the Barbados Blackbelly sheep were all significantly different in the three populations ($p < 0.001$) that were investigated, thus providing evidence that analysis at microsatellite loci is a reliable method for differentiation between populations of Barbados Blackbelly (BBB) sheep. Comparison of the extent to which gene diversity was influenced by polymorphism, heterozygosity and polymorphism information content (PIC) indicates that, of these parameters, PIC is the most reliable means of determining the usefulness of a microsatellite for genetic analysis. The PIC value of 19 of the 20 microsatellite loci investigated was greater than 0.5 and therefore highly informative for the genetic analysis, while the value at four loci was greater than 0.25 but less than 0.5 and therefore reasonably informative. There was a direct linear relationship between PIC and gene diversity at the 20 loci investigated in three populations of the BBB sheep breed with $R^2 = 0.9988$ at $P < 0.001$. At locus MAF214, the PIC value of 0.111 indicates that this locus is not useful for the genetic analysis of the BBB sheep breed. However, this locus is of interest because the BBB sheep analyzed were also fully inbred at this locus (f = 1) and homozygous in the three populations that were investigated. Therefore, of the 20 polymorphic microsatellite loci investigated, 19 are useful for the genetic identification of purebred Barbados Blackbelly sheep and for the differentiation and identification of individuals in different populations of the breed.

Key Words: PIC, gene diversity, polymorphism

P4005 Associations of SNPs in hormone-sensitive lipase-like gene 5' terminal-sequences with fatty acid content in longissimus muscle of Chinese Simmental steers.

X. Fang*, R. Yang, H. Xiao, P. Jiang, Y. Yang, Z. Zhao (College of Animal Science, Jilin University, Changchun, China)

Hormone-sensitive lipase (HSL) is responsible for the hydrolytic action of triacylglycerol (TAG) stored in adipose tissue for free fatty acids (FA) release and considered to be a candidate gene affecting meat quality traits. But the SNPs and function of hormone-sensitive lipase-like gene located at upstream of HSL gene was rarely reported, and its function was not clear. This study detected the 5' terminal-sequences SNPs of hormone-sensitive lipase-like gene (LOC107131427) (–447 to +111) and its association with FA content in longissimus tissue was analyzed in Chinese Simmental steers. The PCR products of 135 Chinese Simmental steers were sequenced by Sanger sequencing, and five SNPs (c.-862T > C, c.-1002T > C, c.-1609A > G, c.-1781T > C and c.-1821T > C) were found in 5' terminal-sequences of hormone-sensitive lipase-like gene which had been reported (rs41887429, rs41887428, rs41887427, rs41887426 and rs41887428). The correlation analysis showed that the c.-1002T > C was associated with content of myristic acid, nutmeg oleic acid, palmitic acid, palmitoleic acid, pearl fatty acid, heptadecenoic

an acid, stearic acid and oleic acid in longissimus ($p < 0.05$); the c.-1609A > G was associated with content of myristic acid, palmitic acid, palmitoleic acid, pearl fatty acid, heptadecenoic an acid, stearic acid, oleic acid and arachidonic acid in longissimus ($p < 0.05$); c.-1781T > C was associated with content of palmitic acid, palmitoleic acid, pearl fatty acid, heptadecenoic an acid, oleic acid and arachidonic acid in longissimus ($p < 0.05$); and c.-1821T > C was associated with content of myristic acid, palmitic acid, palmitoleic acid, pearl fatty acid, heptadecenoic an acid, oleic acid and arachidonic acid in longissimus ($p < 0.05$). A haplotype analysis indicated that five SNPs were in linkage and formed three haplotypes: CCGCC (0.733), TTATT (0.225) and TCGTT (0.021). The transcription factor binding sites (TFBS) were predicted by the PROMO and TFBS were changed by SNPs, respectively. The mutant of c.-1821T > C led to an additional binding site for the transcription factor ZF5, and c.-1781T > C led to the elimination of a LIM1, and a C/EBPα TFBS, c.-1609A > G, c.-1002T > C, c.-862T > C led to the elimination of a TBP, a C/EBPα and a HMG I(Y) TFBS in 5' terminal-sequences, respectively. Our study showed that the SNPs of hormone-sensitive lipase-like gene 5'-terminal sequences may regulate expression levels of hormone-sensitive lipase-like protein which may involve in FA metabolism in cattle muscle.

Key Words: cattle, hormone-sensitive lipase-like gene, fatty acids (FA)

P4006 Equine major histocompatibility complex class II region: Long-read sequencing and annotation of nine bacterial artificial chromosome clones. A. Viluma,* S. Mikko, T. F. Bergström, G. Andersson (Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden)

The Major Histocompatibility Complex (MHC) is one of the most polymorphic regions in the mammalian genome. MHC class I and class II loci encodes antigen presenting molecules binding and presenting foreign peptides. Classical MHC molecules are associated with a large number of inflammatory, autoimmune and infectious diseases in mammals. In the horse, associations have been found with diseases such as Equine Sarcoids and insect bite hypersensitivity. Large repeat element clusters, gene duplications and heterozygous INDELs makes it difficult to sequence MHC region with Sanger and short-read sequencing methods. A bacterial artificial chromosome (BAC) library, CHORI-241, constructed using neutrophil DNA from a male horse called Bravo, has been screened for known MHC genes, and a minimum tiling path is known. To increase the knowledge of equine MHC region and facilitate further research, high-quality genomic sequence is necessary. We used PacBio single molecule Real-Time technology to sequence minimum tiling path of BACs covering the MHC class II region. The obtained consensus sequence was annotated using custom annotation pipeline incorporating existing publicly available recourses (EST, mRNA, RNA-seq data), and results were manually validated with protein homology. The final equine MHC class II consensus sequence consists of two assembled BAC clone contigs joined together by two long-range PCR products that were sequenced using PacBio. An increased base pair mismatch was observed in two BAC clones overlapping a total of 155 kb, and a selection of mismatched positions were investigated with Sanger sequencing. The results suggested that both homologous chromosomes were sequenced and that Bravo is heterozygous for two haplotypes with an estimated divergence time of approximately one MYR. Annotation of sequenced BAC clones allowed us to identify 22 complete MHC class II genes, one incomplete gene and nine pseudogenes. Based on sequence structure analysis and annotation results, we show that there are four duplicated Eqca-*DQA-DQB* blocks, a single-*DRA* gene and six-*DRB* loci (three functional and three pseudogenes). Moreover, the *Eqca-DOB* gene has been duplicated twice. Comparison with the reference genome sequence showed a high SNP and INDEL frequency in the *DRB* region, but not over the-*DQ* region. This in depth analysis of the equine MHC class II region may allow conclusions to be drawn concerning the evolutionary history of the equine MHC as well as a foundation for genetic association studies of immune-mediated diseases in the horse.

Key Words: MHC, horse, PacBio

P4007 High genetic diversity and distribution of Bubu-DQA alleles in swamp buffaloes (bubalus bubalis carabanesis): Identification of new Bubu-DQA loci and haplotypes. S. K. Mishra* (Gautam Buddha University, Gr. Noida, India; National Bureau of Animal Genetics Resource, Karnal, India)

Diversity of MHC class II genes directly influences the immune response against pathogens in a species. Here we analyzed the genetic diversity of MHC class II DQA locus using PCR-RFLP, cloning and sequencing in swamp buffaloes (*Bubalus bubalis carabanesis*). A total of 25 *Bubu*-DQA alleles belonging to eight major types of three allelic groups — DQA1, DQA2 and DQA3 — were identified having different RFLP haplotypic patterns. Based on exon 2 region analysis, 10 total novel DQA alleles were identified, not reported previously in any of the bovine species. Phylogenetic

analysis revealed the distribution of buffalo DQA alleles in two major clusters, sharing evolutionary lineages with cattle DQA1 and DQA2 genes, which could be due to trans-species evolution. Further, a *Bubu*-DQA*2501 allele shared high homology and common lineage with the alleles of cattle (*BoLA*) DQA3 gene, not reported in buffalo previously, indicating existence of a new *Bubu*-DQA3 locus in buffalo. PCR-RFLP and presence of diverged alleles revealed high DQA duplication with different combinations of DQA1, DQA2 and DQA3 loci in swamp buffaloes. Higher dN than dS values and Wu–Kabat variability frequencies observed, particularly at peptide binding regions in both of *Bubu*-DQA1 and-DQA2 alleles indicated the high polymorphism with balancing selection at buffalo DQA locus, similar to cattle. The newly identified DQA alleles with high diversity present in the form of duplicated haplotypes in different combinations in a small buffalo population indicates the genetic richness of bovines in general and buffalo in particular to combat pathogens prevalent in tropical region.

Key Words: *Bubalus bubalis*, MHC-DQA, duplicated haplotype, allelic diversity, peptide binding site, phylogeny

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P4008 Genome-wide copy number variation in the bovine genome detected using low coverage sequence of popular beef breeds. B. N. Keel,* W. M. Snelling, J. W. Keele (USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE)

Genomic structural variations are an important source of genetic diversity. Copy number variations (CNVs), gains and losses of large regions of genomic sequence between individuals of a species, are known to be associated with both diseases and phenotypic traits. Deeply sequenced genomes are often used to identify CNV, but deep sequence is too expensive to collect on many individuals. The random variation in coverage that occurs with current sequencing methods may make low coverage sequence unsuitable for CNV detection. A more complete catalog of important CNV might be obtained from low coverage sequence from several individuals if approaches capable of detecting CNV in low coverage sequence can be identified. Benchmark CNV data sets were simulated and used to test the performance of three modern CNV detection algorithms at varying levels of coverage. Comparison of the three methods determined that a multiple sample read depth approach is most suitable for detecting CNV in low coverage genomic sequence. As part of an effort to identify DNA sequence variation that affects beef cattle performance, this method was applied to low coverage DNA sequence from 154 influential bulls in the U.S. Meat Animal Research Center Germplasm Evaluation (GPE) project. These bulls were purebred sires sampled from seven popular beef breeds in the United States (Angus, Hereford, Simmental, Limousin, Charolais, Gelbvieh, and Red Angus). Sequence reads were mapped to the current bovine genome assembly; a mean of 2.9-fold coverage per bull was obtained. A total of 1532 copy number variable regions (CNVRs) were detected, with each being present in at least eight different bulls. These CNVRs covered approximately 7% of the bovine genome and overlapped 2004 protein-coding genes. A larger-than-expected number of genes involved in immune system processes were affected by CNV. In addition, CNV were shown to overlap several known regions of DNA that correlate with variation in cattle phenotype. Further investigation is needed to assess how much influence the coding sequence CNVs identified from this work might have on cattle performance.

Key Words: copy number variation, beef cattle, genome sequence

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P4009 Diversity of West African dwarf goat in southwestern Nigeria based on allozyme markers. O. K. Awobajo* (Tai Solarin University of Education, Ijebu-Ode, Nigeria)

Effective conservation, rational management and inadequate information on genetic diversity are the major challenges in livestock production. Genetic diversity has been used to reveal the extent of differentiation within livestock species. However, information on the use of allozymes in genetic diversity of the West African Dwarf (WAD) goat is insufficient. Therefore, genetic diversity of the WAD goat populations in southwestern Nigeria was investigated in this study. Three protein loci markers were used. Blood samples(5 mL each) were randomly collected from 20, 20, 40 and 60 goats from Ondo, Oyo, Ogun and Osun states, respectively. The samples were subjected to cellulose acetate electrophoresis to determine the genetic variants at haemoglobin (Hb), carbonic anhydrase (CA) and transferrin (Tf) loci. Another set of blood (5 mL) from 20 different individual animals were randomly obtained from each of Ondo, Oyo, Ogun and Osun states. Allele frequency, observed heterozygosity (H_O), polymorphic information content (PIC), F-statistic ($F_{ST,}$ F_{IT} and F_{IS}), gene flow (Nm), gene diversity (D), number of alleles per loci (A_p), effective number of allele (A_E) and mean number of allele (MNA) were generated from the data obtained. Data were analyzed using Hardy-Weinberg equilibrium (HWE) at $\alpha_{0.05.}$ The allele frequency ranged between 0.11 (Hb^{A+}) and 0.58 (Hb^{B+}), 0.17 (CA^{F+}) and 0.44 (CA^{FS}), and 0.08 (Tf^{A+}) to 0.6 (Tf^{AB}). Deviation from HWE was not

significant in all populations except at Tf locus (0.00). The H_O ranged from 0.43 to 0.62 and Nm and D ranged between 3.68 and 32.40 and 0.34 to 0.5, respectively. The MNA was 0.67, but A_E ranged from 1.52 to 2. The allozymes revealed some level of genetic diversity and a genetic differentiation indicative of the amount of genetic differences among individuals within the West African Dwarf goat population.

Key Words: goat polymorphism, allozyme markers, genetic and biochemical characterization, genetic variability

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P4010 Investigation of maternal lineages and genetic diversity of South African goat (capra hircus) populations using complete mitochondrial DNA sequences. K. T. Ncube* (Agricultural Research Council-Biotechnology Platform, Pretoria, South Africa)

South Africa is one of the leading goat-producing countries and has some well-defined goat breeds. The origins and maternal lineages of these goats is, however, unknown, and most populations still remain uncharacterized. Complete mitochondrial DNA was used to investigate genetic diversity and maternal lineages of 50 South African goat breeds in conjunction with mtDNA goat sequences from the GenBank. Rolling Circle Amplification together with Illumina MiSeq next generation sequencing were used to generate the full length (16.64kb) of the mtDNA of South African commercially developed Boer ($n = 9$), the captive feral Tankwa ($n = 9$), and undeveloped village ($n = 33$) goat populations. The mtDNA was highly polymorphic in the South African goat populations. A total of 184 SNPs and 55 amino acid changes were observed. The percentage of SNPs per mtDNA gene ranged from 0.01% (tRNA-Ala) to 33% (ND3) genes. High within population variation was observed in all the groups ranging from 98.6–99.52%. Low F_{ST} ($F_{ST} = 0.003–0.049$) indicated close relatedness between SA goat populations and suggested gene flow between populations. The 50 sequences yielded 42, 19 and 26 haplotypes for the D-loop, COX1 and complete mtDNA, respectively. A phylogeographic analysis of the SA D-loop sequences together with Chinese sequences from the GenBank representing A–D lineages resulted in six clades and SA goats were represented in A and B lineages. The COX 1 haplotypes clustered in the Wild bezoar and the Bangladesh Black Bengal clade suggesting that the SA goat populations have been genetically influenced by the Bezoar and the Black Bengal, and further suggesting that the Bezoar may be the ancestor of the SA domestic goats. Complete mtDNA haplotypes resulted in six clades and SA goats were represented in the Chinese lineage A clade. Haplotypes and clades observed in the D-loop, COX1 and whole mtDNA network trees demonstrated relationships between South African goat populations. In the D-loop, South African goats clustered with Chinese goats from lineages A and B, suggesting common maternal lineages between the Chinese and South African goat populations. The COX1 results further confirmed that the Bezoar (*Capra aegagrus)* goat is a possible ancestor of the domestic goats. The complete mtDNA also demonstrated a clustering of the South African goats with the Chinese goats from lineage A. The domestication and migration of goats into South Africa was inferred.

Key Words: goats; mtDNA, domestication, genetic diversity, maternal lineages, phylogeographic structure

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P4011 Analysis of Cytochrome *b* gene variations shows that Amami rabbit (*pentalagus furnessi*) and European rabbit (*oryctolagus cuniculus*) have close genetic structure. R. Ashidate* (School of Agriculture, Meiji University, Kawasaki, Japan), T. Kuraishi (Amami Laboratory, The Institute of Medical Science, The University of Tokyo, Kagoshima, Japan), Y. Mizoguchi (School of Agriculture, Meiji University, Kawasaki, Japan)

The Leporidae are a family of mammals that includes rabbits and hares, and they are distributed in many ranges throughout the world. The European rabbit (*Oryctolagus cuniculus*) is a descendant of all domesticated rabbits and has been transferred to many countries. Several species live in Japan, such as the Japanese hare (*Lepus brachyurus*) and the Amami rabbit (*Pentalagus furnessi*). The Amami rabbit is an endangered species that is endemic to Amami Oshima and the Tokunoshima islands in the Ryukyu Archipelago. To investigate the genetic structure of the Amami rabbit, we analyzed Cytochrome *b* gene (1140 bp) polymorphisms in the mitochondrial DNA of 69 rabbits from these islands. We identified eight new single nucleotide polymorphism and seven haplotypes. Additional rabbit sequences were obtained from GenBank. Phylogenetic analysis was performed on 10 haplotypes of Amami rabbit (including three haplotypes of Yamada et al., 2002) and 34 haplotypes of other rabbits and an outgroup (*Ochotona hyperborea*) haplotype. Neighbor joining phylogenetic analysis was implemented using the Kimura 2 parameter model. The phylogenetic tree had two branches: the hare and others. We found that Amami rabbits belong to the European rabbit lineage, which confirms results

in a previous study (Yamada et al., 2002). This study shows that the Amami rabbit and the European rabbit are also closely related in terms of their genetic structure. Future studies could reveal more details about the genetic relationships between Amami and European rabbits by analyzing other mitochondrial DNA variations and microsatellites on autosome.

Key Words: mitochondrial DNA, phylogenetic analysis, rabbit

P4013 Applicability of using bovine, ovine and caprine SNP chips for alpaca and dromedary genomic studies. F. Bertolini* (Department of Animal Science, Iowa State University, Ames, IA), A. Elbeltagy (Department of Animal Science, Iowa State University, Ames, IA; Animal Production Research Institute, Cairo, Egypt), F. A. Ponce de Leon (Department of Animal Science, University of Minnesota, St. Paul, MN), G. A. Gutiérrez (Department of Animal Production, Universidad Nacional Agraria La Molina, Lima, Peru), M. F. Rothschild (Department of Animal Science, Iowa State University, Ames, IA)

The alpaca *(Vicugna pacos)* and the dromedary *(Camelus dromedarius)* belong to the *Camelidae* family. Together, they represent two of the most economically important domesticated species for communities under certain climatic stresses. Alpacas are widely used to produce natural fiber for manufacturing of textile products, while the dromedary camel is widely used for rural transportation and as a precious source of proteins (meat and milk) in arid desert regions. Despite the economic importance of these two species, currently there is a lack of well-defined assembled genomes, and few genetic markers (single nucleotide polymorphisms; SNPs) are available. SNP chips designed for other related livestock species could be a useful initial source to identify common SNPs that could be considered for alpaca and dromedary genomic analyses. In this study, alpaca and dromedary samples were genotyped using 3 Illumina BeadChips: the Bovine 777K-SNP BeadChip, (eight alpaca = ALP-BOV, eight dromedaries = DROM-BOV), the Ovine 600KSNP BeadChip, (12 alpaca = ALP-OV, 17 dromedaries = DROM-OV) and Caprine 53K SNP BeadChip. Due to the lower number of SNPs that had been successfully genotyped with the caprine SNPchip, results focused on the Bovine and Ovine SNP chips. The 10.5% (ALP-BOV), 0.6% (ALP-OV), 20.7% (DROM-BOV) and 4.4% (DROM-OV) of the SNPs were successfully genotyped for all the animals. A total of 31,888 SNPs in the Bovine SNP chip and only 143 in the Ovine SNP chip overlapped in the two species. A total of 0.6%, 1.2%, 0.7% and 53% had no calls for ALP-BOV, ALP-OV, DROM-BOV, DROM-OV, respectively, while the remaining SNPs were partially genotyped on various numbers of animals. The SNPs successfully genotyped in all animals were analyzed with the variant effect predictor using the bovine and ovine annotation references. Approximately 55% of the SNPs for the four SNP sets (ALP-BOV, ALP-OV, DROM-BOV, DROM-OV) were located in intergenic regions, while ~45% were located within or nearby an annotated gene, for a total of 8872 genes for ALP-BOV, 1094 for ALP-OV, 11,769 for DROM-BOV and 7291 for DROM-OV. The number of genes commonly detected in the two SNP chips (Bovine and Ovine) was 823 for alpaca and more than 5000 for the dromedary. These results provide a first SNP list that could be used in genetic studies for these two species. Further studies could evaluate these SNPs in the alpaca and dromedary populations as well as reconsider SNPs not successfully genotyped in all the animals, including the caprine SNPs.

Key Words: alpaca, dromedary, BeadChip

P4014 Global and local admixture analyses of baladi cattle. A. Shabtay (Department of Ruminant Sciences, Agricultural Research Organization (ARO), Newe Ya'ar Research Center, Ramat Yishay, 30095, Israel), M. Soller (Hebrew Univarsity of Jerusalem, Jerusalem, Israel), J. Sölkner (University of Natural Resources and Life Sciences, Vienna, Austria), G. Mészáros (University of Natural Resources and Life Sciences, Vienna, Austria), T. Sonstegard (Recombinetics Inc., St Paul, MN), E. O. Ünal (Namik Kemal University, Agriculture Faculty, Department of Animal Science, Tekirdag, Turkey), H. J. Huson (Cornell University, Ithaca, NY), Y. T. Utsunomiya (UNESP University Estadual Paulista, Jaboticabal, Brazil), E. Lipkin* (Hebrew University of Jerusalem, Jerusalem, Israel)

The Baladi is a native *Bos taurus* breed of the Mediterranean basin known for its hardiness, disease resistance, tolerance of poor care, meager diet and adverse climate conditions. In Israel, and probably in other Mediterranean countries, the Baladi breed is in danger of extinction due to the introduction of larger more productive European breeds. Domestication of cattle occurred independently in two or three sites: Southwest Asia (*Bos taurus*), Southeast Asia (*Bos indicus*), and possibly North Africa (*Bos taurus*). In this study, global admixture analysis of pure Baladi and 15 other cattle breeds around the world were used to investigate the ancestral components of the Baladi genome. The Baladi was revealed as a unique combination of the

three main postulated ancestral cattle domestications, having each component in roughly equal proportions, and thus represents an enormous store of cattle genetic diversity. Local admixture analysis exposed regions characterized by positive deviation for the Indicine and African Taurine genome components of the Baladi genome and mostly negative selection against the Southwest Taurine component. Thus, these regions may harbor genomic elements increasing the Baladi local adaptation. The Baladi share similar global and local admixture profiles with three Turkish breeds. Thus, all four breeds may be considered in large part as independent replicates taken from the same Middle East ancestral mix. This brings enormous potential for high-resolution validated mapping of chromosomal regions contributing to adaptation. Thus, from both a practical and preserving biodiversity views, the Baladi is a prime candidate for conservation.

Key Words: population admixture, domestication, Baladi, coservation

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P4015 Molecular phylogeny and domestication pattern of river buffalo through mitochondrial D-loop DNA analysis. M. Y. Zahoor* (University of Veterinary & Animal Sciences, Lahore, Pakistan)

River buffalo (*Bubalus bubalis*) is a major livestock resource in many Asian countries, particularly in South Asia. The origin, domestication and genetic structure of the river buffalo are poorly understood. Pakistan has the second-largest population of river buffalo, 34.6 million heads, and is also the second-largest producer of buffalo milk after India. It is uncertain whether domestication of river buffalo occurred together with or independent of other buffalo species/subtypes. Sequence analysis of the mtDNA D-loop region of Pakistani buffaloes revealed a total of 24 polymorphic sites and four haplotypes. These haplotypes were compared in phylogenetic trees with 347 additional mtDNA D-loop sequences already reported for river and swamp buffaloes. All phylogenetic trees revealed two major clades: the river buffalo and the swamp buffalo clade. Among the river buffalo clade were present a number of subclades representing distinct matrilineal and geographical origins. These findings indicate that multiple domestication events have occurred in river buffalo, leading to independent expansion of some maternal lineages with minimum introgression from wild populations. The results of the current study lend support to the archeological evidence for the domestication of the river buffalo in the Indian subcontinent.

Key Words: *Bubalus bubalis,* domestication, genetic diversity

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P4016 Paternal genetic characterization of wild boars and domestic pigs in Japan, based on SRY and TSPY gene haplotypes. Y. Sato,* K. Sato, Y. Mizoguchi (School of Agriculture, Meiji University, Kawasaki, Japan)

In Japan, there are two subspecies of wild boar: the Ryukyu wild boar (RWB) (*Sus scrofa riukiuanus*) on the Ryukyu Archipelago and the Japanese wild boar (JWB) (*Sus scrofa leucomystax*) on the Honshu, Kyushu and Shikoku islands. In several previous studies, elucidation of genetic diversity in wild boars and the origin of domesticated pigs have utilized autosomal microsatellites and mitochondrial DNA variations. The aim of this study is to clarify the paternal genetic characteristics of wild boar and domestic pig in Japan. We used two genetic markers residing on the Y chromosome: sex-determining region Y (SRY) and testis-specific protein on Y (TSPY). Samples from 140 male RWBs were collected from five islands (Amami, Tokunoshima, Okinawa, Ishigaki and Iriomote) and compared with reference samples from 28 male JWBs from Honshu and Kyushu, five male Taiwanese wild boars (TWBs) (*Sus scrofa taivanus*) from Taiwan and 19 male domestic pigs (Landrace and Meishan). Our analysis assigned these samples into five haplotypes (HTs): RWB and TWB samples were all assigned to HT4, JWB samples belonged to HT2 and HT3, Asian (Meishan) pigs belonged to HT5, and Landrace belonged to HT1. These results suggest that the genetic isolation of RWB and JWB is due to the Watase line, a major biographical boundary separating the Indomalaya and Palearctic regions. Also the Asian (Meishan) pig and European (Landrace) pig have different processes of domestication based on their paternal genetic characteristics.

Key Words: *Sus scrofa*, genetic diversity, Y chromosome

P4017 Distribution of Y chromosomal haplotypes in Japanese native horse populations. H. Kakoi* (Laboratory of Racing Chemistry, Utsunomiya, Japan), T. Tozaki (Laboratory of Racing Chemistry, Utsunomiya, Japan), M. Kikuchi (Laboratory of Racing Chemistry, Utsunomiya, Japan), K. I. Hirota (Laboratory of Racing Chemistry, Utsunomiya, Japan), S. I. Nagata (Laboratory of Racing

Chemistry, Utsunomiya, Japan), M. Takasu (Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan)

Indigenous horses in Japan, likely originating from the Mongolia-type horses, are distributed across the country and have formed some local populations; however, their population size has significantly decreased since the latter half of 19th century. Currently, their descendants remain only in eight local areas. Until date, autosome-based STR/SNP and matriline-based mitochondrial analyses to elucidate genetic relationship among the populations have been developed for preserving genetic resources and endemic characteristics. Recently, haplotype variation of equine Y chromosome has been identified for worldwide breeds. In this study, the distribution of Y haplotypes in the native populations was investigated to obtain their genetic information to further add to the results of previous studies. Blood DNA samples were obtained from 143 male/gelding horses of seven populations: 13 from Hokkaido; 37, Kiso; 38, Misaki; 7, Noma; 11, Taishu; 17, Miyako; and 20, Yonaguni. Haplotyping was performed by using five Y chromosomal loci. The SNP/indels of these loci were analyzed by direct sequencing of the targeted PCR amplicons. In these analyses, SNP variations were found at two loci, Y-45288 and -50869. The analyzed horse Y chromosomes were classified into three haplotypes, namely JHT-1, −2 and −3; these were identical to the previously reported haplotypes in worldwide breeds. The Hokkaido, Noma, Taishu and Miyako populations showed only JHT-1. On the other hand, both the Kiso and Misaki populations showed only JHT-2. Although JHT-1 and −3 were observed in the Yonaguni population, JHT-3 appeared due to a de novo mutation of JHT-1. Based on this result and previously reported data of Asian horses, it can be assumed that JHT-1 is a major haplotype in ancestral native horses, which then became distributed and integrated into most of the modern horse populations. The fixation of JHT-2 also suggests influence by limited patrilines in the Kiso and Misaki populations. These findings complement the results of studies on the genetic features of Japanese native horse populations.

Key Words: horse, Y chromosome, haplotype

P4018 Effective population size and inbreeding in South African indigenous chicken populations: Implications for management and conservation of unique genetic resources. B. Mtileni* (Tswane University of Technology, Pretoria, South Africa), K. Dzama (University of Stellenbosch, Stellenbosch, South Africa), K. Nephawe (Tshwane University of Technology, Pretoria, South Africa), C. Rhode (University of Stellenbosch, Cape Town, South Africa)

Conservation of locally adapted indigenous livestock breeds has become an important objective in sustainable animal breeding, as these breeds represent a unique genetic resource. Therefore, the Agricultural Research Council of South Africa initiated a conservation program for four South African indigenous chicken breeds. The evaluation and monitoring of the genetic constitution of these conservation flocks is important for proper management of the conservation program. Using molecular genetic analyses, the effective population sizes and relatedness of these conservation flocks were compared to village (field) chicken populations from which they were derived. Genetic diversity within and between these populations are further discussed within the context of population size. The conservation flocks for the respective breeds had relatively small effective population sizes (point estimate range: 38.6–78.6) in comparison to the field populations (point estimate range: 118.9–580). Furthermore, evidence supports a transient heterozygous excess, generally associated with the occurrence of a recent population bottleneck. Genetic diversity, as measured by the number of alleles, heterozygosity and information index, was also significantly reduced in the conservation flocks. The average relatedness among the conservation flocks was high (0.12–0.41), while it remained low for the field populations (0.002–0.02). There was also significant evidence for population differentiation between field and conservation populations. F_{st} estimates for conservation flocks were moderate to high, with a maximum reached between VD_C and VD_F (0.285). However, F_{st} estimates for field population were excessively low between the NN_C and EC_F (0.007) and between EC_F and OV_F (0.009). Significant population differentiation of the conservation flocks from their geographically correlated field populations of origin is further supported by the AMOVA, with 10.51% of genetic diversity ascribed to population differences within groups (F_{SC} = 0.106). Results suggest that significant genetic erosion has occurred within the conservation flocks due to inbreeding, pronounced effects of random drift and selection. It might be necessary to introduce new breeding individuals from the respective field populations to increase the effective population sizes of the conservation flocks and counter the effects of genetic erosion.

Key Words: conservation, effective population size, indigenous chickens

P4019 Statistical analysis of alleles in 4703 thoroughbred racing horses using fifteen microsatellite DNA markers. S.-W. Kang*, S.-Y. Lee, D.-H. Chio, H.-J. Kang, M.-B. Hu, Y.J. Yang (Racing Laboratory, Korea Racing Authority, Gwacheon, 13822, South Korea)

We analyzed 4703 thoroughbred horses using 15 microstellite markers for parentage testing and individual identification. We analyzed number of alleles, allelic frequencies, observed(Ho) and expected(He) heterozygosity, probability of exclusion (PE) and total PE for parentage test. The number of alleles of the markers varied between five and 10, with an average number of 6.6. The heterozygosity ranged from 0.427 to 0.817, and mean expected heterozygosity was 0.682. Observed PIC was from 0.444 (HTG4) to 0.791 (ASB2), and total PE of all markers was 0.9999. These results verify that 15 microsatellite markers used in this study are very powerful for parentage test and individual identification of thoroughbred horse in South Korea.

Key Words: thoroughbred horse, microsatellite, parentage test

P4020 Diversity analysis of transcribed MHC class IIβ loci in Japanese quail. S. Asaji (Tokyo University of Agriculture, Atsugi, Japan), S. Suzuki (Tokai University School of Medicine, Isehara, Japan), T. Ishige (Tokyo University of Agriculture, Setagaya, Japan), K. Hosomichi (Kanazawa University, Kanazawa, Japan), T. Shiina (Tokai University School of Medicine, Isehara, Japan), H. Hara (Tokyo University of Agriculture, Atsugi, Japan), T. Hirano (Tokyo University of Agriculture, Atsugi, Japan), K. Hanzawa* (Tokyo University of Agriculture, Atsugi, Japan)

The major histocompatibility complex class IIβ loci in Japanese quail (CjIIB) are highly repeated; for example, seven CjIIBs map to haplotype*01 (CjIIB-HT*01). Additionally, among five haplotypes (CjIIB-HT*01 to *05), copy number variation (CNV) of transcribed CjIIB genes is evident based on analysis of cDNA clones. However, because of nucleotide sequence similarity over some kb, it has been difficult to determine the exact composition of the CjIIB genes via conventional methods that depend on subcloning and Sanger sequencing. Therefore, the aim of this study was to identify the transcribed CjIIB gene sequences within each CjIIB-HT by 454 GS Junior-based amplicon sequencing and to determine apparent frequencies of CjIIBs via RNA-seq with HiSeq2500. Quails were selected to ensure that all CjIIB-HT*01, *03, *04 and *05 were included in the sample. Total RNA was extracted from peripheral lymphocytes of each quail. A primer pair that amplifies a fragment comprising part of exon 1, exon 2 (hyper variable β1 domain) and part of exon 3, of all known CjIIB (411 bp or 441 bp) was used. Template RNA was amplified via RT-PCR using primers that were each fused to adaptor sequences. In CjIIB-HT*01 homozygote quails, the seven known CjIIBs that map to CjIIB-HT*01 were identified. Furthermore, five new CjIIB sequences were found in all CjIIB-HT*01 quails. In four CjIIB-HTs, the 20 known CjIIB sequences and 12 new CjIIB sequences were confirmed. These 32 CjIIB sequences were classified into eight major CjIIB alleles, each of which had an apparent frequency of more than 10%, and 24 minor CjIIB alleles, each of which had an apparent frequency of less than 5%. Both the class of major CjIIB alleles (from one to three) and the class of minor CjIIB alleles (from 0 to 10) showed remarkable diversity among the CjIIB-HTs. CjIIB-HT*04 had three major CjIIBs and did not have a minor CjIIB. In contrast, CjIIB-HT*05 had only one major CjIIBs and had 10 minor CjIIB. The results of diversity analysis of CjIIB gene alleles suggested that the CNV of CjIIB genes might have resulted from dynamic genetic reorganization, e.g., duplication, fusion, functional deficiencies and function sharing. The apparent frequencies of CjIIBs determined via RNA-seq were similar to the apparent frequencies that were determined via amplicon sequencing. Therefore, the effect of PCR bias during amplicon sequencing on apparent frequencies of functional CjIIB seems to be small.

Key Words: MHC class IIβ, quail, amplicon sequencing, RNA-seq

P4021 Genomic patterns of differentiation in native and introduced populations of the cupped oysters Crassostrea gigas and Crassostrea angulata and in hybrid progenies. S. Lapègue* (Ifremer, SG2M-LGPMM, Laboratoire de Génétique et Pathologie des Mollusques Marins, La Tremblade, France), P. A. Gagnaire (ISEM- CNRS, UMR5554, SMEL, Sète, France), J. B. Lamy (Ifremer, SG2M-LGPMM, Laboratoire de Génétique et Pathologie des Mollusques Marins, La Tremblade, France), F. Cornette (Ifremer, SG2M-LGPMM, Laboratoire de Génétique et Pathologie des Mollusques Marins, La Tremblade, France), S. Heurtebise (Ifremer, SG2M-LGPMM, Laboratoire de Génétique et Pathologie des Mollusques Marins, La Tremblade, France), E. Flahauw (Ifremer, SG2M-LGPMM, Laboratoire de Génétique et Pathologie des Mollusques Marins, La Tremblade, France), L. Dégremont (Ifremer, SG2M-LGPMM,

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The two Asian species, *Crassostrea gigas*, the Pacific oyster, and *Crassostrea angulata*, the Portuguese oyster, display adjacent distributions in their native ranges. Both species have been introduced into Europe accidentally or for aquaculture production at different periods in the recent past. They can be differentiated but are considered as phylogenetically very close. Furthermore they did not show experimentally any barrier to hybridization. The co-introduction has potentially altered the genomic architecture of differentiation by facilitating gene swamping or triggering adaptive gene flow in the new environment. Therefore, oysters offer a particularly interesting model to understand the mechanisms responsible for the buildup and maintenance of species divergence. However, the debate is still open as to whether *these* species still exchange genes in nature. Here, we address this question using two complementary approaches based on the analysis of genomic variation patterns in (1) native and introduced *C. gigas* and *C. angulata* populations and (2) second-generation hybrid progenies. Our aim was to detect regions of the genomes associated with increased differentiation in nature and increased segregation distortion in hybrids. We first generated a pseudo-chromosome assembly of the Pacific oyster genome using a combination of BAC-end sequencing and scaffold anchoring to a new high-density linkage map. We then used RAD-sequencing to characterize genome-wide variation patterns in both native and introduced populations of *C. angulata* and *C. gigas*, and in F2 hybrid progenies. The analysis of segregation distortions in F2s allowed mapping genetic incompatibilities. Furthermore we show that the recent introduction of both species into Europe was not accompanied by a significant reduction in within-species genetic diversity but has facilitated gene flow between species. Despite stronger introgression in Europe, the genomic landscape of differentiation remained highly similar in both native and introduced ranges, suggesting that the environmental transition did not affect the genomic architecture of gene flow between species. Besides, the location of highly differentiated genomic regions was partly explained by chromosomal variation in recombination rate. Finally, inferring the demographic divergence history of the two species revealed that gene flow has eroded past differentiation at different rates across the genome after a period of geographic isolation in the native range. Those results support the view that low-recombining regions help in maintaining genetic differences between species on secondary contact. Finally a panel of SNP markers was developed to distinguish *C. gigas* and *C. angulata* individuals.

Key Words: species divergence, genomic patterns, cupped oysters

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P4022 Genetic diversity of Mexican cattle Lidia breed and its relationships with Spanish populations through bovine SNP 50K BeadChip.

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The Lidia breed refers to a racial grouping of native bovines widely distributed throughout European countries such as France, Portugal and Spain and several American countries, where it takes part in different types of traditional, popular events. This breed is peculiar because it is one of the rare bovine population selected for behavioral traits from five centuries ago, having special management that lead the breed genetically isolated from the rest of domestic bovine breeds. Although there is a desirable pattern of behavior, diverse types of traditional events demanded different types of behavior, generating fragmentation of the racial group into small lineages. Genetic diversity in Spanish populations has been assessed identifying 28 lineages genetically differentiated. Moreover, Mexican populations have not been studied. The breed was brought to Mexico in 1522. Since then, the flow of animals from Spain declined for certain lineages according to preferences of the breeders, thus inducing fragmentation. Both high fragmentation and the reduced effective population size led us to suspect that Mexican populations suffer important losses of genetic variation. The goal of the current work was to assess genetic diversity of Mexican Lidia breed populations and its relationships with respect to Spanish lineages. With this aim, we genotyped 467 individuals: 348 belong to 28 already-classified Spanish lineages, and 119 corresponded to 20 Mexican herds. We used the Bovine 50K Illumina BeadChip and, after excluding individuals with missing genotypes (> 0.20), SNPs with a minimum allele frequency (MAF) < 0.01, markers that departed significantly from the Hardy-Weinberg equilibrium (HWE) ($P < 10^{-6}$) and restricting the level of linkage disequilibrium (LD) ($r^2 < 0.01$), 573 SNPs spanning all bovine autosomal chromosomes were selected. Expected heterozygosities ranged between 0.26–0.44, and there was significant

inbreeding, finding F_{is} higher values of 0.2 for both populations explained mainly by high genetic divergence between herds within lineages, and the lower value of 0–0.17, probably caused by Wahlund effect. High genetic differentiation between lineages within both populations was found with a mean of 0.096 in Mexican herds and 0.192 on Spanish lineages. F_{st} value of both populations was 0.048. Together, correspondence (GENETIX) and a clustering analysis (STRUCTURE) coincide in a clear separation between populations, excepting a few Mexican herds that may have recently introduced Spanish animals. In conclusion, our study could explain genetic diversity of Mexican population finding genetic differentiation from Spanish lineages. Fragmentation of Lidia breed populations explain the great genetic richness of the whole breed.

Key Words: bovine, genetic diversity, SNPs

P4023 Cloned horses: MtDNA heteroplasmy makes difficult the differentiation protocol. M. Costa, B. Elguero, C. Ratti, M. Martinez* (Laboratorio de Genética Aplicada, Sociedad Rural Argentina, Buenos Aires, Argentina)

Polo Argentino is a world-famous breed, with animals ranked according to their handicap in the sport of polo. In the last years, most valuable Argentine stallions and mares have been cloned in the United States or in Argentina. Although genetically identical, donor and cloned horses' performance can be remarkably different, making some clones much more valuable than others obtained from the same donor. Therefore, the Polo Breeder Association requests a protocol to difference cloned horses from the same donor and from the donor itself. The association was also looking ahead to differentiate offspring from cloned female horses. Since the nuclear DNA differentiation is not possible, mitochondrial DNA (mtDNA) has been used for identification purposes. Clones are produced by nuclear transfer from somatic cells into surrogate enucleated oocytes, the source of mitochondria for the new embryos. Equine mtDNA features a hypervariable sequence in the D-loop of the non-coding control region, showing nucleotide diversity among non-related animals. A fragment of 744 bp from this region was analyzed in 39 animals, including cases from one donor–one clone to one donor–eight clones. When no single difference was found in this sequence, an additional fragment was analyzed. While doing this analysis, we found evidence of mitochondrial heteroplasmy in some clone sequences, with bi-allelic polymorphism along the sequence. This finding was confirmed by re-sequencing of mtDNA from blood and hair of some cloned horses. In some cases, when the heteroplasmy was not evident in the first hypervariable sequence analyzed, it became clear when more mtDNA was sequenced. Extra mtDNA seems to come from the donor cell. Previous works have reported mtDNA heteroplasmy as a side-effect of the somatic nuclear cell transfer technology in sheep, pig and calves but not in horses. It has also been described in the heteroplasmy transmission to the next generation, thus challenging the offspring differentiation. Considering the cloning technique and the sequence information collected, heteroplasmy seems to be the rule and not the exception, suggesting that mtDNA analysis is not the right procedure for the unequivocal differentiation of cloned horses.

Key Words: cloned horse, heteroplasmy, mtDNA

P4024 Identification of TLR polymorphisms of the main cattle breeds in russia. K. Novák* (Institute of Animal Science, Prague, Czech Republic), M. I. Dunin (All Russian Research Institute of Animal Breeding, Lesnye Polyany, Russian Federation), A. E. Kalashnikov (L. K. Ernst Research Institute of Animal Husbandry, Dubrovitsy, Russian Federation)

The study of the genetic structure and variability of the Toll-like receptors (TLR) in cattle is aimed to detect polymorphisms, which are responsible for resistance of cattle breeds in Russia for important infections. On the other hand, it is important to know how structural changes of TLR in natural killers (NK) and other immune cells affects the detection of pathogens. The survey comprised Russian breeds Kholmogory — both pure Pechersky type and a hybrid with Holstein, Yaroslavl — both the pure type and a hybrid with Holstein, Simmental-milk type, Yakut, Yakut x Simmental crossbreed, Simmental-meat type, Kholmogory breed of Yakutian origin, Kholmogory x Yakut breed, Black pied and a bovine hybrid with wood bison in a total number of $N = 275$. The target region comprised coding sequences of *TLR1–TLR10* and was amplified in a series of 80 overlapping PCR reactions. The sequencing on PacBio platform provided reads from 400 to 1200 nt long with 3–12x coverage for individual animals. The groups of antibacterial (*TLR1, 2, 4, 5, 6*) and antiviral (*TLR3, 7, 8, 9, 10*) members of the TLR family were processed in separate pools. After primary data processing with the RS Touch and Dashboard software (Pacific Biosciences), an independently developed pipeline for processing long reads, which are characteristic for PacBio technology, was applied. The bioinformatic processing comprised primary data quality check with FastQC (PicardTools)

and filtering quality (Galaxy.org), assembly with Ugene and removal of duplicate with Picard tools, followed by variant calling using SAMtools, Free Bayers and filtration with VCFFilter. The annotation of the found polymorphisms was performed using VeIP. The detected polymorphism was assigned to individual animals with subsequent genotyping. The data on the TLR variant distribution among breeds is treated in the context of breed relatedness and history. The results are also foreseen to be used in further resistance breeding in modern cattle breeds.

Key Words: SNP, TLR, PacBio

P4025 Random forest based approaches identify breed-informative SNPs matching selection signature regions in the pig genome. G. Schiavo (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), F. Bertolini (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy; Department of Animal Science, Iowa State University, Ames, IA), G. Galimberti (Department of Statistical Sciences "Paolo Fortunati," University of Bologna, Bologna, Italy), D. G. Calò (Department of Statistical Sciences "Paolo Fortunati," University of Bologna, Bologna, Italy), D. Matassino (ConSDABI, Benevento, Italy), V. Russo (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), S. Dall'Olio (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), L. Nanni Costa (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), L. Fontanesi* (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy)

Genetic heterogenity present among breeds, populations or lines can be used to allocate animals and in turns their products to the groups they belong to or from which they are originated. Animal allocation in livestock can have several applications that include the definition of management and conservation practices for animal genetic resources, the estimation of admixture between populations, the implementation of appropriate breeding strategies and the possibility to obtain information useful for the authentication of mono-breed brand products. In this study we genotyped with the PorcineSNP60 BeadChip a total of 2691 animals from seven different Italian pig breeds, including three commercial breeds (Italian Large White, Italian Duroc and Italian Landrace) and four local breeds (Casertana, Apulo-Calabrese, Nero Siciliano and Cinta Senese). Several approaches were used to identify breed-informative single nucleotide polymorphisms (SNPs), including a pre-filtering step based on principal component analysis combined with delta (based on allele frequency differences among breeds) and Fst statistics, and followed by random forests computed using different ranking methods (mean decrease in the Gini Index and mean accuracy decrease). Combination of these approaches identified subsets of partially overlapping breed-informative SNPs. Error rates in breed assignment ranged from 0% to about 20%, depending on the methods and breeds, indicating also different levels of admixture between breeds. Many SNPs of these subsets were located in selective sweep regions identified using other approaches, suggesting that informative SNPs are associated with phenotypes or production traits that might differentiate the investigated breeds.

Key Words: breed allocation, random forest, selective sweep

P4026 Discrimination of native chicken breeds using SNP markers selected from the 600K chip data. N. R. Choi*, D. Seo, S. Jin, P. Manjula, S. H. Lee, J. H. Lee (Chungnam National University, Daejeon, Korea)

The Korean native chicken (KNC) is highly favored for the consumers due to their unique taste and meat quality, even though the price is almost two to three times higher than the commercial broilers. Since 1970, these native chicken lines have been maintained for line breeding purposes in the commercial chicken breeding company. To protect intellectual property for commercial breeding lines in Korean chicken industry, this study attempted to develop a traceability system using SNP markers. Using 600K SNP data on base population, highly informative 96 SNPs and 30 SNPs were selected to test breed discrimination analyses in this study. The first attempt was that six lines of native chickens were compared with White Leghorn (WLH), commercial broiler (CBC), and another commercial native chicken line called Woorimatdaq (WM) using 96 SNP markers that were initially selected from 600K SNP chip data. The results showed that 96 SNPs classify each breeds. Based on the further analysis, 30 SNP markers were finally selected. The results indicated that the selected 30 SNP markers can discriminate well between the six native chicken lines and control groups such as WHL, CBC and WM. With further validation study for efficacy of the selected markers in many different breeds, the selected 96 and 30 SNP markers will provide useful basic information for development of traceability system in KNC industry.

Key Words: breed discrimination, chicken, SNP marker

P4027 Systematic profiling of short tandem repeats in the cattle genome. L. Xu (Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD), R. Haasl (University of Wisconsin–Platteville, Platteville, WI), J. Sun (South China Agricultural University, Guangzhou, China), Y. Zhou (Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD), D. Bickhart (Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD), J. Li (Institute of Animal Science of Chinese Academy of Agricultural Sciences, Beijing, China, Beijing, China), J. Song (University of Maryland, Animal Science and Avian, College Park, MD), T. Sonstegard (Recombinetics Inc., St Paul, MN), C. P. VanTassell (Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD), H. Lewin (University of California, Davis, Department of Evolution and Ecology, David, CA), G. E. Liu* (Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD)

Short tandem repeats (STRs), or microsatellites (MS), are genetic variants with repetitive 2–6 base pair motifs in many genomes. Using high-throughput sequencing and experimental validations, we systematically profiled STRs in five Holsteins. We identified a total of 60,106 microsatellites and generated the first high-resolution STR map, representing a substantial pool of polymorphism in cattle. We observed significant STR overlaps with RefSeq genes and quantitative trait loci (QTL). We performed evolutionary and population genetic analyses using over 20,000 common dinucleotide STRs. Besides corroborating the well-established positive correlation between allele size and variance in allele size, these analyses also identified dozens of outlier STRs based on two anomalous relationships that counter expected characteristics of neutral evolution. And one STR locus overlaps with a significant region of a summary statistic designed to detect STR-related selection. Additionally, we showed that only 57.1% of STRs are located within SNP-based linkage disequilibrium (LD) blocks, while the other 42.9% are not. Therefore, a substantial number of STRs are not tagged by SNPs in the cattle genome, likely due to STR's distinct mutation mechanism and elevated polymorphism. This study provides the foundation for future STR-based studies of cattle genome evolution and selection.

Key Words: cattle genome, short tandem repeat (STR), whole genome sequencing (WGS)

P4028 Hematopoietic chimerism in Italian horses. C. Grasso* (UNIRELAB, Settimo Milanese, Italy), M. Bonuglia (UNIRELAB, Settimo Milanese, Italy), M. Dobosz (University of Perugia, Perugia, Italy), V. Chiofalo (UNIRELAB s.r.l., Settimo Milanese, Italy)

A chimera is an organism whose cells derive from two or more zygotes. This phenomenon has been detected in a wide variety of organisms, including mammalians. Chimeras can be phenotypically normal, so most of them have been discovered only by chance and the frequency of spontaneous chimerism might have been greatly underestimated. Cells with the "extra" genotype might be found in any part of the body, according to the mechanism of its development. We found several cases of chimeric horses during routine genotyping: They show more than two alleles for each locus, leading to a false incompatibility between the foal and its parents. Each of these subjects was derived from twins pregnancy, and we hypothesize that they could be hematopoietic chimeras, where an exchange of blood cells between the fetuses occurred in utero and these additional alleles derived from cells contributed by his twin sibling because of the establishment of vascular anastomoses between the developing placentas. This exchange occurs in utero, when the twins are immunologically tolerant and this condition of "mixed" blood" could take place. The number of extraneous cells in every twin can change and decrease over time. In some cases, depending on the moment in which anastomoses takes place, this number could exceed the number of cells of the original genotype. The presence of blood chimerism can lead to a false interpretation of trace analysis in criminal cases or in the investigation of kinship. In suspected cases, it is advisable to determine which profile represents the real one, typing biological samples derived from other body districts.

Key Words: horse, chimerism, STR

P4029 Launching SheepGenomesDB: 100 million variants from nearly 500 sheep genomes. J. Kijas* (CSIRO Agriculture, Brisbane, Australia), R. Brauning (AgResearch, Mosgiel, New Zealand), S. M. Clarke (AgResearch, Mosgiel, New Zealand), A. McCulloch (AgResearch Limited, Mosgiel, New Zealand), N. E. Cockett (Utah State University, Logan, UT), G. Saunders (EMBL-EBI, Hinxton, United Kingdom), M. Naval Sanchez (CSIRO Agriculture, Brisbane, Australia), S. McWilliam (CSIRO Agriculture, Brisbane, Australia),

H. Daetwyler (Department of Economic Development, Jobs, Transport and Resources, Bundoora, Australia)

On behalf of the ovine research community, this project has assembled publically available sheep genomes into a single analytical workflow for the purpose of variant detection. We report the application of a harmonized pipeline for raw read filtering, mapping and variant detection using almost 500 sheep genomes from nearly 50 breeds. This generated nearly 100 million unfiltered variants which have been filtered to high confidence. Summary statistics concerning the performance of two variant callers are provided (accuracy and overlap), along with allele frequency distributions. The data allowed assessment of genetic diversity levels present in sheep sampled from different geographic regions, free from the ascertainment bias inherent in previous, chip-based studies. This confirmed high rates of nucleotide diversity in sheep from the domestication center. To enable SNP annotation, visualization and options for interactive data download, this large variant collection has been entered into the European Variation Archive. We view this dataset as an essential resource for researchers performing gene mutation discovery, imputation and investigations into the consequences of animal breeding and selection.

Key Words: sheep, genomes, VCF, database

P4030 Diversity and linkage disequilibrium in farmed Tasmanian Atlantic salmon. J. Kijas* (CSIRO Agriculture, Brisbane, Australia), P. D. Kube (CSIRO, Hobart, Australia), B. Evans (SALTAS, Hobart, Australia), N. Botwright (CSIRO, Brisbane, Australia), H. King (CSIRO Agriculture, Hobart, Australia), C. Primmer (University of Turku, Turku, Finland), K. Verbyla (Data 61, Canberra, Australia)

Farmed Atlantic salmon (*Salmo salar*) is a globally important production species, including in Australia where breeding and selection has been in progress since the 1960s. As a precursor to genome-wide association and genomic prediction, this study collected genotypes at 218,132 SNP in 777 fish from a Tasmanian breeding population to assess levels of genetic diversity, the strength of linkage disequilibrium (LD) and imputation accuracy. Genetic diversity in Tasmanian Atlantic salmon was much lower than observed within European populations when compared using four diversity metrics. Further, the distribution of allele frequencies also showed a clear difference, with the Tasmanian animals carrying an excess of low minor allele frequency variants. The strength of observed LD was high at short distances (< 25 kb) and remained above background for marker pairs separated by large chromosomal distances (hundreds of kb), in sharp contrast to the European Atlantic salmon tested. Genotypes were used to evaluate the accuracy of imputation from low density (0.5 K to 5 K) up to increased density SNP sets (78 K). This revealed high imputation accuracies (0.89–0.97), suggesting use of low density SNP sets will be a successful approach for genomic prediction in this population. The long range LD, depressed levels of genetic diversity and high imputation accuracy in Tasmanian salmon is consistent with known aspects of their population history, which involved a small founding population and no subsequent introgression. The findings of this study represent an important first step toward the design of methods to apply genomics in this economically important population.

Key Words: salmon SNP

P4031 Ancient DNA analysis of the *MC1R* gene in wild boar specimens from Mesolithic Ertebølle sites. J. Tetens* (Institute of Animal Breeding and Husbandry, Kiel University, Kiel, Germany), D. Ellinghaus (Institute of Clinical Molecular Biology, Kiel University, University Hospital Schleswig-Holstein, Kiel, Germany), M. Nutsua (Institute of Clinical Molecular Biology, Kiel University, University Hospital Schleswig-Holstein, Kiel, Germany), G. Thaller (Institute of Animal Breeding and Husbandry, Kiel University, Kiel, Germany), A. Nebel (Institute of Clinical Molecular Biology, Kiel University, University Hospital Schleswig-Holstein, Kiel, Germany), B. Krause-Kyora (Institute of Clinical Molecular Biology, Kiel University, University Hospital Schleswig-Holstein, Kiel, Germany)

The analysis of ancient DNA (aDNA) from human or animal remains provides unique information about past genetic variation and often allows for the inference about population structures or migration scenarios. Furthermore, known genotype-phenotype relationships (e.g., coat color) can be exploited to reconstruct the appearance of early domesticated animals. The analysis of aDNA is, however, hampered by varying patterns of DNA damage and exogenous DNA contamination. In the current study, two wild boar specimens from Mesolithic Ertebølle sites in northern Germany (Neustadt and Grube Rosenhof) were analyzed. Both specimens were dated to approximately 4500–4000 BC. During this time, Neolithic agriculturalists migrated into northwestern Europe, bringing domesticated animals with them. It has recently been demonstrated that the Ertebølle hunter-gatherers had

ready access to domesticated pigs. The samples, a molar and a humerus, were processed in rooms dedicated to aDNA procedures, following established stringent protocols. DNA was extracted by a magnetic bead-based technology. The *MC1R* gene was enriched using array capture technology, and sequencing was performed on an Illumina HiSeq2500 instrument using HiSeq v3 chemistry. Libraries treated with uracil–DNA–glycosylase (UDG) to remove deaminated cytosine as well as untreated libraries were analyzed. Reads were mapped against the porcine genome assembly Sscrofa 10.2. With non-UDG libraries, 100% and 91% of the ORF were covered in the two samples with a mean depth of 10.9X and 2.9X, respectively. Typical damage patterns were observed. The analysis of the *MC1R* ORF revealed a pattern matching previously described European wild-type haplotypes for one of the samples. The results for the other sample were also consistent with a wild-type allele, but that animal was heterozygous for two variants that have previously been observed only in present-day Asian haplotypes. This finding indicates that the population structure of the suids in the Meso-/Neolithic and their domestication history might be more complex than inferred from modern pig data. In future studies, more ancient samples will be analyzed and further genes will be included.

Key Words: aDNA, pig, *MC1R*

P4032 Polymorphism of 10 microsatellite DNA used for parentage control in pigeons in Poland.

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The aim of this study was to test the polymorphism of 10 microsatellite markers and their usefulness for parentage verification in pigeons. Samples were collected from 69 individuals of Polish, German and Belgian breeds from different locations in Poland. The assay involved the following 10 loci: CliμD01, CliμD16, CliμD32, CliμT13, CliμT17, PG2, PG3, PG 5, PG 6, PG 7 and the bird sex marker CHD. We used genomic DNA extracted from feathers and buccal swab samples. DNA extracts were amplified by PCR for the all microsatellite and CHD marker together in one multiplex reaction. Each of the forward primers was labeled with fluorescent dye (6-Fam, Vic, Ned, Pet). Markers were amplified using the QIAGEN Multiplex PCR Kit, the amplified products were separated on a ABI PRISM® 3100xl Genetic Analyzer and genotyped using GeneMapper software (Applied Biosystems). In the study of 10 microsatellite markers we detected 60 alleles, which number per locus ranged from 2 (PG5) to 8 (CliμD01 and CliμT17). Based on the frequency of identified alleles expected heterozygosity (He) and observed (Ho) were determined as Ho = 0.88 and He = 0.78, respectively. The average negative inbreeding coefficients was Fis = -0.03. The lowest polymorphism in the present study was noted at the PG5 (PIC = 0.34 and H = 0.47) where two alleles identified and PG6 (PIC = 0.43 and H = 0.59) for which two of three alleles occurred with higher frequency in excess of 94%. The average power of discrimination (PD) was 0.83. The combined power of discrimination values for all 10 loci reached the high value as 0.9999999. The cumulative probabilities of parentage exclusion, when one parent is known, and two parents is known (PEc1 and PEc2) were 0.9726 and 0.9982, respectively.

Key Words: pigeon, microsatellite DNA, parentage control

P4033 Characterization and diversity analysis of European local pig breeds and production systems under Treasure project framework.

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Treasure is a multidisciplinary European Union H2020 funded project focused on the research and development of activities for the benefit of sustainable pork chains based on European local pig breeds and their production systems. One of the main objectives of the project is to demonstrate singularity of about 20 untapped local pig populations from nine European countries (Portugal, Spain, France, Italy, Slovenia,

Croatia, Serbia, Germany and Lithuania) through phenotypic, genomic and transcriptomic activities. Genomic approaches include the use of high density SNP data, candidate gene analyses and whole genome sequencing (WGS). Untapped breeds are first characterized at the production system level through a specific survey addressed to collect census data, breed distribution and phenotypic traits and information on breed organizations and production chains. Genomic data analyses bring the possibility for the estimation of different population genetics and population genomics indicators, such as relative homozygosity and observed and expected genetic diversity of the studied local pig breeds, as well as the definition of the structure of meta-populations through the proportion of shared alleles/haplotypes among animals and population admixture parameters. Comparative analyses including commercial pig populations in Europe identify genome regions with evidence of selective sweeps or signatures of demographic events across breeds. WGS analyses are focused on population adaptation and resilience signatures. To complete the local pig breed characterization, identification of population-specific biological processes responsible for specific production traits and product quality will be achieved through transcriptomic and metagenomic assays under specific production systems and management conditions. Together all these analyses are expected to provide useful methods and DNA markers for authentication and traceability of mono-breed products, conservation of local pig genetic resources and development of specific breeding programs for sustainable pork production chains in Europe. The Treasure project is funded under European Union's Horizon 2020 research and innovation program, Grant No. 634476

Key Words: Treasure, Europena local pig breeds, genomics, transcriptomics, metagenomics

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P4034 Milk protein polymorphisms in African indigenous cattle: Opportunity for Sustainable Breeding Program. I. Houaga* (Jomo Kenyatta University Of Agriculture And Technology, Juja-Kenya, Kenya; University of Abomey-Calavi, Abomey-Calavi, Benin)

Milk protein polymorphisms in cattle are important tools for genetic diversity studies, breed characterization, gene evolutionary studies with many applications in human nutrition and animal breeding. Unfortunately, few studies have focused on African indigenous cattle compared with Western dairy breeds. This paper summarizes the available information about the genetic polymorphism of major milk proteins in African indigenous cattle and discusses the opportunity to increase their milk production by implementing a sustainable breeding program using both quantitative and molecular approaches. Moreover, news proteins variants have been discovered in African indigenous cattle, but their effects on milk traits have never been investigated. This strongly suggests the necessity of genetic associative studies on major milk proteins polymorphism, which is deemed necessary for the implementation of rapid and effective genetic improvement program for African indigenous cattle adapted to local environment to avoid loss of genetic diversity.

Key Words: African indigenous cattle, polymorphisms, genetic diversity

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P4035 Exploiting Genomic Data of Spanish Atlantic salmon to identify genes involved in sex determination and to estimate effective population size. M. Saura* (INIA, Madrid, Spain), A. Chtioui (INIA, Madrid, Spain; Universidad Politécnica de Valencia, Valencia, Spain), A. I. Fernández (Departamento de Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain), P. Morán (Universidad de Vigo, Vigo, Spain), M. P. Kent (Center for Integrative Genetics (CIGENE), Department of Animal and Aquacultural Sciences (IHA), Norwegian University of Life Sciences (NMBU), Ås, Norway), B. Villanueva (INIA, Madrid, Spain)

Advances in salmon genomics in recent years have led to the development of high-density single nucleotide polymorphism (SNP) chips opening thus new opportunities for investigating the evolutionary history of populations. In particular, they can be used for detecting genes affecting life history traits and to infer ancestral and current population sizes. In this study, the 220K high-density Affymetrix SNP genotyping array (Aquagene/CIGENE) has been used for identifying genes involved in sex determination and to estimate effective population size in Spanish Atlantic salmon populations. Samples from six rivers covering all the distribution area of the species in Spain were genotyped. After quality control, 187 fish and more than 150,000 SNPs were available for the analyses. A total of 317 significant associations with sex determination were detected representing nine putative QTL regions on Ssa2, Ssa6, Ssa9, Ssa10, Ssa21 and Ssa22. Giving these results, powerful candidate genes to be responsible of the QTL effects are proposed: the *sdY* gene, which is the master male-specific sex-determining gene, maps within our QTL intervals on Ssa2, and the estrogen receptor gene, *esr1*, which is essential for sexual development and reproductive function in females, maps close to our QTL region

on Ssa6. Linkage disequilibrium (LD), measured by the squared correlation coefficient (r^2), was found to be relatively high between closely linked markers ($r^2 > 0.5$ at 5 kb), although it declined rapidly with increasing distance (90% decrease over the first 0.28 Mb). This LD pattern may facilitate the discrimination between independent but proximal QTL and causal genes and mutations. Estimates of effective population size from LD measures of the Spanish metapopulation ranged from about 800 individuals 50 generations ago (before the decrease in census size) to less than 100 at present. Also, our results indicate a restoration of these populations after supportive breeding programs were implemented at the beginning of 1990s. To our knowledge, this is the first study identifying genomic regions associated to sex determination in this species and investigating the magnitude of the effective population size from genomic data in this particular metapopulation, which suffers the most extreme conditions of the distribution range of the species in the world.

Key Words: Atlantic salmon, sex determination, effective population size

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P4036 Estimation of linkage disequilibrium and effective population size in Korean native chicken.
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The linkage disequilibrium (LD) is an important indicator for population genetic parameters such as inbreeding rate and effective population size. The extent of LD also provides information about historical events of population such as past effective population size (*Ne*), and it allows inferences on the genetic diversity of chicken lines. The objective of this study was to estimate the LD and *Ne* in Korean purebred native chicken (NR and NY) and commercial native chicken (CL1–CL8), which are maintained for commercial purposes by commercial company. In this study, eight types of commercial native chicken lines that are derived from two types of Korean purebred native chicken lines were used. As the results of LD analysis, commercial native chicken lines have lower LD than Korean purebred native chicken lines, and we can estimate that they have different ancestors in two of the populations. In addition, purebred native chickens could estimate higher inbreeding level than commercial native chickens, and commercial native chickens have higher effective population size compared with purebred native chickens. The population structure was depicted in a multidimensional scaled plot. In MDS plot, Korean purebred native chicken were positioned in close proximity to each other, far from the other eight commercial chicken breeds. These results can provide useful information for estimation of effective population size and LD structure between the markers in native chicken population. In conclusion, the differences in LD and *Ne* for each breeds reflect historical events and recent selection through the breeding program. The LD and *Ne* values would be useful for sustainable breeding program in commercial native chicken lines, and efforts are needed to maintain genetic diversity for sustainability.

Key Words: linkage disequilibrium, Korean native chicken, effective population size

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P4037 Around the tail of the Khmer cat.
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In Cambodia the local indigenous cat is appreciated by many. They can share flats in the capital city, where they are treated as pets, or in the rural houses of the countryside villages, where they have a functional role. Practically they always originate from stray cats that have conquered humans with their gentle and social behavior. There are different narratives that substantiate a more or less ancient and noble origin, while, based on anthropological observation, they are key to some traditional customs and worshipping. They are characterized by several peculiar features that occur with variable frequencies in the observed subjects. Since 2011, an attempt was made to select some subjects and breed them to have progenies who express all the desired characteristics. So far, this breeding program has reached the third generation, and the process for registering these populations as a breed has started in Italy (Khmer cat). Investigations on the morphology of the tail and on its inheritance has been performed. More than five fancy breeds are bred worldwide having in their standard the "short" tail: Manx, Japanese Bobtail, Kurilian Bobtail, American Bobtail and Pixie Bob. To characterize the Khmer, a three-generation familiar group (seven ancestors) of 73 Khmer cats segregating bobtails and other tail "abnormalities" was reconstructed. No litter size nor occurrences of abortions, newborn defects or deaths were available or reported; therefore, a lethal effect linked to the character was not assessable. The cats were categorized by palpation and X-ray analysis of the backbone and tail. As reported in Japanese bobtail, the transmission of the characteristic short tail was consistent with a dominant inheritance with variable

expression, ranging from anural through short-pom-pom and short-strait tail. Genetic and/or not genetic modifier factors were strongly suspected. Genetic profiling was performed for parentage control. X-ray showed tail abnormalities. Out of the familiar group, a large subset of cats was analyzed to genotype the known causative mutations of the feline bobtail. The data available so far exclude that Khmer share the same mutations described in Manx cats.

Key Words: *Felis catus*, Khmer breed, bobtail

P4038 Whole genome semiconductor based sequencing of farmed European sea bass (dicentrarchus labrax) using a DNA pooling approach identifies putative selection signatures in Mediterranean genetic stocks. C. Geraci* (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), F. Bertolini (Department of Animal Science, Iowa State University, Ames, IA), G. Schiavo (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), M. T. Sardina (Palermo University, Palermo, Italy), V. Chiofalo (Messina University, Messina, Italy), L. Fontanesi (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy)

European sea bass (*Dicentrarchus labrax*) is an important marine species for commercial and sport fisheries and aquaculture production that has increasing economic worldwide value. In this work, taking advantage from the draft reference genome assembly of the European sea bass, we performed a genome-wide single nucleotide polymorphism (SNP) discovery using the chip semiconductor-based next-generation-sequencing technology of the ion proton sequencer. The experimental design was based on two DNA pools constructed from randomly collected fishes, sampled from two different hatcheries located in two Italian islands of the Mediterranean sea. About 98% of the European sea-based genome was covered by mapping about 62 million of generated and filtered reads. A total of about 1.6 million SNPs were identified, spread all over the chromosomes with a Ti/Tv = 1.28 comparable to the Ti/Tv ratio already reported in Salmon species. A pilot signature of selection analysis was performed using an across-genome homozygosity evaluation on genomic windows of 50 kb, combining data from the two DNA pools and hatcheries. This approach identified 17 regions in 11 different chromosomes with high homozygosity indicating putative selection signatures. These regions included 31 annotated genes. Pathway gene analyses and gene ontology term enrichment suggested that selection signatures included genes involved in (1) ion transport and chloride channel functions, as a potential adaptation of the euryhaline regulating systems in farmed conditions, (2) amino acid metabolism, as potential adaptation to artificial feeding and (3) circadian clock and related neurological systems, suggesting a modification of circadian rhythms and behavioral traits that are considered forms of adaptation to faming conditions. This is the first study that reported genome-wide polymorphisms in a fish species obtained with ion proton. These data might help to understand the genetic mechanisms underlying the recent domestication processes that are shaping the European sea bass genome.

Key Words: aquaculture, selection sweep, domestication

P4039 Design of a polymorphic microsatellites set for domestic turkey (meleagris gallopavo) genetics characterization. A. Canales (University of Cordoba, Cordoba, Spain), A. M. Martinez* (University of Cordoba, Cordoba, Spain; Animal Breeding Consulting SL, Cordoba, Spain), V. Landi (University of Cordoba, Cordoba, Spain; Animal Breeding Consulting SL, Cordoba, Spain), P. Cervantes (Universidad Veracruzana, Veracruz, Mexico), J. V. Delgado (Departamento de Genética. Universidad de Córdoba, Cordoba, Spain), M. E. Camacho (IFAPA Centro Alameda del Obispo, Cordoba, Spain)

The domestic turkey (*Meleagris gallopavo*) evolved from wild ancestors who crossed the Bering Strait when Alaska was connected with Eurasia. The domestic turkey is nowadays characterized by a strong, intensive rearing system using only a few genetically improved lines, whereas local breeds are severely endangered. Local turkey populations can be found in several countries such as Mexico, Spain, Italy and several Islamic countries (Iran, Egypt and Nigeria). In Mexico, this species has a great socio-cultural role and can be found in towns and suburban areas where low production system characterized by poor sanitary practices and unbalanced diets are common. Generally, rural people of such countries use this production in traditional parties or special events such as Christmas, the New Year or birthdays. These genetic resources are not properly kneed, and the lack of scientific information could lead to the extinction of the local breeds of important example of genetic variability and environmental adaptation. Although there are several microsatellites markers designed for mapping or QTLs search purposes, there is no standard panel marker for genetic characterization and genetic diversity assessment. For this reason, the aim of this study is to design

a polymorphic set of microsatellites that can be used for population study with the future view to perform a world study of local turkey's populations. Thirty microsatellites were selected according to the availability polymorphism information, DNA sequences, chromosomal position completeness and quality of repeat motif: WT83, TUM20, MNT318, RHT024, MNT374, WT90, MNT331, MNT353, MNT288, MNT264, MNT258, MNT360 MNT379, MNT391, RHT0009, MNT247, MNT393, MNT266, MNT411, MNT274, MNT295, MNT348, MNT297, MNT386, MNT282, RHT0216, MNT321, MNT294, WT54 and MNT389. We used the M13-tailed primer method (Boutin-Ganache et al., 2001) to label amplicons for visualization on the capillary sequencer allowing allocation of all loci in a unique electrophoresis set using Applied Biosystem G5 dye set. The primer sequence was redesigned when possible to achieve an optimal annealing temperature and amplification product size compatibility. The loci were tested on 50 DNA samples from commercial and local turkey breeds. All markers were amplified in a total number of six multiplex and were polymorphic, showing a number of alleles ranging from three and 14.

Key Words: turkey, STRs, biodiversity

P4040 Computer analysis of genetic parentage: application in equine diversity maintenance in Brazil. A. Atomiyama* (LinkGen Biotecnologia, Sao Paulo, Brazil), M. S. Lauretto (Universidade São Paulo (USP)-Zona Leste, Sao Paulo, Brazil), F. Nakano (Instituto Butantan, Sao Paulo, Brazil), J. M. Stern (Instituto de Matemática e Estatística da USP, Sao Paulo, Brazil), D. Levy (Faculdade de Medicina da USP, Sao Paulo, Brazil), S. P. Bydlowski (Faculdade de Medicina da USP, Sao Paulo, Brazil), C. R. Bydlowski (LinkGen Biotecnologia, Sao Paulo, Brazil)

Genotypic STR characteristics of equine breeds in Brazil are not well described, preventing development of computational statistical tests suitable for determination of genetic parentage. With this information, the minimum number of most informative markers in Brazil could be calculated, helping decrease endogamy, maintaining the genetic variability. DNA was extracted from hair equine samples. Offsprings and both parents (LinkGen banking) were examined. For genetic equilibrium test and calculation of markers frequencies, only animals that were not parents of others were considered. This criterion was established to avoid statistical dependencies between animals. This filter resulted in 689 animals, distributed as follows: Arabian Horse (A): 178; Crioulo (C): 164; Brazilian Sport Horse (BH): 163; Quarter Horse (QH): 184. Fourteen dinucleotide ISAG markers were studied: ASB17, VHL20, HTG10, HTG4, AHT5, AHT4, HMS3, HMS6, HMS7, LEX3, LEX33, ASB2, ASB23 and HMS2. Twenty-two other tetra and pentanucleotide markers (called LINK01 to LINK22), present on Chrs 1 to 18, were also studied. A computer system for parentage examination was developed, adapting and extending the Bayesian Networks methodology. For genetic linkage analysis, markers whose descriptive level (*P*-value) was less than 0.05 and also markers containing only one allele were excluded. After this filtering, 16 markers remained. The question was: What is the probability of a tested individual, with certain genotype, to be the biological product of tested parents? Or P (T | A = a, B = b, i = i). P (A) P (B) P (T) P (F | A, B), P (I | M, T) are known. Thus, the probability of genetic linkage, given n markers, is given by: P (T = 1 | A = a, B = b, *R* = i) = P (t = 1) R/(1 + R). Of the 16 valid candidate markers, 10 were considered to make an optimum combination: ASB17, VHL20, AHT4, ASB23, LINK11, LINK12, LINK13, LINK15, LINK16 and LINK08. The following indicators were obtained: sensitivity: 0.93; specificity: 0.98; area under the ROC curve: 0.96. Regarding breeding markers, the combination of 14 was informative: ASB17, AHT4, ASB23, HMS2, LINK09, LINK10, LINK11, LINK12, LINK15, LINK16, LINK02, LINK05, LINK07 and LINK08. The estimated overall accuracy was 69%, with fluctuations (BH and QH: 63%, C: 69%; A: 80%). In conclusion, we described six new genetic markers with discrimination power for linkage calculation in Brazil. Ten new genetic markers were shown to contribute to breeding discrimination power.
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Key Words: equine markers, Brazil, breeding discrimination

P4041 Pooled whole-genome sequencing reveals molecular signatures of natural adaptive selection in Djallonke sheep of Ghana. M. Yaro* (Curtin University, Perth, Australia), K. A. Munyard (Curtin University, Perth, Australia), E. Morgan (Curtin University, Perth, Australia), R. J. Allcock (University of Western Australia, Perth, Australia), M. J. Stear (University of Glasgow, Glasgow, United Kingdom), D. M. Groth (Curtin University, Perth, Australia),

The Djallonke sheep is of high socioeconomic and cultural importance in at least 14 countries within the sub-Saharan region of Africa, mainly because it is well adapted to the harsh environmental conditions and major livestock disease within this region.

The larger-framed Sahelian sheep breed that cohabits large parts of the region is less adapted to all these challenges. The most prominent among these adaptive attributes is resistance to trypanosomiasis and helminthosis, two parasitic diseases, which collectively cost the worldwide animal production industry billions of dollars annually. Here we sequenced and pooled whole genomic DNA from five individuals each of Djallonke and Sahelian sheep breeds sampled from Ghana, at greater than 22-fold average coverage on an ion proton sequencer. A total of approximately 404 million (97%) and 343 million (97%) sequence reads from the Djallonke and Sahelian, respectively, were successfully mapped to the sheep reference genome OviAri3. Preliminary analysis of the sequenced data of Djallonke breed showed several potential selective sweeps, some of which are co-localized within genomic regions known to harbor genes that mediate immune response in sheep. To the best of our knowledge, this is the first-ever whole-genome sequencing of these two sheep breeds. Our ongoing analysis of the sequenced data will provide a valuable resource for elucidating the underlying mutations and mechanisms of the numerous adaptive traits of the Djallonke sheep. This knowledge will not only ensure the sustainable breeding and utilization of the Djallonke sheep but will also have a long-term implication for the food security and poverty alleviation of all the 14 countries in the sub-Saharan African region.

Key Words: whole genome sequencing, selective sweeps, sub-Saharan Africa

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P4042 Design of a polymorphic microsatellite set for domestic turkey (*meleagris gallopavo*) genetic characterization. A. Canales (University of Cordoba, Cordoba, Spain; Animal Breeding Consulting SL, Cordoba, Spain), A. M. Martinez* (University of Cordoba, Cordoba, Spain; Animal Breeding Consulting SL, Cordoba, Spain), V. Landi (University of Cordoba, Cordoba, Spain; Animal Breeding Consulting SL, Cordoba, Spain), P. Cervantes (Universidad Veracruzana, Veracruz, Mexico), J. V. Delgado (Departamento de Genética, Universidad de Córdoba, Cordoba, Spain), M. E. Camacho (IFAPA Centro Alameda del Obispo, Cordoba, Spain)

The ancestors of the domestic turkey (*Meleagris gallopavo*) evolved from an ancestor who crossed the Bering Strait when Alaska was connected with Eurasia. The domestic turkey is nowadays characterized by a strong intensive rearing system using only a few genetic improved lines, whereas local breeds are severely endangered. Local turkey populations can be found in several countries such as Mexico, Spain and Italy, and several Islamic countries (Iran, Egypt and Nigeria). In Mexico, this species has a great sociocultural role and can be found in towns and suburban areas where low production system characterized by poor sanitary practices and unbalanced diets are common. Generally, rural people of such countries use this production in traditional parties or special events such as Christmas, the New Year or birthdays. These genetic resources are not properly kneed, and the lack of scientific information could lead to the extinction of the local breeds of important example of genetic variability and environmental adaptation. Although there are several microsatellites markers designed for mapping or QTLs search purposes, there is no a standard panel marker for genetic characterization and genetic diversity assessment. For this reason, the aim of this study is to design a polymorphic set of microsatellites that can be used for population study with the future view to perform a world study of local turkey's populations. Thirty microsatellites were selected according to the availability polymorphism information, DNA sequences, chromosomal position completeness and quality of repeat motif: WT83, TUM20, MNT318, RHT024, MNT374, WT90, MNT331, MNT353, MNT288, MNT264, MNT258, MNT360 MNT379, MNT391, RHT0009, MNT247, MNT393, MNT266, MNT411, MNT274, MNT295, MNT348, MNT297, MNT386, MNT282, RHT0216, MNT321, MNT294, WT54 and MNT389. We used the M13-tailed primer method (Boutin-Ganache et al., 2001) to label amplicons for visualization on the capillary sequencer allowing allocate all loci in a unique electrophoresis set using Applied Biosystems G5 dye set. The primer sequence was redesigned when possible to achieve an optimal annealing temperature and amplification product size compatibility. The loci were tested on 50 DNA samples from commercial and local turkey breeds. All markers were amplified in a total number of six multiplex and were polymorphic, showing a number of alleles ranging from 3 to 14.

Key Words: STRs, biodiversity, local breeds

P4043 Construction of the SNP panel for hucul horse parentage control based on the openarray platform. A. Fornal,* (National Research Institute of Animal Production, Balice, Poland), A. Piestrzynska-Kajtoch (National Research Institute of Animal Production, Department of Animal Genomics and Molecular Biology, Balice n. Krakow, Poland), A. Radko (National Research Institute of Animal Production, Department of Animal Genomics and Molecular Biology, Balice n. Krakow, Poland)

SNP markers could be an effective tool for parentage testing of horses. Due to the cases of questionable parentage verification (single-marker exclusion), we decided to undertake the SNPs set verification on QuantStudio 12K Flex as an alternative and supporting method. One hundred twenty SNPs were designed and tested on Hucul horse (non-related representatives) for the construction of an alternative tool for parentage verification. Hucul horse is a regional and endangered horse breed with a small number of individuals. Therefore, the recommended parentage testing STRs set could be not adequate for some difficult issues. In the presented study we verified 67 SNPs selected from the single nucleotide polymorphism database (dbSNP) and the 53 JPN system. We used TaqMan allelic discrimination for the samples genotyping on OpenArray platform with QuantStudio 12K Flex. Microsatellite markers (including loci recommended in parentage testing of horses) were used for the SNPs comparison. STRs were detected in capillary electrophoresis by 3130xl Genetic Analyzer and typed. SNPs were selected to cover all chromosomes: 115 SNPs were located in autosomes and 5 SNPs in allosomes. Most of the selected and detected SNPs were polymorphic. The SNPs set seems promising and could be implemented as a supporting tool for parentage testing of Hucul horse.

Key Words: parentage testing, SNP, STR

P4044 Search for polymorphisms through next-generation sequencing of genes involved in reproductive development in Guzerat bulls.

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Reproductive development is regulated by genetic and environmental factors. Among reproductive characters, puberty is an important factor in cattle breeding, and strong differences exist between the age of arrival to puberty between and within breeds. Guzerat is one of the most important zebuine breeds raised in South American tropical regions for meat production, but unfortunately, it possess several reproductive disadvantages when compared with European breeds, including later age at puberty. The identification of genes and polymorphisms explaining the variation in this character could be useful in the early selection of precocious bulls, increasing the genetic progress in the improvement programs of this breed. The objective of this work was to detect polymorphisms in genes associated to sexual development in Guzerat bulls. To do this, 690 amplicons belonging to coding, promoter and regulating (5´UTR y 3´UTR) regions in 72 genes were sequenced in 96 males through next-generation sequencing technology using MiSeq sequencer (Illumina Inc.). First, raw data was filtered by sequence quality. Subsequently, sequences were aligned to the reference *Bos taurus* genome, and detection and genotyping of SNP and indels were performed. From the total polymorphisms detected in the approximately 300,000 bp sequenced, and after applying alignment quality filter and minimun allele frequency filter of 0.05, 798 SNPs (one every 375 bp) and 39 insertions and 35 deletions were obtained. The latter belong to one indel every approximately 4000 pb; however, these results should be studied deeply, as some of them could be artifacts. Using a program for predicting the effects of different types of variants, polymorphisms were classify by genomic location, type, functional class (50.4% missense, 1.5% nonsense and 48.1% silent) and impact on the future protein (high, low or moderate). Furthermore, some of them are novel SNPs, because they were not previously reported. A continuation of this work, the effects of the detected variants, will be studied in detail, and association of these polymorphisms with age at puberty estimated through scrotal circumference and sperm

motility in the studied breed will be analyzed. The results obtained will contribute to the understanding of genetic regulation of puberty.

Key Words: puberty, beef cattle, candidate gene, next-generation sequencing, polymorphisms

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P4045 Genetic characterization of three Korean native cattle breeds using the Bovine 640K Affymetrix Axiom Arrays. J. Kim,* Y. Kim, Y. Lee, A. Iqbal (Yeungnam University, Gyeongsan, South Korea)

Yellow, brindle and black Korean native cattle (Hanwoo) breeds have been raised in Korea, among which the yellow Hanwoo has been commercially selected for the needs of Korean beef consumers, while the breeding of the latter two breeds have been focused on much less. Three sets of samples were collected randomly, i.e., 250, 18 and 18 individuals for the respective breeds, and genotyped with the 648K Affymetrix Axiom arrays. After quality control tests (MAF > 0.05, H-W > 0.001, call rate > 0.9), the numbers of available SNPs were 401K (62%), 343K (53%), and 328K (51%), with average distances (standard deviation) between adjacent SNPs 7.0 (9.7), 8.2 (11.8), and 8.6 (12.3) kbp, respectively. Among the available SNPs, 1143, 2518 and 16,155 SNPs were found with allele frequency difference (> 0.5) between yellow-brindle, yellow-black and brindle-black Hanwoo breeds, respectively. Also, Linkage disequilibria (LD) were measured using r2 values (GOLD program). The average values were 0.28, 0.37 and 0.40 between SNPs within 1 kbp, and 0.21, 0.31 and 0.34 within 1–10 kbp distance for the yellow, brindle and black Hanwoos, indicating the greatest LD for the black and smallest LD for the yellow Hanwoo breeds. A set of 287 SNPs with great allele frequency differences between the three breeds were selected to test breed identification, for which Structure program (v 2.3.4) was applied such that 97%, 86% and 87% of the yellow, brindle and black Hanwoo individuals were correctly identified as their respective Korean native breeds.

Key Words: Hanwoo, breed identification, SNP chip

P4046 Development and evaluation of a set of 100 SNP markers for DNA typing in the domestic horse. H. Holl* (Etalon Inc., Menlo Park, CA), J. Vanhnasy[2] (Agena Bioscience, San Diego, CA), R. Everts (Agena Bioscience, San Diego, CA), D. Cook (Etalon Inc., Menlo Park, CA), S. Brooks (Etalon Inc., Menlo Park, CA), M. Carpenter (Etalon Inc., Menlo Park, CA), C. Bustamante (Etalon Inc., Menlo Park, CA), C. Lafayette (Etalon Inc., Menlo Park, CA),

Genetic markers are important resources for individual identification and parentage assessment. Short tandem repeats (STRs) have been the traditional DNA marker of choice in many species. However, technological advances have led to single nucleotide polymorphisms (SNPs) becoming an attractive alternative marker. SNPs can be highly multiplexed and automatically scored, which allows for easier standardization and sharing among different laboratories. The domestic horse currently uses STR based DNA typing. Although there is a community interest in the possibility of SNP testing in ISAG parentage labs, only one panel has been developed. The set of 53 SNPs was found to be as efficient as the STR panel for the thoroughbred breed, but it was not evaluated in other horses. We obtained a publicly available data set of 729 horses representing 32 diverse horse breeds genotyped on the equine SNP50 to select SNP markers for DNA typing. Minor allele frequencies were used to identify highly polymorphic markers shared by multiple breeds. The resulting markers were filtered by repeat content and proximity to other SNPs to generate a set of SNPs amenable to genotyping with multiple platforms. A proposed set of 100 SNPs was analyzed for DNA typing suitability. The overall MAF of the panel across all 32 breeds was 0.375 (range 0.293–0.417), with per-breed probability of identities (PI) ranging from 5.21×10^{-34} to 5.14×10^{-42}. If only one parent was available, exclusion probabilities (PE2) ranged from 0.9998 to 0.999995, although when both parents were available, all breeds had exclusion probabilities (PE1) > 0.9999999. A test set of 20 horses from the 2015 HCT was genotyped in two multiplexes using the MassArray platform. The developed panel includes one SNP marker for sex as an internal control. The overall genotyping rate of the 100 parentage markers was 97%. Non-exclusion probabilities within the HCT samples were higher for the SNP panel than for the StockMarks microsatellite parentage set (9.0×10^{-6} for one parent and 3.0×10^{-9} for two parents, versus 6.0×10^{-4} and 2.7×10^{-6}). Our developed marker set is both present on current generation SNP chips and can be highly multiplexed in standalone panels, and thus is a promising resource for SNP based DNA typing.

Key Words: horse, parentage, SNPs

P4047 Studies on genetic diversity and phylogenetic relationships of Korean native chicken using the microsatellite marker. J. H. SEO* (Genomic Informatics Center, HanKyong

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In this study, the genetic diversity and phylogenetic relationships of Korean native chickens from five different breeds, namely, Korean native chicken (NC), Leghorn (LH), Cornish (CS), Rhode Island Red (RIR) and Hanhyup commercial line, were studied by performing genotyping using 20 microsatellite (MS) markers. Among the 20 MS markers selected for the genotyping, the number of alleles ranged from five (ADL0268) to 20 (MCW0127). The observed heterozygosity (H_{obs}) ranged from 0.349 (GCT0016) to 0.683 (MCW0145). MCW0104 showed the highest expected heterozygosity (H_{exp}) and (polymorphic information content) PIC values of 0.878 and 0.866, respectively, whereas ROS0083 showed the lowest H_{exp} and PIC values of 0.742 and 0.708, respectively. Among these markers, estimation of fixation index for Fst, Fit, and Fis values ranged from 0.048 to 0.182, 0.200 to 0.551, and 0.111 to 0.442, respectively. The correspondence analysis showed close relationship among individuals belonging to the NC, CS and HH lines. The expected probabilities of identity values using 20 MS markers were calculated in random individuals (PI), random half-sibs ($PI_{half\text{-}sibs}$) and random sibs (Pi_{sibs}), and were estimated as 3.88×10^{-60}, 4.86×10^{-39}, and 1.38×10^{-12}, respectively. In conclusion, this study showed useful genetic diversity and phylogenetic relationship data that can be utilized for Korean native chicken breeding and development by the commercial chicken industry to meet consumer demands.

Key Words: Korean native chicken, microsatellite, polymorphism

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P4048 Comparison of three methods to discover copy number variants in Nellore and Angus cattle. Y. Xing* (Interdisciplinary Graduate Program in Genetics, Texas A&M University, College Station, TX), C. A. Gill (Department of Animal Science, Texas A&M University, College Station, TX)

Copy Number Variants (CNV) are insertions and deletions of 1 kb or larger in a genome that are present in a variable number of copies compared with a reference genome. Differential expression of genes, in part due to gene dosage effects, can affect phenotypic variation. Our objective was to discover CNV using whole genome sequences of the Nellore and Angus founders of our mapping population. Illumina paired-end 100 bp reads of seven Nellore (*Bos taurus indicus*) bulls and six Angus (*Bos taurus taurus*) cows (33–88x coverage) were aligned with BWA and GATK 3.2 to the UMD3.1 bovine assembly of a Hereford (*Bos taurus taurus*) cow. We compared CNV-seq, RAPTR-SV and BreakDancer for CNV discovery. CNV-seq identifies CNV based on differences in read depth after normalization for depth of coverage across the genome; one Angus cow was the reference individual for all pairwise comparisons. BreakDancer identifies CNV based on read pair information, and RAPTR-SV combines read pair and split read approaches for CNV discovery. CNV-seq and RAPTR-SV both detected insertions and deletions, whereas BreakDancer only detected deletions. Putative CNV regions (CNVR) were detected on all 29 autosomes and the X chromosome. CNV-seq was the most conservative detection method, and CNV counts per animal ranged from 141 to 1914. There were 492 CNVR found by all three applications. We used DAVID for CNVR gene ontology (GO) enrichment analysis and applied a within-category Benjamini-Hochberg correction to control the false discovery rate. Of the enriched GO terms in Nellore animals, 75% were enriched in Angus animals too. Enriched GO terms were olfactory transduction, phosphate metabolic process, MHC protein complex and nucleotide binding.

Key Words: Copy number variants, CNV-seq, gene ontology enrichment

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P4049 Molecular and genetic characterization of DGAT1 gene in Sudanese dairy cattle Kenana and Butana. S. A. M. A. A. Mohammed Ali (Alneelain University, Khartoum, Sudan)

The objectives of the current study were characterization of some Sudanese dairy cattle types Kenana and Butana (on farm and molecular characterization) and their dairy production systems, adopted management practices, breeding objectives and constraints of dairy development in Sudan. A structured questionnaire was administered to 101 Kenana and Butana owners randomly selected from homelands of both breeds. Personal interviews and repeated field visits were conducted. Kenana and Butana farmers adopted different management systems. The traditional nomadic system was prevalent in Kenana area (98%), while all Butana owners used a transhumant system (100%). Kenana and Butana cattle are kept in a mixed crop-livestock production system and livestock species kept by farmers comprise cattle, sheep and goats. Cattle are the dominant species, mainly used for draft power followed by milk production. The purpose of keeping

cattle in the study area was to generate income from the sale of milk which was the main source of income for herders in the Kenana area (100%) and up to 50% of Butana owners. However, surplus milk is sold at farm gate to middlemen at low prices, and animals are sold live in the village or nearest markets. The general appearance and body conformation were the criteria for selection of breeding bulls used by bot Kenana and Butana owners (72% and 80.4%, respectively). Disease prevalence was important in both production systems and almost all farmers in both areas reported incidences of diseases. Trypanosomosis was the main problem reported by Kenana herders (61.8%) while Butanan owners complained mainly of ticks. Veterinary services in the country at large have declined in recent years and in some areas have witnessed a degree of collapse. The two breeds were also characterized by genotyping the (*DGAT1*) gene. In both breeds, *DGAT1* Lysine variant (232K) is associated with high fat and protein content and high fat yield in other breeds was the most frequent allele. The frequencies of 232K allele were 96.3% and 84.6% in Kenana and Butana breeds, respectively. At the *DGAT1* promoter VNTR locus, four alleles containing four to seven repeats of the 18 bp motif were found in both breeds. Most frequent allele was VNTR allele 3 containing five repeats with a frequency of 60.4% and 57.5% in Kenana and Butana breeds, respectively. In conclusion, two examined Sudanese dairy cattle breeds do not differ in allele frequencies at the *DGAT1* locus.

Key Words: dairy cattle, DGAT1, Kenana, Butana

P4050 Inference of population structure of purebred dairy and beef cattle using high density genotype data. M. M. Kelleher (Irish Cattle Breeding Federation, Bandon, Co. Cork, Ireland), D. C. Purfield (Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland), F. Kearney, R. Evans (Irish Cattle Breeding Federation, Bandon, Co. Cork, Ireland), and D. P. Berry (Teagasc, Moorepark, Fermoy, Co. Cork, Ireland)

Genomic breed prediction may be of interest in commercial mating programs, especially within crossbreeding schemes. Verifying the genetic diversity and population structure between cattle breeds could be used to maximize the phenotypic production potential of offspring by exploiting the most diverse breed groups with good combining ability, while simultaneously benefiting from heterosis. The strong use of artificial selection to increase the frequency of favorable alleles at the loci affecting phenotypic performance (e.g., milk production), coupled with intense selection that featured heavily in some breeds, has resulted in reduced genetic diversity and has affected the extent of genomic homozygosity. In this study high-density genotypic data on a large number of individuals of different breeds was available to make inferences of population structure for the different breeds used in temperate cattle production systems. The objective of present study investigated the genetic diversity and population structure of the most predominant dairy and beef breeds used in Ireland. Illumina high-density genotypes (777,962 single nucleotide polymorphisms; SNPs) were available on 4623 purebred bulls from 9 breeds; Angus (n = 430), Belgian Blue (n = 298), Charolais (n = 893), Hereford (n = 327), Holstein-Friesian (n = 1261), Jersey (n = 75), Limousin (n = 943), Montbéliarde (n = 33), and Simmental (n = 363). Principal component analysis revealed that Angus, Hereford, and Jersey formed non-overlapping clusters, representing distinct populations. In contrast, overlapping clusters suggested geographical proximity of origin and genetic similarity between Limousin, Simmental, Charolais and Montbéliarde and to a lesser extent between Holstein, Friesian and Belgian Blue. The observed SNP heterozygosity averaged across all loci was 0.379. The Belgian Blue had the greatest mean observed heterozygosity (H_O = 0.389) between individuals within breed while the Holstein-Friesian and Jersey populations had the lowest mean heterozygosity (H_O = 0.370 and 0.376, respectively). The correlation between the genomic-based and pedigree-based inbreeding coefficients was weak (r = 0.171; $P < 0.001$). Mean genomic inbreeding estimates were greatest for Jersey (0.173) and least for Hereford (0.051). The pair-wise breed fixation index (F_{st}) ranged from 0.049 (Limousin and Charolais) to 0.165 (Hereford and Jersey). In conclusion, substantial genetic variation exists in breeds commercially used in Ireland. Thus custom-mating strategies would be successful in not only increasing the variation within breed, but across-breeds by maximizing the exploitation of heterosis in crossbreeding strategies.

Key Words: admixture, genotype, fixation index, phylogenetic

P4051 Genetic relationships between Iberian and Criollo horse breeds. J. L. Vega-Pla (Laboratorio de Investigacion Aplicada. Ministry of Defense, Cordoba, Spain), O. Cortés (Universidad Complutense de Madrid, Madrid, Spain), L. T. Gama (CIISA–Faculdade de Medicina Veterinaria. Universidade Tecnica de Lisboa, Lisboa, Portugal), J. Canon (Universidad Complutense, Madrid, Spain), M. C. Penedo (Veterinary Genetics Laboratory, School of Veterinary Medicine, UC

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Criollo horse populations, which essentially result from horses brought from the Iberian Peninsula over the period of colonization, are spread throughout America, and may have received influences from other horse breeds in more recent past. A total of 25 autosomal microsatellites was used to investigate the genetic diversity, population structure, breed relationships and possible genetic contributions in a broad representation of Criollo horses. The analyses included DNA samples of 2385 animals from 50 horse breeds, representing Criollo populations from 12 American countries (27 breeds), breeds from the Iberian Peninsula representing the Celtic and Iberian groups (19 breeds), one breed from France, one from Morocco, and two cosmopolitan horse breeds (Thoroughbred and Arabian). Observed and unbiased expected heterozygosities per breed ranged from 0.62 (Sorraia) to 0.82 (Cr. Colombiano) and from 0.64 (Sorraia) to 0.81 (Monchino), respectively, with slight differences among overall means for observed (0.74) and expected (0.75) heterozygosity. The amount of differentiation (FST) among the 50 populations studied was 0.071 (95% CI 0.066–0.075). Bayesian cluster analysis implemented by STRUCTURE showed, for k = 2, a subdivision of breeds with a well-known Thoroughbred influence. Setting k = 3 a new cluster further separated the Paso Fino Colombiano lineages. Iberian-type horse breeds were grouped in a new cluster for k = 4 while Criollo breeds (except the ones from the Unites States) and Celtic populations were further subdivided in two separate clusters when k = 5. In the Factorial Analysis of Correspondence the first and second axes (accounting for 9.7% and 8.3% of the total inertia, respectively) differentiated among the Thoroughbred breed, breeds from the USA, Paso Fino Colombiano lineages and the Celtic group of breeds. The majority of the Criollo populations were grouped together, in a position intermediate between breeds belonging to the Celtic and Iberian groups. In conclusion, the majority of the Criollo horse breeds analyzed in our study show clear signs of the influence of breeds originating from the Iberian Peninsula, even though some other breeds may have also been introduced in more recent years. Celtic-type horse populations from the Iberian Peninsula had the greatest genetic influence in the development of the Criollo horse populations, followed by Iberian-type horses. Globally, three genetic clusters are well differentiated among Criollo populations, corresponding to the majority of the USA breeds, Colombian Paso Fino lineages and the remainder Criollo breeds, with some degree of genetic substructure among them.

Key Words: horse biodiversity, genetic structure, microsatellites

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P4052 The Swine Leukocyte Antigen (SLA) nomenclature system of the International Society for Animal Genetics (ISAG) and the International Union of Immunological Societies (IUIS): Update 2016.

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The SLA system is among the most well characterized MHC systems in non-human animal species. The ISAG/IUIS-VIC SLA Nomenclature Committee was formed in 2002, with the primary objectives: 1) to validate newly identified SLA sequences according to the guidelines established for maintaining high quality standards of the accepted sequences; 2) to assign appropriate nomenclatures for new alleles as they are validated; and 3) to serve as a curator of the IPD-MHC SLA sequence database (http://www.ebi.ac.uk/ipd/mhc/sla/), which is the repository for all recognized SLA genes, their allelic sequences and haplotypes. The IPD-MHC Database is currently undergoing major revisions on its infrastructure and website, aiming to significantly improve the performance and allow for a simpler submission process and more frequent update. To date, there are 176 class Ia (SLA-1, SLA-2, SLA-3), 16 class Ib (SLA-6, SLA-7 and SLA-8), 5 class I-like (SLA-12) and 190 class II (DRA, DRB1, DQA, DQB1, DMA, DMB, DOA and DOB1) alleles officially designated. Additionally, there are 43 class I and 33 class II haplotypes designated at the allele level resolution. Recent evidence has suggested other loci in the SLA system, previously recognized as pseudogenes (e.g., SLA-9, SLA-11, DQB2 and DOB2), may be expressed at the transcript level for some haplotypes. Specifically, alignment of transcripts to the genomic sequence showed that SLA-11 appears to be a protein coding gene with alternative splicing. Its full length transcript

appears to encode a canonical class I-like protein with 8 exons while two other variants encode proteins either lacking exon 3 (α 2 domain) or exons 3 and 4 (α 2 and 3 domains). These data suggest that SLA-11 could be an expressed class Ib-like gene within the class Ia gene cluster. The committee will consider reclassifying SLA-11 as a putative functional gene as additional evidence accumulates. A systematic nomenclature for the genes, alleles and haplotypes of the swine MHC is critical to the research in swine genetic diversity, immunology, health, vaccinology, and organ or cell transplantation. Continuous efforts on characterizing SLA alleles and haplotypes and studying of their diversity in various pig populations will further our understanding of the architecture and polymorphism of the SLA system and their role in disease, vaccine and allo- or xeno-graft responses.

Key Words: alleles, haplotypes, swine leukocyte antigen nomenclature

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P4053 Prospects for whole genome sequencing of ancient Finnish cattle. M. B. Weldenegodguad (Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland; Natural Resources Institute Finland (Luke), Jokioinen, Finland), C. Der Sarkissian (Centre for GeoGenetics, University of Copenhagen, Copenhagen, Denmark), A. Bläuer (Natural Resources Institute Finland (Luke), Jokioinen, Finland; Department of Archeology, University of Turku, Turku, Finland), K. Pokharel (Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland; Natural Resources Institute Finland (Luke), Jokioinen, Finland), J. P. Taavitsainen (Department of Archeology, University of Turku, Turku, Finland), L. Orlando (Centre for GeoGenetics, University of Copenhagen, Copenhagen, Denmark), and J. Kantanen (Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland; Natural Resources Institute Finland (Luke), Jokioinen, Finland)

Genetic diversity and population structure of prehistorical and historical cattle populations have been typically investigated by analyzing partial D-loop of mitochondrial DNA and Y-chromosomal DNA-markers. However, autosomal nuclear markers would offer more detailed information on ancient animals than uniparentally inherited markers. By comparing ancient animals with modern animals one could also investigate temporal changes in genetic diversity occurred through different time periods. We have applied the next generation sequencing (NGS) technology to decipher for the first time genome sequences of ancient cattle specimens in Finland. Four ancient cattle bones from three different excavation sites were selected for the sequencing study. We successfully extracted the DNA and built indexed Illumina libraries from ancient DNA extracts from 2 specimens: a sample of Late Iron Age Western Finland (coded as 'Mulli-2'; approximately 1200 y old) and that of Medieval Eastern Finland ('Viipuri-4'; approximately 500 y old). Shallow sequencing revealed minimal proportions of reads aligning uniquely against the cattle reference genome, providing $\leq$ 0.1X genome coverage. Read alignments showed typical DNA damage signatures, consisting of an excess of "C→T and G→A transitions" toward read termini. This pleads in favor of the sequence authenticity. Further sequencing aimed at recovering the whole genome sequence is ongoing, but the data already available, especially at sites overlapping the OMIA database, revealed for example the presence of disease-related allele in ancient Finnish cattle. As this is absent in contemporary breeds, this suggests possible recent genetic changes in the Finnish livestock.

Key Words: ancient DNA, Finnish cattle, genetic diversity

P4054 Resolving misassembled cattle immune gene clusters with hierarchical, long read sequencing. D. Bickhart (Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD), J. A. Hammond (The Pirbright Institute, Guildford, United Kingdom), J. C. Schwartz (The Pirbright Institute, Woking, United Kingdom), D. Harrison (The Pirbright Insitute, Woking, United Kingdom), and T. P. L. Smith (USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE)

Animal health is a critical component of productivity; however, current genomic selection genotyping tools have a paucity of genetic markers within key immune gene clusters (IGC) involved in the cattle innate and adaptive immune systems. With diseases such as Bovine Tuberculosis and Johne's disease costing the UK and US industries an annual £50 million and $200 million, respectively, identifying genetic markers associated with disease resistance will greatly assist producers. The high genetic diversity and highly repetitive nature of IGCs also means that the cattle reference genome assembly contains many mistakes or greatly underrepresents the true diversity of alleles in these clusters. To properly identify and annotate the breadth of IGC alleles, we use a hierarchical assembly approach that sequences bacterial artificial chromosome (BAC) library clones that span target sites with long read sequencing. The sequencing of

46 such BACs has already identified an alternative allele for the natural killer cell (NKC) cluster that is currently not represented on the cattle reference genome. In total, replacement NKC sequence fills 10 existing sequence gaps on the genome and removes an improperly assigned contig containing olfactory receptor genes. Further assembly polishing using this approach will finally enable the interrogation of functional variants within IGC regions, thereby enabling future genomic selection of animal health traits.

Key Words: cattle genome, assembly, immune genes

P4055 Assessing the genomic status of South African mutton, pelt and dual purpose sheep breeds using genome-wide single nucleotide genotypes. E. F. Dzomba (University of KwaZulu-Natal, Pietermaritzburg, South Africa), M. A. Snyman (Grootfontein Agriculture Development Institute, Middelburg, South Africa), M. Chimonyo (University of KwaZulu-Natal, Pietermaritzburg, South Africa), and F. C. Muchadeyi (Agricultural Research Council-Biotechnology Platform, Pretoria, South Africa)

South Africa has a vibrant sheep industry which contributes significantly to livestock gross domestic product through meat, wool and pelt production. Several industrial breeds are reared mainly on commercial farms with extensively raised breeds found mainly in the smallholder areas. Most breeds have been developed for adaptive and functional traits leading to a diverse array of phenotypically-distinct breeds. To gain an insight into the genome diversity of the various sheep breeds, we undertook a study to assess their breed history and population genetic structure. The study used the Illumina OvineSNP50 BeadChip to Genotype 376 animals belonging to 10 breeds representing mutton (23 Dorper, 8 Blackhead Persian, 48 Meatmaster, 30 Nguni, 10 South African Mutton Merino and 4 Namaqua Afrikaner animals), pelt (96 Swakara animals) and mutton and wool dual purpose (56 South African Merino, 50 Dohne Merino and 51 Afrino animals) breeds. Nguni sheep samples obtained from Makhathini Research Station (KwaZulu-Natal, South Africa) were included as a representative of indigenous breeds that are reared in smallholder farming areas while the Swakara, sampled from private farms in Namibia and were partitioned into four groups based on their coat color and the sub-vital genetic disorder phenotypes. The rest of the sheep samples were obtained from the biorepository at Grootfontein Agricultural Development Institute (GADI) in Middelburg, South Africa where tissue samples of the major sheep breeds are kept for biodiversity research and conservation. Across breeds, genetic diversity ranged from $H_E = 0.621$ (Dohne Merino) to $H_E = 0.742$ (Namaqua Afrikaner) with an overall mean of 0.633. Namaqua Afrikaner ($F = 0.330$) and Nguni and Blackhead Persian ($F = 0.274$) were the most inbred breeds with Dohne Merino ($F = 0.0162$), SA Merino ($F = 0.0570$) and Afrino ($F = 0.0667$) the least. The first principal component grouped the Merinos, Swakara, and the other breeds into separate clusters. The second principal component explained approximately 80.55% of the total variation and clustered the breeds according to their function and historical origin, splitting the different Merino specialized breeds and distinguishing the Nguni, Namaqua Afrikaner, Blackhead Persian, Afrino and Dorper breeds. The optimal cluster $K = 9$ for ADMIXTURE revealed various sources of within and among breeds genomic variation associated with purpose, adaptation and history of the breeds. These results are useful in understanding the current status of the sheep genetic resources of South Africa.

Key Words: sheep breeds, SNP genotypes, diversity, population structure, South Africa

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P4056 Evaluation of single nucleotide polymorphism (SNP) markers for canine parentage analysis. J. Qiu (GeneSeek, a Neogen Company, Lincoln, NE), B. Simpson (GeneSeek, a Neogen Company, Lincoln, NE), L. Kock (GeneSeek, a Neogen Company, Lincoln, NE), J. Donner (Genoscoper Laboratories, Helsinki, Finland), C. Cole (Mars Veterinary, Portland, OR), S. Davison (Mars Veterinary, Portland, OR), M. Dunn (American Kennel Club, Raleigh, NC), D. Bannasch (School of Veterinary Medicine, Univ. California Davis, Davis, CA), and A. Boyko (Cornell University, Ithaca, NY)

Microsatellite markers (a.k.a. short tandem repeats, STRs) have traditionally been used to determine pedigree or parentage in canines. However, some parentage issues involving closely related sires can be difficult to resolve using current STRs due to limited allelic variations. The use of SNP markers has been shown to be very effective in resolving parentage issues in other species such as cattle and has been officially adopted by the ISAG/ICAR. Recently, a number of genome mapping studies have identified hundreds of informative SNPs that could be potentially used for canine parentage analysis. The objectives of this study were 1) to evaluate minor allele frequencies (MAF) of a few hundred selected canine SNPs for major dog breeds, 2) to design multiplex SNP panel(s) using a subset of these SNPs for evaluating their effectiveness

in parentage analysis using well-defined trios, and 3) to determine the concordance of parentage results obtained by both STR and SNP independently. MAF of candidate SNPs were determined from nearly 6000 canine samples from over 112 breeds genotyped on Illumina-Infinium® based CanineHD chips. Some of the most informative SNPs (based on the MAF) were selected to develop multiplex panels using Agena's MassARRAY® technology. Comparative parentage analysis of ~180 trio families was performed using STR (either the ISAG or the American Kennel Club recommended microsatellite marker panels) and the SNP panels. Our preliminary results indicate an average MAF across 238 select SNPs ranging from 0.28 to 0.50 for the panel of 112 dog breeds examined. These SNPs can be effectively multiplexed in 2–3 panels that allow equal or better determination of canine parentage compared to traditional STR markers. These results provide valuable genetic data to support future adoption of using SNPs as a standard method for dog parentage analysis worldwide.

Key Words: SNP, STR, canine

P4057 Characterization of MITF coding region in llamas. M. Anello, M. Silbestro (Instituto Multidisciplinario de Biología Celular (IMBICE)-CIC-CONICET-UNLP, La Plata, Argentina), F. Veiga, V. Trasorras (Facultad de ciencias veterinarias, Universidad de Buenos Aires, Buenos Aires, Argentina), L. Vidal Rioja, and F. Di Rocco (Instituto Multidisciplinario de Biología Celular (IMBICE)-CIC-CONICET-UNLP, La Plata, Argentina)

The llama (*Lama glama*) is a fiber producer species that presents a wide variety of coat colors, among which white is one of the most valued. Microphthalmia-associated transcription factor (MITF) regulates the differentiation and development of melanocytes and is responsible for pigment cell-specific transcription of the melanogenesis enzyme genes. MITF consists of at least five isoforms with first specific exons, but only MITF-M is melanocyte-specific. Mutations in this isoform have been associated with white and white spotting phenotypes in many species of mammals. Previous studies of our laboratory excluded mutations in KIT coding region as responsible for these phenotypes. The aim of the present study is to describe and characterize the complete coding region of MITF-M and to detect mutations that could be associated with withe and white spotting phenotypes. For this purpose, fiber samples and skin biopsies from animals with different coat color phenotypes were collected. Then, RNA was extracted and total cDNA was obtained. cDNA was used as template for PCR reactions that fully covered the coding region and flanquing 5´and 3´UTR. We sequenced the MITF-M complete coding region of three white, two white spotting and five colored llamas. Two distinct isoforms were found in both colored and white/white spotted animals. MITF-M (−) which consists of a coding region of 1242 bp, corresponding to 414 amino acids, and MITF-M (+) with an insertion of 18 pb in nucleotides 564–582. Only four mutations were observed: two SNPs that represent synonymous mutations and two non-synonymous SNPs (c.110 C > A and c.575 C > T). Mutation c.575 C > T was observed in both, colored and white phenotypes; therefore it is unlikely to be coat-color-associated. On the contrary, mutation c.110 C > A introduces a Tyrosine instead of a Serine in a highly conserved position. Since allele A was only observed in a white animal and was not found in colored llamas, this SNP deserves a more thorough study. However, it appears that mutations in the MITF coding region are not mainly responsible for white/white spotting phenotypes. Regulatory mutations affecting expression or different genes could explain these phenotypes in llamas.

Key Words: MITF, llama, coat color

P4058 Fecundity genes polymorphism in indigenous sheep of eastern Ethiopia. H. Nigussie (Ambo University, Ambo, Ethiopia), M. Agaba (Arusha, Tanzania), Y. Mekasha (International Livestock Research Institute, Addis Ababa, Ethiopia), and S. Abegaz (Ethiopian Institutes of Agricultural Research, Debre Zeit, Ethiopia)

In Eastern Ethiopia, indigenous sheep contribute significantly to the livelihood of most pastoralist, agro-pastoralists and smallholder farmers. The increasing demand for mutton both in the domestic and export market outstrip the current productivity of sheep. Increasing prolificacy could be an option for improving reproduction rate and production efficiency. Fecundity gene polymorphism in eastern Ethiopia indigenous sheep has not been previously performed; therefore, three breeds (Afar, Black Head Somali and Hararghe Highland) were genotyped at 5 microsatellite markers (BM1329, BMS2508, OarAE101 and TGLA68 and TGLA54) linked with Booroola gene (FecB) and Bone morphogenetic protein 15 (BMP15) X linked gene (FecX gene), respectively. A total of 300 individual sheep were tested to assess fecundity gene polymorphism in the three breeds of sheep from ten different locations and three production system (Mixed crop-livestock, agro-pastoral and pastoral). Effects of breed type and production

systems on heterozygosity of the gene were also assessed using the GLM procedure of SAS. The polymorphic information content (PIC) values ranged from 0.43 (for marker TGLA68) to 0.76 (marker BMS2580) with an average value of 0.54, showing that the microsatellite panel used was polymorphic. The overall observed and expected heterozygosity values were 0.46 and 0.54, respectively. Higher heterozygosity (He = 0.76) was observed in the BMS2508 marker whereas lower heterozygosity(He = 0.43) was observed in the TGLA54 marker. Sheep managed under mixed crop-livestock system showed significant heterozygosity ($P < 0.05$) at BM1329, TGLA68 and OarAE101 loci compared to those sheep breeds managed under pastoral and agro-pastoral production systems. The current result indicated that fecundity gene polymorphism has a positive relationship with the production systems where the sheep were managed. However, further study will be required to substantiate the presence fecundity genes and associated factors in the three indigenous sheep breeds. The fecundity gene polymorphism found in the current study would be used as baseline information for further study in the relationship of reproductive trait and fecundity gene in indigenous sheep of eastern Ethiopia.

Key Words: Booroola FecB, FecX gene and microsatellite marker

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P4059 Origins and genetic structure of Creole cattle inferred from Y-chromosomal variation.

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American Creole cattle originated from animals brought from the Iberian Peninsula in the early years of discovery and colonization (15th century), with additional influences of cattle from other regions over the centuries. The paternal lineages of Creole cattle were investigated by using a comprehensive sample of American Creole populations and a broad representation of the breeds that may have influenced them. Y-chromosome markers are useful to detect recent demographic events, such as founder effects, population expansions and bottlenecks. The study included DNA samples from a total of 1758 males belonging to 95 breeds representative of the following geographic groups: Creole ($N = 31$ breeds), Iberian ($N = 32$), Continental European ($N = 6$), British ($N = 6$), Atlantic and Balearic Islands ($N = 5$), African ($N = 10$), and Indicine cattle ($N = 5$). Genetic polymorphisms located on the non-recombining region of the Y-chromosome were genotyped, including five STRs (*DDX3Y1*, *BM861*, *INRA189*, *UMN0103* and UMN0307), two *indels* (*ZFY10*, *USP9Y*) and one SNP (*UTY19*). Multiplex PCR was done using the QIAGEN multiplex PCR kit, and the fragments analyzed on ABI 3730 instruments (Applied Biosystems). A negative and a female DNA controls were included in all assays. Allele sizes were standardized via known fixed alleles from several samples or breeds to match published data. SNP and STR alleles were combined into haplotypes. GENALEX v6.5 was used in Excel to determine distributions of haplotype frequencies and diversities. Median-joining (MJ) networks were constructed to investigate phylogenetic relationships among haplotypes with NETWORK v4.2.0.1. The markers allowed to identify major Y-haplogroups among the 1758 bulls analyzed, such as Y1 (587) and Y2 (824) which are known to be predominant in Northern and Southern European cattle, respectively, and the Y3-lineage (347) of Indicine cattle. Y-STRs allowed to detect the genetic diversity of paternal lineages within major haplogroups, *i.e.* at the breed level, with a total of 58 haplotypes (Y1 = 12; Y2 = 35; and Y3 = 11) detected. African cattle contained unique paternal lineages, with 13 and four exclusive Y2 and Y3 haplotypes, respectively. Y-haplotype diversity in Creoles was high, with several Y1 (7), Y2 (9) and Y3 (7) haplotypes represented. The sharing of specific patrilines corroborates influence of Iberian (two Y1 and one Y2 haplotypes) and African (one Y2 haplotype) cattle in American Creoles, even though the major influence was from Indicine haplotypes.

Key Words: American Creole cattle, genetic diversity, Y-chromosome

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P4060 Runs of homozygosity reveal natural selection footprints of some African chicken breeds and village ecotypes.

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University, Ames, IA), C. M. Ashwell (Department of Poultry Science, North Carolina State University, Raleigh, NC), C. J. Schmidt (Department of Animal and Food Sciences, University of Delaware, Newark, DE), S. J. Lamont, and M. F. Rothschild (Department of Animal Science, Iowa State University, Ames, IA)

The earliest evidence of domestic chicken (*Gallus gallus*) introduction into Africa was in Egypt in the New Kingdom, 19th Dynasty (1307–1196 BC) through the ancient cinnamon trade. Chickens were introduced in Eastern Africa (EA) later via Egypt or direct introduction via Indian Ocean trading. With the absence of genetic improvement schemes and breeding associations, EA rural chicken populations are likely under natural selection, and most are considered as ecotypes, not breeds. A few populations, e.g., Egyptian Fayoumi have fixed criteria and are registered as a breed. The current study aims to assess probable co-ancestry among six African chicken populations, in comparison with a highly inbred US Fayoumi line, to investigate the inter-population genomic variation due to natural selective pressure and to determine functional variants associated with Runs of Homozygosity (ROH). The populations studied included three indigenous African ecotypes from Uganda (UGN), Rwanda (RWN) and Egyptian naked-neck (ENN); two Egyptian registered breeds; Fayoumi (FEG) and Dandarawi (DEG), one hybrid developed in India and sampled in Uganda, Kuroiler (KRL), and a highly inbred US Fayoumi (FUS). A total of 290 birds were randomly sampled from villagers in Egypt, Rwanda, and Uganda, representing indigenous populations, and 6 birds were sampled from the inbred FUS flock. Birds were genotyped using the Affymetrix 600K Axiom® Genome-Wide Chicken Genotyping Array. ROH were defined using PLINK v1.9, for a minimum ROH length of 500kb, with no more than one missing SNP and one heterozygous SNP genotype per window. A total number of 15,277 ROH were detected across the studied populations. ROH length ranged between 500kb to around 50Mb. ROH were classified into three length categories; short (500kb to < 1Mb, $n = 9970$; 65.3%); medium (1Mb to < 5Mb, $n = 4960$; 32.5%) and long ($\geq$ 5Mb, $n = 347$; 2.3%). The average number of ROH per bird was highest for the inbred FUS (240.7), and the least for the DAN (19.9) indicating outbreeding. Intra-breed regions with a high occurrence of ROH were identified on several chromosomes indicating ROH landscaping, where several genes involved in survivability and tolerance (i.e., reproduction, metabolism, muscle formation, ion-exchange, and heat and oxidative stresses tolerance) were detected. This study illustrates the distribution of ROH and functional variants within ROH in some African breeds and ecotypes. Detecting genomic regions involved in traits under natural selection contributes to our understanding of regions of importance for selection and distribution of functional variants in the chicken genome.

Key Words: runs of homozygosity, natural selection, African chicken

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P4061 The ramification of meiotic recombination differences in sheep. K. M. Davenport and B. M. Murdoch (University of Idaho, Moscow, ID)

The production of viable gametes is an integral part of reproduction and therefore a critical aspect for the sustainability of the livestock industry. Homologous recombination or crossovers (CO) contribute to genetic variation and ensures proper chromosome segregation. In virtually all organisms studied thus far, it is clear that at least one CO per chromosome arm is necessary to avoid mis-segregation. Furthermore, the locations of CO are not random, exhibiting some preferences (called hotspots) and with the presence of one CO "interfering" with the proximity of a second. Importantly, failure or improper placements of recombination represent a significant contribution to fetal loss and infertility. Despite the importance of these issues, we know very little about meiotic recombination rates in livestock species. Although global recombination rates are known to differ between strains of mice, this has not been evaluated in livestock breeds. In this study we characterized and quantified the number of recombination events in males from different breeds of sheep. Characterizing recombination differences between breeds of sheep will greatly enhance breed specific genetic predictions. Testicular tissue samples were taken from mature rams of different breeds and spermatocytes were spread and fixed on slides. Immunofluorescent staining was used to identify the synaptonemal complexes (SYCP3) and CO events (MLH1) of pachytene stage prophase cells. The total number of CO per meiocyte and their locations on the chromosomes were quantified. Our data suggests that global recombination rates are 10% higher in Targhee than in Suffolk rams. Despite having a similar number of chromosome arms and genome size, the number of recombination events in sheep spermatocytes are approximately 20% higher than in cattle. This research provides important information regarding recombination rates in sheep spermatocytes and has a direct impact on genetic breed predictions. Moreover, this research contributes valuable information toward a greater understanding of the factors that control meiotic recombination to enhance reproduction, improve genetic predictions, and advance selection strategies

toward the sustainability of the livestock industry.

Key Words: recombination, selection, variation

P4062 DNA sequencing and genetic polymorphism discovery in the canine monoamine oxidase A (MAOA) gene. J. Sacco, A. Ruplin, P. Skonieczny, and M. Ohman (Drake University, Des Moines, IA)

Monoamine oxidase type A (MAOA) is an enzyme that degrades neurotransmitters. In humans, reduced activity of the MAOA enzyme due to genetic polymorphisms within the *MAOA* gene leads to increased neurotransmitter levels in the brain which may result in aggressive behavior. Our overall hypothesis is that, in dogs, aggression, a common behavioral problem, is influenced by variation within the canine *MAOA* gene. Therefore, the aim of this preliminary study was to identify novel alleles in functionally important regions of the canine *MAOA* gene, located on chromosome X. Genomic DNA was collected via cheek swabs from 50 non-aggressive pure-bred dogs (22 females, 28 males) representing diverse genetic clusters (ancient, herding, mastiffs, modern European, mountain). Following DNA purification, target regions of the canine *MAOA* gene were amplified, sequenced, and screened for polymorphisms. All genetic polymorphisms that were found have not been reported previously. Seven were single nucleotide polymorphisms (SNPs; two exonic, two intronic and three in the promoter) and four were repeat intronic variations. Two synonymous coding SNPs, c.1254 C > T and c.1567 C > T, were discovered in exons 12 and 15, respectively. Two highly heterozygous microsatellites (ATTT and TTTA repeats) were found in introns 1 and 10, respectively. The microsatellite region in intron 10 was represented by three alleles, representing variable numbers of TTTA repeats (10, 11, or 12). The polymorphism in intron 1 was an ATTT sequence inserted within a short interspersed nuclear element (SINE) that is unique to canids. Haplotype analysis indicated strong evidence of recombination between several alleles. Comparative genomic analysis demonstrated that a proximal promoter SNP (−212A > G), which was found in only two of the dogs sequenced, is the major allele in wolves and other related mammalian species not subject to domestication. This −212G allele is predicted to alter the binding of several transcription factors in the MAOA promoter. These novel MAOA polymorphisms provide information for follow-up behavioral genetic studies in aggressive dogs.

Key Words: canine, MAOA, aggression

P4063 Genotyping of ApaLI RFLP at heat shock transcription factor 1 in Creole and Holstein cattle differing in coat type and associations with molecular breeding value and DHI traits. Y. R. Velez (University of Puerto Rico, Mayagüez Campus, Mayagüez, Puerto Rico), J. Patino, E. Soto-Moreno, B. Velez (University of Puerto Rico, Mayaguez, Puerto Rico), T. S. Sonstegard (USDA, ARS, BFGL, Beltsville, MD), and M. Pagán-Morales (Department of Animal Science, University of Puerto Rico, Mayaguez Campus, Mayaguez, Puerto Rico)

Heat stress in dairy cattle is one of the major problems that contribute to farmer's economical losses in tropical climates. Dairy cattle under heat stress show lower milk yields, deficiency in reproductive traits and immune depression. Genetic selection of animals that present higher thermotolerance in high temperatures helps offsets the adverse effect of heat stress. In this study genotyping of ApaLI RFLP reported at the Heat Shock Transcription Factor 1 (HSF1) gene was performed in Puerto Rican Creole (n = 21) and Holstein [Slick (SH; n = 36) vs. Wild-Type (WT; n = 56)] cattle. Segregation of the three genotypes (TT, TC and CC) associated with this RFLP was observed in the three cattle groups. Allelic frequencies were 0.79 T/0.21 C and 0.68T/0.32 C for SH and WT Holsteins, respectively. In Creole, similar allelic frequencies were observed (0.69 T/0.31 C; Chi Square $P > .05$). A significant association of this HSF1-RFLP with a molecular breeding value (Igenity, Neogen Corp.) for milk (TT, CT > CC; $P < .05$) but was not phenotipically confirmed with milk adjusted at 305d (DHI; $P > .05$). Conversely, an interaction between HSF1 and coat type was observed in calving interval ($P = 0.0358$). In that regard, SH-TT presented a calving interval 52 d lower than WT-CT ($P = 0.0240$). Also, WT-CC has 72.5 d lower calving intervals when compared with SH-CT ($P = 0.0475$). These results suggest that the reproductive usefulness of this HSF1 polymorphism is dependent on the type of coat in Holstein cattle and that both natural (Creole) and artificial selection (SH/WT Holstein) favors the T allele.

Key Words: HSF1, slick, Creole

P4064 Genetic diversity and population structure of wild and semi-domesticated reindeer (Rangifer tarandus) inhabited in northeastern Siberia based on single nucleotide polymorphism markers. V. R. Kharzinova, A. V. Dotsev (L.K. Ernst Institute of Animal Husbandry, Moscow, Russian Federation), I. M. Okhlopkov (Science Institute of Biological

Problems Cryolithozone, Yakutsk, Russian Federation), E. A. Gladyr (L.K.Ernst Institute of Animal Husbandry, Moscow, Russian Federation), V. I. Fedorov (Federal Government Budget Scientific Institutions Yakut Scientific Research Institute of the Agriculture Federal Agency Scientific Institutions, Yakutsk, Russian Federation), G. Brem (Institute of Animal Breeding and Genetics, VMU, Vienna, Austria), and N. A. Zinovieva (L.K. Ernst Institute of Animal Husbandry, Moscow, Russian Federation)

Reindeer is one of the most important species of farm animals in the northern parts of Russia. Wild populations are also tightly associated with the life of indigenous people. The study of reindeer genetic diversity has always interested scientists worldwide. One of the modern approaches for biodiversity assessment in non-model species (species whose genomes have not been sequenced yet) is screening for single nucleotide polymorphisms using DNA BeadChips, designed for their related model species. Our study aimed at characterizing genetic diversity and population structure of wild and semi-domesticated reindeer using commercially available SNP chips, developed for cattle. A total of 47 of wild and 38 of semi-domesticated reindeer individuals were included in this study. The samples of wild reindeer were collected in 14 different sites in northeastern Siberia (the Republic Sakha-Yakutia) and the samples of semi-domesticated reindeer were taken from two farms of the same region. DNA was extracted from tissue samples using Nexttec column (Nexttec Biotechnology GmbH, Germany) according to recommendation of manufacture. Genotyping was performed using Bovine SNP50 v2 BeadChip (Illumina Inc., San Diego, CA). Statistical analysis was performed with PLINK 1.07, Arlequin 3.5.2.2, HP-Rare 1.1 and GenAlEx 6.5.1 software. In total, 625 SNPs were selected after quality filtering as the set of markers for further analyses. Pairwise F_{st} value between wild and semi-domesticated reindeer was 0.0497 ($p < 0.001$). ANOVA indicated that the genetic variation mainly occurred within populations (95.06%) and the variance among populations was only 4.94%. The average number of effective alleles was 1.942 ± 0.009 for wild reindeer in comparison with 1.781 ± 0.017 alleles for semi-domesticated reindeer, the number of informative alleles was 1.285 ± 0.012 and 1.288 ± 0.013, respectively. Allelic richness was significantly higher in wild population as compared to semi-domesticated population: 1.92 ± 0.01 and 1.78 ± 0.02, respectively. The observed heterozygosity was 0.178 ± 0.006 in wild reindeer and 0.181 ± 0.007 in semi-domesticated reindeer. Insignificant differences in the levels of expected heterozygosity were obtained: 0.184 ± 0.006 and 0181 ± 0007 in wild and semi-domesticated reindeer, respectively. Inbreeding coefficients were higher in wild reindeer (F = 0.029 ± 0.008) than in semi-domesticated reindeer (F = 0.014 ± 0.008). The group of wild reindeer was characterized by minor heterozygote deficiency, whereas no differences between observed and expected heterozygosity values were observed in semi-domesticated reindeer. Our study demonstrates that the commercially available DNA chip designed for cattle might be successfully applied for biodiversity assessment of reindeer (*Rangifer tarandus*). The study was supported by the Russian Science Foundation within Project no. 14–36–00039.

Key Words: biodiversity, SNP, reindeer *Rangifer tarandus*

P4065 MicroGBS: High-throughput microsatellite genotyping using Illumina sequencing platforms.

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The use of microsatellite markers in animal agriculture is less frequent with the advent of high-throughput SNP genotyping platforms. Though available for genomic selection in channel and blue catfish, these SNP platforms are not cost effective for the thousands of animals that require genotyping to resolve parentage and sibship because catfish cannot be efficiently tagged for identification. Therefore we expanded on a genotyping-by-sequencing (GBS) protocol for high-throughput SNP genotyping to perform high-throughput microsatellite genotyping. We designed 400–500 bp amplicons that contained microsatellite loci known to be polymorphic in channel and blue catfish based on resequencing analysis. Amplicons were multiplex amplified in 96-well format for 10 cycles based on locus-specific primers that were tailed with Illumina sequencing primers. Diluted products were then amplified with primers that matched the Illumina primer sequences and were tailed with 8 bp barcodes and the Illumina binding sequences. Barcoded amplicons were pooled from each plate, purified from lower molecular weight contaminants using magnetic beads, quantified for each 96-well plate, then equimolar plate pools were re-quantified and sequenced on the MiSeq platform using a single 300 bp read with dual index reads. Sequences from individual samples were demultiplexed using bcl2fastq. A bash script extracted locus-specific reads from each sample, defined the microsatellite repeat region, and determined the length of the repeat. We genotyped 37 loci each in 576 samples on the MiSeq platform. These loci contained di-, tri-, tetra-, or pentanucleotide repeats. The 300 bp read lengths eliminated allele sizing errors due to non-template adenylation

at fragment ends, tolerated a broad range of sequence quantities between loci, and also permitted genotyping of long repeat spans. MicroGBS eliminated interference with allele calling between loci that can limit the number of multiplexed loci for fluorescent fragment analysis, especially for loci that have a long range of alleles– one catfish locus contained up to 52 tetranucleotide repeats. Genotypes from fluorescent fragment analysis and MicroGBS were compared for six loci. Of 2953 total genotypes, 34 genotypes were divergent at one allele and only 16 were divergent at both alleles. Extension of MicroGBS to the HiSeq platform will permit higher-throughput genotyping of thousands of samples per lane. MicroGBS provides flexibility to add or substitute loci, and can also be used to genotype microsatellites, other indels, and SNP loci. The latter can be useful for transitioning established microsatellite genotyping standards to SNP-based genotyping standards.

Key Words: genotype-by-sequencing, microsatellite, high-throughput

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P4066 Genetic diversity of pig populations from the US mainland, Pacific islands and China: Autosomal SNP evaluation. H. Blackburn (National Animal Germplasm Program ARS-USDA, Fort Collins, CO), D. A. Faria (National Animal Germplasm Program, Fort Collins, CO), C. Wilson (National Animal Germplasm Program ARS-USDA, Fort Collins, CO), and S. R. Paiva (EMBRAPA-LABEX US-Secretariat International Affairs, Brasilia, Brazil)

Genetic diversity for 19 pig populations (n = 500) that had entered the US gene bank were evaluated using a commercially available 70K SNP chip for pigs. Berkshire, Duroc, Hampshire, Landrace, Yorkshire, Chester White, and Spotted represented commercially vibrant breeds; Fengjing, Guinea Hog, Hereford, Large Black, Mangalista, Meishan, Minzhu, Ossabaw Island, and Tamworth were among the minor breeds; and feral hogs from the Pacific islands of Guam, Kauai (Hawaii, HI), and Hawaii (HI) were included in the study. All breeds, with the exception of Mangalista, have been well established in the US for one century or longer, or they were developed in the US. After basic quality control procedures a total of 8765 SNPs were used for analysis. Observed heterozygosity ranged from 0.29 (Meishan) to 0.62 (Mangalista). A Bayesian genetic structure analysis suggested 7 main clusters: 1.) Chester White–Landrace; 2.) Meishan–Fengjing–Minzhu; 3.) Guam–Kauai–Hawaii; 4.) Tamworth–Duroc–Hereford; 5.) Berkshire; 6.) Hampshire; and 7.) Yorkshire. All breeds evaluated were aligned on four vectors in a three dimensional principal components (PC) analysis. The first principal component (PC) indicated a distinct separation among breeds derived from Asia and the US, with the Pacific island populations being intermediate. The second and third PCs placed Hampshire, Duroc and Yorkshire at extreme positions corroborating their unique genetic variability. Also aligned on those three vectors were breeds that tended to be red, black or white in color. The Landrace and Chester White were closely placed and distinctly separated from Yorkshire. The rare breeds Guinea Hog, Large Black, and Mangalista were found to be at or near the origin of the three PCs. To better understand the genetic structure of Pacific islands populations and rare breeds a new Bayesian analysis was performed with nine populations. Two Chinese breeds and seven of the more widely sampled US breeds were discarded. With 5 clusters identified the Pacific islands populations were unique and showed little admixture with the other populations evaluated. The data suggested populations from Kauai and Hawaii are different from each other. In addition, pigs sampled on Guam were different from those from the Hawaiian Islands and China (Minzhu). This assessment suggests US pig populations have substantial genetic variability for future use and further exploration of genetic diversity among feral island populations in the Pacific is warranted.

Key Words: genetic diversity, pigs, feral populations

P4067 Comparing genetic diversity of pig populations on the US mainland, Pacific Islands and China: Y chromosome evaluation. D. A. Faria (National Animal Germplasm Program, Fort Collins, CO), S. R. Paiva (Embrapa-Labex US-Secretariat International Affairs, Brasilia, Brazil), C. Wilson (National Animal Germplasm Program ARS-USDA, Fort Collins, CO), and H. Blackburn (National Animal Germplasm Program ARS-USDA, Fort Collins, CO)

Genetic diversity unpins a country's ability to adapt its livestock populations to varying environmental, production and consumer changes. Conservation of genetic resources in gene banks supports national conservation efforts. Previous work with ruminants suggests US populations held in the national gene bank are genetically diverse. Here we evaluate the genetic variability captured in the US gene bank focusing on differences among Y chromosomes for different pig populations. Pigs provide an interesting model for such a study in that mitochondrial analyses suggested pigs and wild boars have a worldwide

distribution with multiple domestication centers. This study analyzed 485 boars belonging to 16 breeds (7 commercial: Berkshire, Duroc, Hampshire, Landrace, Yorkshire, Chester White, Spotted; 6 rare: Guinea Hog, Hereford, Large Black, Mangalista, Ossabaw Island, Tamworth; 3 from China: Fengjing, Meishan, Minzhu) and 3 feral populations (Pacific islands of Guam, Kauai and Hawaii) by 14 SNP markers on the Y chromosome in a commercial 70K chip. After quality control (SNP call rate, heterozygosity and absence of polymorphism), 6 of the 14 markers remained in the analysis. Five different haplotypes were found among the animals sampled. The H3 was present in 278 samples (57%). Except for Large Black and Mangalista, this haplotype was observed in all breeds that came to the US via the Atlantic. H3 was also the only haplotype observed in Duroc, Yorkshire, Tamworth, Hampshire and Guinea Hog breeds. The haplotypes H1 and H2 were present in pigs from the Hawaiian Islands, China, and the US mainland. If this represents admixture between Asian and European populations it would seem to have taken place a relatively long ago. The H4 haplotype was exclusive to animals from China and the Pacific islands. Pigs from Guam and Kauai islands were the only populations exhibiting the H5 haplotype. Median joining network analysis has shown that haplotypes H4 and H5 (China and Pacific islands) are closer than the remaining three haplotypes. However, there seems to have been no human migration between Guam and Kauai Islands but there may have been exchange between Guam and Polynesia. We find it interesting that 10 of the populations sampled only exhibited one haplotype and this lack of variation was inclusive of both rare, commercially important, and Chinese breeds.

Key Words: animal genetics resources, molecular markers, conservation genetics, gene banks, phylogeography

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P4068 Interaction of STAT1 and PGR specific genotypes affects milk production in slick and normal coat Holsteins. B. Velez, J. Patino (University of Puerto Rico, Mayaguez, Puerto Rico), Y. R. Velez (University of Puerto Rico, Mayagüez Campus, Mayagüez, Puerto Rico), T. S. Sonstegard (USDA, ARS, BFGL, Beltsville, MD), and M. Pagán-Morales (Department of Animal Science, University of Puerto Rico, Mayaguez Campus, Mayaguez, Puerto Rico)

Candidate genes polymorphisms (SNPs) interactions can unveil functional relationships between genes and production traits. Recently, special attention has been given to Holsteins that inherit a novel slick coat mutation that provides adaptation to high temperature and humidity conditions characteristic of the tropics. DNA and phenotypic records (DHIP) from 186 Holstein cows (107 slick and 79 normal coat) were collected. Animals were genotyped for SNPs at the progesterone receptor (PGR) and signal transducer and activator of transcription 1 (STAT1) using restriction fragment length polymorphism (RFLP). It has been documented that a AdeI-RFLP in PGR (at least one copy of allele G) increased fertilization rate and embryo survival in vitro. On the other hand, PagI-RFLP in STAT1 (at least one copy of allele C) gene had been associated with significant increases in milk, fat, and protein yields. Both SNPs, found in PGR and STAT1, were used for an association study with milk production, somatic cell count, and calving interval. These genes showed a significant interaction ($P < .0011$) for 305 d adjusted milk. Favorable genotype combinations for that trait (CC for PGR; CT for STAT1) in both types of cattle (slick and normal coat) were observed and phenotipically validated. Meanwhile, slick resulted in higher milk yields than normal coat even when the same genotype combination was observed (GC-PGR/CT-STAT1). The percentage of favorable genotypic combination for milk production (CC for PGR, CT for STAT1) seemed to be greater in slick (70%) than normal coat cattle (30%; $P < .0001$)). Conversely, the percentage of the reproductive favorable genotype (GG for PGR) was greater in normal coat cattle (78.95%) than slick (21.05%; $P < .0001$). This marvel may be due to mutations associated with slick coated cattle's adaptation to high temperatures. Studying gene interaction with production traits could provide a better understanding of molecular markers and their importance in dairy herd improvement particularly in slick and non-slick cows.

Key Words: PGR, STAT1, slick

P4069 Genetic differences in a Colombian Paso horse breed by gait selection. M. A. Novoa (Genetica Animal de Colombia Ltda., Bogota, Colombia; Universidad Nacional de Colombia, Bogota, Colombia) and L. F. García (Universidad Nacional de Colombia, Bogota, Colombia)

The Colombian Paso breed CCC is the main horse breed in the country, with more than a half million animals, and includes three different subpopulations defined by gaits. It descended from some horse populations which Columbus brought to the Americas in the XVI century. These populations were selected by local breeders for different farming activities considering the variety of Colombian topographic landscapes; therefore, for centuries those breeders were looking

for animals with particular movements that were able to correctly perform on those places. We tested the hypothesis whether or not there is a genetic structure based on gait selection.

This approach was made through an exhaustive analysis of genetic information including 14–16 autosomal microsatellite markers (140,000 genotypes), X chromosome microsatellites (1000 genotypes), mitochondrial d-loop sequences (200 animals), genealogical information (226,000 records approx.), and phenotypic data (morphometric and gait information). All data were analyzed by using statistical tools, population genetics approaches and phylogenetic reconstruction.

The analyses show a single breed with a genetic structure based on the selection of different gaits through the history of this population. That is supported by significant phenotypic differences among the populations defined by gaits. Genetic differences have been increasing in the last 20 yr, so it is possible that if artificial selection provides the reproductive barriers among gaits, it could be possible in the near future that the CCC breed would become two or more different breeds. Finally, the phylogenetic and genealogical reconstructions show a single common origin of these subpopulation which demonstrates a single substructured breed.

Key Words: horse gait substructured

P4070 SNP discovery and allele frequency estimation in indigenous breeds of South Africa. A. Zwane (Agricultural Research Council, Pretoria, South Africa), A. A. Maiwashe (ARC-Animal Production Institute, Irene, South Africa), and E. van Marle-Koster (University of Pretoria, Pretoria, South Africa)

Indigenous breeds generally perform poorly as compared to other commercial breeds. This is because there have not been in-depth studies that focus on determining the important production traits at a genome-wide level that will help to improve them. This is due to lack of genomic data that allows these breeds to be thoroughly investigated. SNP arrays have revolutionized the ability of genome-wide studies to detect regions harboring sequence variants that affect complex traits. Extensive numbers of validated SNPs with known allele frequencies have rather mainly focused on breeds other than South African breeds, and were essential to construct genotyping assays with broad utility. This biasness makes the use of existing assays in local breeds less efficient in studies such as genome-wide association studies due to lower minor allele frequencies exhibited by these breeds as described in previous studies. The aim of this project was to discover SNPs in Afrikaner, Drakensberger and Nguni indigenous breeds and estimate their allele frequencies. 90 cattle representing these three populations were used to identify more than 30 000 putative SNPs and predicted their allele frequencies. The data from 90 individuals validated about 90% of 10 000 SNPs selected genome-wide with a genotypic and allele frequency correlation of $r = 0.42$. Analysis included mapping of the sequence to the available *Bos taurus* reference genome. Identification of high frequency markers in indigenous breeds suggests the utility of whole genome SNPs as a potential resource for identifying naturally selected trait-regulating genomic targets and functional allelic variants adaptive to diverse climatic regions, for genetic enhancement of the gene-pools.

Key Words: SNP discovery, allele frequency, indigenous breeds

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P4071 Extensive functional class I MHC diversity in sheep. K. Ballingall (Moredun Research Institute, Edinburgh, United Kingdom), S. Goh (Royal Veterinary College, Hatfield, United Kingdom), J. M. Pemberton, and K. Dicks (The University of Edinburgh, Edinburgh, United Kingdom)

We have previously described functional class I MHC allelic and haplotype diversity associated with four MHC haplotypes from a Scottish blackface sheep flock. This provided reference sequences representing at up to three classical class I and up to six other class I loci. However, only limited information is available on the extent of MHC class I allelic diversity at these loci in sheep. To begin to explore the relationship between class I diversity and immune function we need to be able to genotype animals across the range of different class I loci. To do so we first need to understand the level of diversity at each of the previously identified class I loci in sheep. Using a set of pan-class I specific primers designed to amplify a 500 bp fragment representing the polymorphic second and third exons from all transcribed class I loci, we amplified, cloned and sequenced the range of class I alleles from 38 sheep of 9 different breeds from the UK and continental Europe. From these 38 animals, 104 different class I transcripts were sequenced. Phylogenetic analysis of these sequences with the previously described locus specific reference sequences identified clusters which are likely to represent alleles at each locus. Unlike diversity at the highly polymorphic class II MHC *DRB1* locus where a substantial proportion of alleles are shared between breeds, only two of the 104 class I sequences was shared between animals of different breeds. This suggests that substantial breed-specific diversity remains to be identified at the class I loci and that diversity at class I loci

is generated more rapidly than class II.

Key Words: sheep, MHC class I, diversity

P4072 Development of a 55K SNP array for oysters (*C. gigas* and *O. edulis*). A. P. Gutierrez (The Roslin Institute and R(D)SVS, University of Edinburgh, Midlothian, United Kingdom), F. Turner (Edinburgh Genomics, University of Edinburgh, Edinburgh, United Kingdom), T. P. Bean (Center for Environment Fisheries and Aquaculture Science, Cefas Weymouth Laboratory, Weymouth, United Kingdom), K. Gharbi (Edinburgh Genomics, University of Edinburgh, Edinburgh, United Kingdom), and R. D. Houston (The Roslin Institute and R(D)SVS, University of Edinburgh, Midlothian, United Kingdom)

Pacific oyster (*C. gigas*) is one of the most important aquaculture species worldwide, with a production in 2013 of > 550,000 tonnes. The European flat oyster (*O. edulis*) is farmed in smaller volumes, and native wild populations are under threat partly due to the *C. gigas* introduction. Therefore, from an aquaculture and an ecological perspective, it is important to develop genomic resources for these species. The goals of the current study were to discover a large number of genome-wide SNPs in *C. gigas* and *O. edulis* populations, and to create the first high-density SNP array for oyster species. To achieve this, we performed whole genome sequencing (WGS) of eight pools of genomic DNA from *C. gigas* oysters sampled from diverse geographic locations, and Restriction-site Associated DNA (RAD) sequencing of pooled samples from 11 diverse *O. edulis* populations. For *C. gigas*, sequence data were aligned to the reference genome detecting approximately 12 million putative SNPs. For *O. edulis*, RAD loci were assembled *de novo* and ~590K SNPs were identified in ~215K loci. SNP filtering based on criteria including minor allele frequency and read coverage across populations, monomorphic flanking regions, even distribution across the genome, and Affymetrix prediction of SNP conversion score has been applied to identify a final set of suitable SNPs. The final oyster SNP array contains ~55K SNPs, comprising ~40K *C. gigas* SNPs and ~15K *O. edulis* SNPs. The first application of the array will be to map genes affecting resistance to Oyster Herpes Virus in a challenged commercial *C. gigas* population. The oyster SNP array will be publicly available, and should enable high resolution genetics studies in these commercially and environmentally important species.

Key Words: oyster, SNP array, whole genome sequencing, RAD sequencing

POSTERS: GENETIC MARKERS AND SELECTION

P5000 Confirmation of genome-wide associations for clinical mastitis in German Holstein cattle. H. Abdel-Shafy(Department for Crop and Animal Sciences, Humboldt-Universität zu Berlin, Berlin, Germany; Department of Animal Production, Faculty of Agriculture, Cairo University, Cairo, Egypt), R. H. Bortfeldt, M. Reißmann (Department for Crop and Animal Sciences, Humboldt-Universität zu Berlin, Berlin, Germany), and G. A. Brockmann (Albrecht Daniel Thaer-Institut for Agricultural and Horticultural Sciences, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Berlin, Germany)

Our initial genome-wide association study (GWAS) identified six genomic regions associated with somatic cell score (SCS) in German Holstein cattle as a surrogate trait for mastitis susceptibility. Since SCS is not completely correlated with mastitis, a further study was necessary to directly address clinical mastitis (CM) and to validate if the statistical evidence provided by GWAS for SCS were true-positive associations. The current study was performed with a population consisting of 1702 German Holstein cows from three herds. The data for CM in the first three lactations were classified according to whether animals were infected or not based on visual examination at the time of test day. We tested twelve single nucleotide polymorphisms (SNP) representing six genomic regions that were associated with SCS in the previous GWAS. Association analyses were performed using a generalized linear mixed model (GLMM) approach as implemented in SAS software. In this model, age at calving, lactation number, genotypes for each SNP, and regression coefficient associated with fixed lactation curve were considered as fixed effects, while the environmental differences between consecutive lactations and between test days within lactation were treated as random and we accounted for population stratification by fixing the sire as a random effect. Out of twelve tested SNPs, seven were significantly associated with CM located on *Bos Taurus* autosomes (BTA) 6, 13, 18, and 19. Four SNPs had the same direction of effect as those previously reported in the initial GWAS for SCS. The major allele of the two SNPs on BTA6 and the minor allele of the two SNPs on BTA13 were favorable for decreasing CM cases. The other three significant SNPs had minor allele effects in the opposite direction. The SNPs on BTA6 coincided with previously reported QTL for CM in Danish Holstein. The

SNPs on BTA13 have not been reported before, neither in Holstein nor other cattle breeds. Verified SNPs are located within or very closed to several promising candidate genes. In conclusion, our confirmation study using CM data in the cow population provides evidence for the functional role of the linked genomic regions for immune response and could contribute to identification of causative mutations. In particular, SNPs with minor frequency of the favorable allele possess high potential to reduce mastitis incidence in German Holstein cattle by genomic selection.

Key Words: clinical mastitis, validation, GWAS

P5001 Molecular exploration of genetic resistance in riverine buffalo. M. Javed and A. Nadeem (University of Veterinary & Animal Sciences, Lahore, Pakistan)

Bovine tuberculosis (bTB) is a neglected endemic zoonoses, causing lot of mortalities every year. Previous methods of controlling bTB, like vaccination, antibiotics, have raised the concern of animal product consumers regarding microbial resistance. These measures have not proved effective in developing countries, so disease prevalence in these areas is increasing continuously. In a report from WHO, Pakistan have been declared eight out of ten countries with the highest incidence of bTB. So, there is dire need to find new therapeutic and preventive measures against Mycobacterium infection. Identification of selection signatures for genetic resistance is a promising new alternate to combat bTB. Many of countries have opted for this new approach and have reported useful data in cattle, but very limited efforts have been put into the river buffaloes. River buffaloes of the Indo-Pak region are world famous for their superior genetic potentials and inter-breed variations that provide substantial basis for identification of significant selection signatures. Present research was planned to explore IFNg gene in river buffalo for its association with bTB. Interferon γ (IFNg) is a key responder cytokine in Mycobacterium infection. For its genetic characterization, blood was collected from tuberculin negative (n = 267) and tuberculin positive (n = 194) animals. DNA was extracted and Sanger's method of DNA sequencing was used. Sequence comparison of two groups provided a total of five variations. Significance of each variation was tested by Hardy Weinberg equilibrium ($P < 0.05$). Association was performed by one way ANOVA. Results illustrated only one variation found significantly associated with better immunity against bTB. For the purpose of some additional and supportive information phylogenetic analysis was also being performed by neighbor joining method with bootstrap value-1,000. Tree indicated that river buffaloes are in closest proximity to *Bos taurus* and its genetic distance from other species may also be seen in the figures provided. Genetic markers identified in this study can be useful in future breeding selection programs against bTB resistance.

Key Words: bTB, IFNg, buffalo, polymorphism, association, phylogenetic tree

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P5002 You say variation, they say mutation, we say confusion: Genetic communication, why it needs standardization! G. Sofronidis (Orivet Genetic Pet Care, Melbourne, Australia)

The world of genetics is evolving and growing at an exponential rate. The number of new tests available sees genetics being used by breeders worldwide seeking scientific ways to manage their breeding programs. What a breeder demands with regards to information can sometimes be frustrating for laboratories. "Where are my results?" "What does that result mean?" "What colour is my cat?" "Where can I get that test done?" These are just some of the common questions laboratories receive. We see breeders having different priorities for the use of tests with an increased demand in phenotypic tests. Although most institutions have focused on standardizing scientific reports with regards to profiling no focus has been placed on standardizing the language used to report genetic disease and trait results. Words like deletion, insertion, stop codon and variants tend to make up a scientist's vocabulary. The breeder or user of genetic tests tends to focus on what a result such carrier, Bb or Nn means and how they can use this in their breeding program. A laboratory tends to focus on turnaround times, reliability of data, affordability, costs and providing reporting in a consistent and easy way to understand. It is strange how the same scientific result can be reported as normal or clear or wild-type or NN or BB or DD and the list can go on depending on which laboratory has done the test. This presentation will focus on demonstrating how and why it should be a priority for institutions to look at a standardized way of reporting genetic results; thus allowing laboratories to compare and use another laboratory's results and ensure identical science is used. It will be a case of the scientist vs. the breeder vs. the laboratory and who dares wins! In the end if we standardize reporting in this area we can all win.

Key Words: genetics interpretation standardization

P5003 Genome-wide association for calving interval in buffaloes. G. M. de Camargo (Universidade Tecnológica Federal do Paraná, Brazil, Dois Vizinhos, Brazil), R. R. Aspilcueta Borquis (State University of São Paulo, Faculty of Agriculture and Veterinary Sciences, Jaboticabal, Brazil), D. J. A. Santos (Sao Paulo State University, Jaboticabal, Brazil), D. F. Cardoso (Fundação de Amparo à Pesquisa do Estado de São Paulo-FAPESP(Bolsista), Sao Paulo, Brazil), N. Hurtado-Lugo, and H. Tonhati (State University of São Paulo, Faculty of Agriculture and Veterinary Sciences, Jaboticabal, Brazil)

Genome-wide association is a very promising tool to identify genes with major effect in a specific trait. Identifying these genes, we are able to better understand the physiology behind the trait as well as the prospect of major genes for fine-mapping. The aim of the study was to do genome-wide association for calving interval (CI) in dairy buffaloes. A total of 452 buffaloes (57 sires and 395 dams) were genotyped using the 90K Axiom ® Buffalo Genotyping (Affymetrix). The associations were done with the deregressed breeding values. The most important genes identified were *TPCN1* (SNP info = AX-85069024, 17: 63515708) and *LOC101903522, MGMT* (SNP info = AX-85067656, 26: 49118646). *TPCN1* is a gene involved in spermatozoa acrosome reaction identified in humans. Spermatozoa acrosome reaction is necessary for fertilization and tends to be studied in the context of male fertility. The association of *TPCNI* with CI suggests an interesting thought: a gene related to male fertility might be more relevant to herd performance (in terms of CI) than genes related to female fertility. Increased conception rates after calving, and, as consequence, decreased CI may also reflect fertilization ability of bulls in the studied population. As a complex trait, CI may be influenced by several component traits linked to both male and female fertility, including spermatozoa quality andrological parameters. This is a promising candidate gene for causative mutation future studies. Moreover, the results found, helped to better understand the physiology behind the trait.

Key Words: *Bubalus bubalis*, SNP, reproduction

P5004 Identification of signatures of selection and assessing the diversity of East African Shorthorn Zebu mitochondrial DNA. H. Bahbahani (Kuwait university, Kuwait, Kuwait), J. Mwacharo (International Livestock Research Institute, Addis Ababa, Ethiopia), and O. Hanotte (School of Life Sciences, University of Nottingham, Nottingham, United Kingdom)

The mitochondrial DNA (mtDNA) is an important genetic marker commonly used to study the evolution of the bovidae family. The availability of the full bovine mtDNA sequence has further cleared the domestication process of this species. Moreover, given the importance of the mitochondria in cell energy production, mtDNA is expected to be a target of natural selection. In this study we have compared the full mtDNA sequences of indigenous East African Shorthorn Zebu cattle (EASZ) with cattle breeds from Europe, Africa and Asia to evaluate the extent of EASZ mtDNA genetic diversity and identify signatures of selection within African cattle mtDNA. Our results indicated that the EASZ mtDNA sequences are all of the taurine type and members of T1a, T1b and T1b1 sub-haplogroups. Nineteen taurine-zebu non -synonymous variants were detected, but none seem to be associated with a selective advantage for taurine mtDNA. Based on ω ratio analyses, purifying selection is the main selection pressure targeting EASZ mtDNA with less selective constrains at the ATP6 and ATP8 genes. Interestingly, within African cattle, we identified a positive selection signal in the Cox-2 gene in the T1b/T1b1 sub-haplogroups, together the most common sub-haplogroups on the continent. This may indicate a probable advantage for these sub-haplogroups in Africa.

Key Words: mitochondrial DNA, signatures of selection, haplogroup

P5005 Hitchhiking effects influence allele frequencies and exclusion probabilities of microsatellites used for parentage control in Holstein Friesian cattle. B. Brenig and E. Schütz (Institute of Veterinary Medicine, Georg-August-University, Göttingen, Germany)

Methods for parentage control in cattle have changed since their initial implementation in the late 1950s from blood group typing to single nucleotide polymorphism determination nowadays. In the early 1990s, a panel of 12 microsatellites was selected as international standard for parentage control and has been used since then, accompanied by international comparison tests ensuring permanent validity for the most common cattle breeds. Although nearly every parentage can be resolved using these microsatellites, cases with very close relatives became an emerging resolution problem during recent years. Thus, it must be presumed that although microsatellites have been selected based on their polymorphism information content and exclusion probabilities, and no direct selection against their variability was applied, other effects have induced a trend to the fixation of alleles and monomorphism. To

determine changes of allele frequencies and exclusion probabilities, we analyzed the development of these parameters for the 12 microsatellites from 2004 to 2014. 168,000 recorded Holstein Friesian cattle genotypes were evaluated. During this time certain alleles of nine microsatellites increased significantly (t-values > 5). When calculating the exclusion probabilities for 11 microsatellites, reduction was determined for the three situations, i.e., one parent is wrongly identified (p = 0.01), both parents are wrongly identified (p = 0.005), and the genotype of one parent is missing (p = 0.048). With the addition of BM1818 to the marker set in 2009, this development was corrected leading to significant increases in exclusion probabilities. Although, the exclusion probabilities for the three family situations using the 12 microsatellites are > 99%, the clarification of 142 relationships in 40,000 situations where one parent is missing will still be impossible. 25 sires were identified that are responsible for the most significant microsatellite allele increases in the population. The corresponding alleles are mainly associated with milk protein and fat yield, body weight at birth and weaning, as well as somatic cell score, milk fat percentage, and longissimus muscle area. Our data show that most of the microsatellites used for parentage control in cattle are not neutral DNA markers and are influenced by hitchhiking effects.

Key Words: parentage control, microsatellite, exclusion probability

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P5006 Genome-wide linkage analysis of fatty acid composition in the F2 intercross between Landrace and Korean native pigs. I. C. Cho (NIAS, Jeju, Korea), S. H. Han (Educational Science Research Institute, Jeju National University, Jeju, Korea), and H. B. Park (NIAS, Jeju, Korea)

The objective of this study was to locate quantitative trait loci (QTL) influencing fatty acid (FA) composition in an F_2 intercross between Landrace and Korean native pigs. Eighteen FA composition traits were measured in F_2 progeny. All experimental animals were genotyped with 173 microsatellite markers located throughout the pig genome. We detect that a total of 112 QTLs for the FA composition were revealed on autosomes, exception chromosome X; forty seven QTLs reached the genome-wide significant threshold. We identified a cluster of highly significant QTLs for FA compositions on SSC12. QTL on SSC12 that accounted for up to 16.9% of phenotypic variance, among them, PUFA, which was the highest test statistic (F-value = 97.2 under additive and dominance model, nominal P-value 3.6×10^{-39}) observed in this study. Also, on the similar position, four more QTLs (i.e., QTL for C18:1, C18:2, C20:4, and MUFA) explained more than 10% of phenotypic variance.

Key Words: fatty acid, quantitative trait locus, genome-wide linkage analysis, Landrace and Korean native pigs

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P5007 Systems biology approach provides novel insights into gene networks controlling tenderness and meat quality traits across French beef breeds. Y. Ramayo-Caldas, G. Renand (GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France), M. Ballester (Departament de Genètica i Millora Animal, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Torre Marimon, Caldes de Montbui, Spain), R. Saintilan (INRA, UMR 1313, GABI, Jouy-en-Josas, France), and D. Rocha (GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France)

Several studies focusing on Warner-Bratzler shear force (WBSF) have been performed to identify markers associated with beef meat tenderness. However, these studies have not explored the interactions among genes associated with WBSF. The main goal of our study was to identify a common set of interacting genes associated with tenderness and meat quality traits across Charolais, Limousin and Blonde d'Aquitaine beef breeds by combining genetic and transcriptomics information. First, the Association Weight Matrix (AWM) approach was used to identify genes co-associated with tenderness and meat quality traits across the previously mentioned breeds. This multi -trait approach was initially applied for each breed and 17 phenotypes. Afterward, across breeds' comparisons were performed at functional and AWM levels. Genetic heterogeneity was observed, and most of the variants segregated within rather than across breeds. A total of 206 genes (~ 8% in each breed) were shared across the three breeds, genetic variants mapping within those common genes explained around the 28–30% of the WBSF phenotypic variance. In a second step, we integrated *longissimus dorsi* muscle RNASeq data considering only 121 of the 206 genes expressed in muscle. To prioritize the candidate gene selection, integrative approaches were also implemented to systematically compare the covariation of gene expression with the phenotypic variation of WBSF. Given confidence in the reliability of the results, well-known candidate genes, as well as new candidate genes, regulators and pathways affecting tenderness and meat quality traits were identified. Our results suggest that systems biology approaches increase the power to identify and prioritize candidate genes beyond the "traditional" one-dimensional approach used to identify genes affecting

single traits. Further studies targeting genetic variants within the common genes, their pathways, regulators and interactions might lead to the discovery of functional mutations to guide genomic selection in a beef multi-breed context.

Key Words: tenderness, beef, systems biology

P5008 An initial exploration on the genetic variability of a selection sweep region on OAR6 by exploiting massive genome sequencing of dairy and meat breeds. P. K. Chitneedi, B. Gutiérrez-Gil (University of León, León, Spain), C. Esteban-Blanco (Supercomputing Center of Castilla y León, Leon, Spain), and J. J. Arranz (University of León, León, Spain)

In this study the genomic region encompassing the interval 30.760–38.243 Mb of sheep chromosome 6 (OAR6), which has been previously suggested as a signature of selection when comparing dairy and non -dairy sheep breeds, has been studied at high resolution. Hence, whole genome sequencing information from two Lacaune and three Merino DNA samples were analyzed. These two breeds had shown a remarkable genetic differentiation in that genomic region based on the 50K-chip genotypes of the SheepHapMap dataset. The raw whole genome sequences generated with Illumina pair-end technology were obtained from the Sequence Read Archive. After a quality control of the whole genome raw reads, the alignment against the reference genome assembly (Oar_v3.1), and different cleaning steps, we performed a variant calling analysis following the recommended protocols steps of the GATK software and using the HaplotypeCaller function. For all the seven analyzed samples, the number of variants (Indels and SNPs) identified with the GATK work-flow across the genome was 29,013,784, with 78,490 of them being included in the target interval. Considering the 67,847 SNPs identified in the studied interval, we used the PLINK software to identify the SNPs showing the most divergent allele between the two studied breeds. Among the 133 SNPs identified as divergent variation and based on the sheep genome annotation, the divergent markers were predicted to cause 115 intragenic variants distributed across a total of 7 unique annotated genes (*GRID2, MMRN1, PPM1K, SNORA57, HERC3, CCSER1* and *FAM184B*). The Ensemble Variant effect predictor (VEP) software used to perform a functional annotation of these intragenic SNPs classified the resulting variants as 92 intronic variants, 17 downstream gene variants, three synonymous variants and four missense variants. All the missense variants were found in the *MMRN1* (multimerin 1) gene which is a soluble protein found in platelets and in the endothelium of blood vessels although it also has function as an extracellular matrix or adhesive protein. Among the genes harboring intronic variants, we would highlight the gene *PPM1K,* which has been found to be an essential regulator of normal lactation, whereas the *FAM184B* gene has been suggested to influence carcass yield. Based on the initial stage of the annotation of the sheep genome, additional efforts should confirm the consequences of these mutations and try to explore their possible relationship with the selection sweep studied here.

Key Words: massive sequencing, selection sweep, divergent variation

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P5009 A genome-wide association analysis for carcass and meat quality traits in Duroc pigs. R. Gonzalez (Center for Research in Agricultural Genomics, Bellaterra, Spain), P. G. Eusebi (Universitat Autónoma de Barcelona, Faculty of Veterinary, Bellaterra, Spain), R. Quintanilla (IRTA, Caldes de Montbui, Spain), T. Figueiredo, A. Manunza (Center for Research in Agricultural Genomics, Bellaterra, Spain), J. L. Noguera (IRTA, Lleida, Spain), A. Clop (Center for Research in Agricultural Genomics (CRAG), Cerdanyola del Valles (Barcelona), Spain), and M. Amills (Center for Research in Agricultural Genomics, Bellaterra, Spain)

In the current work, we have analyzed the genomic architecture of carcass and meat quality traits in a Duroc commercial population of 350 individuals distributed in five half-sib families. The following carcass phenotypes were recorded: backfat thickness between third and fourth ribs (mm, BFT34R), backfat thickness at last rib (mm, BFTLR), lean meat percentage (LE%), carcass weight (kg, CW), and ham weight (kg, HW). With regard to meat quality traits, we measured in the gluteus medius (GM) and longissimus dorsi (LD) muscles the following parameters: electric conductivity (CE) and pH at 24 h (pH24) as well as lightness (L*), redness (a*) and yellowness (b*). Pigs were typed with the 60K Illumina Porcine BeadChip by using standard protocols. Single-SNP association analysis were performed under an additive genetic model with the Genome-wide Efficient Mixed-Model Association (GEMMA) software. The proportion of phenotypic variance explained by the SNPs ranged between negligible (L* at GM and LD) to moderate (HW, BFT34R, a* at GM and LD, and b* at LD), evidencing a considerable amount of missing heritability for some of these traits. We found genome-wide significant associations for LD CE on pig chromosomes (SSC) 4 (104 Mb), 5 (15 Mb) and 13 (137 Mb). We also identified chromosome-wide significant associations

for CW (SSC11), HW (SSC11), BFTLR (SSC12), BFT34R (SSC12), LE% (SSC13 and SSC16), GM CE (SSC5), LD pH24 (SSC16), GM pH24 (SSC17), LD a* (SSC10), GM a* (SSC3), GM L* (SSC16). In keeping with previous QTL results obtained in the same population, we observed different trait-associated regions for the GM and LD muscles indicating that meat quality traits are regulated, at least in part, by muscle-specific factors. Moreover, we compared our data with similar datasets obtained in other purebred pig populations and we observed a limited positional concordance among trait-associated regions, suggesting the existence of a high level of genetic heterogeneity for these phenotypes among porcine breeds.

Key Words: GWAS, pig, genetic heterogeneity

P5010 Optimization of a genomic breeding program for a moderately sized dairy cattle population. J. I. Weller (ARO, The Volcani Center, Bet Dagan, Israel), A. Reiner-Benaim, and E. Ezra (Israel Cattle breeders Association, Caesarea, Israel)

Although it is now standard practice to genotype thousands of female calves, genotyping of bull calves is still limited to progeny of elite cows. In addition to genotyping costs, increasing the pool of candidate sires requires purchase, isolation and identification of calves until selection decisions are made. We economically optimized via simulation a genomic breeding program for a population of ~120,000 milk-recorded cows, corresponding to the Israeli Holstein population. All 30,000 heifers and 60,000 older cows of parities 2–4 were potential bull dams. Animals were assumed to have genetic evaluations for a trait with heritability of 0.25 derived by an animal model evaluation of the population. A pseudo phenotype was generated, consisting of the animal's genetic value plus a residual with variance set to obtain the assumed reliability for each group of animals. Genetic values of founder heifers were simulated by sampling from a normal distribution with a mean of zero, and a variance of unity. Genetic values of older cows were generated with the same variance, but with means decreasing by 0.2 for each additional parity, to reflect the assumed genetic gain per year. Reliabilities increased with cow age. Forty founder sires were simulated in a similar manner assuming reliabilities of 0.9, and a mean genetic value of zero. From 4 to 20 bulls and from 200 to 27,000 cows with the highest pseudo phenotypes were selected as candidate bull parents. For all progeny of the founder animals, genetic values were simulated as the mean of the parental values plus a Mendelian sampling effect with a variance of 0.5. A probability of 0.3 for a healthy bull calf per mating, and a genomic reliability of 0.43 were assumed. The 40 bull calves with the highest genomic evaluations were selected for general service for 1 yr. Cost included genotyping of candidate bulls and their dames, purchase of the calves from the farmers and identification. Costs of raising culled calves were recovered by resale for beef. Annual costs dependent on the number of candidate bulls were estimated as: \$10,650 + \$300*candidate bulls. Annual profit per cow per genetic SD was \$106. Economic optimum with a discount rate of 5% and a profit horizon of 15 yr was obtained with 1650 to 1850 candidate bulls. Annual response to selection ranged from 0.4 to 0.35 genetic SD for 4 to 20 bull sires.

Key Words: genomic selection, economic optimization, dairy cattle breeding

P5011 Fine mapping the QTL for growth traits in outbred chicken advanced intercross lines by improved ddGBS. Y. Wang, X. Cao, X. Gu, and X. Hu (China Agricultural University, Beijing, China)

Deciphering the genetic architecture of complex traits is the key objective of animal science. Growth traits of broilers have significant economic value in the chicken industry. Because of the extensive linkage disequilibrium, QTL-mapping using F_2 populations or backcrosses can only obtain a large confidence interval. To generate a higher resolution QTL map, more recombination events and higher SNP marker density are needed. In this study, a nine-generation AIL pedigree was constructed from two Chinese chicken lines, Huiyang Beard chicken (slow-growing) and High Quality chicken Line A (fast-growing), with distinct growth traits. Growth traits were measured at hatching and every other week until 14 wk of age. We performed improved double-enzyme digestion genotyping by sequencing (ddGBS) method on 824 individuals, comprising 31 F_0 individuals, 191 F_8 animals, and 602 F_9 progeny. A total of 292k SNPs were identified by ddGBS according to the strict filter criteria and the SNPs were evenly distributed across the chicken genome. GWAS and linkage analysis in F_0–F_9 population showed that there was a 3-Mb major effect QTL interval that affects body weight at 2–14 wk of age, located at the distal end of chicken chromosome 1. Haplotype-sharing approach was used to refine the map position of the QTL. We genotyped 48 additional parental specific SNP markers in this region and confirmed their parental origin in F_9 individuals. IBD (Identical-by-descent) analysis identified four recombination breakpoints that divided the QTL interval into five haplotype blocks. Only one block showed significant body weight difference between two alleles and narrowed the major QTL down to a 600-kb interval. According to genome

annotations, several functional SNPs have nearly fixed differences between two groups of extreme phenotype individuals in F_9 generation. Motilin is a peptide hormone involved in gastrointestinal motility and motilin receptor (*MLNR*) is a special receptor for motilin. For motilin is a key regulatory hormones for appetite, we consider *MLNR* as a functional candidate gene for body weight. Meanwhile, we identified a narrow region of chicken chromosome 27 to be strongly associated with shank length at 6–12 wk of age. *PHOSPHO1* is a phosphoethanolamine/phosphocholine phosphatase and is involved in initiating bone matrix mineralization. It has been reported that Phospho1−/− mice displayed poor weight gain, growth plate and skeletal abnormalities, and smaller body size. Considering the important role of *PHOSPHO1* in initiating bone matrix mineralization, we identify *PHOSPHO1* as a candidate gene for shank length.

Key Words: chicken, growth-traits, fine-mapping

P5012 Integrative analysis of metabolomic, proteomic and genomic data to reveal functional pathways and candidate genes for drip loss in pigs.

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In genetic analyses new types of functional traits can be used to increase the information density between genes and phenotypes. The consideration of different omics levels, like protein and metabolite abundance allows us to elucidate the black box of phenotype expression and to identify potential candidate genes. Aim of this study was to integrate the data of the genome, proteome and metabolome to characterize underlying functional pathways, biochemical processes and corresponding candidate genes of the meat quality parameter drip loss in pigs. Generally, this trait is strongly influenced by environmental effects and therefore our hypothesis was that metabolites and proteins, significantly associated with drip loss, are more accurate and reliable phenotypes than drip loss itself. We applied an untargeted (holistic) metabolomics approach and a targeted protein profiling to determine and quantify the metabolite and protein profiles in *Musculus longissimus dorsi* (LD). Therefore, LD samples of 97 Duroc × Pietrain pigs were collected and phenotypes as well as genotypes were recorded. In total, 126 and 40 KEGG annotated metabolites and proteins were identified in the tissue samples. An enrichment analysis resulted in 206 detected pathways and 10 pathways showed a significant meaning for drip loss. Besides drip loss itself, 18 metabolites and four proteins that belong to the significant metabolic pathways, namely sphingolipid metabolism, pyruvate metabolism, glycolysis/gluconeogenesis and methane metabolism were analyzed as phenotypes within a genome-wide association study (GWAS). For drip loss, two proteins and 11 metabolites we detected in total 430 significantly associated genetic markers (SNPs) located on *Sus scrofa* chromosomes (SSC) 1, 2, 3, 4, 6, 7, 8, 10, 13, 14, 16, 17, and 18. The functional annotation of these SNPs allowed discovering promising genomic regions as well as potential candidate genes. As one interesting example, on SSC 18 the genes PTN, CREB3L2 and LRGUK were identified based on significant SNPs for muscle physiology associated with drip loss, protein phosphoglycerate mutase 2 (PGAM2) and metabolite glycine. These genes are involved in the homoeostasis of muscle energy metabolism and metabolic disorders. Because of the increased information density due to the consideration of proteome and metabolome, our results lead to the conclusion that a combined omics analysis has an advantage compared to 'classical' GWAS. Therefore, we expect that GWAS approaches based on metabolic traits induce a smaller percentage of false-positive results and contribute to identify reliable candidate genes.

Key Words: metabolomics, proteomics, drip loss

P5013 Evaluation of gene interactions affecting carcass yield and marbling in beef cattle.

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Genotype-specific management of beef cattle in feedlots has the potential to improve carcass uniformity. Gene variants affecting marbling include *LEPc.73C > T, ADH1Cc.-64T > C, TG5,* and *GALR2c.-199T > G* while those in *CRHc.22C > G, POMCc.288C > T, MC4Rc.856C > G* and *IGF2c.-292C > T* influence lean yield. The purpose of the current study was to assess combinations of marbling gene variants with those associated with lean yield. Gene variants were initially genotyped in 386 crossbred steers and evaluated for associations with carcass traits (hot carcass weight, average fat, grade fat and rib-eye area). The goal was to select a subset of variants to genotype in 2000 steers (1000 implanted and 1000 hormone free) with camera graded carcass data (vision grade USDA yield, vision grade marbling, rib-eye area and fat thickness). We selected seven gene variants to proceed with (*TG* was discontinued) as they either had an association

or were involved in gene interactions affecting a trait. Associations between gene variants with traits were made simpler due to the fact that some genotypes could be pooled, as least squares means (LSM) were not significantly different, indicating a dominant effect of one allele. Interestingly the mode of action of a gene changed depending on the trait. For example, in the implanted steers *GALR2* affected rib-eye area ($P = 0.002$) where it exhibited an additive effect (*TT* = 12.98 in^2, *TG* = 13.07 and *GG* = 13.47) however there was a dominant effect of the T allele for marbling ($P = 0.0001$; *TT/TG* = 397.83 and *GG* = 378.27) and fat ($P = 0.001$; *TT/TG* = 8.38 mm and *GG* = 7.31). This same association with marbling ($P < 0.0001$; *TG/TT* 463.52 mm and *GG* = 430.90) and fat ($P = 0.006$; *TT/TG* = 10.23 mm and *GG* = 9.14) was also observed in the hormone free steers where again the T allele showed dominance. Gene interactions affecting a trait were only observed in the hormone free steers: *LEPc.73C > T* and *IGF2c.-292C > T* with fat ($P = 0.05$) and a trend with marbling ($P = 0.07$); *MC4Rc.856C > G* and *POMCc.288C > T* with marbling ($P = 0.05$); and *GALR2c.-199T > G* and *POMCc.288C > T* with rib-eye area ($P = 0.03$). The ability to pool genotypes not only simplified the interactions, it resulted in a larger number of animals with combined genotypes. The gene SNP networks generated using EPISNP support the mode of action between gene variants. For example, the gene interaction that was a 3 by 2 was also determined to be Additive-Dominance. The gene SNP networks were affected by implant status of the animals.

Key Words: beef, gene interactions

P5014 Porcine β-casein: A new selection marker?.
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Genes encoding major milk proteins are used worldwide in marker assisted selection (MAS) in dairy species: α*S1-casein* in goats–associated with milk protein content and flavor; κ-*casein* and β-*lactoglobulin* in cattle–associated with milk quantity and quality, bovine β-*casein*–associated with human health, etc. Taking into consideration the fact that milk protein content varies widely within and between porcine breeds (highlighting the potential for selection) and the fact that the gene encoding the most abundant protein in sow milk, namely β-*casein*, is highly polymorphic, we critically reviewed the body of evidence supporting the testing of this marker for MAS. Several porcine β-*casein* mutations were identified, the most likely to impact milk protein content being a SNP involving an intron/exon junction (leading to alternative splicing, and the formation of new isoform) and a SNP in the gene's TATA box (leading to decreased promoter activity), with possible subsequent implications in piglet growth dynamics during the suckling period. Two relatively recent studies investigating associations between porcine β-*casein* polymorphisms and milk protein content were also identified. However, the methodology of the two studies and/or the polymorphisms investigated make the results difficult to interpret. In light of the recent interest toward such investigations, we conclude that porcine β-*casein* is a promising candidate for MAS, and that the two mutations mentioned here warrant further attention.

Key Words: *Sus scrofa*, *CSN2*, MAS

P5015 A genome-wide association study for quantitative trait loci of speed index in the racing line of Quarter Horses.
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With a smaller population size in Brazil, but of great economic representativeness, the racing line of the Quarter Horse breed is characterized by animals that can reach high speeds over short distances and within a short period of time. Although the athletic performance of horses is probably influenced by a large number of genes, few genetic variants have so far been related to this trait and this was done exclusively in Thoroughbred horses. In view of that the DNA polymorphisms effects on characteristics are intrinsic parameters of each line or breed in a given environment, the purpose of this research was to conduct a study of association between performance on races (Speed Index maximum–IS max) and genetic markers in Quarter Horses, using single-step genomic association study (ssGWAS). Phenotypic information from 345 horses were used, in which 112 were genotyped with 50k chip (Equine SNP50 BeadChip) and 233 with 70k (Equine SNP70 BeadChip). Exclusive markers of each panel were imputed in two ways through FImpute software, resulting in 345 genotyped animals and 59,549 SNPs. The rounds of ssGWAS were conducted through BLUPF90 family programs. The component PreGSf90 of the BLUPF90 program was used to prepare the genotypes, including quality control.

The PostGSf90 program was used to convert from GEBV to SNPs effects and to calculate the variance explained by 18 SNPs adjacent windows. The GEBVs were re-estimated using the matrix G based on the weight (D), which was recalculated at each iteration (two iterations were considered). The model used for the analysis included hippodrome (from 1 to 28) and sex as fixed effects, and the year of the race (from 1988 to 2015) and the age at the race (from 2 to 4 yr old) as co-variables. Eleven genomic regions were found explaining 23.3% of the characteristic variation, each one explaining more than 1%. These regions are located on the equine chromosomes 1 (2 regions), 4 (2), 11 (3), 15 (2), 18 (1), and 24 (1). The top 20 and 30 windows explained 29.99 and 35.57% of the trait variation, respectively. These results showed the absence of major genes controlling the performance in races in Quarter Horses, unlike the situation observed, for example, for the optimal racing distance in the Thoroughbred. Financial support: FAPESP.

Key Words: equine, performance, SNP

P5016 Golden milk: Increasing β-carotene content in developing countries: First step. F. Bertolini and M. F. Rothschild (Department of Animal Science, Iowa State University, Ames, IA)

Vitamin A is essential for human health and current intake levels in many developing countries such as India are considered to be too low due to malnutrition. Vitamin A deficiency can be addressed through delivery of foods that contain β-carotene which is then metabolized to vitamin A. Recently three key genes have been identified in cattle: *BCMO1* and *BC02*, which are involved in the cleavage of β-carotene to form vitamin A and *SCARB1* which is involved in cellular transport of β-carotene. Genetic variation in *B. taurus* has been shown to be associated with the amount of β-carotene in milk, increasing its content up to 80%. To investigate the genetic variation of the three genes among three species/subspecies commonly milked in India (*Bos taurus, Bos indicus, Bubalus bubalis*), Single Nucleotide Polymorphism (SNP) discovery was performed with the Next Generation Sequencing information provided by the Sequence Nucleotide archive and other output reads provided by collaborators. More than 200 animals belonging to the 3 different species and subspecies were investigated. *B. indicus* and *B. taurus* reads were both aligned against the same reference UMD3.1, due to the high genomic similarity and to the lack of a *B. inducus* reference genome. *B. bubalis* reads were aligned to the reference *MD_CASPUR_WB_2.0.* A preliminary filter step was applied for the *B. indicus* samples to discard all reads with mapping quality < 10. Then, the standard pipeline of Samtools or GATK was applied to call the variants in all the samples and only SNPs with SNP quality $\geq$ 30 in at least 1 animal (or at least 2 for *Bos taurus*) were considered. A total of 1443 SNPs were detected in the 3 genes for *B. Taurus*, 1659 for *B. indicus* and 2297 in *B. bubalis.* The number of SNPs in exonic regions (synonymous, missense, stop codon) detected were approximately 23 for each of the three species/subspecies, with nearly no overlap between them. This first list of SNPs, with particular attention for the SNPs in the exonic regions, will be used in a plan of genotyping thousands of Indian buffalo and cattle to confirm existing and find new associations with β-carotene production and develop genetic tools to select cattle and buffalo that will naturally increase the potential for increased β-carotene in milk. Funding for this project kindly provided by the Bill & Melinda Gates Foundation.

Key Words: β-carotene genes, cattle, buffalo

P5017 Identifying genetic regions to spring ewes to lamb out of season. C. J. Posbergh, M. L. Thonney, and H. J. Huson (Cornell University, Ithaca, NY)

Sheep are one of the few livestock species that are seasonally polyestrous therefore limiting lamb production to specific times of the year. However there are certain breeds, such as the Dorset and Polypay, which have been selected for the ability to lamb outside the traditional spring lambing period. Even within these breeds there is substantial variation in the ability of an ewe to lamb out of season in the U.S. Out of season lambing has a low estimated heritability of 0.08. Trait expression generally requires multiple years of breeding to determine both reproductive success and out of season success. Lastly, it is only expressed in females. These properties make selection progress difficult to achieve based on phenotype alone. For this study, Dorset and Dorset x Finn ewes were classified as Seasonal (controls) or Aseasonal (cases) based on their historical out of season breeding success. Success was defined as the percentage of times an ewe lambed divided by the number of times she was exposed to a ram. Seasonal success applied to lambings occurring from January through May while Aseasonal success applied to lambings occurring from August to November. All ewes had a seasonal reproductive success rate of 66% ensuring that overall reproductive success was not a limiting factor for Aseasonal potential. All ewes were exposed for out of season breeding at least 3 times. Ewes were defined as Aseasonal if their out of season lambing success rate was greater than 65%. Seasonal ewes were defined as ewes with an out of season lambing success

rate less than 35%. Individuals were genotyped using the Illumina Ovine Infinium HD SNP BeadChip providing 606,006 SNP markers spanning the genome for analysis. Fifty-four Individuals (26 cases and 28 controls) and 478,494 SNPs passed quality control measures for use in a genome-wide association study. The most significant SNPs (FDR $p < 0.05$) were discovered using EMMAX to correct for population stratification and a dominant inheritance model. These SNPs are located on chromosomes X, 25, 7, and 4. Potential candidate genes include *NREP,* involved with neuron function, *LRRN3,* involved with synapse assembly, and *IMMP2L,* involved with follicular development. This study provides preliminary data which is being followed up with additional Dorset and Polypay sheep for validation. It is the first step toward identifying major genes responsible for out of season lambing sheep and developing markers to assist selection.

Key Words: sheep, aseasonality, GWAS

P5018 Genome-wide association studies for dry-cured ham quality traits in Italian Large White and Italian Duroc pigs. L. Fontanesi, G. Schiavo (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), G. Galimberti (Department of Statistical Sciences "Paolo Fortunati", University of Bologna, Bologna, Italy), S. Bovo (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), F. Bertolini (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy; Department of Animal Science, Iowa State University, Ames, IA) M. Gallo (ANAS, Roma, Italy), V. Russo (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), and L. Buttazzoni (Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Roma, Italy)

Protected denomination of origin (PDO) dry cured hams, like Parma and San Daniele hams, are produced from the hind legs of heavy pigs, according to Consortia rules. Seasoning of the legs lasts at least 12 mo with the only addition of salt. This simple production technology cannot correct possible original defects of the green legs. A specific breeding program able to produce animals with the requested characteristics of the meat and legs is therefore needed. For this reason, two specific traits, linked to the quality of the final products, have been defined by ANAS and used in the selection programs of Italian heavy pigs: visible intermuscular fat (VIF) and ham weight loss at first salting (HWLFS). VIF is a subjective evaluation of the level of fat deposition between leg muscles. An excess in intermuscular fat negatively affects the acceptability of hams by consumers and depreciates the final product. HWLFS is correlated to total weight loss of the hams during the entire seasoning period. In this study we performed genome-wide association studies (GWAS) for these two traits in the Italian Large White and Italian Duroc pig breeds. About 1350 and 430 performance tested pigs of these two breeds respectively were genotyped by the PorcineSNP60 BeadChip. Results of the GWAS identified quite a large number of QTL regions affecting these traits in both breeds with poor overlapping between traits and breeds. These results suggest that VIF and HWLFS are mainly affected by different genetic factors supporting their combined use in the definition of a Total Merit Genetic Index in Italian heavy pigs.

Key Words: dry-cured ham, GWAS, meat quality

P5019 Identification of QTLs for the fatty acid composition in chicken. S. Jin (Chungnam National University, Daejeon, Korea), H. B. Park (NIAS, Rural Development Administration, Jeju, Korea), D. Seo, N. R. Choi (Chungnam National University, Daejeon, Korea), C. K. Yoo[3] (Division of Applied Life Science (BK21 program), Gyeongsang National University, Jinju, Korea), S. Jung (Chungnam National University, Daejeon, Korea), C. Jo (Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul, Korea), P. Manjula, S. H. Lee, and J. H. Lee (Chungnam National University, Daejeon, Korea)

The fatty acid composition is one of the important traits in meat because of its contribution to meat quality. In this study, the leg and breast meat samples from Korean native chicken (KNC) were separately used for the QTL analysis for fatty acid composition traits. A total of 18 traits were investigated from the meats at 20 wk of age in 595 F1 birds using 167 informative DNA markers on 26 autosomes. Using the CRI-MAP software, the map order and genetic distances were determined. The half-sib and full-sib QTL analyses were performed using GridQTL and SOLAR programs. The initial analysis showed that 30 QTLs (12 for the leg and 18 for the breast meat) were detected by the half-sib analysis and 7 QTLs were identified by the full-sib analysis. With further verification of the QTL regions and the possible candidate genes, this study can provide valuable information for the variations affecting fatty acid composition and eventually help for the selection of birds having favorable fatty acid composition to the customers.

Key Words: fatty acid composition, Korean native chicken, QTL

P5020 Gene networks driving meat quality and palatability of beef. R. Mateescu (University of Florida, Gainesville, FL), J. W. Buchanan (University of California-Davis, Davis, CA), D. J. Garrick, and J. M. Reecy (Iowa State University, Ames, IA)

Tenderness, juiciness and flavor are the major determinants of beef palatability and are often used to measure eating satisfaction. Eating satisfaction is of great interest to the beef industry as improving these traits should lead to increased beef demand. The objective of this study was to utilize partial correlation and information theory algorithm (PCIT) to analyze an input matrix with SNP data across multiple meat quality and palatability phenotypes to derive gene networks associated with these traits in Angus beef cattle. Samples of LM from 1720 Angus cattle were analyzed by a trained sensory panel for tenderness, juiciness, connective tissue and beef flavor intensity. Eight trained panelists evaluated samples in duplicate for overall tenderness, sustained juiciness and connective tissue using an 8-point scale and for cooked beef flavor intensity using a 3-point scale. The average score of all panelists for each animal was used in analysis. The Bayes-B statistical model was utilized to perform a genome-wide association study to estimate effects between 54K SNP genotypes and each phenotype. Additive SNP effects explained 28.6% of the variation in panel tenderness, 5% in juiciness, 22% in connective tissue, and 13.3% in beef intensity flavor. The phenotypic measures, pedigree information, and Illumina 54K SNP chip genotypes were utilized to derive an annotated gene network associated with beef palatability in 1720 Angus beef cattle. Posterior means of the effects were estimated for each of the 54K SNP and for the collective effects of all the SNP in every 1-Mb genomic window in terms of the proportion of genetic variance explained by the window. Partial correlations were used to identify correlated regions of the genome for that set of largest 1 Mb windows that explained up to 35% genetic variation in either palatability trait. SNP were allocated to windows based on the bovine UMD3.1 assembly. Results were used in conjunction with network scoring and visualization software to analyze correlated SNP across palatability phenotypes to identify SNP of significance. Networks derived from partial correlation analysis captured up to 67.9% of the genetic variance explained by all SNPs. Significant pathways implicated in meat quality through GO term enrichment analysis included proteolysis of cytoskeletal remodeling, cartilage biogenesis, membrane protein-cytoskeleton interactions, and signal transduction. Further investigations are needed to identify causal variants harbored within the identified genomic regions to create new opportunities for identification of animals with desirable eating quality attributes.

Key Words: gene networks, meat quality, beef

P5021 Indel polymorphism in 3‘-UTR of RXFP2 does not segregate with horns status in sheep breeds with a variable and/or sex-limited horns status. G. Lühken (Department of Animal Breeding and Genetics, Justus Liebig University, Gießen, Germany), S. Krebs (Laboratory for Functional Genome Analysis, Gene Center, Ludwig Maximilians University, Munich, Germany), S. Rothammer (Chair of Animal Genetics and Husbandry, Ludwig Maximilians University, Munich, Germany), J. D. Küpper (Department of Animal Breeding and Genetics, Justus Liebig University of Gießen, Gießen, Germany), B. Mioč (Department of Animal Science and Technology, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia), I. Russ (Tierzuchtforschung e.V. München, Grub, Germany), and I. Medugorac (Chair of Animal Genetics and Husbandry, Ludwig Maximilians University, Munich, Germany)

The inheritance mode of the horns status in sheep is more complicated than it may appear at first glance. It is influenced by sex, but also differs between breeds. Previous studies postulated that a 1.8-kb insertion within the 3‘-UTR of the relaxin/insulin-like family peptide receptor 2 gene (*RXFP2*) on sheep chromosome 10 is causing polledness in sheep.

We re-sequenced a region of about 250,000 bp covering the *RFXP2* gene locus and the flanking upstream region in a total of 24 sheep from 6 completely horned and 6 completely polled breeds. We identified the same indel polymorphism published before segregating perfectly with horns status in these breeds. After establishment of a multiplex PCR for efficient genotyping, we tested for the indel polymorphism in a total of 435 sheep with breed-specific or individually known horns status from 1) 17 completely polled breeds (208 sheep), 2) 8 completely horned breeds (84 sheep), 3) single or multiple crossings of completely polled with completely horned breeds (18 sheep), and 4) 9 breeds with sex-limited and/or variable horns status (125 sheep). The *RXFP2*3’-UTR indel polymorphism segregated perfectly in completely polled and completely horned breeds. A sex-dependent horns status of sheep heterozygous for the indel polymorphism was observed for completely polled breeds and for their crossings with completely horned breeds: Male sheep carrying the genotype del/ins were horned, whereas female sheep with this genotype were polled. However, one heterozygous multiple crossed ewe with horn rudiments disputes complete association. Unexpectedly, this segregation pattern was not or at least not completely reproducible in breeds with sex-limited and/or variable horns status. For the breeds Alpine Steinschaf

and Bavarian Forest, it can be summarized that the occurrence of the del/ins genotype on the one hand, and a scurs/small horns phenotype on the other hand showed no regular pattern. Moreover, our samples originating from even more southern European regions (Bovec sheep, Cres sheep, Travnička Pramenka and Walachenschaf) as well as from Africa (Dorper and Kamerun sheep) clearly disclaim causal relationship or segregation of the *RXFP2*3'-UTR indel polymorphism with horns status. In this group of sheep, we observed almost all combinations of indel genotypes, sex and phenotypes of horns status. Therefore we conclude that the 3'-UTR *RXFP2* indel polymorphism is not a useful marker for horns status in sheep breeds with sex-limited and/or variable horns status.

Key Words: sheep, horns status, horned, polled, insertion, deletion, indel, RXFP2, segregation

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P5022 Identification of genetic markers associated with feeding efficiency in fattening Holstein calves, using targeted sequence capture.

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Ecological and economic concerns drive the need to improve feed utilization by domestic animals. Residual Feed Intake (RFI) is one of the most acceptable measures for feed efficiency (FE). However, phenotyping RFI related traits is complex, expensive, and requires special equipment. Advances in marker technology allow the development of various DNA-based selection tools. To assimilate these technologies for the benefit of RFI-based selection, reliable phenotypic measures are prerequisite. In the current study, we used genomic DNA of individuals presenting RFI phenotypic consistency across different ages and diets (stages 1–3), for targeted sequencing of chromosomal regions associated with FE and RFI related traits. Forty-eight top single nucleotide polymorphisms (SNPs), significantly associated with at least one of three stages were identified. Eleven of these SNPs were harbored by the fatty acid binding protein 4 (*FABP4*). While ten significant SNPs found in *FABP4*, were common for stage 1 and stage 3, one SNP (FABP4_5; A < G substitution), in the promoter region of the gene, was significantly associated with all three stages. As the three stages reflect changing diets and ages with concomitant RFI phenotypic consistency, the above polymorphisms and in particular FABP4_5, might be considered possible markers for RFI-based selection for FE in the Holstein breed, following a larger scale validation.

Key Words: feed efficiency, RFI, targeted sequence capture, SNPs, *FABP4*, Holstein breed

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P5023 Searching imputed sequence for mutations influencing fatty acid composition of beef fat.

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Dietary fatty acids that constitute a large proportion of bad cholesterol in humans are mainly derived from fatty components of milk and beef with genetic selection providing a pathway for reduction. This study investigated imputed genomic sequence to identify a candidate causal mutation influencing Pentadecanoic acid (C15:0). C15:0 represents less than 1% of fatty acids found in beef and is investigated as a model region due to a strong GWAS signal not previously reported. The animal population was a designed backcross experiment with 406 heifers and steers born in 1996–1997 with phenotypes, sired by three Jersey (J) x Limousin (L) bulls out of J and L dams. Animals were raised on pasture and slaughtered at 22–28 mo with subcutaneous fat from over the *longissimus dorsi* muscle used to extract nine fatty acids which were presented as a percentage of total fatty acids including C15:0. A GWAS with the Illumina BovineHD SNP array identified SNP 2:5601419 as the most significant. A surrounding ~200kb region (2:5500178–5698040) was investigated containing 61 SNP from the BovineHD panel. Sequence data were imputed for 350 animals (Fimpute software) in this region using reference sequence from 103 bulls acquired from the 1000 bull genomes project including 66 J, 35 L and 2 of the JxL sires from this population. Genotype concordance rate was 96% for this ~200kb region for the 2 sires with sequence data. GWAS was performed using 242 progeny of the two sequenced sires with 664 remaining SNPs after removing 2612 loci that were homozygous. The five most significant SNPs were 2:5601419 (FDR = 9.20E-08), 2:5604335 (FDR = 1.31E-07), 2:5596919 (FDR = 1.38E-07), 2:5575950 (FDR = 1.53E-07) and 2:5565799 (FDR = 1.84E-07) with the first, second and third SNPs overlapping with the BovineHD panel. Although a large proportion of the 664 SNPs were significant ($P <$

0.001) in the GWAS, when the GWAS was run with a fixed adjustment for 2:5601419 genotype, there were no remaining significant ($P < 0.001$) SNPs, indicating that this SNP is accounting for the genetic control of C15:0 in this region. The SNP 2:5601419 is within the coding sequence of gene A7MB61_BOVIN. With the top 3 SNPs all overlapping with the BovineHD panel, the imputation to sequence did not reveal any additional variants of interest. The ability of imputed sequence to identify new mutations causing variation in meat quality traits in this population requires further study, including more regions with better sequence coverage of all 3 sire families.

Key Words: genetic, selection, genomic

P5024 Investigating the molecular regulation and control of spawning performance in domesticated *Penaeus monodon* broodstock. J. Goodall (The University of Queensland, St Lucia, Australia; CSIRO, Integrated Sustainable Aquaculture Production, St Lucia, Australia) N. Botwright, N. Wade (CSIRO, Integrated Sustainable Aquaculture Production, St Lucia, Australia), D. Merritt (The University of Queensland, St Lucia, Australia), G. Coman, and M. Sellars (CSIRO, Integrated Sustainable Aquaculture Production, St Lucia, Australia)

The Giant Tiger Prawn, *Penaeus monodon*, represents an important global food resource, however captive rearing and domestication remains problematic as significant reproductive performance issues exist within selectively bred stocks. Although production from captive reared populations has been successful, nauplii production has not yet reached a level where it can sustain aquaculture demand; thus wild-type stocks are the industries preferred source of seedstock. Previous work has established that current broodstock conditioning practices do not appear to be negatively impacting the reproductive performance of male stocks; however requirements for high performance female conditioning remain unknown. To better understand the mechanisms governing spawning performance in female *P. monodon* broodstock, we used Illumina RNA-seq to examine the differential gene expression profile of ovarian tissue across multiple spawning events. Analyses of the variations in spawning performance with particular emphasis on gene pathways related to lipid metabolism and regulatory control of prostaglandins, lipid compounds with significant hormone like control of maturation and ooctye development will be discussed.

Key Words: Giant Tiger Prawn, *Penaeus monodon*, domestication, reproduction, prostaglandin

P5025 Production of chickens with high body weights, low amounts of abdominal fat, and a high thigh meat yield using DNA microsatellite marker-assisted selection. K. Tatsuda (Hyogo Prefectural Institute of Agriculture, Forestry and Fisheries, Kasai, Japan)

We developed a special chicken called the "Hyogo-Ajidori" (HA) via a 3-way cross between a White Plymouth Rock and a Hyogo, which was itself produced through a 2-way cross between a Satsumadori (SD) male and a Nagoya female 25 yr ago. Initially, the productive performance of the HA was very poor. Its body weight (BW) at 16 wk of age was 2.8 kg, its abdominal fat weight to live body weight ratio (%, AFR) was 7.6%, and its thigh meat weight to live body weight ratio (%, TMR) was 20%. To improve the performance of the HA, we performed a quantitative trait locus (QTL) analysis of BW and the AFR, and performed association tests between marker alleles and the TMR. A QTL affecting BW was mapped at 123 cM on chromosome 1. The closest locus to the QTL was ADL0019 (alleles: A, B, C, and D). In SD males, the DD genotype resulted in a higher BW (1.92 kg) than the CC genotype (1.66 kg) ($P = 0.06$). A QTL that influences abdominal fat deposition was mapped at 147 cM on chromosome 7. The closest loci to the QTL were MCW0316 (alleles: A and B) and ADL0169 (alleles: A and B). For both markers, the AFR of birds with 4 A alleles was 41% higher than that of birds with no A alleles. Association tests between 104 marker alleles and the TMR indicated that the alleles of the markers ADL0019 (alleles: A, B, and C) and LEI0068 (alleles: A and B) were significantly associated with the TMR. Regarding ADL0019, the A allele was associated with a higher TMR ($p = 0.014$). As for LEI0068, the A allele was associated with a higher TMR ($p = 0.044$). The TMR of HA chickens that exhibited AA genotypes for both ADL0019 and LEI0068 was 24.8%. These results show that it is possible to simultaneously perform DNA microsatellite marker-assisted selection for three traits (BW, the AFR, and the TMR) in HA chickens. We produced HA chickens based on the above marker data. At the present time, the HA chickens are 14 wk of age, and their BW, AFR, and TMR are 3.9 kg, 5.4%, and 25%, respectively.

Key Words: chicken, DNA microsatellite marker-assisted selection, productive performance

P5026 Genetic analysis of conformation traits in Icelandic horses. K. Jäderkvist Fegraeus, M. Shrestha (Department of Animal Breeding and Genetics, Swedish University of Agricultural

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Originating from Iceland, the Icelandic horse breed has been bred without any introduction of foreign genetic material since 900 AD. These small, robust horses are typically known for their gaits tölt and pace. However, within the breed, estimated breeding values (EBVs) are used to aid in selecting the best animals not only based on gaits, but also on conformation. With the aim to investigate the genetic background of conformation in Icelandic horses, a genome-wide association study was performed using EBVs for the different conformation traits head, neck, withers and shoulders, back and croup, proportions and harmony, leg quality, mane and tail, gracefulness, leg correctness and hooves, in 349 Icelandic horses. The EBVs were obtained from the Icelandic horse studbook Worldfengur. The horses were genotyped on the 50K SNP chip (n = 209) and the 70K SNP chip (n = 140), and after quality control 33 577 SNPs remained. The data were analyzed using a mixed model method in the GenABEL package in R. A significant association for a SNP on chromosome 20 ($p = 5.5\text{x}10^{-7}$) and a borderline significant association for a SNP on chromosome 4 ($p = 8.60\text{x}10^{-6}$) was observed for the trait "head" ($\lambda = 1.01$) with a Bonferroni significance level of $1.49\text{x}10^{-6}$. The results from this study indicate that there are genomic regions that are significantly associated with head conformation in Icelandic horses. We are currently genotyping an additional 300 Icelandic horse samples for the significant marker on chromosome 20 and the borderline significant marker on chromosome 4. The additional results from the aforementioned genotyping will also be presented at the meeting.

Key Words: genomic, EBV, selection

P5027 Transcriptome profiling of Arabian horse blood tissue during training regime using Next Generation Sequencing method. K. Ropka-Molik (National Research Institute of Animal Production, Balice, Poland), M. Stefaniuk-Szmukier (Department of Horse Breeding, Institute of Animal Science, University of Agriculture in Cracow, Cracow, Poland), K. Zukowski, K. Piorkowska, and A. Gurgul (National Research Institute of Animal Production, Balice, Poland)

In a horse, intensive training initiates the long-term adaptation processes which are involved in establishment of a new homeostasis of the body. One of the most important adaptive responses to exercise are changes in gene expression responsible for metabolism modification, cell cycle progression and finally resulting in a return to homeostasis. Thus, the aim of our research was the identification of the genetic basis of changes occur in blood of Arabian horses during a training regime which can pinpoint the main metabolic pathways related with adaptation to exercise and will be associated with athletic performance. Whole transcriptome profiling of blood was performed for 6 untrained horses (2,5 yr old) and 12 horses from which samples were collected during 3 different periods of training procedure (T_1–during intense training period, March; T_2–before racing season, May; and T_3–after flat racing season, October). In total, 42 blood samples were analyzed using RNA-seq approach. All horses were entered into the accounting Polish Arabian Stud Book (PASB). The RNA sequencing was performed with 75 single-end cycles on HiScanSQ platform (Illumina). The raw data were qualitatively and quantitatively evaluated, and trimmed by using FastQC and Flexbar software. The RSEM with combination of STAR aligner were used to quantify read counts and next, differentially expressed genes (DEG) were determined using Deseq2 software. The highest number of significant differentially expressed genes (FDR < 0.05; fold change < 1.5) was identified between untrained horses (C) and horses in last training phase (T_3)–520 DEGs. During training period, the amount of DEG between subsequent phases increased (T_1:T_2 −97; T_2:T_3 −356; T_3:C- 520), while between untrained horses and horses at the beginning of the training (C:T_1) only 25 gene were differentially expressed. The most abundant exercise upregulated transcript were genes involved in pathways important in regulating the cell cycle (PI3K-Akt signaling pathway), signaling pathway regulating pluripotency of stem cells and regulation of actin cytoskeleton as well as pathway regulated gluconeogenesis (FoxO signaling pathway), glycerophospholipid metabolism and calcium signaling. Our research confirmed changes in gene expression profiles in response to an increase of training intensity. The observed transcriptional activation of genes such as: *LPGAT1, AGPAT5, GPD2, FOXN2, FOXO3, ACVR1B* and *ACVR2A* can be a base for further research to identify genes potentially associated with race performance in Arabian horses. The study was supported by the Polish Ministry of Science and Higher Education (project

no. 2014/15/D/NZ9/05256).

Key Words: Arabian horse, training, RNA-seq.

P5028 A genome-wide association study for growth rate in commercial pigs. J. Horodyska (Teagasc Food Research Centre, Dublin, Ireland; Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany) R. M. Hamill (Teagasc Food Research Centre, Dublin, Ireland), H. Reyer (Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany), P. Varley (Hermitage Genetics, Kilkenny, Ireland), and K. Wimmers (Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany)

Growth and related traits (e.g., average daily gain) are of main interest in pig breeding ensuring progress toward a reduced number of days to market. Hence, the objective of this study was to identify genomic regions associated with growth rates in pigs represented by the trait 'Days to 110 kg (D110)'. A total of 952 commercial line Maxgro boars (Hermitage Genetics) were genotyped using Illumina Porcine SNP60 BeadChips. After quality control, the remaining 51,661 SNPs were tested for an association with estimated breeding values (EBV) for D110. In total 124 SNPs reached the threshold of suggestive significance (p-value $\leq 5 \times 10^{-5}$) for an association with EBV of D110. The largest number of associated SNPs was located on SSC1 (16 SNPs), SSC4 (15 SNPs), SSC3 and 15 (14 SNPs), followed by SSC13 (12 SNPs), and SSC5, 10, and 11 (6 SNPs). Moreover, 12 of these SNPs mapping to 7 porcine autosomes crossed the Bonferroni-adjusted genome-wide significance threshold (p-value $\leq 1 \times 10^{-6}$). A list of positional candidate genes, closest to significantly associated SNPs, was created allowing a maximum distance of 1 Mb between the marker and genes. Subsequently, the list of genes was used to identify enriched pathways and biological functions for D110. Most promising QTLs were detected on SSC 10 and 15. On SSC10, association analyses revealed *AKR1C3* as the positional candidate gene. On SSC15, 5 SNPs reaching the genome-wide significance were located within a 682 Kb segment between 2.64 and 3.32 Mb. Three of these SNPs mapped within intronic regions of *KIF5C*. The two remaining SNPs were located next to *MBD5* and *LYPD6B*. A number of positional candidate genes for EBV of D110 (AKR1C3, MBD5, ACVR2A, AKR1C1/AKR1C2 and AKR1C4) were clustered in an endocrine system function and development category. Additionally, these genes were significantly overrepresented in lipid metabolism and energy production. In summary, the present study demonstrated a number of chromosomal regions significantly associated with EBV for D110 in pigs. The identified positional candidate genes indicate for a complex functional basis of growth traits comprising endocrine regulations, resource allocation and cellular metabolism.

Key Words: GWAS, growth rate, pig

P5029 The use of Bayesian methods, biological priors and sequence variants to identify genomic regions associated with dairy cow fertility. D. C. Purfield (Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland), I. M. MacLeod, B. J. Hayes (Department of Economic Development, Jobs, Transport and Resources, Bundoora, Australia), S. Butler (Animal & Grassland Research and Innovation Centre, Teagasc Moorepark, Fermoy, Co. Cork, Ireland), S. G. Moore (University of Missouri, Columbia, MO), B. Moran (Animal & Grassland Research and Innovation Centre,Teagasc, Grange, Dunsany, Co. Meath, Ireland), F. Kearney (Irish Cattle Breeding Federation, Bandon, Co. Cork, Ireland), and D. P. Berry (Teagasc, Moorepark, Fermoy, Co. Cork, Ireland)

Genetic improvement for bovine female fertility has been hampered by antagonistic correlations with milk production and low accuracy genetic evaluations. An adaptation of BayesR, known as BayesRC, incorporates biological prior information such as gene expression data and genome annotation such as non-synonymous coding, to improve the accuracy of genomic predictions and associations. The present study compares the accuracy of BayesR and BayesRC for female fertility using high density (HD) SNPs and imputed sequence variants in a total of 4265 Holstein-Friesian bulls. Imputed whole genome sequence data were available on all animals. To reduce the number of variants for analysis, edits were applied including removing rare variants (minor allele frequency < 0.0005), intronic/intergenic variants and retaining only one variant of any pair of variants in strong LD ($r^2$of > 0.9); preference was given to non-synonymous coding variants, variants in regulatory regions and finally variants on the Bovine HD beadchip. After edits, 873,192 variants remained. To quantify the impact on genomic predictions of using sequence variants, a HD dataset of 660,255 SNPs was retained for each animal. Deregressed predicted transmitting abilities for calving interval were available and only sires with > 30% reliability, following the removal of parental contribution, were retained. BayesRC uses the same approach as BayesR except prior biological information is used

to allocate each variant to specific classes used in the analysis. Classes were defined based on variant annotation and candidate genes associated with female fertility. Candidate genes were identified from two sources; 1) 575 genes previously reported to be differentially expressed in the bovine endometrium and corpus luteum and 2) 1097 genes with fertility-related annotation or whose mutations are known to cause reproductive defects in female mice. The validation set ($N = 499$) were the most genetically distinct sires identified from principal component analysis, ensuring none were sires/sons of the training set. The accuracy of genomic prediction, defined as the correlation (adjusted for heritability) between predicted genetic values and phenotypes, with BayesR increased from 0.39 using HD variants to 0.43 with sequence variants. A similar accuracy of 0.41 using sequence variants was also obtained for BayesRC. This increase in accuracy from the sequence variants may be due to the inclusion of causal mutations and rare variants which may be in LD with other causal mutations. It is expected that this analysis could benefit from the inclusion of more phenotypes, and more bovine specific biological information for BayesRC.

Key Words: fertility, genomic prediction

P5030 Dietary carotenoid levels and stearoyl-coA haplotype exert a complementary action over fat content and composition in pig. R. N. Pena, E. Henríquez-Rodríguez, A. R. Seradj, M. Tor (University of Lleida-Agrotenio Center, Lleida, Spain), P. Christou (University of Lleida-Agrotenio Center, Lleida, Spain; ICREA, Barcelona, Spain), and J. Estany (University of Lleida-Agrotenio Center, Lleida, Spain)

In fattening pigs, vitamin A restriction promotes deposition of monounsaturated fat, both in muscle and backfat, without any clear effect on total fat content. In past experiments we identified a haplotype group (H1) in the stearoyl-coA desaturase (*SCD*) promoter associated with increased subcutaneous and intramuscular monounsaturated fat with no effect on total fat content. One of the mutations of this *SCD* haplotype affects a potential binding site for transcription factors responding to retinoic acid. In follow up experiments, we investigated the interaction between these dietary and genetic factors in growing pigs. Thirty two castrated Duroc pigs (ca: 100 kg live weight) reared previously under a standard commercial system were distributed in a 2 × 2 factorial design considering (i) two concentrates (free of synthetic vitamin A) containing 25% of a carotenoid-enriched (*Carolight*^{R}) or carotenoid-depleted corn (wild-type near isogenic line) and (ii) two *SCD* haplotypes (H1H1 and H2H2). After slaughter (at 120 kg live weight) fat content and composition were measured in *longissimus dorsi*, *gluteus medius*, liver and subcutaneous fat. As expected, the *SCD* haplotype did not affect the fat content of the tissues. In contrast, the restriction of dietary carotenoids increased fat content in liver ($P < 0.05$) and *gluteus medius* ($P < 0.01$), but did not affect backfat thickness. In muscle, fat composition was more influenced by the *SCD* haplotype than the diet. In particular, monounsaturated fat content, which is considered a healthier fat type, was higher in H1H1 pigs ($P < 0.01$) at the expense of saturated fat content ($P < 0.05$). The effect of the *SCD* haplotype was also evident in backfat and liver. In addition, fat composition was influenced by the dietary carotenoid content, particularly in back fat and liver. The carotenoid-rich diet significantly increased monounsaturated fat content at the expense of polyunsaturated fat in back fat ($P < 0.05$) while the complete opposite effect was detected in liver ($P < 0.001$). In agreement with the above, *SCD* expression was enhanced by the carotenoid-rich diet in back fat and liver and repressed in the *longissimus dorsi* and *gluteus medius* muscles. We are currently assaying the expression of retinol-activated transcription factors and retinol transmembrane transporters in these tissues. In conclusion, dietary carotenoid restriction and SCD haplotypes could be combined to effectively modify pork fat composition without undesirable back fat deposition.

Key Words: oleic acid, fat composition, vitamin A restriction

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P5031 Runs of homozygosity highlight candidate genes and biological pathways related to athletic performance in Alaskan sled dogs. H. J. Huson, A. Valenti, and A. Boyko (Cornell University, Ithaca, NY)

Modern Alaskan sled dogs illustrate the development of a unique working dog breed and their genetic selection for athletic attributes. For over 200 yr, Alaskan sled dogs have been bred to perform at extreme levels physiologically and mentally, and to do so within harsh arctic conditions. Our previous research established that Alaskan sled dogs are a distinct genetic breed selected solely on their performance and investigated the role of various ancestral breeds. Current research exploring signatures of selection identifies genomic regions and biological pathways likely contributing to athletic ability. 325 dogs were genotyped on the Illumina Canine High-density beadchip using 113,113 single-nucleotide polymorphisms. Signatures of selection were identified using marker based F_{ST}, ROH, and genome-wide association tests. Alaskan sled dogs ($n =$

158) were compared to ten purebred breeds (n = 167) identified through either pedigree or genetic research as representing breeds admixed with Alaskan sled dogs. This analysis approach was aimed at identifying selection unique to Alaskan sled dogs. In addition, Alaskan sled dogs were partitioned into two groups based on the competitive racing style in which they perform. "*Distance*" runners (n = 65) compete in events traversing ~1,000 miles at an average speed of 8–12 mph. "*Sprint*" runners (n = 93) compete in events traversing 6–30 miles at an average speed of 18–25mph. Genetic population structure reflects these racing styles which have selected dogs for either endurance or speed reflectively. Comparison of Sprint and Distance dogs aimed at identifying selection reflective of these racing styles and possibly influencing traits of endurance and speed. F_{ST}results identified population informative markers on chromosome 11 comparing Alaskan sled dogs to the purebred breeds and on chromosomes 1, 18, 20, 28, and 29 distinguishing Sprint and Distance dogs. ROH were determined within all individuals and numeric association tests were run to identify differential ROH patterns among the populations. Principle component analysis was used to correct for population stratification in the association tests which identified over 91 associated ROH patterns (FDR < 0.05). PANTHER Gene ontology software was used to explore biological significance of genes located within these associated ROH regions and highlighted pathways relating to locomotion, blood circulation, muscle contraction/development, skeletal and nervous system development, response to external stimuli, and metabolism. An additional 116 Alaskan sled dogs have recently been genotyped and are being added to the original dataset of 325 dogs to validate the current findings and explore candidate genes.

Key Words: runs of homozygosity, Alaskan sled dogs, athletic performance

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P5032 A genome-wide association study of young horse test traits in Swedish Warmblood.

S. Eriksson, Å. Viklund, and S. Mikko (Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden)

Tests of 3-yr-old Swedish Warmblood (SWB) horses have traditionally used subjective assessments on a ten point scale of the quality of the horses in relation to the breeding goal. In 2013 a complementary linear scoring of the horses on a nine point scale from one extreme to the other was introduced. The horses are assessed for traits related to conformation, gaits and jumping ability. Also, for some traits, deviations from normality are indicated as 0/1. In this study we aimed to find genomic regions associated with these test traits. In total, 380 SWB horses tested as 3-yr-olds in 2013 or 2014, were genotyped using the Affymetrix 670K SNP-chip. After quality control the data set included 379 horses and 467,606 SNPs. A fast score test for association in the R software GenAbel was used for a first set of analyses of 97 traits. The uncorrected lambda value was > 1 for most traits so a genomic control was used where the model included year and place of the test as well as sex of the horse. Preliminary results validate findings in other horse breeds of a region on ECA3 highly significant for height at withers. SNP-associations significant after Bonferroni correction were also found for traditionally scored type of the horse, and linear measures of type (light–heavy built). Because the data were particularly stratified for jumping traits, analyses were also done within clusters of genomic relationship. Within one of the two clusters, significant associations were also found for jumping technique. Suggestive significant associations were found for several traits and continued analyses including polygenic effects are in the pipeline.

Key Words: horse, linear scoring, GWAS, performance traits

P5033 A GWAS of teat number in pigs.

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Number of functional teats is an important trait in commercial swine production. As litter size continues to increase, the number of teats must also increase to supply nutrition to all piglets. The pig displays considerable variation for number of teats; therefore, a genome-wide association (GWA) analysis was conducted to identify genomic regions that affect this trait in a commercial swine population. Genotypic data from the Illumina Porcine SNP60v1 BeadChip were available for 2951 animals with total teat number (TTN) recorded. A subset of these animals (n = 1828) had number of teats on each side recorded. From this information, the following traits were derived: number of teats on the left side (LTN), number of teats on the right side (RTN), the maximum number of teats on a side (MAX), the difference in LTN–RTN (L-R) and the absolute value of L-R (DIF). After data editing, 41,148 SNP markers were included in the analysis implementing the Bayes C option of GENSEL (version 4.61) and 1 Mb windows. Fixed effects fitted in the model were season of birth and a regression coefficient for the number of copies of the ancestral vertnin allele. Marker heritabilities were highest for TTN

(0.233), intermediate for individual side counts (0.088 to 0.115) and virtually nil for difference traits (0.002 for L-R and 0.006 for DIF). Each copy of the mutant vertnin allele increased teat count by 0.35 (TTN), 0.16 (LTN and RTN) and 0.19 (MAX). The number of 1 Mb windows explaining more than 1% of the genomic variation detected was 15 for TTN, 18 for LTN, 13 for RTN and 18 for MAX. These regions cumulatively accounted for over 50% of the genomic variation of LTN, RTN and MAX, while only explaining 30% of the genomic variation of TTN. Ten 1 Mb windows were associated with more than one trait. Most notable was SSC 10:52 Mb which was associated with all four traits, while SSC 10:60 Mb and SSC 14:54 Mb were associated with three of the four traits. Further research on these regions should yield markers that could be used to increase the number of functional teats in commercial pigs.

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Key Words: GWAS, teat number, pigs, swine

P5034 Differential proportion of ancestral MHC haplotypes in Brangus breed. D. Goszczynski, C. Corbi, H. Morales, D. Posik, E. Villegas Castagnasso (IGEVET–Instituto de Genetica Veterinaria Ing. Fernando Noel Dulout (UNLP-CONICET La Plata), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Argentina), S. Munilla, P. Peral García (IGEVET–Instituto de Genetica Veterinaria Ing. Fernando Noel Dulout (UNLP-CONICET La Plata), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Argentina), A. Rogberg (IGEVET–Instituto de Genetica Veterinaria Ing. Fernando Noel Dulout (UNLP-CONICET La Plata), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Argentina; Departamento de Produccion, Facultad de Agronomia, Universidad de Buenos Aires, Buenos Aires, Argentina) R. J. C. Cantet (Departamento de Produccion, Facultad de Agronomia, Universidad de Buenos Aires, Buenos Aires, Argentina; INPA–Unidad Ejecutora UBA-CONICET de Investigaciones en Produccion Animal, Buenos Aires, Argentina), and G. Giovambattista (IGEVET–Instituto de Genetica Veterinaria Ing. Fernando Noel Dulout (UNLP-CONICET La Plata), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Argentina)

Brangus breed was developed to use the superior characteristics of both founder breeds. The breed maintains the high adaptability to tropical and sub-tropical environments, disease resistance and rusticity from Zebu cattle, and the high reproductive capability, production and meat quality traits from Angus. It has been studied that the major histocompatibility complex (MHC), located on BTA23, encodes genes implicated in the adaptive immune response, and may be responsible for the adaptation to those environments. The objectives of this work were to study the MHC ancestral haplotypes in a Brangussample to detect the breed of origin (Angus or Brahman) of each genotype, and then determine if there is a divergence from the overall genomic proportion. For this, a total of 169 animals (99 Brangus, 48 Angus and 22 Brahman) were genotyped using Affymetrix BOS1 (640K) Chip. Structure software v2.3.4 was used to estimate the whole genome fraction of each founding breed that remained in the sampled Brangus animals, with a subset of 5K SNPs evenly spaced over the 29 autosomes. The SNPs included in the MHC BTA23 region (5585 SNPs within 7013,913–28,998,760 bp) were primarily phased with ShapeIT2 algorithm, for further haplotype origin assessment with LAMP-LD package. Two other regions demonstrated for coat color (MC1R gene on BTA18: 1682,065–2,046,164bp) and polled trait (BTA1: 14,038,121–14,991,286bp) were selected for Proof of Concept, as those traits were selected in Brangus for the Angus phenotype (solid coat color and polled). The result obtained for the whole genome composition of Brangus was 34.7% Brahman, ranging from 22.3% to 81.1%, while for the MHC region, the haplotypes appear to have originated from Brahman in 55.3% of the chromosomes. As expected, the Proof of Control regions showed that the Angus haplotypes were nearly fixed, which supports the hypothesis that the divergence of the haplotypes of the MHC region may have originated in a selection process to promote adaptation to tropical and sub-tropical environments.

Key Words: MHC, Brangus, ancestral haplotype, selection

P5035 Fine mapping of a distal chromosome 4 QTL affecting growth and muscle mass in a chicken advanced intercross line. S. Lyu, D. Arends (Albrecht Daniel Thaer-Institut for Agricultural and Horticultural Sciences, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Berlin, Germany), M. K. Nassar (Albrecht Daniel Thaer-Institut for Agricultural and Horticultural Sciences, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Berlin, Germany; Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt), and G. A. Brockmann (Albrecht Daniel Thaer-Institut

for Agricultural and Horticultural Sciences, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Berlin, Germany)

Previously, a genome-wide quantitative trait loci (QTL) analysis in an F_2 cross between the inbred lines New Hampshire (NHI) and White Leghorn (WL77) revealed a growth QTL in the distal part of chromosome 4 (from 61.5 to 88.4 Mb, Nassar et al. 2015, *Journal of Animal Genetics, 46: 441–446*). In this study, we performed fine mapping to physically reduce the chromosomal interval and the number of potential candidate genes. An advanced intercross line (AIL) has been established from the initial F_2 mapping population, in which we used generations F_{10} to F_{12}. Nine single nucleotide polymorphism (SNP) markers within the QTL confidence interval region were selected to perform an association analysis with several growth traits from hatch to 20 wk and body composition traits at 20 wk. The confidence interval of the QTL has been reduced from 26.9 to 3.4 Mb. Within the fine mapped region, markers *rs14490774*, *rs314961352* and *rs318175270* were in full linkage disequilibrium (D' = 1.0) and showed the strongest effect on growth and muscle mass (LOD $\geq$ 4). This reduced region contains 30 genes, compared to 292 genes in the original region. Chicken 60K and 600K SNP chips combined with DNA sequencing of the parental lines were used to call mutations in the reduced region. In the narrowed-down region 489 sequence variants were detected between NHI and WL77. The most deleterious variants are a missense variant in *ADGRA3* (SIFT = 0.02) and a frameshift deletion in the functional unknown gene *ENSGALG00000014401* in NHI chicken. In addition, five synonymous variants were discovered in the genes *PPARGC1A*, *ADGRA3*, *PACRGL*, *SLIT2* and *FAM184B*. In our study, the confidence interval and the number of potential genes were reduced 8- and 10-fold, respectively. Further research will focus on functional effects of mutant genes.

Key Words: chicken, advanced intercross line, fine mapping, sequence variants, growth, bone mass, muscle, GGA4

P5036 Selection signatures in commercial Duroc pig populations revealed by high density SNP chip. K. S. Kim, Z. Edea (Chungbuk National University, Cheongju, Korea), J. K. Hong (National Institute of Animal Science, Cheonan, Korea), Y. C. Jung (Jung P&C Institute, Yongin, Korea), E. S. Kim (Recombinetics, Saint Paul, MN), and M. F. Rothschild (Department of Animal Science, Iowa State University, Ames, IA)

Duroc breed is known for its superior performance in terms of growth, carcass and meat quality traits. The breed is imported into South Korea for commercial production and has been under selection for economic traits during the last decades. However, selection signatures in Korean Duroc populations have not been yet investigated at the genome level. In this study, we detected selection signatures in the genome of four commercial Duroc populations (n = 488) and crossbred pigs (Duroc x Korean native pig, n = 155) sampled from five pig farms and genotyped using 50,862 autosomal markers. By applying the F_{ST} and extended haplotype homozygosity (EHH-*Rsb*) methods, we screened the highly differentiated SNPs and associated genes. These include known growth associated genes (*DOCK7, PLCB4, HS2ST1*, *FBP2* and *TG*), carcass and meat quality genes (*ANXA13*,*TG, COL14A, FBXO5, NR3C1, TG, SNX7, ARHGAP26* and *DPYD*), ear morphology gene (*SOX5*) and immune response genes (*CADM1, IL18* and *INPP5D*) most of which are near or at fixation. The detection of genes related to production traits substantiate the analyses that pigs have been subjected to strong human mediated selection following domestication. These results can serve as a basis to investigate the underlying mutations associated with observed phenotypic variation in the genome of Duroc breed. Validation via genome-wide association analysis will also facilitate the inclusion of some of these markers in breeding programs.

Key Words: Duroc pigs, genome-wide, selection signatures

P5037 Targeted-enrichment next generation sequencing in the estimation of QTL variants associated with meat quality on SSC15 in pigs. K. Piórkowska, K. Zukowski, K. Ropka-Molik, and A. Gurgul (National Research Institute of Animal Production, Balice, Poland)

The chromosome region on SCC15 in pigs between microsatellites SW1683 and SW906 (79.3–89.3 cM) is rich in quantitative traits loci (QTLs) associated with meat quality such as juiciness, meat color, pH, cooking and drip loss, firmness, shear force, thawing loss and an intramuscular fat. The goal of the study was to identify the genetic markers on chromosome 15 in pigs, which could be helpful in breeding programs leading to faster breeding progress focusing on the improvement of meat quality. The study was conducted on 16 pigs of two breeds: Polish Landrace (n = 8) and Puławska (n = 8) differing in meat quality traits and as a method the targeted-enrichment strategy for next generation sequencing was used. The enrichment technique applied in this approach was RNA hybrid capture (1x tiling). DNA was isolated by

Wizard® Genomic DNA Purification Kit (Promega) from whole blood stabilized by EDTA. 200 ng of total DNA was used to prepare the DNA library by SureSelectXT Custom 3–5.9 and SureSelectXT Reagents (Agilent) according to a protocol (Index A1-H2). The quality and quantity of DNA libraries were measured by TapeStation 2200 (Agilent) and Qubit Fluorometer (Invitrogen). The final concentration of the DNA libraries was normalized to 10 nM, diluted to 2 nM and pooled altogether according to a cluster generation protocol, loaded into a v3 Illumina flowcell (16 samples) and clustered by cBot (Illumina) using a TruSeq SR Cluster Kit v3-cBot-HS. Pair-end sequencing, with a read length of 75 bp in two technical replicates was performed on the HiScanSQ System using TruSeq SBS Kit v3-HS chemistry (Illumina). To process the raw data FastQC tool, Flexbar (quality score $\geq$ 20, size reads $\geq$ 36 bp). Filtered sequences were aligned to the pig genome (Sscrofa10.2 assembly) with BWA aligner, and SAMtools/Picard tools and GATK were used to base quality score recalibration, INDEL realignment, duplicate removal, and performed SNP and INDEL with GATK Best Practices approach. The alignment statistics were obtained with Qualimap. An average 80% reads per sample to chromosome 15 were aligned. Moreover, a high coverage was obtained for 10 unmapped contigs: JH118564.1, GL893143.2, JH118558.1, JH118457.1, GL893450.2, GL893465.2, GL892871.2, GL894011.1, JH118559.1, GL892452.1, in which are encoded a few novel genes and two known BCS1L and IGFBP2. In further analysis will be performed the analysis of polymorphic variant for SSC15 regions rich in QTLs associated with meat quality in pigs to identify the candidate genes.

Key Words: targeted-enrichment sequencing, meat quality, genetic markers

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P5038 Quantitative trait loci for backfat thickness in an F2 population between Landrace and Jeju Black pigs. S. H. Han (Educational Science Research Institute, Jeju National University, Jeju, Korea), Y. K. Kim, H. S. Oh (Faculty of Science Education, Jeju National University, Jeju, Korea), H. B. Park, and I. C. Cho (NIAS, Rural Development Administration, Jeju, Korea)

The quantitative trait loci (QTL) for backfat thickness were screed using porcine 60K SNP panels in an F2 population produced between Landrace and the Jeju Black pig. The levels of backfat were measured at three different positions on the carcass: between fourth and fifth thoracic vertebrae (BF4_5), between 11th and 12th thoracic vertebrae (BF11_12) and between last thoracic and first lumbar (BF_TL). The results of genome-wide association study (GWAS) showed that the QTLs of backfat thickness at three positions found on different chromosomal regions. QTLs of BF4_5 were found on the *Sus scrofa* chromosome 1 (SSC1), SSC2 and SSC9. Those of BF11_12 were found only on the SSC1 and those of BF_TL were found on SSC1, SSC5, SSC6 and SSC19 (not assessed the specific chromosomal location or mitochondrial SNPs). For the BF4_5, among five significant SNPs detected, MARC0022036 of SSC2 showed the highest value (-log$P$ = 8.82) of associations. For the BF11_12, a total of nine significant SNPs were detected, and ALGA0007305 of SSC1 showed the highest value (-log$P$ = 7.70). A total of one hundred and ninety-five SNPs showed the significant association with the phenotype levels of backfat and genetic variations, and ALGA0031489 of SSC5 showed the highest value (-log$P$ = 14.26). These findings suggest that the developmental levels of subcutaneous backfat may be affected by different chromosomal genes according to their locations. In addition, these findings provide that we can employ the different approaches for controlling the backfat thicknesses at different locations of the pigs related to the Jeju Black pig.

Key Words: association, backfat, Jeju Black pig, QTL, SNP

P5039 A landscape genomic approach to unravel the genomic mechanism of adaptation in indigenous goats of South Africa. K. Mdladla[*1,2], E. F. Dzomba[2], F. C. Muchadeyi[1] ([1]Agricultural Research Council-Biotechnology Platform, Pretoria, South Africa, [2]University of KwaZulu-Natal, Pietermaritzburg, South Africa)

South African indigenous goat populations are kept under heterogeneous production systems and varied environmental conditions. There are indications of local .5adaptation of South African indigenous goats amid several unknowns regarding the adaptive potential of these populations to local environmental conditions. The current study used a correlation-based landscape genomics approach spatial analysis method (SAM) to quantify the association between genomic variation [48,126 single nucleotide polymorphisms (SNPs)] and geographical location and climatic conditions among 194 indigenous goats collected across 5 major goat-producing provinces, and different production systems in South Africa. Samples were collected across 5 major goat-producing provinces and production systems. To test the effects of the environment on genetic variability, environmental data at sampling locations were required. Environmental data shown previously to be important for feed and

water availability, tick-borne disease profile, and shape phenotypic characteristics within agro-climatic zones were compiled, using geographical coordinates and elevation, where goats were sampled. Climatic data accounted for mean annual range in temperature and total rainfall, as well as the mean annual range in temperature during summer and winter months. Using univariate models, Samßada identified significant associations for 619 SNPs (1.29%) potentially subject to selection for the environmental variables. Highest number SNPs (n = 428) were associated with longitude, 163 with winter mean minimum temperature, 15 with annual mean minimum temperature, and 13 with altitude. No SNP associations were apparent for the other variables. The current results provide a valuable insight into the links between environmental variation and potential adaptive genetic divergence. Further analysis using other landscape approaches that account for population structure, in addition to the use of a multivariate analysis, is being undertaken to provide insight on the influence of population structure on allele frequencies to identify climatic variables most correlated with the genomic variation.

Key Words: goat, SAM, adaptive loci

P5040 Multiple genes on SSC7 affect the variation of vertebrae numbers in the pigs. S. H. Han (Educational Science Research Institute, Jeju National University, Jeju, Korea), Y. K. Kim (Faculty of Science Education, Jeju National University, Jeju, Korea), H. B. Park (NIAS, Rural Development Administration, Jeju, Korea), Y. J. Kang (Subtropical Livestock Research Institute, National Institute of Animal Science, RDA, Jeju, Korea), I. C. Cho (NIAS, Rural Development Administration, Jeju, Korea), and H. S. Oh (Faculty of Science Education, Jeju National University, Jeju, Korea)

The number of vertebrae is a variable trait ranged from thirteen to eighteen in the pig populations. Among the vertebrae, rib number has a great impact on the economic success of pork production because that is closely related to body length and meat productivity. To better understand the underlying genetics of body composition, especially in rib numbers, a genome-wide association study (GWAS) was undertaken. Traits included total number of vertebrate (TNV), cervical vertebra (CER), rib number identical to thoracic vertebra (THO) and lumbar vertebra (LUM). A total of 30,917 SNP were tested using a Bayesian approach. From the analysis, of the available 8814 QTLs, 613 SNPs were found to be statistically significant ($P <$ 0.01). Multiple testing was considered using the probability of false positives. Statistical testing for TNV and THO identified a single QTL on SSC7. On the other hand, no QTL was identified for CER or LUM. From the further analyses using genome re-sequencing and SNP validation, 6 SNPs (three non-synonymous mutations and three synonymous mutations) were found in the protein coding regions of three genes, *vertnin* (*VTRN*), *latent transforming growth factor-β-binding protein 2* (*LTBP2*), and *Niemann-Pick disease type C2* (*NPC2*) on SSC7 which closely related to TNV and THO variations. These QTLs when combined with information on genes found in the same regions should provide useful information that could be used for marker assisted selection, marker assisted management, or genomic selection applications in the Jeju Black pig-related crossbreeding systems.

Key Words: GWAS, vertebrae, thoracic, lumbar, Jeju Black pig, QTL, SNP

P5041 Searching for allelic distortion in RNA-seq data from boar's mature sperm. M. Gòdia (Center for Research in Agricultural Genomics (CRAG), Cerdanyola del Valles (Barcelona), Spain, F. Mayer (Center for Research in Agricultural Genomics (CRAG), Cerdanyola del Valles (Barcelona), Spain; Instituto de Pesquisas Veterinarias Desiderio Finamor, Fundasao Estadual de Pesquisa Agropecuaria, Porto Alegre, Brazil), J. Nafissi (Center for Research in Agricultural Genomics (CRAG), Cerdanyola del Valles (Barcelona), Spain), J. E. Rodríguez-Gil (Unit of Animal Reproduction, Department of Animal Medicine and Surgery, Universitat Autonoma de Barcelona, Cerdanyola del Valles (Barcelona), Spain), S. Balasch (Grup GEPORK, Masies de Roda, Spain), A. Sánchez, and A. Clop (Center for Research in Agricultural Genomics (CRAG), Cerdanyola del Valles (Barcelona), Spain)

Boars have been historically selected for meat and carcass quality. However, breeders are starting to pay attention to additional traits that could be included as selection goals. This is the case for sperm quality, as up to 30% of the boars that enter insemination centers are rejected due to poor values on sperm phenotypes. We have previously performed RNA-seq using mature sperm samples from 6 adult boars and profiled their transcriptome. The objective of the present study was to analyze another layer of information of this RNA-seq experiment by calling single nucleotide polymorphism (SNPs) and Insertion/Deletions (INDELs) and searching for statistically significant allelic distortions in sperm relevant genes. RNA libraries were prepared with two different kits to compare performances: (1) SMARTer Universal Low Input RNA (Clontech)

and (2) TruSeq (Illumina), sequenced on an Illumina HiSeq 2000 platform. RNA-seq reads were mapped onto the swine genome (ssc10.2) with TopHat 2. Variants were called with SAMtools/BCFtools and filtered with VCFtools. Then, the Variant Effect Predictor tool (VEP) was used to predict the effect of the coding variants on the affected protein. The low amount and quality of RNA molecules in mature sperm was reflected in the RNA-seq mapping data. Both the SMARTer and the Truseq sequencing reads poorly mapped to the swine genome. For SNP calling, we used a total of 804,328 and 141,288 reads for the SMARTer and the TruSeq, respectively. Preliminary results showed better performance of the TruSeq for SNP/INDEL calling, with an average of 393 SNPs/INDELs per sample called with a minimum read depth of 5, compared to 156 reads obtained with the SMARTer. To assess potential allelic distortion, we screened SNPs with a read depth above 10, allelic ratios (number of reads with reference alleles/alternative allele reads) < 0.4 or > 0.6 and a Chi-squared Test with a p-value < 0.05. Results showed some SNPs with significant allelic read differences in at least one pig in genes related to spermatogenesis and sperm motility as for example the *ODF1* gene (*Outer Dense Fiber Of Sperm Tails 1*). Our preliminary results suggest the utility of RNA-seq to identify SNPs potentially relevant to sperm quality. RNA-seq with higher sequencing depth, in more samples and combined with genome-wide association scan are needed to better exploit allelic distortion analysis in the search for DNA markers of sperm quality in boars.

Key Words: RNA-seq, sperm, allelic distortion

P5042 Comparative analysis of gene expression during postnatal growth in Czech Fleckvieh cattle. J. Kyselova, L. Barton, D. Bures (Institute of Animal Science, Prague, Czech Republic), and J. Simunek (Institute of Animal Physiology and Genetics AS CR, Prague, Czech Republic)

The aim of this study was to analyze changes of the relative gene expression of genes involved in fatty acid (FA) metabolism (*ACACA, DGAT1, FABP4*) and myogenic transcription factors (TF) genes (*MYF5, MYOD1 and MYOG*) in Czech Fleckvieh cattle throughout its life cycle. Biopsy samples of the *longissimus lumborum muscle* were collected at age of 6, 12, and 18 mo from six bulls and six heifers. Relative levels of mRNA were determined using 2-step real-time RT qPCR with specific TaqMan Gene Expression Assays. The relative expression was calculated as a ratio of the target gene mean *Cq* to the reference genes mean *Cq* using the Pfaffl formula with qBase+ Premium software. geNorm analysis selected as optimal reference targets *EIF3K*, *CLN3* and *UXT* genes (stability value M < 0.5). In general relative target gene expression was measured to be higher in the biopsy samples of 6 and 12 mo old heifers, moreover the expression levels of the 18 mo heifers were the lowest and the most balanced throughout the whole study. Gene expressions ascertained in bulls´ samples were the highest in their earliest age and at the 18 mo and lowest at the age of 12 mo. Expression of the FA metabolism genes was consistent in heifers but at the same time a considerable decrease was apparent in bulls of 12 mo. The oldest bulls showed again moderately elevated expression of the FA metabolism genes. The RNA transcripts of the myogenic TF genes were measured on a higher level in the first taken biopsy samples of heifers and then also at 18 mo bulls. In the other samples the myogenic TF genes were expressed in a similar level and were comparable to the relative expression of the FA metabolism genes. Pearson's correlation test revealed a strong positive correlation (*r*) among relative quantities of the particular myogenic TF gene transcripts, ranging from 0.788 to 0.953. Moreover a linear correlation between the relative gene expressions of *ACACA* and *DGAT1* or *FABP4* was in overall positive and it reached middle levels with the *r* value fluctuating around 0.5. On the other side there was no relationship between *FABP4* and *DGAT1* gene expressions. Acknowledgment: The work was supported by the Czech Ministry of Agriculture; the grant MZERO 0716.

Key Words: biopsy, cattle, fatty acids, gene expression, muscle, transcription factor

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P5043 Association analysis between STAT5A and PROP1 genes and milk production in Czech National dairy goat breed: Preliminary results. J. Rychtarova (Institute of Animal Science, Prague, Czech Republic), Z. Sztankoova, J. Schmidova (Institute of animal science, Prague, Czech Republic), and J. Kyselová (Institute of Animal Science, Prague, Czech Republic)

PROP1 is expressed in the pituitary gland, and plays an important role in the morphogenesis of the pituitary gonadotropes as well as lactotropes, somatotropes and caudomedial thyrotropes. It also controls the expression of growth hormone (GH) prolactin (PRL) and thyroid stimulating hormone (TSH) subunits, through regulatory PIT1 factor. *STAT5A* was discovered initially as a PRL-induced transcription factor. It is a key intracellular mediator of prolactin signaling and can activate transcription of milk protein genes in response to prolactin. Therefore, *PROP1* and *STAT5A* genes could be considered as a candidate gene for milk

production. PCR-RFLP methods were used to identify 2 SNPs: *g.6852C > T* in *STAT5A* gene and *g.1795C > T* in *PROP1* gene. For these SNPs a preliminary association analysis with milk production traits was performed by using phenotypic measurements (milk yield, protein and fat content, somatic cell score) obtained from 100 lactating goats. The statistical results showed significant associations for *PROP1* and somatic cell score. Further analyses, including larger goat's populations, are needed to understand the possible relationship between the *STAT5A* and *PROP1*genes and milk production traits in Czech national dairy goat (White Shorthaired and Brown Shorthaired goats).

This work was supported by the project no. NAZV QJ1510137 and MZE RO0716.

Key Words: *PROP1, STAT5A,* milk production

P5044 Association of acetyl-coenzyme A carboxylase á, lipoprotein lipase and fat acid synthase genes with milk parameters in Czech East Friesian breed. Z. Sztankoova (Institute of animal science, Prague, Czech Republic), J. Rychtarova (Institute of Animal Science, Prague, Czech Republic), J. Schmidova (Institute of animal science, Prague, Czech Republic), J. Kyselová (Institute of Animal Science, Prague, Czech Republic), M. Milerski, and T. Kott (Institute of animal science, Prague, Czech Republic)

In the Czech Republic, ewe milk is mainly processed to cheese. Milk yield and composition are of great economic importance for dairy sheep industry. Fat acid composition of milk is of great interest because of its implications for human health. More than 60% of the fatty acids in sheep's milk are saturated fatty acids (SFA), while monounsaturated and polyunsaturated fatty acid (MUFA and PUFA) are present at much lower concentrations. Associations between polymorphism at 3 candidate genes and milk production traits in East Friesian sheep farmed in Czech Republic were investigated in present study. Considered genes were acetyl-coenzyme A carboxylase α (ACACA), the major regulatory enzymes of fatty acid biosynthesis; lipoprotein lipase (LPL), which play a central role in plasma triglyceride metabolism, and fat acid synthase (FASN) is the central enzyme of the de novo fatty acid biosynthesis pathway. Association analysis of the 214 observed animals, confirmed that genotypes (SNPs at position 1168A/G, 1330G/T, 1338C/G and 1430C/T) of ACACA locus were associated with observed milk parameters: milk yield, protein and fat content. Significant statistical a result was implied that locus LPL; genotype combination TTTTTT was associated only with milk yield. Allele T has positive effect on increasing milk yield. Locus FASN (SNP at position 257C/T) was associated only with protein content. The C allele has positive effect on increasing protein content. These results indicate that loci should be used for genetic improvement of East Friesian dairy breed as well as local dairy sheep breeds for increasing milk production. This work was supported by the national Agency of Agriculture of the Czech Republic (NAZV) project no. QJ1310107.

Key Words: ACACA, LPL, FASN

P5045 Expression of CYP2C49, CYP7A1, CYP2B22, ACSL5 and APOA4 genes in the liver of Pietrain and Landrace pigs. M. Oczkowicz (National Research Institute of Animal Production, Department of Animal Genomics and Molecular Biology, Balice n. Krakow, Poland), K. Ropka-Molik (National Research Institute of Animal Production, Balice, Poland), M. Wojtaszek, and J. Warzecha (National Research Institute of Animal Production, Cracow, Poland)

The aim of our study was to evaluate expression level of several genes responsible for lipid metabolism: *CYP2C49, CYP7A1, CYP2B22, ACSL5* and *APOA4* in the liver of pigs representing two different breeds: Pietrain and Polish Landrace. We have chosen these genes for the analysis because our previous study had shown that expression of a few of these genes was changed after diet treatment in the liver of pigs (data not shown). We wanted to examine if the genetic background may influence the expression of these genes. The samples of liver were collected from 13 adult Pietrain animals and 23 Polish Landrace pigs. RNA was isolated using Trizol Reagent (Thermofisher Scientific) and cDNA was produced with cDNA Archive Kit (Thermofisher Scientific). qPCR was performed on ECO illumina Instrument, with GoTaq Probe qPCR Master Mix (Promega) and predesigned assays containing primers and probes (Thermofisher Scientific). *RPL27* gene was used as an endogenous control and relative mRNA expression. Relative mRNA expression of target genes was calculated according to the equation described by Pfaffl (2001). The expression of *CYP2C49, ACSL5* and *APOA4* genes was significantly higher in Landrace than in Pietrain pigs ($p < 0.005$, $p < 0.018$, $p < 0.0098$ respectively), while the expression of *CYP7A1* and *CYP2B22* was the same in both breeds. Expression of *CYP2C49* was almost fourfold higher in Landrace pigs, while *ACSL5* and *APOA4* were expressed approximately threefold higher in this breed when compared to Pietrain. Our results suggest that the expression of *ACSL5, CYP2C49* and *APOA4* may be modulated by unknown polymorphisms within

regulatory regions of these genes. In the future we are going to sequence these regions to find causative mutations. It would also be interesting to perform a nutrigenetic experiment in which animals with different genotypes within these polymorphisms would obtain different diets.

Key Words: pig, gene expression, qPCR

P5046 A genome-wide association study for natural antibodies measured in blood of Canadian Holstein cows. B. de Klerk (Animal Breeding and Genomics Centre, Wageningen University, Wageningen, Netherlands), K. A. Thompson-Crispi (Trouw Nutrition Agresearch, Guelph, ON, Canada), M. Sargolzaei (Semex Alliance, Guelph, ON, Canada), J. J. van der Poel, B. J. Ducro, J. A. M. van Arendonk (Animal Breeding and Genomics Centre, Wageningen University, Wageningen, Netherlands), and B. Mallard (Department of Pathobiology, OVC, University of Guelph, Guelph, ON, Canada)

Currently, no parameters in the breeding index select for the overall health status (immune capacity) of a cow, therefore, there is a demand of finding other parameters associated with overall natural resistance in dairy cows. Natural antibodies (NAb) are an important component of the innate immune system, and fight infections as a first line defense. Natural antibodies are poly-reactive and can respond non-specifically to naive antigens. Therefore, natural antibodies may be a key trait when evaluating an animal's potential natural disease resistance. Variation in natural antibodies is caused by both genetic and environmental factors. In this study genetic parameters of NAb were estimated and a genome-wide association study (GWAS) was performed to gain further understanding on the genes that are responsible for the observed genetic variation in natural antibody levels. In total blood samples of 727 cows from 7 farms were studied. Natural antibody levels binding keyhole limpet hemocyanin (KLH) were determined via indirect ELISA. Both immunoglobulin isotype IgG and IgM were tested. Cows were genotyped for 45.187 SNP markers. After removing outliers for both IgG- and IgM isotype, by Median Absolute Deviation method, the final data set consisted of 681 cows with both genotypes and phenotype. Each individual marker was tested to detect genetic variation in natural antibody levels. Results show heritabilities of 0.13 ± 0.062 (IgG) and 0.27 ± 0.076 (IgM). Furthermore, only significant associations (FDR < 5%) were found for immunoglobulin isotype IgG, and all significant associations were located on chromosome 21. Genomic regions that were identified in this study contained immune-response related genes like IgH, TRAF3 and TP53BP1. Therefore, these regions are suggested to contain candidate gene(s) involved in natural antibody expression in dairy cows, both from the gene positional and gene functional perspective.

Key Words: genome-wide-association study, natural antibody, dairy cattle

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P5047 Male fertility evaluation by a candidate gene approach. W. Liu, X. Yue, T. C. Chang (The Pennsylvania State University, University Park, PA), H. A. Adams (CRI International Center for Biotechnology, Mount Horeb, WI), and K. B. Krieger (Genex Cooperative, Inc., Shawano, WI)

Infertility or subfertility is a common problem in humans and other mammals. Approximately 10–15% of human couples worldwide are affected by reduced rates of fertility, with 30–50% of cases being due to male factors. In the dairy industry, a variation of up to 25% of non-return rate (NRR) is evident within a population of AI bulls that meet the normal commercially acceptable standards. In the beef industry, 18–30% of beef bulls used in natural service are reproductively deficient. Recent progress in genome-wide association studies (GWAS) with SNP markers makes it possible to design genetic diagnostic assays for bull fertility selection. Here we report the bull fertility evaluation by a candidate gene approach. We generated and validated a custom-made bovine 384-SNP chip using the Illumina VeraCode GoldenGate technology. This chip contains 237 autosomal, 4 X-linked, and 143 Y-linked SNPs, from 192 autosomal, 4 X-linked and 17 Y-linked genes/families that have been confirmed to play a role in spermatogenesis, sperm function, and/or semen quality. The chip was validated among 935 AI bulls (from Holsteins, Jersey and Angus) with detailed phenotypic records in sperm morphology 1–3 (Morph1, 2, 3) and sperm motility. The genotypic data were analyzed using the Illumina Genome Studio software. Associations of genotypes with sperm quality traits were calculated using linear model procedures in R: $Y_i = \mu + G_i + e_i$. By a breed-combined approach, a total of 37 significant SNPs ($p < 0.05$) were identified across all traits with corresponding effects and false discovery rate (FDR)-corrected p-values. Twenty-three significant SNPs for Morph1, 8 for Morph2, 22 for Morph3, and 14 for sperm motility were identified. When individual breed approach was applied, a total of 51 significant SNPs ($p < 0.05$) were identified, 16 of which overlapped with the markers identified from the breed-combined approach, the remaining 35 were new makers. Taken together, the breed-combined and individual breed approaches discovered a total of 72 SNPs from 65 candidate genes that have been confirmed to

be involved in spermatogenesis and male fertility. The potential of using this chip for male fertility selection at an early age in cattle is under evaluation.

Key Words: candidate gene, SNP, male fertility, sperm morphology, sperm motility, cattle

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P5048 Wide genome involvement in response to long-term selection for antibody response in an experimental population of White Leghorn chickens. M. Lillie (Swedish University of Agricultural Sciences, Uppsala, Sweden)

Long-term selection experiments provide a powerful approach to gain empirical insights into adaptation by uncovering the targets of selection and inferring how these contribute to the mode and tempo of adaptation. Here we report a pooled genome approach to investigate the consequences of 39 generations of bidirectional selection in White Leghorn chickens on a humoral immune trait: antibody response to sheep red blood cells. We observed wide genome involvement in response to this selection regime, with over 200 candidate sweep regions characterized by spans of high genetic differentiation (F_{ST}). These selection signatures, encompassing almost 20% of the chicken genome, are the result of bidirectional selection on different haplotypes present in the base population. This profound response highlights the extent of standing genetic variation at immune loci available at the onset of selection, and also the extent of gene involvement that has contributed to selection response.

Key Words: Virginia antibody chicken lines, long-term selection, pooled genome sequencing, selective sweeps

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P5049 Application of DNA marker-assisted selection using SNPs of reproduction related genes and their genotype combination effects on litter size in black pigs of Jeju island. J. H. Kang, E. A. Lee, S. H. Lee (Korea University, Seoul, Korea), Y. C. Ryu (Jeju National University, Jeju, Korea), Y. I. Oh (Gilgal agricultural association corporation, Jeju, Korea), and K. C. Hong (Korea University, Seoul, Korea)

In South Korea, commercial black pigs in Jeju island are one of the most popular indigenous products and also selling at a high price. However, black pigs usually have poorer reproductive ability than other commercial breeds. The aim of this study was to increase the reproductive ability in black pig farms in Jeju island using well known DNA markers. First parity reproductive traits of a total of 273 sows were recorded: total number of piglets born (TNB), number of piglets born alive (NBA), number of weaned piglets (NW) and number of teats (NT). Two single nucleotide polymorphisms (SNP) in estrogen receptor (*ESR*) gene and prolactin receptor (*PRLR*) gene were used. According to previous reports, the favorable allele of the *ESR* gene was B and that of the *PRLR* gene was A. All animals were genotyped by digital SNP genotyping analysis. In a base population, favorable allele frequency of the *ESR* gene was 0.08 and that of the *PRLR* gene was 0.68. Because favorable allele frequency of *ESR* was too low, *ESR* was considered more than *PRLR* when DNA marker-assisted selection. After the second generation, B allele frequency of *ESR* had more than doubled than that of the 0th generation (G0: 0.08 → G2: 0.19) and A allele frequency of *PRLR* slightly decreased (G0: 0.68 → G2: 0.65). Although favorable gene frequency was increased (especially *ESR* gene), litter size of each generation has no significant changes. Therefore, we analyzed the effect of genotypes of these two genes on reproductive traits of the entire population to see those DNA markers that were actually effective in our population. The genotype that has both favorable alleles does not exist. ABAA genotype combination group (*ESR*: AB heterozygote & *PRLR*: AA homozygote), which has 3 favorable alleles, has significantly higher TNB ($P = 0.0537$), NBA ($P = 0.0088$), NW ($P = 0.0052$) than the AAGG genotype combination group (does not have the favorable allele). It was thought that *ESR* had a major effect and *PRLR* had a minor effect (not no effect) on this population and it seems that an increase of favorable gene frequency during 3 generations is not high enough to make reproduction ability different between generations. In future work, if DNA marker-assisted selection were continued, favorable gene frequency would be increased and the improvement of reproduction ability of black pigs in Jeju pigs would be expected.

Key Words: DNA marker-assisted selection, Black pigs in Jeju island, litter size

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P5050 A genome-wide scan for signature of positive selection in some Iranian sheep breeds. Z. Manzari, H. Mehrabani Yeghaneh, A. Nejati-Javaremi (University of Tehran, Tehran, Iran), M. Gholizadeh (Sari Agricultural Sciences and Natural Resources University, Sari, Iran), and M. H. Moradi (Arak University, Arak, Iran)

The sheep is the earliest grazing animals to be domesticated that produces a great scale of animal-based protein for human consumption and plays an essential role in the modern agricultural economy. Iran is one of the important centers for sheep genetic resources and breeding in the world. The aim of the present study was

to investigate the selective sweeps between three Iranian sheep breeds, namely Baluchi, Lori-Bakhtiari and Zel using the Illumina ovine SNP50k BeadChip. The Weir and Cockerham's F_{ST} (Theta) method was applied to detect the selection sweeps across the genome. In total, thirty eight genomic regions were identified on 1, 2, 3, 4, 5, 7, 8, 10, 11, 12, 13, 15, 16, 18 and X chromosomes with high population differentiation between different Iranian sheep breed comparisons. Study of the genes has already been reported in these regions and revealed that almost all of these loci are associated with the genes involved in reproduction traits, cytology cells and nervous system, immune system, muscular system, sugar and energy metabolism. Our results reported genomic scans for selective sweeps in Iranian sheep breeds and could help detect functional candidate genes under positive selection and also better understand the relationship between genomic composition and phenotypic diversity for further genetic and breeding research in Iranian sheep breeds.

Key Words: Iranian sheep breeds, population differentiation, positive selection, Weir and Cockerham's F_{ST} (Theta) test

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P5051 Transcriptome hallmarks of musculoskeletal fatigue in blood of Arabian horses under racing training regime. M. Stefaniuk-Szmukier (Department of Horse Breeding, Institute of Animal Science, University of Agriculture in Cracow, Cracow, Poland), K. Ropka-Molik, K. Zukowski, and K. Piórkowska (National Research Institute of Animal Production, Balice, Poland)

The Arabians are commonly believed to be one of the oldest and the most influential horse breeds in the world and have been widely used to improve several horse breeds. Since the eighteenth century they have a very special place in Polish horse breeding. The origin of founding mares traces back to 1800 and since then the breeding is documented as pure breed. That is why Polish population are excellent example of a closed population, strongly affected by selection. The race track performance is considered as one of the selection criteria of Arabian horses which favorably influences the development of young horses. On the other hand racing training is considered to be aggravating for a developing organism, thus the aim of presented research is identification of the transcriptomic signatures of musculoskeletal fatigue in the blood of Arabian horses under a racing training regime. The RNA-seq analysis of blood has been performed for 6 2,5 y.o. horses. Total of 12 samples has been collected in two periods: I–unbroken and II–after heavy canters. The RNA sequencing was performed in 75 single-end cycles on HiScanSQ platform (Illumina). The cDNA libraries were prepared by using TruSeq RNA Kit v2 kit (Illumina) and their quality and quantity were estimated using Qubit 2.0 (Invitrogen) and TapeStation 2200 (Agilent). Validation of obtained results was performed by the real-time PCR method. The differentially expressed genes (DEG) were determined using Deseq2 software. Between two investigated training periods we detected the high number of significant differentially expressed genes (FDR < 0.05; fold change < 1.5): 2544 DEGs. Apart from a large number of transcripts corresponding to the general biological processes, the pathways indicating musculoskeletal fatigue have been observed. The identified pathways regarding regulation of proteoglycans, regulation of actin cytoskeleton, thyroid hormone signaling pathways together with training mediated activation of transcriptional response of muscle burden (ATPase gene family–e.g., *ATP5*, ubiquinone gene family–e.g., *NDUFA3*, hyaluronic maintain pathways–e.g., *CD44*) demonstrate the impact of the racing training regime on musculoskeletal system in young developing horses.

Key Words: Arabians, training, RNA-seq

P5052 Genetic investigation of sheep families demonstrating the entropion eye condition. T. Hadfield and N. E. Cockett (Utah State University, Logan, UT)

Lambs are sometimes born with a condition called entropion in which the lower eyelid is inverted, causing the bottom eyelashes to rub on the cornea which can lead to blindness if not treated. Treatment is commonly done by unrolling the eyelid and surgically stapling it in correct alignment for a few weeks. Previous reports on entropion have indicated that it is genetically controlled. In this study, samples from five paternal half-sibling families segregating for entropion were collected in 2014 and 2015. Two of the five sires were born at the Utah State University sheep facility; one was from a flock with high incidence of entropion and born with the condition while the other sire, normal at birth, was from a flock with no recorded entropion births in the last 7 yr. The other three sires were purchased and their eye condition at birth is unknown. Forty eight of the 159 lambs produced by these five rams were born with entropion. In an attempt to identify genetic regions involved with the entropion eye condition, genomic DNA was extracted from all lambs, sires and dams in the five families and the DNA samples genotyped with the Illumina HD SNP chip. Analysis of the SNP genotypes and entropion was done using *SNP & Variation Suite v8. (Golden Helix, Inc.)*. Preliminary results suggested associations

between the entropion condition and SNP markers on ovine chromosomes 1 and 3. In 2016, 25 ewes that showed the entropion condition will lamb. These ewes were bred to rams that also were born with entropion. The lambs from these matings will be added to the 159 lambs and additional analyses are underway to localize the significant regions and identify underlying genes or genetic regulatory factors.

Key Words: ovine, entropion eye

P5053 Test duration for feed and water intake in beef cattle using an Insentec system.

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To understand the effect water has on beef performance and drought adaptability, individual animal water intakes need to be collected. The Beef Improvement Federation has established guidelines for feed intake test duration, but no such guidelines exist for water intake. To establish a preliminary test duration for water intake, individual daily feed intake (FI) and water intake (WI) records were collected for a total of 70 d on 236 crossbred steers using an Insentec system at the Oklahoma State University Willard Sparks Beef Research Unit. Steers were fed in two groups from May to August in 2014 and 2015 and were individually weighed every 14 d. Within each group, steers were blocked by weight (low and high) at the beginning of the study and randomly assigned to one of four pens containing approximately 30 steers per pen. Each pen provided 186.5 m^2 of shade and included an Insentec system containing six feed bunks and 1 water bunk. Steers were fed a constant diet throughout the study. Intake records were filtered for reasonableness using parameters for feed and water bunk starting weight, ending weight, and duration. Records collected on weigh dates and days where equipment malfunctioned were filtered to maintain data quality. Average intakes for each animal were computed for increasingly large test durations (7, 14, 21, 28, 35, 42, 49, 56, or 63 d) to determine the optimum test duration for feed and water intake in this dataset. Phenotypic correlations for each shortened test duration as compared to averages for the full 70 d test were calculated using the correlation procedure in SAS. Minimum test duration was determined when both the Pearson and Spearmen correlations were above 0.90 for each trait. Our results indicated that minimum test duration for both water and feed intake is 28 d. While no values exist for WI tests, the test duration for FI is slightly shorter than other studies within the literature, which may be due to homogeneity in the study population, bunk competition, reduced variation due to missing data points or small sample size. While this calculation should be augmented with additional data as it becomes available, the data would suggest that WI records can be collected within the same time frame as FI records, so cattle can be tested simultaneously for feed and water intake without extending test duration to collect the additional phenotype.

Key Words: water intake, beef cattle, feed intake

P5054 Characterization of a region within bovine chromosome 6 associated with gray coat color in a Nellore-Angus cross.

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Breeds of cattle native to hot and humid climates often have light, gray, or white coats. Although the hide of Nellore cattle is generally black, the coat ranges from white to dark gray. This variation in hair color is likely due to irregular deposition of pigment within individual hairs. In other species, gray is caused by mutations affecting formation of dendritic cells, resulting in malformed dendritic cells depositing melanin in clumps rather than uniformly along the hair shaft. The objective of this study was to identify the locus that causes gray in a Nellore-Angus F_2 cross population. There were 779 cattle available for this study, each with 34,957 SNP genotypes. Each individual was scored for gray, and through genome-wide association analysis, gray was mapped to the same region of bovine chromosome 6 that has previously been associated with reddening and spotting phenotypes. We subsequently imputed this region to sequence scale and characterized haplotypes from recombinant individuals to define the critical interval for gray. We will also present evidence for epistatic interactions across this region that may contribute to misclassification of the gray phenotype in this cross.

Key Words: gray, melanin, Nellore

P5055 Association between g.98535683A > G:BTAU7 marker the CAST gene and meat characteristics of Nellore cattle (*Bos indicus*) and their crosses with *Bos taurus*.

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In meat science, single nucleotide polymorphisms (SNPs) have contributed to the rapid identification of genetic markers in the selection and improvement of quality characteristics. The calpastatin protein (CAST) inhibits the activity of calpain and it regulates the tenderization of the meat during the ageing process. The SNP g.98535683A > G:BTAU7 the *CAST* gene was associated with meat tenderness in the *Bos taurus* breeds. However, before transferring the populations' molecular marker where they were identified to other populations, corroboration of their effects on characteristics of interest is required in different breeds and environments. The aim of this study was to estimate allelic and genotypic frequencies of the SNP g.98535683A > G:BTAU7 the *CAST* gene and evaluate the occurrence of associations between this polymorphism and meat characteristics in *Bos indicus*. Were used five hundred Nellore cattle (n = 300) and crosses (n = 200) with *Bos taurus* from six different genetic groups and genotyped for the SNP g.98535683A > G:BTAU7 and phenotyped to shear force (SF), myofibrillar fragmentation index (MFI) rib eye area (REA), backfat thickness (BFT) and total lipids (LIP). Both alleles from this SNP were found, the A allele and the G allele with their genotypes AA, AG and GG. In the six genetic groups studied the A allele showed higher frequency than the allele G. There were no differences in allele frequencies between the genetic groups studied, showing that should be similar in *Bos indicus* and *Bos taurus*. Significant and close association between the SNP g.98535683A > G:BTAU7 and tenderness (MFI, p = 0.0044; SF, p = 0.0598) was observed where the AA genotype was better for this characteristics. The SNP g.98535683A > G:BTAU7 showed no significant association with the characteristics of REA, BFT and LIP. These results showed the occurrence of the SNP g.98535683A > G:BTAU7 the *CAST* gene in Nellore (*Bos indicus*) and its potential application in animal selection for the tenderness in this breed and its crosses. Financial support: FAPESP.

Key Words: beef cattle, meat quality, calpastatin

P5056 Genome-wide association study for stayability measures in Nellore-Angus crossbred cows. B. N. Engle, A. D. Herring, J. E. Sawyer, D. G. Riley, J. O. Sanders, and C. A. Gill (a Department of Animal Science, Texas A&M University, College Station, TX)

Beef cow stayability is traditionally defined as the probability that a cow will remain in the herd through 6 yr of age with a perfect weaning record. The objective of this study was to identify genes associated with reproductive longevity as a proxy for beef cow stayability. A population of 287 Nellore-Angus crossbred cows in central Texas was evaluated. Cows were culled from the herd after the second incidence of failure to wean a calf. Reproductive longevity was scored as a binary trait (0 = left herd, 1 = in herd through 6 yr) and adjusted for the fixed effect of contemporary group (birth year and season of birth). There were 7 heifers that never had a calf, another 9 heifers that never weaned a calf, and 222 cows that remained in production through 6 yr of age. Of these, 113 cows had a perfect weaning record. Genotypes were obtained with the Illumina BovineSNP50v1 chip, which were filtered in PLINK to remove SNP with completion rates < 90%, minor allele frequencies < 0.05, and those deviating from Hardy-Weinberg equilibrium proportions at P < 0.0001, leaving a total of 34,651 SNP for analysis. A genome-wide association study for beef cow reproductive longevity was performed using the univariate procedures of GEMMA that fitted the genomic relationship matrix to account for genetic covariance among animals. The Benjamini and Hochberg false discovery rate was constrained to 0.15 to correct for multiple tests. Single nucleotide polymorphisms associated with beef cow reproductive longevity were found on bovine chromosomes (BTA) 4, 5, 15, and 19. Although this is the first report of SNP associated with beef cow stayability, the associations are near dairy QTL for calving interval and non-return rate on BTA 5, productive life span on BTA 15, and fertility index on BTA 19.

Key Words: GWAS, stayability, beef cattle

P5057 Use of genomics to simultaneously improve feed efficiency and meat quality in grow-finish pigs. C. Zhang (University of Alberta, Edmonton, AB, Canada), R. A. Kemp, N. J. Boddicker (Genesus Inc, Lethbridge, AB, Canada),

J. C. M. Dekkers (Department of Animal Science, Iowa State University, Ames, IA), Z. Wang (University of Alberta, Edmonton, AB, Canada), and G. Plastow (Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada)

For the pig industry, feed is the largest cost of production, with the largest proportion (approx. 74%) consumed in the grow-finish phase. Efforts to improve grow-finish feed conversion will significantly reduce production costs and consequently increase total profitability. Feed efficiency is usually defined as lean growth efficiency evaluated through feed intake, daily gain, loin depth and backfat thickness, but without any focus on pork quality. Better pork quality is another high priority for the pork industry to satisfy consumer demand for an enhanced eating experience. Selection based on pedigree and phenotype have shown that high emphasis on lean growth efficiency improves feed efficiency (lower feed intake and higher lean growth), but also reduces pork quality in terms of less marbling and tenderness, and lower pH and lighter meat color (*Suzuki et al. J. Anim. Sci. 2005, 83: 2058–2065; Gilbert et al. J. Anim. Sci. 2006, 85: 3182–3188; Cai et al. J. Anim. Sci. 2008, 86: 287–298; 2008; Lefaucheur et al. J. Anim. Sci. 2011, 89: 996–1010*). Improving lean growth efficiency without deterioration of pork quality is now a priority. We have performed large scale genomic studies with industry on both feed efficiency and meat quality for grow-finish pigs. We are utilizing whole genome sequence and different densities of SNP genotypes (60K, 80K and 650K) for genome-wide association studies. Our aims are to investigate the genetic architectures and relationships between these economically important traits and to develop genomic tools to increase genetic gain for both feed efficiency and meat quality. Significant genomic regions and markers have been identified for feed intake, loin depth, backfat thickness, meat color, pH, drip loss and marbling. Preliminary genomic prediction results show high accuracy for most feed efficiency component traits, but somewhat lower for meat quality. We are continuing to optimize the genomic selection methods by making good use of such abundant information to improve prediction power and validate the accuracy of the genomic estimated breeding values.

Key Words: genomic studies, pig feed efficiency, pork quality

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P5058 The Ovar-DRB1 *0404 haplotype is associated with growth and lifetime prolificacy ewe traits. M. U. Cinar (Washington State University College of Veterinary Medicine, Pullman, WA; Erciyes University Faculty of Agriculture, Kayseri, Turkey), M. R. Mousel, J. O. Reynolds (USDA, ARS, Animal Disease Research Unit, Pullman, WA), L. M. Hermann-Hoesing (Washington State University College of Veterinary Medicine, Pullman, WA), J. B. Taylor (USDA, ARS, Rangeland Sheep Production Efficiency Research, Dubois, ID), D. P. Knowles (USDA, ARS, Animal Disease Research Unit, Pullman, WA; Washington State University, Pullman, WA), and S. N. White (USDA, ARS, Animal Disease Research Unit, Pullman, WA; Center for Reproductive Biology, Washington State University, Pullman, WA)

The major histocompatibility complex (MHC) is an organized cluster of tightly linked vertebrate genes with immunological and non-immunological traits. While many infectious diseases have been investigated in regard to DRB1, only one study based on microsatellite markers has previously examined a relationship to any sheep production traits. Furthermore, to our knowledge, no studies have examined the relationship of this region with lifetime ewe prolificacy traits. Therefore, this study analyzed association between DRB1 coding SNP haplotypes and aproduction traits including individual growth and ewe lifetime prolificacy in 372 Columbia, Polypay and Rambouillet sheep by using mixed model. The DRB1 *0404 haplotype was associated with weaning and mature weights, as well as average daily gain after multiple testing (Sidak $P < 0.05$). Interestingly, the *0404 haplotype also showed a trend toward association with total number of lifetime lambs born (nominal $P = 0.0097$; Sidak $P = 0.084$) and number of lambs born alive (nominal $P = 0.01$; Sidak $P = 0.084$). Since the *0404 haplotype was present in all three breeds, these results suggest there is at least one functional mutation in the region that influences growth and prolificacy traits that may be broadly present across several breeds. While there have been reports of genetic associations considering growth trait near DRB1, to our knowledge this is the first report of an association with ewe lifetime prolificacy on ovine chromosome 20. Furthermore, the *0404 multi-marker haplotype may improve identification of relevant animals for use in mutation discovery. If undesirable mutation alleles can be identified, genetic or genomic selection incorporating selective pressure against one or a small number of undesirable alleles could be used to improve production with limited impact on MHC genetic diversity and susceptibility to infectious disease.

Key Words: Ovar-DRB1, production traits, association study

P5059 Ovine MYADM-like repeat gene association with lifetime cumulative ewe production and wool traits. M. U. Cinar (Washington State University College of Veterinary Medicine, Pullman, WA), M. R. Mousel (USDA, ARS, Animal Disease Research Unit, Pullman, WA), M. V. Gonzales (Washington State University College of Veterinary Medicine, Pullman, WA), J. O. Reynolds (USDA, ARS, Animal Disease Research Unit, Pullman, WA), J. B. Taylor (USDA, ARS, Rangeland Sheep Production Efficiency Research, Dubois, ID), D. P. Knowles (USDA, ARS, Animal Disease Research Unit, Pullman, WA; Washington State University, Pullman, WA), and S. N. White (Center for Reproductive Biology, Washington State University, Pullman, WA)

The sheep HapMap included a scan for signatures of historical selection among global sheep breeds, and this scan identified a region on ovine chromosome 18 that includes a large tandem repeat of MYADM-like genes that are unique to artiodactyls. Specifically, domestic pigs have a relatively small repeat region containing < 20 genes/pseudogenes, while ruminants including sheep, goats, and cattle each have 35 or more genes/pseudogenes in their expanded MYADM-like repeat locus. Recent work in our lab showed the same region was associated with both ewe lifetime weight of lamb weaned and red blood cell traits, suggesting potential basis for the signature of intense selection in domestic sheep. In particular, ewe lifetime weight of lamb weaned is one of the most important selection criteria for sheep worldwide. However, in our original study with more than 1000 sheep one homozygote class could not be analyzed due to low minor allele frequency in the investigated population. The aim of the present work was to expand the examined population to more than 2000 sheep to better investigate the association of the MYADM-like gene region with ewe lifetime and individual production traits, especially in underrepresented homozygote animals. We are also expanding the range of production traits examined to incorporate more wool data. These data should validate and expand the previously observed association in a larger population composed of Columbia, Polypay, Rambouillet, Targhee and Suffolk sheep breeds.

Key Words: MYADM-like gene, sheep, association study

P5060 The effect of selection over years on breed composition in tropical composite cattle. L. R. Porto-Neto (CSIRO Agriculture, Brisbane, Australia), S. Harburg (North Australian Pastoral Company, Brisbane, Australia), R. Bunch (CSIRO Agriculture, Brisbane, Australia), R. E. Lyons (University of Queensland, Gatton, Australia), S. A. Lehnert, and A. Reverter (CSIRO Agriculture, Brisbane, Australia)

Cross breeding is common practice in livestock production to improve productivity. Hybrid vigor confers a performance advantage over straight-bred lines or breeds, which is not as evident after a few generations. The use of crossbreds also provides the opportunity to retain beneficial traits (or alleles) from ancestral breeds in subsequent generations, e.g., the parasite resistance of Zebu breeds, or the shorter post-partum anoestrous interval observed in Angus cattle. Thereafter, breed composition often has a significant effect on production traits, which is even more pronounced when comparing across different populations. By combining genotype data from a target population with genotype data from a panel of reference breeds, we can replace pedigree-based breed composition with an estimated SNP-based breed composition. We used 50K SNP data and a model-based approach to estimate the breed composition from a stable composite crossbred herd formed by crosses between five breeds around 20 yr ago, which has not had additional animals introduced to it since then. There were more than 6700 animals available for analyses, these were a representative subset of the herd, born between 2001 and 2013. The estimated SNP-based breed composition resembled the pedigree-based composition from the formation of the breed. On average, the estimated genome-wide breed composition did not change substantially over several generations of selection, and it was not significantly associated with genomically-enhanced breeding values for various production traits. Nevertheless, there is still variation, and we obtained evidence that inbreeding in this herd has been effectively controlled. We hypothesize that selection pressure on production traits would result in the maintenance of relatively small genomic fragments carrying the favorable alleles from a given ancestral population, rather than shifting the overall breed composition. This would impact on the local ancestry of genomic fragments, but would be diluted in a genome-wide analysis. Preliminary analyses supported the hypothesis. The selection of relatively small genomic fragments might have been facilitated in this case by the intensive and diverse crossbreeding during the formation of this composite breed. Further analyses using sequence data to explore the ancestry of genomic segments and their association to production traits should assist the identification of favorable alleles kept in the selected population.

Key Words: cattle, composite, tropical, selection

P5061 Association study between SNPs of the genes within bovine QTLs and meat quality of Hanwoo. D. Yoon and E. Ko (Department of Animal Science, Kyungpook National University, Sangju, Korea)

There have been many studies of bovine QTL and genomic information. Also, we previously detected selective sweep regions (BTA2 & BTA21) through the next generation sequencing (NGS) of 12 Hanwoo (Korea cattle). In these regions, we selected twelve genes (*MYH9, FAM174B, MIPOL1, CHD2, PTPN4, EPB41L5, RALB, INHBB, ETFA, ISL2, RCN2, PST-PIP1*) as candidate genes for causal variation of carcass traits. This study was performed to analyze association with SNPs on candidate genes and carcass traits. Carcass traits record of back fat thickness (BFT), eye muscle area (EMA), carcass weight (CW), marbling score (MS), and maturity (MA) were obtained from 44 Hanwoo steer and a total of 4 SNPs were genotyped using the PCR-RFLP (Group A) and from 278 Hanwoo (141 steer and 137 cow) and total 167 SNPs were genotyped using the Fluidigm SNPtype Assay (Group B). The association analyses for five genetic modes were performed using the SNPassoc package in the R program. The rs132694895 SNP in *FAM174B* was a significant difference in EMA, MS and MA, the rs42603506 SNP in *MIPOL1* was associated with EMA and MS in Group A. Sixty-two SNPs (3 SNPs of BFT, 7 SNPs of EMA, 18 SNPs of CW, 3 SNPs of MS and 32 SNPs of MA) were significantly associated with at least one of genetic mode in Group B. In single SNP analysis using the general linear model, eighteen SNPs on BFT, three SNPs on EMA, twenty SNPs on CW, four SNPs on MS and sixty-one SNPs on MA were significant, respectively. These results indicate that significant SNPs detected in this study may be the genetic markers for carcass traits in Hanwoo population.

Key Words: bovine QTL, selective sweep, association study

P5062 Comparative genomics reveal common diversity and signature of positive selection in West African taurine cattle populations. A. Tijjani (School of Life Sciences, University of Nottingham, Nottingham, United Kingdom; National Biotechnology Development Agency (NABDA), Abuja, Nigeria), J. Kim (National Human Genome Research Institute, National Institutes of Health, Bethesda, MD), H. Kim (Seoul National University, Seoul, Korea), R. Mrode (International Livestock Research Institute, Nairobi, Kenya), and O. Hanotte (School of Life Sciences, University of Nottingham, Nottingham, United Kingdom; International Livestock Research Institute, Nairobi, Kenya)

African cattle possess a unique adaptation to the tropical conditions such as high temperatures and tropical parasitic and infectious diseases challenges. West African *taurine cattle* populations include Muturu, a local breed mostly found in the southern part of Nigeria and N'dama, originally from Guinea, which are particularly unique due to their known resistance/tolerance to trypanomiasis, hence called "trypanotolerant" cattle. The genome diversity as well as the genetic basis of their adaptive traits remains poorly understood. In this study, we report the analyses of genetic variation and detection of loci under positive selection by whole-genome re-sequencing of 20 indigenous West African *taurine* populations, equal sample size for the Muturu and N'dama, with each animal sequenced to about 10 fold coverage using the Illumina platform. In comparison to UMD3.1 bovine reference assembly, we identified a total of 17,096,191 single nucleotide polymorphisms (SNPs) and 4423,966 Insertion/deletions (InDels) in both populations, of which about 18% and 49% of these variants respectively are new. Following the pool heterozygosity (*Hp*) approach, 34 regions of the genome have been identified as possible candidates for positive selection in our study populations. 76 genes were found within these regions based on *Ensembl*Genes 83 database. The genes were further classified into five enriched functional annotation clusters, 'Innate immune response', 'immune response' and 'defense response' were revealed as the most significantly enriched functional term cluster (Enrichment Score: 1.43) of genes mapped within the candidate region intervals. Further characterization of the genes identified may reveal causal variants that are associated with tropical adaptive traits.

Key Words: African *taurine*, re-sequencing, SNPs

P5063 The effect of the IGF2 gene on pork and fat quality traits in two populations of the South African Landrace and Large White pig breeds. P. D. Soma (Agricultural Research Council, Animal Production Institute, Pretoria, South Africa)

The Large White and Landrace are the predominant pig breeds used in the South African industry. Due to consumer demands, selection of leaner pigs receive attention in selection programs. *IGF2* is an imprinted gene, paternally expressed with a positive association with an increase in lean yield. The aim of this study was to investigate the effect of the *IGF2* gene in the two South African populations with regard to meat and fat quality traits. For the majority of meat

quality traits (pH_u, water-holding capacity, color, eye muscle area, drip loss, thiols and thiobarbituric acid reactive substances), the *IGF2* genotypes did not differ significantly ($P < 0.05$). There was a significant genotype effect on warm and cold carcass weights where the A/A genotype had lower weights compared to the G/G genotype. The pH_u values varied from 5.8 to 6.10, indicating the absence of pale, soft exudative meat. The G/G genotype displayed differences ($P < 0.05$) with a mean value of 5658 mm^2 for eye muscle area where the G allele is associated with more fat. Color measurements at 24 h *post mortem* were not different between the *IGF2* genotypes ($P > 0.05$). The color measurements from 1 to 7 d *post mortem* increased across all breed*genotype combinations for b* and Chroma measurements. Warner Bratzler shear force displayed differences ($P < 0.05$) between the *IGF2* genotypes. The G/G genotype displayed more tender pork. Fat free dry matter of belly fat was the only significant measurement with a genotype effect where the A/A genotype had the highest percentage of 9.18% in comparison to the A/G genotype with a percentage of 8.15%. The G/G genotype had a higher mean value for fat content in muscle compared to backfat in this study, although not significant in the number of samples tested. Interesting to note that the A/A genotype tended to have more fat in the belly. The *IGF2* genotypes were significant for the SFA's (C15:0, C16:0, C17:0 and C20:0) in belly fat where the A/A was higher compared to A/G and G/G genotypes. The double bond index and iodine values were higher in A/A genotypes which is consistent with leaner animals. Results of this study are comparable with other studies showing that *IGF2* did not have any negative effect on meat and fat quality traits. This study has highlighted that there is potential for using *IGF2* gene as a genetic tool for selection of leaner pigs.

Key Words: pigs, IGF2, meat and fat quality

P5064 Accuracy of genome-wide predictions of heterosis in beef cattle using 50K genotypes.

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Commercial beef cattle production makes use of crossbreeding to exploit heterosis and breed complementarity. Developing a reliable method of heterosis prediction would greatly improve the efficiency of crossbreeding schemes. Also, there is potential for improving accuracy of crossbred breeding values by accounting for heterosis in the genetic evaluation model. The objective of this study was to evaluate the accuracy of genomic prediction of heterosis using a cross-validation approach and to test the impact on genomic estimated breeding value (GEBV) accuracies in beef cattle. A total of 6794 multi-breed and crossbred beef cattle with phenotype and Illumina BovineSNP50 (50K) genotype data were used. Details of breed description, population structure and data editing were reported by [1]. The studied traits included growth and carcass traits as defined in Table 1. Three methods that utilized genome-wide SNP data were applied to predict heterosis: 1) average heterozygosity across loci (H), 2) dominance deviations from dominance relationship matrix (D) and 3) deviation of crossbred phenotype from mid-parent value using information from genomic breed proportions (HV) obtained from the admixture software [2]. A mutually exclusive random sampling of all animals was performed to form 5-groups replicated 5 times with an average of 1359 animals per group. In each analysis within a replicate, one group was dedicated as the validation set while the remaining four groups were combined to form the reference set. The phenotype of the animals in the validation set was assumed to be unknown, thus it resulted in every animal having heterosis predicted without using its own phenotype, allowing their phenotype to be used for validation. The same approach was applied for testing the accuracy of GEBV when accounting for heterosis predicted from the three methods. Our results showed that the best predictor of genomic heterosis for beef carcass and growth traits was HV (Table 1) with accuracy ranging from 0.46 to 0.99. Inclusion of heterosis from the HV method in genomic evaluation improved accuracy of GBV up to 20%. Thus, the opportunity exists for predicting heterosis, improving accuracy of genomic selection and subsequently, optimizing crossbreeding program in beef cattle.

[1.] Lu, D., Akanno, E. C. Crowley, J. J., Plastow, G., et al. 2016. J. Anim. Sci. doi:10.2527/jas.2015-0126.
[2.] Alexander, D. H., Novembre, J., and Lange, K. 2009. Genome Research, 19:1655–1664.

Key Words: beef cattle, heterosis, genomic prediction

P5065 Comparison of SNP and haplotype models for genome-wide association studies for feed efficiency traits in crossbred beef cattle.
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Feed costs comprise the majority of variable expenses in beef cattle systems making feed efficiency an important economic consideration within the beef industry. Due to the expense of recording feed intake phenotypes, identification of genomic regions associated with feed efficiency traits is advantageous for facilitating selection programs. Genome-wide association studies were performed using 748 crossbred steers and heifers representing seven sire breeds with phenotypes for ADG and DMI. Animals were genotyped with the BovineSNP50v2 BeadChip. Both traits were analyzed independently through SNP (BayesC) and haplotype association studies and together in a bivariate analysis with a haplotype model (BayesIM). In brief, a hidden Markov model (HMM) of variable length haplotype segments is built where haplotypes are mapped to haplotype clusters based on local haplotype similarity. The estimated HMM was then used to assign haplotype cluster genotypes, instead of SNP genotypes, as latent covariates in a Bayesian mixture model. Haplotype cluster effects at loci with non-zero effects were modeled as normal random variables. In the bivariate model, loci where both traits had non -zero effects, cluster effects were modeled as bivariate normal random variables. The number of haplotype clusters at each location was assumed to be either 8 or 16, resulting in a total of three univariate analyses for each trait and two bivariate analyses. Chromosomal regions were defined as 1-Mb windows from the BayesC analyses or 900kb QTL regions produced by the haplotype models. Posterior genomic heritability estimates (SD) for ADG were 0.39 (0.11), 0.42 (0.13), 0.42 (0.13), 0.39 (0.11) and 0.40 (0.15) for BayesC, BayesIM 8 clusters, BayesIM 16 clusters, BayesIM bivariate 8 clusters and BayesIM bivariate 16 clusters, respectively. Dry matter intake posterior genomic heritability estimates (SD) were 0.27 (0.08), 0.35 (0.10), 0.33 (0.10), 0.31 (0.11) and 0.31 (0.12) for the same analyses. Three pleiotropic chromosomal regions in common with all univariate (SNP and haplotype) and bivariate analyses were identified on BTA 1 at 157 Mb, 9 at 4 Mb, and 15 at 68 Mb. These results verify that SNP and haplotype associations yield similar heritability estimates and chromosomal regions. The USDA is an equal opportunity employer.

Key Words: beef cattle, feed efficiency, genome-wide association study

POSTERS: GENETICS AND DISEASE

P6000 Whole genome sequencing of canine family trios to identify rare alleles for Mendelian diseases.
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Genetic variants associated with autosomal recessive diseases are responsible for over 250 Mendelian traits and disorders in the domestic dog (*Canis familiaris*). Although dog breeds show remarkable interbreed heterogeneity and disease prevalence, small founder populations of breeds and selective breeding have led to extensive linkage disequilibrium over long genomic regions within breeds, resulting in enrichment of rare mutations in individual breeds. Genome-wide association studies (GWAS) have been successfully used in mapping the responsible genomic regions when sufficient number of cases and controls are available. The mutations associated with increased risk for disease development in these regions can then be identified with fine-mapping and targeted re-sequencing. However, the method is often laborious and time consuming. As the costs of next-generation sequencing are coming down due to the rapid technological development, whole genome sequencing of individuals without prior GWAS has become a possible approach in detecting rare variants. In rare diseases where only a few individuals are available, this approach can be used to sequence family trios consisting of an affected individual and its healthy parents. With an average depth coverage of 10X per individual, 98 to 99% of the reads can be aligned to the reference genome. Variant discovery is then made with an effective bioinformatics pipeline to filter single nucleotide variants (SNVs) and indels and candidate mutations are validated using Sanger sequencing. Whole genome sequencing of family trios will enable the identification of causative variants underlying the monogenic canine diseases, most of which have human homologs, using far less samples than required for GWAS studies. The identification of the candidate mutations enables the development of genetic testing to avoid unintentional use of affected dogs in breeding.

We have applied whole-genome sequencing (WGS) of family trios without prior GWAS to identify genetic

variants associated with autosomal recessive diseases. Using this approach, a missense variant *FAM83G* (c155G > C, p.R52P) associated with hereditary footpad hyperkeratosis (HFH) was identified in Kromfohrländer dogs. The results from this study and the potential of whole genome sequencing of canine family trios will be presented.

Key Words: dog, monogenic disease, next-generation sequencing

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P6001 Effectively managing bovine genetic disease risk via genotyping the Irish national herd.

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Historically, an animal's genetic disease carrier status was only determined after the observation of affected offspring. Once identified, the livestock producer typically had two choices 1) cull any ancestor of the affected progeny, or 2) risk producing another affected calf. As molecular tests for causative mutations became available carrier animals could be identified, but often only AI bulls or elite pedigree animals were tested due to the high cost of testing. Commercial producers tried to minimize their genetic disease risk by purchasing bulls assumed to be free of genetic diseases. Very few commercial producers, especially smaller enterprises, tested their own breeding stock. For national breeding programs the disease risk of using a known carrier sire was determined from the estimated allele frequency in the national herd and pedigree relatedness. Both of these variables contain a level of error due to low sampling rates and unvalidated parentage.

To aid cattle genomics a low cost, custom bovine Illumina single nucleotide polymorphism (SNP) genotype panel (International Dairy & Beef panel; IDB) was developed which contains 50 validated probes for Mendelian diseases and undesirable traits. Currently, ~500,000 Irish beef and dairy animals have been genotyped using the IDB, representing 25% of the total bovine breeding stock. An additional 30,000 Irish cattle have been genotyped on other commercial SNP chips. While traditionally more dairy than beef animals are genotyped nationwide, Ireland has ~400,000 beef animals genotyped, representing ~40% of the national beef herd. SNP imputation using FImpute is used to decrease genotyping errors and remove missing genotypes to provide a comprehensive genetic disease profile on all genotyped animals. This level of genotyping allows a more accurate estimation of the disease frequency in the national herd as allele frequencies between AI, pedigree, and commercial herds can and does vary. Informed breeding decisions, via farm reports, can be made available to maximize genetic gain while avoiding carrier by carrier matings of lethal or undesirable traits/diseases based on the animals true carrier status instead of its hypothetical risk. Additionally, all animals are processed for SNP based parentage validation and parentage prediction (for non-validated parents) using an 800 parentage SNP set, which contains the ISAG parentage SNP, developed to effectively remove the risk of A) validating a false parent, B) predicting > 1 parent, and C) predicting a false parent which would occur if using the ISAG parentage SNP panel alone.

Key Words: genetic disease, cattle, diagnostic, parentage SNP

P6002 Evaluating the metagenome of nasal samples from cattle with bovine respiratory disease complex (BRDC).

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Bovine respiratory disease complex (BRDC) is a multi-factor disease, and disease incidence may be associated with an animal's commensal microbiota (metagenome). Therefore, evaluation of the animal's resident microbiota in the upper nasal cavity may help us to understand the impact of the metagenome on incidence of BRDC in cattle. Nasal swabs from approximately 700 calves were collected each year at various time points including preconditioning, weaning, and when the animal enters the feedlot and is diagnosed with BRDC. Samples from healthy cohorts were also collected for each time point evaluated in the feedlot to compare metagenome profiles of healthy and sick animals. Samples from animals diagnosed with BRDC in the feedlot were pooled in groups of ten based on when the animal was diagnosed with BRDC (1 wk, 2 wk, 3 wk, or 4 wk after weaning). Samples from these same animals were also evaluated at the time points previous to entering the feedlot (preconditioning and weaning) to evaluate changes in the metagenome across time. Additionally, samples from the preconditioning and weaning time points were pooled in groups of ten based on location origin of the animals before entering the feedlot, as calves came from four pasture locations before being weaned and comingled in the feedlot. To evaluate and compare the metagenome of each pooled sample, the variable region along the 16S ribosomal RNA gene was amplified by PCR. These amplified

products were then sequenced using next-generation sequencing (Pacific Biosciences RSII instrument or Illumina MiSeq) and sequence reads were analyzed by WebMGA and GreenGenes to identify the bacterial populations present. Overall, metagenomic populations differed across time points evaluated including feedlot, preconditioning and weaning. Additionally, metagenome profiles differed across animal location origin before entering the feedlot. These results demonstrate a change in the metagenome of the nasal cavity across different time points of production.

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Key Words: bovine respiratory disease complex, metagenome, 16S sequence

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P6003 The use of Kosher phenotyping for mapping QTL affecting susceptibility to bovine respiratory disease. E. Lipkin (Hebrew Univarsity of Jerusalem, Jerusalem, Israel), M. G. Strillacci (Università degli Studi di Milano, Milano, Italy), H. Eitam, M. Yishay (Department of Ruminant Sciences, Agricultural Research Organization (ARO), Bet-Dagan, Ramat Yishai, Israel), F. Schiavini (Università degli Studi di Milano, Milano, Italy), M. Soller (Hebrew Univarsity of Jerusalem, Jerusalem, Israel), A. Bagnato (Università degli Studi di Milano, Milano, Italy), and A. Shabtay (Department of Ruminant Sciences, Agricultural Research Organization (ARO), Newe Ya'ar Research Center, Ramat Yishay, Israel)

Bovine respiratory disease (BRD), caused by multiple pathogens that become more virulent in response to stress, is the leading cause of morbidity and mortality in feedlot cattle. As various preventive strategies failed, marker or gene assisted selection for resistance becomes attractive. In the present study, selective DNA pooling was applied in a genome-wide association study to map BRD QTLs in Israeli Holstein male calves. Kosher scoring at the abattoir was used to allocate 122 and 62 animals to High and Low BRD resistant groups, respectively. Kosher scoring involves examination of the lungs for adhesions, a consequence of secondary bacterial infection in BRD, and hence a marker for individual history of BRD infections. Animals are graded Glatt (adhesions absent; assigned to High group), Kosher (moderate adhesions) and Treif (severe adhesions; assigned to Low group). All pools were genotyped by Illumina BovineHD BeadChip. Moving average of-logP was used to map QTLs and 1 Log drop was used to define QTL boundaries (QTLRs). The combined procedure was efficient for high resolution mapping. Nineteen QTLRs distributed over 13 autosomes were found, some overlapping previous studies. The QTLRs contain polymorphic functional and expression candidate genes, with putative immunological and wound healing activities that might affect kosher grade. Kosher grading was shown to be a low cost, easily collected phenotype for mapping QTLs affecting BRD morbidity.

Key Words: bovine respiratory disease, BRD, QTL, selective DNA pooling, GWAS

P6004 Estimation of heritability for fracture in the Thoroughbred racehorse. T. Tozaki (Laboratory of Racing Chemistry, Utsunomiya, Japan), T. Miyake (Comparative Agricultural Sciences, Kyoto University, Kyoto, Japan), M. Kikuchi, H. Kakoi, K. I. Hirota, and S. I. Nagata (Laboratory of Racing Chemistry, Utsunomiya, Japan)

Thoroughbred racehorses can damage several joints and bones due to rigorous training and racing, and develop various fractures during their athletic life. These fractures cause considerable wastage of racing Thoroughbreds. The fracture risk has been shown to be heritable in several species, although fractures in racehorses are generally believed to be influenced by various environmental factors such as speed and truck surface conditions. In this study, we estimated the heritability of the fracture risk in the Thoroughbred racehorse to clarify the genetic factors involved, by using Bayesian analysis with Gibbs sampling based on a categorized model. The clinical data of 3927 racehorses diagnosed by veterinarians of the Racehorse Clinics of Japan Racing Association were used. The health status regarding fractures was categorized as non-fracture, chip-fracture in the carpus, and other types of fractures. The heritability estimate (h^2) for non-fracture versus fracture (all types of fractures), obtained from a nonlinear model, was 0.0911. Genetic factors were suggested to be involved in the fracture risk of Thoroughbred racehorses. The heritability estimate for chip-fracture in the carpus ($h^2 = 0.2598$) was much higher than that for the other types of fractures ($h^2 = 0.0319$). These results show that genetic factors relatively contribute to chip-fracture in the carpus, while environmental factors contribute to the other types of fractures. Based on the above results, we are conducting a genome-wide association study (GWAS) to identify candidate genes for chip-fracture in the carpus. At the presentation, we will also report the advances in the GWAS.

Key Words: Thoroughbred, fracture, heritability

P6005 Diversity of Toll-like receptor genes in the indigenous Czech cattle breeds. K. Novák, V. Czerneková (Institute of Animal Science, Prague, Czech Republic), A. E. Kalashnikov (L.K. Ernst

Research Institute of Animal Husbandry, Dubrovitsy, Russian Federation), and V. Mátlová (Institute of Animal Science, Prague, Czech Republic)

The variability of disease resistance genes in traditional breeds is considered to be a reservoir of functional variants for breeding programs. The population equilibrium is supposed to reflect local infection pressure while the rare variants are useful in counteracting the gene pool erosion. Screening for the diversity of innate immunity genes has been performed in two conserved breeds of cattle, Czech Red and Czech Red Pied. The survey comprised ten members of bovine *TLR* gene family coding for Toll-like receptors involved in early pathogen recognition. Population of 115 animals included most of living individuals of each breed and archived samples. Along with Sanger sequencing, polymorphism has been discovered in pooled amplicons using the PacBio NGS platform and subsequently validated with designed genotyping assays. An independent pipeline for processing long PacBio reads has been developed which combined primary data quality check with FastQC, assembly with Ugene and removal of duplicate PCR with Picard Tools followed by variant calling using SAMtools and filtration with VCFtools. The polymorphism revealed in a small population of historical breeds was unexpectedly high. The numbers of detected polymorphisms and reconstructed haplotypes (in brackets) in the antibacterial series were 6 (4) in *TLR1*, 24 (6) in *TLR2*, 8 (18) in *TLR4*, 26 (6) in *TLR5*, and 4 (6) in *TLR6*, while the mostly antiviral *TLR3*, *TLR7*, *TLR8*, *TLR9*, and *TLR10* harbored another 70 SNPs and indels. The observed diversity approaches general diversity in *TLR*s as reported for the panel of world breeds (Fisher et al., Plos One 6:11, 2011). Breed specificity can be exemplified in *TLR4* where three haplotypes were shared in similar frequencies while the other haplotypes showed preferences for one of the local breeds. Namely, haplotype B1, otherwise common in European breeds, was greatly reduced in Czech Red. Although the haplotype frequencies might have been distorted by the bottleneck in the history of both populations, the presence of specific features coincides with the phenotypic distinctness. The diversity in conserved genetic resources also contrasted to the paucity of *TLR* variants in the commercial populations of Simmental type. The effect of intensive breeding on *TLR*diversity is being evaluated in the background of commercial Czech Red Pied as well as the health effects of individual haplotypes as revealed with a panel of 60 tag SNPs.

Key Words: cattle, TLRs, diversity

P6006 The cytokines expression in the milk somatic cells of goats infected with small ruminant lentivirus. J. Jarczak (Institute of Genetics and Animal Breeding, Jastrzebiec, Poland), E. Kościuczuk (Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL), J. Kaba (Warsaw University of Life Sciences, Faculty of Veterinary Medicine, Warsaw, Poland), D. Słoniewska, and E. Bagnicka (Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Jastrzebiec, Poland)

The aim of this study was to determine the level of mRNA and protein expression of selected cytokines in milk somatic cells of dairy goats infected with small ruminant lentivirus. The study was performed on 26 dairy goats, Polish White Improved and Polish Fawn Improved breeds, which were from their second to the sixth lactation. The animals were divided into two groups: control, with animals free from infection and experimental, with animals infected with small ruminant lentivirus (SRLV). Selection of animals for both groups was based on the results of at least two serological analyses. The level of mRNA in milk somatic cells was determined using real-time PCR method for: interleukin-1α (*IL-1α*), interleukin-1β (*IL-1β*), interleukin-2 (*IL-2*), interleukin 4 (*IL-4*), interleukin-6 (*IL-6*), interleukin-10 (*IL-10*), interleukin-12 (*IL-12*), interleukin 16 (*IL-16*), interleukin 18 (*IL-18*), interferon α (*IFNa*), interferon β (*IFNβ*), interferon γ (*IFN γ*), and tumor necrosis factor α (*TNF α*). The protein level was measured in protein extracts from milk somatic cells using specific ELISA tests for: IL-1α, IL-1β, IL-6, IFNβ, IFNγ and TNF α. An increase in gene expression levels of *IFNβ, IFNy, TNFα* and *IL-16* in milk somatic cells from infected individuals as compared to goats free from infection has been stated. The presence of transcripts of *IL-2, IL-4* and *IL-12* in milk somatic cells, regardless of whether animals were infected with SRLV or free of the infection has not been found. In protein extracts from milk somatic cells the presence of IL-1α, IL-6 and IFNβ has been confirmed. Expression of IL-1α and IFNβ was increased in the group of animals infected with SRLV. The expression level of IL-6 was at the border of detection, however, a slight but statistically confirmed increased was observed in infected goats. The presence of IL-1β and IFNγ, was not observed in milk somatic cells regardless of health status of animals, their age or stage of lactation. TNFα was present only in the first stage of lactation, 1 wk after birth in a few animals from both groups. The virus probably suppresses the immune response of the body, not allowing the activation of defense mechanisms. The immune

response of the mammary gland of goats, one of the target organs of small ruminant lentivirus is local and independent of the systemic.

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Key Words: gen=e expression, SRLV infection, cytokines

P6007 Cell-type dependent immune response post porcine reproductive and respiratory syndrome virus infection. M. J. Pröll, C. Neuhoff, C. Grosse-Brinkhaus (Institute of animal science, University of Bonn, Bonn, Germany), M. A. Müller, C. Drosten (Institute of Virology University of Bonn Medical Centre, Bonn, Germany), M. J. Uddin (School of Veterinary Science, The University of Queensland, Gatton campus, Gatton, Gatton, Australia), D. Tesfaye (Institute of animal science, University of Bonn, Bonn, Germany), E. Tholen, and K. Schellander (Institute of Animal Science, University of Bonn, Bonn, Germany)

The porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases of the global swine industry. The understanding of the responses to porcine reproductive and respiratory syndrome virus (PRRSV) as well as of the genetic elements and functions involved in the immune response to PRRSV was lacking. Therefore the aims of this study were to investigate the expression profiles and protein profiles of candidate genes which have a high impact on the host's disease response to PRRSV in different respiratory cell types of two pig breeds (Pietrain and Duroc). To improve the understanding of genetic components and functions in the responses to PRRSV as well as to characterize changes in the immune gene expression a RNA-sequencing analysis of PRRSV infected Pietrain and Duroc lung DCs was performed and differently expressed candidate genes were obtained. The gene expression analyses of these candidate genes were done by qRT-PCR at six time points (0 h, 3, 6, 9, 12, 24 hpi) in three respiratory cell types: dendritic cells (DCs), pulmonary alveolar macrophages (PAMs) and trachea epithelial cells. Additionally, the cytokine concentrations of four (IFN-γ, IL-8, IL-1β and TNF-α) cytokines in cell culture supernatants were measured and were set in relation to the cytokine gene expression profiles. The gene expression trends with regard to 24 hpi proceeded for all respiratory cells contrarily. Investigations of the differently expressed genes showed a common reduced expression trend of the cytokines and chemokines for lung DCs. Other trends could be detected for PAMs as well as for trachea epithelial cells. There were more up-orientated gene expression trends for PAMs in comparison to the common down-orientated gene expression trends for lung DCs. Furthermore, the cytokine concentrations varied between Pietrain and Duroc and between DCs, PAMs and trachea epithelial cells. In conclusion, these various cell-type responses to PRRSV showed that there were different cell-type susceptibilities to PRRSV. With regard to time point 24 hpi the expression profiles of lung DCs led to the suggestion that these cells did not have enough power to stimulate other immune reactions. In contrast, PRRSV infected PAMs seemed to have enough capacity to give necessary signals to the immune system. These observed cell-type dependent differences should be taken into account for following investigations about immunity traits in pig breeding and about more effective vaccines.

Key Words: PRRSV, dendritic cells, pig

P6008 Genomic basis of Lipomatous Myopathy in Piedmontese beef cattle. S. Peletto (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy), M. T. Capucchio (Università degli Studi di Torino, Torino, Italy), M. G. Strillacci (Università degli Studi di Milano, Milano, Italy), C. Boin (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy), E. Biasibetti (Università degli Studi di Torino, Torino, Italy), P. Modesto (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy), F. Schiavini (Università degli Studi di Milano, Milano, Italy), P. L. Acutis (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy), and A. Bagnato (Università degli Studi di Milano, Milano, Italy)

In Piedmontese cattle breed, the sporadic detection of lipomatous myopathy (LM) is reported. The disease expression consists in degeneration/infiltration of the muscular tissue characterized by replacement of myofibers with adipose tissue. The aim of this study was to investigate the existence of genetic loci associated with LM in Piedmontese cattle breed through a genome-wide association study based on DNA pooling design. Samples used for the study were collected from the meat cutting plant of a local consortium, pairing cases and controls within farm. Samples of different muscles (diaphragm, superficial and deep pectoral, intercostal, sternocleidomastoid group and vastus lateralis) were histopathologically and enzymatically classified as cases and controls. Pools were constructed after evaluations of DNA integrity, purity and total concentration. Equal amounts of DNA were

pooled from individuals for the constitution of 4 pools (2 independent biological replicates for cases and 2 for controls). Technical duplicates were also built and all pools genotyped with the Illumina BovineHD BeadChip three times each, for a total of 24 chip array positions. SNPs positions were based on the UMB 3.1 bovine assembly. The B-allele frequencies for each array replicate were obtained from the Illumina Genome Studio software® and used in a specific pipeline in R software to perform a multiple marker test. The test statistic used for each SNP was *Ztest = Dtest/SD(Dnull)* where Dtest is the difference of the B-allele frequencies means among tails and Dnull is the difference of the B-allele frequency means within tails. The test statistic was distributed as X^2 with one degree of freedom under the null hypothesis of equal allele frequencies. The analysis was performed after excluding the 5% of SNPs showing the highest BAF variability from the replicate arrays within tail as well as the monomorphic SNPs. A total of 123 significant SNPs were identified on the 29 bovine autosomes, and 57 on the X chromosome. A subset of the identified markers falls inside or nearby the genes *LARGE*, *PDZRN3* and *DMD*. The biological role of these genes in the onset of LM has been identified looking at the known functions of the encoded proteins on the GeneCards database. In particular, a strong association has been identified on the X chromosome with the *DMD* gene, coding for dystrophin and being responsible for Duchenne muscular dystrophy in humans.

Key Words: Myopathy, Piedmontese, GWAS

P6009 Focus on atherosclerosis and the pig as a model to identify genes affecting cholesterol and other plasma lipid levels. P. Karlskov-Mortensen, S. D. Frederiksen, S. D. Pant, S. Cirera, C. B. Jørgensen, C. S. Bruun, T. Mark, and M. Fredholm (Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark)

Cholesterol is a ubiquitous steroid and a vital component of cellular membranes in vertebrates. At the same time, subendothelial deposition of cholesterol and other lipoproteins is the culprit of atherosclerotic lesions leading to a range of cardiovascular diseases which together represent the most frequent causes of death in the industrialized world. The balance between plasma levels of low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) is critical for disease development. A high level of LDL-C is atherogenic whereas a high level of HDL-C is cardio protective. Heritability of LDL-C and HDL-C levels in humans are estimated to be up to 70%. However, loci identified by large-scale genome-wide screens in humans explain only a small fraction of the genetic variation. Few GWAS and QTL mapping studies have been performed in mice to identify loci affecting blood lipid levels. More often, spontaneous dyslipidemic and genetically engineered mice models have been used to study the effect of dyslipidemia associated genes identified in human GWAS studies. Here we employ the pig as a full size animal model for atherosclerosis. The pig has a close similarity to humans in physiology, organ development and disease progression. We have established F2 pedigrees using Göttingen Minipig as the parental boar line and Duroc and Yorkshire as parental sow lines. Whereas Duroc and Yorkshire represent lean, fast growing production breeds, the Göttingen Minipig is an obesity prone pig breed often used in studies of obesity, diabetes and metabolic syndrome. Levels of LDL-C, HDL-C and several other blood lipids were measured at two age points in a total of 564 F2 animals. GWAS, LD and haplotype analyses identified seven loci with effect on different blood lipid levels. Five of these loci are clustered in a 12 Mb region on chromosome 3. Interestingly, the haplotypes associated with an atherosclerosis protective blood lipid profile are in general found to originate from the Göttingen Minipig.

Key Words: pig, GWAS, atherosclerosis

P6010 Identification of novel genetic variants in the equine collagenous lectin genes through targeted, next generation re-sequencing. R. S. Fraser (Department of Pathobiology, University of Guelph, Guelph, ON, Canada), A. Meyer (Ontario Institute for Cancer Research, Toronto, ON, Canada), L. G. Arroyo (Department of Clinical Studies, University of Guelph, Guelph, ON, Canada), J. D. Hammermueller, and B. N. Lillie (Department of Pathobiology, University of Guelph, Guelph, ON, Canada)

Infectious diseases are an important source of welfare and economic burden in horses. Collagenous lectins are a family of soluble pattern recognition receptors that play an important role in innate immune resistance to infectious disease. Through recognition of carbohydrate motifs on the surface of pathogens, some collagenous lectins can activate the lectin pathway of complement, providing an effective means of defense. They may also opsonize, agglutinate, or directly neutralize pathogens. Genetic polymorphisms in collagenous lectins have been shown in other species to predispose animals to a variety of infectious diseases. In this case-control study, we used a high-throughput, targeted

re-sequencing approach to investigate the relationship between genetic variation in equine collagenous lectin genes and susceptibility to disease. DNA was isolated from the liver of normal (n = 35) and diseased (n = 54) horses submitted for post-mortem examination to the Ontario Veterinary College and the Animal Health Laboratory at the University of Guelph. Animals were grouped together by dominant pathological process and their DNA was pooled in equal amounts, for a total of 21 groups, each containing 4–5 horses. The exons, introns, upstream (up to 50 kb) and downstream (up to 3 kb) regulatory regions for the 11 equine collagenous lectin genes and related MASPs were targeted for re-sequencing. A custom-made Roche Nimblegen EZ Developer kit was used to prepare the library, which was subsequently sequenced on an Illumina MiSeq. In total, 3.4 Gb of sequence data was obtained with a mean read depth of 39x per horse. After implementing quality control filters, 5145 single nucleotide variants (SNVs) were identified, 4174 (81%) of which had not been previously reported in dbSNP (build 144). Of these, 82 were present in the coding regions (35 missense, 47 synonymous), 1530 in introns, 3509 in the upstream regulatory region, and 279 in the downstream regulatory region. *In silico* analysis of the missense SNVs identified 13 mutations with potential to disrupt collagenous lectin protein structure or function. The putative impact of SNVs on potential promoters was investigated, and allele frequency was compared between normal and diseased groups. This study contributes to the growing body of evidence that pooled, high-throughput sequencing is a viable strategy for cost-effective SNV discovery. The SNVs discovered in this experiment represent potential genetic contributions to disease susceptibility of horses, and will serve as candidates for further population-level genotyping.

Key Words: equine, collagenous lectins, SNVs

P6011 Transcriptome profiling of the peripheral blood mononuclear cells following PRRSV vaccination in Pietrain pig. A. Islam, C. Neuhoff (Institute of animal science, University of Bonn, Bonn, Germany), C. Große-Brinkhaus (Institute of Animal Science, University of Bonn, Bonn, Germany), M. J. Pröll (Institute of animal science, University of Bonn, Bonn, Germany), M. J. Uddin (School of Veterinary Science, The University of Queensland, Gatton campus, Gatton, Gatton, Australia), S. Aqter Rony, D. Tesfaye (Institute of animal science, University of Bonn, Bonn, Germany), E. Tholen (Institute of Animal Science, University of Bonn, Bonn, Germany), M. Hölker (Institute of animal science, University of Bonn, Bonn, Germany), and K. Schellander (Institute of Animal Science, University of Bonn, Bonn, Germany)

Porcine reproductive and respiratory syndrome (PRRS) is a devastating viral disease affecting swine production, health and welfare throughout the world. Vaccination has been considered as one of the most economic tools for PRRS control. A synergistic action of the innate and the adaptive immune system of host is essential for developing a durable protective immunity to vaccine antigen. The peripheral blood mononuclear cells (PBMCs) play central role in immune system and are able to display gene expression patterns characteristics for certain infection. Therefore, the current study aimed to investigate the global transcriptome profiles of PBMCs to characterize the innate and the adaptive immune response to PRRS Virus (PRRSV) vaccine in Pietrain pigs. We employed nine Affymetrix gene chip porcine gene 1.0 ST array for the transcriptome profiling of PBMCs collected from three female piglets at immediately before (D0), at one (D1) and 28 d (D28) post PRRSV vaccination given at 4 wk (D0) of their age. Two pairwise contrasts were tested to characterize transcriptome alterations associated with the innate immune response (D1 *vs.* D0) and the adaptive immune response (D28 *vs.* D0). Normalization and statistical analysis of microarray data was performed with the 'oligo' and *'limma'* R/Bioconductor package. With FDR < 0.05 and log2 fold change ± 1.5 as cutoff criteria, 83 and 53 transcripts were found to be differentially expressed in PBMCs during innate and adaptive response, respectively. The microarray expression results were technically validated by qRT-PCR. The gene ontology (GO) terms such as viral life cycle, regulation of lymphocyte activation, cytokine activity and inflammatory response were enriched during the innate immune response. The GO terms enriched during adaptive response includes cytolysis, T cell mediated cytotoxicity, immunoglobulin production. Significant enrichment of cytokine-cytokine receptor interaction, signaling by interleukins, viral mRNA translation, IFN-γ pathway and *AP-1* transcription factor network pathways was indicating the involvement of altered genes in the antiviral defense. Network analysis has detected four module were functionally involved with functional network of innate immune transcriptional response and five modules were detected for adaptive immune responses. The innate immune transcriptional network found to be regulated by *LCK, STAT3*, *ATP5B, UBB* and *RSP17*. While *TGFβ*, *IL7R, RAD21, SP1* and *GZMB* are responsible for coordinating the adaptive immune transcriptional response to PRRSV vaccine in PBMCs. Further work is required to determine whether polymorphisms linked to these genes

affect the immune response to PRRSV vaccine in pigs.

Key Words: PRRSV, pig, immunity

P6012 Use of targeted next generation re-sequencing in the identification of polymorphisms in the bovine collagenous lectin gene family. R. S. Fraser, J. D. Hammermueller, J. S. Lumsden, M. A. Hayes, and B. N. Lillie (Department of Pathobiology, University of Guelph, Guelph, ON, Canada)

Collagenous lectins bind carbohydrate motifs on pathogens, leading either to the activation of the lectin complement pathway or to the opsonization or agglutination of pathogens. They play an important role in innate immunity against a variety of bacteria, viruses, and fungi. Functionally altered proteins resulting from genetic mutations in collagenous lectins have been shown in other species to predispose animals to infectious disease. This study aimed to 1) identify genetic variation in the bovine collagenous lectin genes; and 2) determine whether polymorphisms were associated with an increased susceptibility to infectious disease. We used pooled, targeted next generation re-sequencing to identify variants in the bovine collagenous lectin genes. Cattle submitted for post-mortem examination at the University of Guelph were classified as normal (n = 40) or diseased (n = 80) based on the presence or absence of infectious disease. Cattle were placed into groups of 5 based on the similarity of diagnosis, and an equal amount of DNA from each animal was pooled. The collagenous lectin genes (including three unique to bovids) along with 3 kb of downstream DNA and up to 50 kb of upstream DNA were targeted for re-sequencing. The sequencing library was prepared with a Roche Nimblegen EZ Developer kit and sequenced on an Illumina MiSeq. In total, 4.6 Gb of usable sequence data was obtained with an average read depth of 42x/cow over the target region. Following application of quality control filters, 6525 single nucleotide variants (SNVs) were found, including 510 not reported in dbSNP. This included 3948 upstream region SNVs, 2672 downstream region SNVs, and 411 intronic SNVs. Within exons, 107 SNVs, including 54 missense mutations, were identified. *In silico* analysis of the missense mutations identified 16 SNVs with significant potentially disruptive effects on protein structure. A mutation in *MBL2*, resulting in a P42Q change in the collagen-like domain, holds particular interest, as similar mutations in orthologous genes have been shown to have impact on susceptibility to disease. Allele frequencies between the normal and diseased populations were compared and the potential impact of promoter SNVs investigated. This study demonstrates that pooled, targeted re-sequencing is a cost effective method of polymorphism identification and discovery in cattle. We identified 510 previously unreported SNVs, as well as 16 mutations potentially affecting collagenous lectin structure or function. These SNVs will help in our understanding of the genetics of disease susceptibility, and represent potential candidates for genetic selection.

Key Words: collagenous lectins, SNVs, cattle

P6013 Identifying driver mutations for Marek's disease lymphomas in chicken using integrated genomic screens. A. Steep (Michigan State University, East Lansing, MI), H. Xu, Y. Zhang (Technische Universität München, Freising, Germany), A. Black Pyrkosz (USDA, ARS, ADOL, East Lansing, MI), M. E. Delany (University of California-Davis, Davis, CA), D. Frishman (Technische Universität München, Freising, Germany), and H. H. Cheng (USDA, ARS, ADOL, East Lansing, MI)

Marek's disease (MD), a virally-induced lymphoproliferative disease of chickens epitomized by T cell lymphomas, maintains as a premier threat to the world's poultry industries. Despite control measures like widespread usage of MD vaccines and biosecurity, new and more virulent strains of Marek's disease virus (MDV), the causative agent, have repeatedly arisen resulting in disease outbreaks. With the most efficacious MD vaccine in use for 10–15 yr, there is fear that more virulent field strains will emerge soon as well as the need for improved control strategies. Therefore, there is a critical need to understand MD tumorigenesis and the genomic landscapes inside tumors to utilize genetic resistance as a sustainable control MD measure. To identify somatic mutations driving tumorigenesis, highly inbred experimental White Leghorn lines that were MD resistant or susceptible were intermated to produce a cohort of F_1 chickens that were challenged with MDV, and the most homogenous tumors and matching normal tissues collected. To date, most samples have been screened to generate four primary datasets: (1) whole genome sequencing analysis, (2) whole transcriptome sequencing analysis, (3) 15K SNP microarray analysis, and (4) cytogenetic analysis to identify MDV integration sites. Collectively these data should allow for the robust characterization of somatic cancer-associated variants including single nucleotide variants (SNVs), short insertions and deletions (indels), structural variations (SVs), copy number variations (CNVs), and loss-of-heterozygosity (LOH). Thus far with DNA resequencing of 26 MD tumors (and

matched normal tissues), 6 different somatic single nucleotide variant callers have repeatedly identified ~300 SNPs per tumor sample, many of which are associated cancer-genes in humans. The low number of variants increases our chances of finding causative driver gene(s). Furthermore, the low somatic mutation rate (~0.3 SNPs/Mb), which is similar to human juvenile cancers, also suggests a significant genetic component. Further refinements in the computational pipelines as well as integration with the other datasets should enable the identification of key driving genes for MD as well as information on somatic mutation frequency, somatic mutation signatures, and significantly mutated regions. Collectively these cancer-associated inferences can be used to understand the genetic forces and players involved in tumorigenesis and tumor progression to develop sustainable genetic resistance strategies, e.g., genomic selection. While a work in progress, the latest results and analyses will be presented as well as the challenges to implement computational tools developed primarily for the biomedical community.

Key Words: lymphoma, somatic mutations, genetic resistance

P6014 Mapping and exome sequencing of a weak calf syndrome with premature birth. T. Hirano, A. Okazaki (Tokyo University of Agriculture, Atsugi, Japan), S. Sasaki (National Livestock Breeding Center, Fukushima, Japan), Y. Suzuki (Graduate School of Frontier Sciences, University of Tokyo, Kashiwa, Japan), H. Hara (Tokyo University of Agriculture, Atsugi, Japan), Y. Sugimoto (Shirakawa Institute of Animal Genetics, Odakura, Nishigo, Fukushima, Japan), and K. Hanzawa (Tokyo University of Agriculture, Atsugi, Japan)

Weak calf syndrome (WCS) indicates cryptogenic weaknesses, and some WCS affected calves die. It has been reported that a part of the WCS is IARS (isoleucyl-tRNA synthetase) disorder in Japanese black cattle. However, causes of other WCS are unclear. In offspring sired by Bull B, WCS characterized by premature birth was found. To determine a mutation causing the WCS, we performed a mapping and exome sequencing. Bull B, 5 affected calves and 21 normal cattle sired by Bull B were collected. The affected calves were diagnosed with WCS, and their gestation periods were < 275 d. To determine a critical region for the WCS, these 27 animals were genotyped with BovineSNP50 BeadChip (illumina), and homozygosity mapping was performed. To identify a candidate variant for the WCS, whole-exome sequencing was performed with Bull B, 2 affected calves and 1 normal cattle used for the mapping. The critical region was determined on the 60.0 Mb-75.6 Mb region of BTA 4 with homozygosity mapping. It suggested that the WCS was an autosomal recessive disorder. Furthermore, exome sequencing was performed with 1 paired-end 100-bases read length run. In each sample, more than 80% of exon region were covered with > 10 reads. With these reads data, 162 variants that the 2 affected calves were homozygous were detected. Six SNPs and 1 indel of these variants located on the known genes, mRNAs or ESTs, and it was indicated that these variants were non-synonymous and frameshift variants. We concluded that further analysis was needed to these 7 variants as candidates of a causative mutation. To determine a possible causative mutation or identify a variant strongly linked with the WCS from the 7 variants, we are performing a genotyping of the 7 variants for 27 animals used for the mapping and a normal population unrelated to Bull B.

Key Words: weak calf syndrome, mapping, exome sequencing, Japanese black cattle

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P6015 An intronic MBTPS2 variant results in a splicing defect in horses with brindle coat texture. L. Murgiano (Institute of Genetics, University of Bern, Bern, Switzerland), D. Waluk (Department of Dermatology, University Hospital of Bern, Bern, Switzerland), R. Towers (Institute of Medical Genetics, Cardiff University, Cardiff, United Kingdom), N. Wiedemar, J. Dietrich, V. Jagannathan, M. Drögemüller (Institute of Genetics, University of Bern, Bern, Switzerland), T. Druet (University of Liège, Liège, Belgium), A. Galichet (Department of Dermatology, University Hospital of Bern, Bern, Switzerland), M. C. Penedo (Veterinary Genetics Laboratory, School of Veterinary Medicine, UC Davis, Davis, CA), E. Müller (Department of Dermatology, University Hospital of Bern, Bern, Switzerland), P. Roosje (Division of Clinical Dermatology, Department of Clinical Veterinary Medicine, University of Bern, Bern, Switzerland), M. Welle (Institute of Animal Pathology, University of Bern, Bern, Switzerland), and T. Leeb (Institute of Genetics, University of Bern, Bern, Switzerland)

We investigated a family of horses exhibiting vertically striped patterns in their hair coat texture. This phenotype is termed “brindle” by horse breeders. Pedigree analyses were suggestive of a monogenic X-chromosomal dominant mode of inheritance. The striped pattern in affected female horses followed the lines of Blaschko thought to be a consequence of female X chromosome inactivation with subsequent

expansion of cell clones expressing either the wild-type or mutant allele. Thus the striped pattern in the brindle phenotype supports the putative X-chromosomal dominant mode of inheritance. We analyzed whole genome sequences of 4 brindle and 62 non -brindle horses. The analysis revealed a private variant in intron 11 of the *MBPTS2* gene encoding the membrane bound transcription factor peptidase, site 2. The variant was absent from 457 control horses of diverse breeds. Different missense mutations in the *MBPTS2* gene lead to three related genodermatoses in human patients: ichthyosis follicularis, atrichia and photophobia (IFAP, OMIM #308205), Olmsted syndrome (OMIM #300918), and keratosis follicularis spinulosa decalvans (OMIM #308800). As the equine variant was very close to an exon/intron boundary (c.1437+4T > C), we analyzed *MBPTS2* transcripts in skin RNA from an affected and a control horse. The control sample yielded only the expected RT-PCR band whereas in the affected animal we observed an additional aberrant transcript lacking the entire exon 10 and parts of exon 11. Our genetic data and the previous knowledge on MBTPS2 function suggest that the *MBTPS2* intronic variant leads to partial exon skipping and causes the brindle phenotype in horses.

Key Words: horse, Equus caballus, non-coding, splicing, dermatology, whole genome sequencing, X chromosome

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P6016 Epistatic interactions of more than two loci are involved in the rat-tail phenotype in cattle.

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The rat-tail syndrome (RTS) is a bovine congenital, inherited hypotrichosis characterized by various degrees of sparse, curled malformed hair and by missing hair at the animal's tail switch. The defect in hair conformation is restricted to pigmented sections of the pelage and has been observed in crosses between black cattle breeds (e.g., Angus and Holstein) and some European breeds with the specific feature of coat color dilution (e.g., Simmental, Charolais and Hereford). Due to partially controversial results in the literature, the full causal genetic background of RTS is still under debate. Thus, the aim of this study was to monitor the genetic architecture of RTS and to map the locus (or loci) epistatically interacting for RTS. Taking advantage of a resource cross population from German Holstein and Charolais cattle breeds, which was segregating for RTS, we proved that epistatic effects of at least three independent genetic loci are required for the expression of the "rat tail" phenotype. We found that in our population, RTS is exclusively expressed on a eumelanic background with the dominant E^D allele at the *Extension* locus (*MC1R* gene) located on BTA18. In addition, only individuals heterozygous for the *Dilution* locus on BTA5 (*c.64G > A* at the *PMEL* or *SILV* gene) were classified as rat-tail phenotype. However, the results of our segregation analysis prove that a two locus model including the *Extension* and the *Dilution* locus is obligatory but not sufficient to fully explain the rat-tail phenotype. Our results provide evidence that epistatic interaction with at least a third independent locus is required for its expression. Applying linkage and whole genome association analyses with different models and in different pedigrees, the third locus essential for the expression of the rat-tail phenotype was mapped consistently to the chromosomal region 14–22 Mb on BTA5, obviously affecting hair structure as well as hair pigmentation. We clearly demonstrated that this third locus is distinct from the *Dilution* locus represented by the *PMEL* gene. Finally, the results of our study exclude several loci reported to be associated or underlying hair conformation or pigmentation traits as causal mutation for RTS and also promising functional and positional candidate genes associated with hypotrichosis in humans. RTS with its three locus interaction is a prime example for epistatic interaction of several independent loci required for the expression of a distinct phenotype.

Key Words: pigmentation, rat-tail, hair defect

P6017 A high density genome-wide scan for genetic risk factors of insect bite hypersensitivity (IBH): A Horsegene Project Initiative.

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Leuven, Belgium), and G. Lindgren (Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden)

Insect bite hypersensitivity (IBH), a common allergic skin disease in horses caused by biting midges, has been estimated to affect as much as 60% of some breed populations. The result of both genetic and environmental factors, IBH is pruritic and can lead to extensive scratching followed by hair loss, self-excoriation, and skin thickening. Previous studies on IBH suggest both common and breed specific genomic regions associated with IBH. As such, breed specific information regarding the genetic mechanisms involved in IBH development is needed to enable efficient and accurate selection against IBH across multiple breeds. The current study uses Exmoor ponies, a highly inbred breed of horse known to frequently suffer from insect bite hypersensitivity, to identify genomic regions associated with this type I and type IV hypersensitive reaction. A total of 110 cases and 170 controls were genotyped on the 670K Axiom Equine Genotyping Array. Quality control resulted in 452,457 SNPs and 268 individuals being tested for association. Genome-wide association analyses were performed using the GenABEL package in R and resulted in the identification of two regions of interest on Chromosome 8. The first region contained the most significant SNP identified, which was located in an intron of the DCC netrin 1 receptor gene. The second region identified contained multiple top SNPs and encompassed the *PIGN*, *KIAA1468*, *TNFRSF11A*, *ZCCHC2*, and *PHLPP1* genes. While the region consists of five genes, *TNFRSF11A* stands out as a potential candidate gene. Not only is *TNFRSF11A* the closest gene to SNP AX-104330407, but screening for *TNFRSF11A*has already been suggested as a potential diagnostic test for autoinflammatory disorders in humans. At the meeting, we will present the results of our genome-wide association analyses and provide additional information on the frequencies of the top SNPs in other IBH prone breeds.

Key Words: allergy, exmoor, horse

P6018 Revealing the importance of SLA-DRB1 to post-weaning piglet survivability by a case-control analysis and subsequent validation using in silico epitope binding analysis and molecular structural modeling. M. T. Le, H. J. Lee, J. Lee, and C. Park (Konkuk University, Seoul, Korea)

We performed a case-control study to evaluate the effects of polymorphisms of two porcine MHC class II genes, SLA-DQB1 and DRB1 on the post-weaning survivability of piglets. We collected tissues from 388 F1 animals from Landrace-Yorkshire crosses comprised of 187 healthy piglets and 201 with symptoms comparable to wasting diseases including weight loss, emaciation, diarrhea, respiratory distress or sudden death. We typed both *SLA-DQB1* and *DRB1* exon2 in high resolution. A total of 16 and 18 alleles were identified from *SLA-DQB1* and *DRB1*, respectively, resulting in 37 different *DQB1* and *DRB1* haplotypes. We analyzed the associated effect of each SNP from the identified alleles on the piglet survivability. A total of 43 and 83 SNPs were identified and analyzed for *DQB1* and *DRB1*, respectively. The results showed that the most strongly associated SNPs were located to the critical region of the antigen biding groove of MHC class II molecules. More interestingly, in-silico analysis which allows the prediction of the epitope binding affinity to MHC molecules against pig specific pathogenic antigens selected from IEBD database also suggested that SLA-DRB1 functions as a main factor in restricting putative pathogens and *SLA-DRB1*0602* and **04kn05* as favorable alleles for post-weaning survivability with much stronger binding affinity to epitopes of viral pathogens, particularly PRRSV and PCV2. In contrast, the associated alleles with the disease phenotype presenting group showed much weaker binding affinity to MHC class II molecules like *DRB1*0101* and **0201*. Furthermore, we constructed the protein models against significant alleles as well as simulated alleles bearing the critical SNPs of the favorable alleles. The results showed that charges are important for the pocket 9 of the antigen binding site and the structure of the epitope contact area for the pocket 6, which is in agreement with the results of genetic association study. In conclusion, our results suggested that the difference in epitope binding affinity of each MHC allele of any individual may contribute to the difference in post-weaning piglet survivability through the difference in the degree of immune activation against specific antigens. Therefore, the high resolution typing results of the epitope binding region of MHC molecules could be used to predict the capacity of individual animals to become resistant against specific pathogens, and can be considered as important information for controlling disease resistance and susceptibility to certain pathogens in animal breeding

Key Words: pig, wasting disease, SLA, DQB1, DRB1, SNP, epitope binding

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P6019 Host genetics of resistance to bovine tuberculosis infection in dairy cows. S. Wilkinson, S. C. Bishop (The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, United Kingdom), A. R. Allen, S. H. McBride (Agri-Food

and Biosciences Institute Stormont, Belfast, United Kingdom), R. A. Skuce (Agri-Food and Biosciences Institute Stormont, Belfast, United Kingdom; Queen's University Belfast, Belfast, United Kingdom), M. Bermingham, J. A. Woolliams, and L. J. Glass (The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, United Kingdom)

Genetic variation for bovine tuberculosis (bTB) resistance exists in cattle, thus breeding for resistance is a viable complementary control option. bTB infected cattle vary in signs of pathology, suggesting bTB presents as a spectrum of phenotypes. This study investigated the host genetics under-pinning different bTB infection outcomes to increase understanding of biological mechanisms involved in bTB resistance. A case-control design was set up and dairy cows were sampled from Northern Ireland herds. Using the diagnostic skin test and post-mortem examination, infected skin test positive cases were divided into two sub-categories: those with disease (visible lesions detected (VL cases)) and those showing no visible lesions (NVL cases). Resistant controls were those with multiple negative skin test readings. 1966 cows, defined by these 3 phenotypes, were genotyped at 538,231 SNPs. The contribution of each chromosome (BTA) to heritability (h^2_c) was estimated using a linear mixed model and genomic regions of variation (h^2_r) were identified using regional heritability (RH) mapping with 100-SNP overlapping windows. Analyses considered controls against each case phenotype. Chromosome heritability analysis revealed a total of 5 and 14 chromosomes respectively contributed to heritability for VL cases ($2.1\% \leq h^2_c \leq 5.1\%$) and NVL cases ($1.7\% \leq h^2_c \leq 7.7\%$). Of the 5 chromosomes contributing to heritability in VL cases, 4 were also detected in NVL cases. RH mapping identified 1 genomic region in the VL cases at 71.5–71.8 Mb on BTA13 ($h^2_r = 2.1\%$) and 3 genomic regions in the NVL cases at 19.3–19.8 Mb on BTA17 ($h^2_r = 5.3\%$), 57.2–57.5 Mb on BTA22 ($h^2_r = 3.9\%$) and 6.6–7.1 Mb on BTA23 ($h^2_r = 3.5\%$). Genes associated with these regions were identified. Of interest was the bovine leukocyte antigen (*BoLA*) *class IIb* region located at 6.97–7.53 Mb near the region found on BTA23. *BoLA* genes play a major role in immune response to infection, being involved in processing and presenting of foreign antigens. The results suggest there may be at least two bTB infection outcomes controlled by distinct and overlapping genetic variants. Breeding values can incorporate both bTB infection states as they share underlying chromosomal variance.

Key Words: bovine tuberculosis, host genetics, resistance loci

P6020 Deficiency of Trim63 leads to hypertrophic cardiomyopathy in pig. Y. Hu (The State Key Laboratory for Agro-biotechnology, China Agricultural University, Beijing, China), Y. Xing (The State Key Laboratory for Agro-biotechnology, China Agricultural University, Beijing, China), X. Hu, and N. Li (China Agricultural University, Beijing, China)

Hypertrophic cardiomyopathy (HCM) is a common inherited cardiomyopathy, which characterized as myocardial hypertrophy. The morbidity of HCM is 0.2%, 1% of which is linked with sudden cardiac death every year. More than 900 mutations of 15 genes have been identified to associate with HCM, and most of the mutations in genes encodes for muscle sarcomere proteins. Cardiac hypertrophy in HCM is considered secondary to activation of a diverse array of intracellular signaling pathways that collectively promote protein synthesis. The role of protein degradation, the opposite end of the spectrum from protein synthesis, in the pathogenesis of HCM is less well recognized. This work aims to study the effects of atrophy gene *Trim63* on HCM. We knocked out *Trim63* by CRISPR/Cas9 system in pig. Several *Trim63*$^{-/-}$ founders died of heart dysfunction with asymmetric hypertrophy in left ventricular. Higher level of serum myocardial enzymes was detected in *Trim63*$^{-/-}$ vs. control. Our data demonstrate that *Trim63* plays important roles in development of heart structure. Deletion of *Trim63* will cause HCM in pig.

Key Words: pig, Trim63, hypertrophic cardiomyopathy

P6021 The potential of serum IL-10 as a diagnostic biomarker of resilience in the domestic chicken to infection from *Eimeria* Spp. K. Boulton, Z. Wu, A. Psifidi, and D. Hume (The Roslin Institute, Edinburgh, United Kingdom)

The protozoan parasite *Eimeria* causes coccidiosis, a debilitating disease affecting intestinal function and overall health, most notably in the domestic chicken (*Gallus gallus*). Three species (*E. maxima, E. tenella, E. acervulina*) are largely responsible for coccidiosis incidence, costing the international poultry industry over $2Bn per annum. The sustainability of commercial production is further threatened by considerable political pressure in some countries to restrict the use of coccidiostat drugs. Breeding birds that are resilient to disease can contribute to coccidiosis control, and therefore aid poultry production as a sustainable source of protein for the ever-expanding global population. Selective breeding for improved resilience is

dependent on accurate and informative phenotypes. Prognostic biomarkers for *Eimeria* susceptibility are already known, such as lack of body weight gain and parasite replication, while post-mortem intestinal lesion scores indicate the severity of infection. However, diagnostic biomarkers that can predict resilience to *Eimeria* spp. in very young birds are desirable. We have performed a series of challenge experiments in commercial broilers to investigate the genetic basis of resilience, including evaluation of novel phenotypes. These include a level of circulating interleukin-10 (IL-10), an anti-inflammatory cytokine, that is positively correlated with caecal lesion score and negatively correlated with overall body weight gain ($p < 0.001$, $n = 1000$). IL-10 production may contribute to the known immunosuppressive effect of *Eimeria* infections, and its utility as a diagnostic biomarker is under investigation. This novel trait is being used in genome-wide association studies to develop genetic marker predictors of resilience, using whole genome resequencing of birds with disparate phenotypes. Once genomic markers associated with resilience have been identified, they can be applied in selective breeding programs to help control coccidiosis.

Key Words: resilience to *Eimeria*, diagnostic biomarkers, IL-10

P6022 Transcriptomic study of bovine macrophages infected in vitro with *Streptococcus agalactiae*.

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Mastitis is a costly disease in dairy cattle, impairing animal welfare and increasing the use of antibiotics. It is a multi-factorial disease, affected by the load and virulence of the infecting pathogen, the milking hygiene, and the immune status and genetics of the host. *Streptococcus agalactiae* has re-emerged as a potential threat to udder health in Norway, especially in large herds and herds using automatic milking systems. Some cows develop chronic subclinical mastitis with high somatic cell count (SCC). Such animals shed the bacteria and contribute to spreading the infection to other cows and herds. Hence, investigation of why these become chronically infected is important. Macrophages are critical effectors and regulators of inflammation serving as the first line of defense against invading pathogens. Intramammary infections will activate macrophages to produce proinflammatory cytokines required to kill intracellular pathogens, however the balance between pro- and antiinflammatory signals is crucial for immune regulation of inflammation and preventing chronic conditions. The objective of this study is to increase the understanding of the host genetics and immune response to *Streptococcus agalactiae* causing chronic subclinical mastitis in Norwegian Red (NRF) dairy herds. In genotyped cows we will examine the transcriptomic responses of macrophages infected with high and low virulent strains of *Streptococcus agalactiae*. Seven animals were selected for a pilot study of in vitro challenge of blood monocyte-derived macrophages with two strains of *Streptococcus agalactiae* differing in virulence. Transcript analyses of several selected cytokines and chemokines were performed by qPCR. Four thousand NRF cows is currently genotyped using a customized 55K bovine SNP-chip (a typing tool for 55 000 Single Nucleotide Polymorphism markers) and genomic breeding values (GEBVs) for SCC will be estimated to evaluate the contrasts between the groups. Based on this genotyping cows with long duration of high SCC (= chronically infected animals) and unfavorable GEBV for SCC, and cows with very low SCC (= control) and favorable GEBV for SCC will be selected for in vitro challenge of blood monocyte-derived macrophages with the two bacterial strains. For each selected individual transcriptome analyses, i.e., mRNA sequencing, will be performed to identify the macrophage response to the infections in challenged and control sets of cells. This will generate information on genes and splice variants involved in immune responses during the infection with high and low virulent strains of *Streptococcus agalactiae*.

Key Words: macrophages, Norwegian Red, subclinical mastitis, *Streptococcus agalactiae*, transcriptome

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P6023 Novel miRNA involved in host response to avian pathogenic *Escherichia coli* identified by deep sequencing and integration analysis.

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Avian pathogenic Escherichia coli (APEC) causes one of the most common bacterial diseases in the poultry industry worldwide. Effective control methods are, therefore, desirable, and will be facilitated by a better understanding of host response to the pathogen. Currently, miRNAs involved in host resistance to APEC are unknown. Here we applied RNA sequencing to explore the changed miRNAs and deregulated genes in spleen among groups of non-challenged (NC), challenged-mild pathology (CM), and challenged-severe pathology (CS) broilers. Twenty-seven differentially expressed miRNAs were identified, including 13 miRNAs between NC and CM, 17 between NC and CS, and 14 between CM and CS. Through analysis of these miRNA targets, 12 immune-related biological processes were significantly enriched. Using an integrated analysis of miRNA and mRNA expression profiling, 43 miRNA-mRNA pairs displayed significantly negative correlations ($r < -0.8$) among each group. Notably, only miR-429 was greatly increased in CS compared to both CM and NC. In vitro, miR-429 directly repressed luciferase activity via binding to 3'-untranslated regions of TMEFF2, NTRK2 and SHISA2. Overexpression of miR-429 in the HD11 macrophage cell significantly inhibited TMEFF2 and SHISA2 expression, which are involved in the lipopolysaccharide-induced PDGF and Wnt signaling pathways. In summary, we provide the first report characterizing the miRNA changes during APEC infection, which may help to shed light on the mechanism of APEC resistance and susceptibility.

Key Words: APEC miRNA spleen

P6024 Holstein Friesian lethal haplotype 5 is caused by a 138kbp deletion on chromosome 9. C. Wehrhahn, E. Schütz (Institute of Veterinary Medicine, Georg-August-University, Göttingen, Germany), M. Wanjek, R. Bortfeld (Institute for Livestock Reproduction GmbH, Schönow, Germany), J. Beck (Chronix Biomedical GmbH, Göttingen, Germany), and B. Brenig (Institute of Veterinary Medicine, Georg-August-University, Göttingen, Germany)

With the introduction of genomic selection in modern cattle breeding programs, a huge amount of SNP data became available. A thorough analysis of these data led to the identification of significantly underrepresented or almost absent homozygous haplotypes in the genome of different cattle breeds, so-called recessive (lethal) haplotypes. In Holstein Friesian (HF) more than 15 such lethal haplotypes (HHx) have been detected, however, only a few have been elucidated so far. Among the unsolved haplotypes, HH5, reported to be located in the interval between 91.8–93.8Mb on BTA9, has been traced back to Thornlea Texal Supreme born in 1957. In the German HF population a population frequency of as high as ~4.8% was determined. To clarify the underlying mutation, DNA of BAC clones spanning the reported region were hybridized with genomic DNA of known imputed haplotype carriers and unaffected controls. After target enrichment, samples were subjected to massive parallel sequencing on a NextSeq500 platform. The HH5 target region was sequenced with an average 150-fold base coverage per animal (STD:78). Data were aligned to the bovine genome build UMD_3.1 and evaluated for polymorphisms and copy number variations. The latter revealed a deletion of ~138kb with a breakpoint and fusion between BTA9:93,232,651 and BTA9:93,370,998. The deletion breakpoints are flanked by bovine long interspersed nuclear elements Bov-B, located upstream, and L1ME3, located downstream, suggesting a homologous recombination/deletion event. The deleted region harbors only one reference gene, i.e., dimethyl-adenosine transferase 1, or transcription factor B1 mitochondrial (*TFB1M*), encoding for an essential protein for synthesis and function of the small ribosomal subunit of mitochondria, and therefore mitochondrial protein translation. Knock-out experiments in mice have shown that *TFB1M* is irreplaceable, as *TFB1M*$^{-/-}$ mice die at Day 8.5 during embryonal development. The allelic frequency in randomly selected ~2,000 HF cattle of the German population was 2.8%, which calculates to a carrier frequency of 5.5%. To reduce the amount of carrier in the HF population it is advisable to directly genotype the deletion underlying HH5.

Key Words: lethal recessive haplotype, BTA9, Holstein Friesian

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P6025 Network-based integration of gene expression and genome-wide association data to prioritize genomic variants associated with susceptibility/resistance to bovine tuberculosis. K. E. Killick, K. E. McLoughlin (School of Agriculture and Food Science, University College Dublin, Dublin, Ireland), N. C. Nalpas (Proteome Center, University of Tubingen, Tubingen, Germany), L. Burkitt-Gray (School of Agriculture and Food Science, University College Dublin, Dublin, Ireland), I. W. Richardson (Department of

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Mycobacterium bovis, the causative pathogen of bovine tuberculosis (BTB), is responsible for an estimated $3 billion losses to global agriculture annually. The impacts of *M. bovis* infection are manifold, including risks to animal and public health, disruptions to trade, and reduced agricultural productivity, particularly in developing countries. Previous microarray and RNA-seq transcriptomics experiments have facilitated reconstruction of gene regulatory networks and cellular pathways underlying the bovine host response to infection with *M. bovis*. In addition, it is well established that intra- and inter-population genetic variation exists for BTB susceptibility/resistance, with documented heritability estimates ranging from 0.08 to 0.19. For the present study, we therefore used a network-based approach to integrate gene expression data with high-density single-nucleotide polymorphism (SNP) genome-wide association (GWA) data to enhance detection of genomic variants for susceptibility/resistance to *M. bovis* infection. A range of bovine host RNA-seq data have been generated by our research group; these include data from an ex vivo experimental *M. bovis* animal infection time course and in vitro macrophage challenge experiments using *M. bovis*. These gene expression data were superimposed on a base network of the mammalian host response to mycobacterial infections. Following this, systems approaches identified key subnetworks and contextual hub genes. These gene subsets were then used to rank and prioritize SNP variants from a GWA study of individual animal estimated breeding values (EBVs) for BTB susceptibility with SNP genotype data (Illumina® Bovine HD Genotyping BeadChip-597,144 filtered SNPs) generated for 842 Holstein-Friesian dairy bulls. These prioritized statistically robust SNP variants will provide an important reference for genome-enabled breeding programs for BTB resistance traits. Importantly, the approach and methods described here may also be applied to comparable transcriptomics and GWA data generated for studies of the human response to infection with *Mycobacterium tuberculosis*.

Key Words: bovine tuberculosis, cattle, GWAS, integrative genomics, network, transcriptomics

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P6026 Using diverse U.S. beef cattle genomes to identify missense mutations in EPAS1, a gene associated with high-altitude pulmonary hypertension. M. P. Heaton, T. P. L. Smith, J. K. Carnahan (USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE), V. Basnayake, J. Qiu, B. Simpson (GeneSeek, a Neogen Company, Lincoln, NE), and T. S. Kalbfleisch (University of Louisville, Louisville, KY)

The availability of whole genome sequence (WGS) data has made it possible to discover protein variants *in silico*. However, bovine WGS databases comprised of related influential sires from relatively few breeds tend to under represent the breadth of genetic diversity in beef cattle. Thus, our first aim was to use 96 beef sires sharing minimal pedigree relationships, to create a searchable, publicly viewable set of mapped genomes from 19 breeds of U.S. cattle. Our second aim was to use these genomes to identify protein variants, like those encoded by the endothelial PAS domain-containing protein 1 gene (*EPAS1*). This gene encodes the hypoxia inducible transcription factor 2A (HIF2A) and has been associated with high-altitude pulmonary hypertension (HAPH) in American Angus cattle. For each bull sequenced, the identity and quality of its mapped WGS data were evaluated by comparing its single nucleotide polymorphism genotypes to those derived from other genotyping methods. These comparisons showed the average read depths, scoring rates, and accuracies exceeded 12, 99%, and 99%, respectively. We then used the genomes to identify protein variants. Six *EPAS1* amino acid variants were observed among the 96 bulls, including those associated with HAPH (T606 and S610). The *EPAS1* codon genotypes were independently confirmed with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry assays. A network of seven distinct HIF2A polypeptide sequences was inferred by maximum parsimony, resulting in 28 possible diploid combinations. This network of protein variants provides a framework for evaluating the impact of *EPAS1* alleles on the adaptive response to hypoxia in U.S. cattle. Thus, this public, whole genome resource facilitates *in silico* identification of protein variants in diverse types of U.S. beef cattle, and provides a means of translating WGS data into a practical biological

context for hypothesis testing.

Key Words: beef cattle, whole genome sequence, EPAS1, protein variants, pulmonary hypertension

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P6027 Study of the mutant MDR1 allele in four Collie breeds in Italy. S. P. Marelli, G. Minozzi, M. Longeri, R. Rizzi, G. Gandini, and M. Polli (Università degli Studi di Milano, Milan, Italy)

The P-Glycoprotein is an ATP-dependent drug transponder (P-gp) encoded by the MDR1 gene, also known as ABCB1. Lack of the P-gp protein causes multidrug resistance 1 in the hematoencephalic barrier, leading to neurotoxicosis after administration of several drugs (ex: ivermectin and P-glycoprotein substates). Within this study we analyzed a 4 bp deletion mutation that causes the introduction of several stop codons that eventually lead to the premature truncation of P-gp. In the absence of mutations, the P-Gp protein binds to a variety of compounds/drugs in the endothelial cells, transporting them back into the bloodstream and consequently preventing their diffusion in the brain. The aim of the present work was to analyze the frequency of the mutant MDR1 allele in the four recognized Collie breeds (FCI; *Canis familiaris*). The four collie breeds analyzed where: Bearded C. (BEA), Border C. (BOR), Rough C. (ROU) and Smooth C. (SMO) for a total of 135 individuals (mean sex ratio M/F = 3/4). Allelic frequencies in the four breeds and their comparison were calculated using the SAS software. In the BEA group 100% of the subject were wt/wt, in the BOR 98.53 were wt/wt and 1.47% wt/del. In the ROU breed we recorded 16.13% wt/wt, 48.39% of wt/del and 35.48% of del/del. The tested SMO was wt/del. The differences were significantly different ($P \leq 0.05$), however the BEA and SMO where represented only by 5 individuals in total. No differences were recorded in frequency of mutation in tested males and females analyzing the entire population. In conclusion, our results demonstrated that notwithstanding the common phylogenetic origin of the breeds analyzed the MDR1 mutation has significant different, leading to differential risk of drug response. These results further demonstrate, that the deletion can be easily tracked in pure breed collie of the different breeds allowing the planning of selective strategies aimed to improve animal health and genetic variability in small size canine populations.

Key Words: MDR1, Collie, mutations

P6028 A frameshift mutation in MOCOS is associated with familial renal syndrome in Tyrolean Gray cattle. L. Murgiano* (GIGA, Liege, Belgium, and Institute of Genetics, Bern, Switzerland), V. Jagannathan (Institute of Genetics, University of Bern, Bern, Switzerland), C. Piffer (Azienda Sanitaria Alto Adige, Bozen, Italy), C. Drögemüller (Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland), A. Gentile (Department of Veterinary Medical Sciences, Bologna, Italy)

Sporadic cases of renal syndromes are reported in domestic animals. We noticed two affected Tyrolean Gray identical twin calves showing inexorable weight loss, skeletal abnormalities and delayed development associated with kidney stones and other kidney abnormalities. The observed renal syndrome in Tyrolean Gray cattle resembled inherited renal tubular dysplasia in Japanese Black cattle associated with mutations in the *claudin-16* gene. Despite striking phenotypic similarities, no obvious presence of pathogenic variants in this candidate gene indicated locus heterogeneity. The family history of the cases suggested an autosomal recessive inheritance. We performed homozygosity mapping, sequenced the whole genome of one case, and detected two associated nonsynonymous private coding variants: A homozygous missense variant in the uncharacterized *KIAA2026* gene (g.39038055C > G; c.926C > G), located in a 15 Mb sized region of homozygosity on BTA 8; and a homozygous 1 bp deletion in the *molybdenum cofactor sulfurase* (*MOCOS*) gene (g.21222030delC, c.1881delG, and c.1782delG), located in an 11 Mb region of homozygosity on BTA 24. Pathogenic variants in *MOCOS* have been associated with inherited metabolic syndromes and xanthinuria in different species including Japanese Black cattle. The identified deletion is predicted to be highly disruptive, creating a frameshift and premature termination of translation resulting in severely truncated MOCOS proteins that lack two functionally essential domains. The variant *MOCOS* allele was absent from cattle of other breeds, and about 4% heterozygous carriers were detected among 1201 genotyped Tyrolean Gray cattle. The identified *MOCOS* loss of function variant highly likely causes the renal syndrome in the affected animals. We suggest that the phenotypic features of the renal syndrome were related to an extremely severe early onset form of xanthinuria, which highly likely led to the progressive defects. The identification of this candidate causative mutation thus widens the known phenotypic spectrum of *MOCOS* mutations and enables selection against this pathogenic variant in Tyrolean Gray cattle.

Key Words: rare disease, hereditary, kidney, rearing success

P6029 Congenital cataract formation in Holstein Friesian cattle. A. K. Hollmann* , W. E. Wemheuer, B. Brenig, E. Schütz (Institute of Veterinary Medicine, Georg-August-University, Göttingen, Germany), and J. Beck (Chronix Biomedical GmbH, Göttingen, Germany)

Congenital cataracts are opacities of the lens present from birth. Affected individuals suffer from loss of vision differing from moderately impaired up to total blindness. Until today, more than 255 genes have been described to harbor causative mutations for hereditary cataracts in humans. Identified mutations mostly affect genes coding for lens crystallins, but also membrane, cytoskeleton, and gap junction proteins, beaded filaments, growth, and transcriptional factors. Cataracts in cattle have been observed in different breeds as Holstein Friesian, Jersey, Hereford, Aberdeen Angus, Shorthorn, Ayrshire, and Romagnola. However, the genetic basis of cataract formation remained unknown in most cases. We investigated 31 cases of bilateral congenital cataract in Holstein Friesian cattle. Pedigree analysis revealed that 26 of 31 cases were paternal half siblings. 28 of 31 cases shared one common ancestor on the dam and sire site of the pedigree three to five generations earlier. A case-control study was performed using genotyping data of 26 cases and 88 controls to check for associations with the cataract phenotype. A genome-wide analysis using the Illumina BovineSNP50v2 BeadChip revealed an association on bovine chromosome 7 (BTA7) at position 12.4 Mb (Bonferroni-adjusted p-value: 1.85×10^{-32}) and 6.2 Mb (p-value: 5.44×10^{-30}). Regarding the proposed autosomal recessive inheritance, we searched for regions of extended homozygosity in cases. A 7.4 Mb interval from 5,675,621 to 13,146,547 on BTA7 (UMD_3.1) was detected covered by 80 single nucleotide polymorphism (SNP) loci, and does not contain any genes previously described as cataract causing. To assess the population frequency, the region was phased (Beagle v. 3.3.2) and inferred for the putative disease related haplotype. The frequency of carriers deduced from haplotyping was ~0.4% in 25,000 randomly tested Holstein Friesian cattle. Within and around the region of extended homozygosity, we searched for positional-functional candidate genes. Four potential functional candidates strongly linked to lens development (*NOTCH3*, *NXNL1*, *SMARCA4*, *DNMT1*) were identified and analyzed by Sanger sequencing. Furthermore, 21 positional candidates solely based on their location and four genes weakly linked to eye development were selected. Nevertheless, all these genes had to be excluded as potentially disease causing, because none of the detected variants were unambiguously associated with the phenotype, and/or the detected variants had no effect on the amino acid sequence. Due to the lack of further candidate genes in the associated region, additional studies, such as whole genome resequencing of affected and related cattle, are planned to elucidate the disease-causing mutation.

Key Words: cataract, BTA7, Holstein Friesian

P6030 Three diverse mutations underlying canine xanthine urolithiasis. N. M. Tate* , K. M. Minor, J. R. Mickelson, K. Peterson, J. P. Lulich, and E. Furrow (University of Minnesota, Saint Paul)

Hereditary xanthinuria in people is an autosomal recessive disease caused by mutations in xanthine dehydrogenase (XDH) or molybdenum cofactor sulfurase (MOCOS). There are rare reports of hereditary xanthinuria in dogs, but genetic investigations have not previously been described. The purpose of this study was to uncover mutations underlying risk for canine xanthine urolithiasis by sequencing *XDH* and *MOCOS* in genomic DNA from affected dogs. The affected dogs included two Toy Manchester Terriers (TMT), two Cavalier King Charles Spaniels (CKCS), and a mixed breed dog. Three putative causal mutations were found. The TMT dogs had a homozygous splice site mutation in *MOCOS*, the CKCS dogs had a homozygous nonsense mutation in *MOCOS*, and the mixed breed dog had a homozygous splice site mutation in *XDH*. cDNA sequencing verified aberrant splicing for the two splice site mutations. Mutation assays were developed to determine the allele frequencies of the mutations in populations of TMT and CKCS dogs without a history of xanthine urolithiasis. Of 49 TMT dogs tested, 37 were clear, 10 were carriers, and 2 were homozygous for the TMT mutation. Urine was analyzed from the 2 homozygous TMTs and revealed xanthinuria. Of 108 CKCS dogs tested, 105 were clear, 3 were carriers, and none were homozygous for the CKCS mutation. In conclusion, diverse mutations were found to be responsible for hereditary xanthinuria in dogs, and we have developed genetic tests for these forms of the disease. Genetic testing can help inform breeders and identify dogs that may benefit from preventative therapies.

Key Words: xanthinuria, XDH, MOCOS

P6031 Confirmation of genome wide analysis of transcriptional responses to Porcine reproductive and respiratory syndrome virus infection in a pregnant gilt model. L. Hong* and J. K. Lunney, (USDA ARS BARC APDL, Beltsville, MD), J. W. Wilkinson and H. Bao (University of Alberta, Edmonton, AB, Canada), A. Ladinig (University of Veterinary Medicine, Vienna, Austria), P. Stothard and G. Plastow (Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada), and J. C. S. Harding (University of Saskatchewan, Saskatoon, SK, Canada)

Transplacental infection of pregnant pigs with Porcine reproductive and respiratory syndrome virus (PRRSV) can cause late-term abortions, an increase of dead and mummified fetuses, and weak-born piglets. The mechanism of PRRSV-induced reproductive failure is poorly understood, especially the molecular events at the maternal-fetal interface and in the fetus itself. Our group had investigated differences in the gene expression profiles of uterine endothelium with adherent placental tissue, and in fetal thymus on Day 21 postchallenge in four groups of fetuses selected from a large PRRSV challenge experiment of pregnant gilts: control (CON), uninfected (UNINF), infected (INF), and meconium-stained (MEC; n = 12/group) by using RNA sequencing. The objective of this study was to validate the differential expression from RNA-seq of genes selected on the basis of their association with biological pathways, functions, and regulators implicated in host response to PRRSV infection using TaqMan real-time PCR assays. Both endometrial and thymic RNAs were tested for expression of T or NK cell marker genes (CD3D, CD8B, and GZMA), four interferon-regulated genes (CXCL10, GBP1, MX1, OAS1), a chemokine (CCL2) and housekeeping genes (PPIA, RPL32). Endometrial RNAs were also assayed for expression of one immunoglobulin gene (IGJ), two TWIST1-regulated genes (FBN2, PAMR1), and an inflammation-associated gene (ITIH4); thymus RNAs were also assayed for inflammation-associated genes (CASP1, MPO), and one apoptosis-associated gene (TNFSF10). Differential expression by RT-qPCR required a significant difference ($P < 0.05$, t test) between groups. In endometrium, differential expression was confirmed for 53% of the genes identified by RNA-seq; however, the direction of expression (i.e., which group in the contrast exhibited the greater expression of that gene) was confirmed for all cases. In thymus, differential expression was confirmed for all cases. Overall, the immune response to infection in endometrium was mainly adaptive in nature, with the most up-regulated genes associated with humoral or cell-mediated immunity. In contrast, the expression profile of infected fetal thymus revealed a predominantly innate response to infection with 2.1- to 4.3-fold increases in expression of genes regulated by Type I interferon and proinflammatory cytokines. This affirms that the immune responses to PRRSV infection at the maternal-fetal interface and in the fetal thymus were substantially different, possibly reflecting the longer duration of infection in endometrium compared with a more acute infection of fetal thymus. Funding: Genome Canada, Genome Prairie, Saskatchewan Ministry of Agriculture; individual support from China Scholarship Council, Alberta Livestock and Meat Agency, and Alberta Innovates Bio Solutions.

Key Words: RNA-sequencing, RT-PCR, PRRS

P6032 Identification and characterization of a novel pathogen causing bovine abortion. B. T. Welly, M. R. Miller, J. L. Stott, M. T. Blanchard, A. Islas-Trejo, S. M. O'Rourke, A. E. Young, J. F. Medrano, A. L. Van Eenennaam* (University of California, Davis)

Epizootic bovine abortion (EBA), commonly known as *foothill abortion*, is the leading cause of beef cattle abortion in California, responsible for the loss of an estimated 45,000 to 90,000 calves per year. In 2005, a novel deltaproteobacterium was discovered as the etiologic agent of EBA (aoEBA). Thus far, it is not possible to grow this organism in culture using traditional microbiological techniques; rather, it can only be grown in experimentally-infected severe combined immunodeficient (SCID) mice. This led to the development of a live bacterial vaccine consisting of a quantifiable number of aoEBA-infected mouse spleen cells. Difficulties and costs associated with production of this live bacterial vaccine motivated our investigation into the development of a recombinant vaccine as an alternative approach to help prevent EBA. The objectives of this study were to perform a de novo genome assembly for the novel aoEBA deltaproteobacterium, and subsequently identify and validate potential antigenic proteins as candidates for the future development of a recombinant vaccine. DNA and RNA were extracted from spleen tissue collected from experimentally-infected SCID mice following their exposure to the aoEBA deltaproteobacterium. This combination of mouse and bacterial DNA was sequenced and aligned to the mouse genome. Mouse sequences were subtracted from the sequence pool and the remaining sequences were de novo assembled at 50× coverage into a 1.82 Mbp complete closed circular deltaproteobacterial genome, containing 2250

putative protein coding sequences. The phylogenetic analysis of aoEBA predicts that this bacterium is most closely related to the organisms of the order Myxococcales, referred to as Myxobacteria. In silico prediction of vaccine candidates was performed using a reverse vaccinology approach, resulting in the identification and ranking of candidate proteins that were likely to be antigenic. Proteins translated from the top nine candidate antigenic genes were run on Western blots and tested against serum from mice and cattle that had been infected by the bacterium. Six of the top nine candidates were bound by antibodies in the serum from infected mice, and the top candidate was strongly bound by serum from infected mice, cows and calves, but not serum from controls. This study provides the basic background information required to further the development of a recombinant vaccine for this orphan disease, which is California's leading cause of abortion in beef cattle.

Key Words: reverse vaccinology, de novo assembly, bovine abortion

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P6033 Genome association of domestic sheep eosinophils with known parasite resistance QTL. M. R. Mousel* , S.N. White, and D. P. Knowles (USDA, ARS, Animal Disease Research Unit, Pullman, WA), S.N. White, D. P. Knowles, M. V. Gonzalez, and J. O. Reynolds (Washington State University, Pullman), M. V. Gonzalez (Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA), J. B. Taylor (USDA, ARS, Rangeland Sheep Production Efficiency Research, Dubois, ID)

Eosinophils are white blood cells that are often associated with internal parasite burden in mammals. Reducing internal parasite burden is of high priority for livestock producers worldwide. Many quantitative trait loci (QTL) have been identified in domestic sheep that are associated with varying internal parasite resistance traits, and whole genome selection for reduced internal parasite load has been successful. Although eosinophil count can vary greatly within animal from day to day, internal parasite burden should consistently increase this value. A genome-wide association study (GWAS) was performed with eosinophil counts as the trait of interest. Eosinophil and total white blood cell count (WBC) were collected over 3 yr (2011, 2013, and 2014), but only the first record for each animal was evaluated. Eosinophil count, percentage of eosinophils, and eosinophil count divided by WBC count were evaluated in separate GWAS. There were 332 ewes of Polypay, Rambouillet, and Suffolk breeds represented from one large range flock with unknown, but expected to be low, internal parasite burden. Breed was a covariate within the linear model. Significant ($P < 0.05$, Bonferroni corrected) markers with 10% or greater minor allele frequency were compared with genomic regions of known parasite resistance QTL. There were 17 genomic regions on 12 chromosomes which were associated with eosinophil traits (eosinophil count or eosinophil percentage) in this study and internal parasite burden in other studies. Ovine chromosomes represented were 1, 2, 3, 4, 8, 13, 14, 15, 16, 17, 23, and X. Studies are ongoing to elucidate underlying genes or mechanisms which influence eosinophil count or percentage and internal parasite burden.

Key Words: sheep, eosinophil, internal parasite QTL

P6034 Associations between *cis*-expression quantitative trait loci markers and host response to Porcine reproductive and respiratory syndrome virus infection. H. Bao, and A. Kommadath (University of Alberta, Edmonton, AB, Canada), I. Choi, and J. K. Lunney* (USDA ARS BARC APDL, Beltsville, MD), J. M. Reecy, E. Fritz-Waters, C. J. Eisley, and C. K. Tuggle (Iowa State University, Ames, IA), J. E. Koltes (University of Arkansas, Fayetteville, AR), R. R. R. Rowland (Kansas State University, Manhattan, KS), J. C. M. Dekkers (Department of Animal Science, Iowa State University, Ames, IA), L. L. Guan (Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada), P. Stothard, G. Plastow (Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada)

Porcine reproductive and respiratory syndrome (PRRS) is economically the most important disease of pigs with annual U.S. losses of $664 M. Understanding transcriptional responses to viral infection should reveal mechanisms to help control PRRS. The PRRS Host Genetics Consortium (PHGC) was established to combine efforts of scientists from university, government, commercial pig genetics and animal health companies to assess the role of genetics in determining pig resistance or susceptibility to PRRS virus (PRRSV) infection, pathology, and growth effects. The PHGC used a nursery pig PRRSV infection model with deep sampling for phenotypic analyses, extensive genotyping (60K SNPchip) and a shared database (www.animalgenome.org/lunney/, verified 26 May 2016). We probed the blood transcriptome of PHGC pigs using RNaseq to address disease resistance mechanisms and

the underlying genetic architecture of gene expression in response to PRRSV infection. We integrated genome-wide genotype, gene expression, viremia, and weight gain data to identify differential expression and genetic variation controlling expression levels and association to phenotypes in response to Type 2 PRRSV infection. RNA was prepared from Tempus tube preserved peripheral blood samples collected just before experimental challenge (Day 0) and at 4, 7, 10, and 14 d postinfection from 44 pigs, and globin RNA depleted before library production. RNA-seq analysis revealed 6430 differentially expressed genes after infection. We mapped genetic variation that is associated with interindividual differences in expression at each day and found evidence of *cis*-acting expression quantitative trait loci (*cis*-eQTL) for 1201 genes. Associations between *cis*-eQTL markers and phenotypes using 383 pigs indicated host genotype-dependent reduced expression of four genes, including GBP5 as expected from our earlier mapping results. These *cis*-eQTLs contribute to differences in viremia levels or weight gain in response to PRRSV infection. We also showed evidence of allele-specific expression for those four genes. This study expands our understanding of disease control mechanisms and provides potential new diagnostic tools for pig breeders. Further studies are needed to define the causal mutations that regulate expression of these candidate susceptibility genes and test how changes in expression of these genes can directly affect the traits. This work was funded by Genome Canada, Genome Alberta, Alberta Livestock and Meat Agency, PigGen Canada, and USDA ARS.

Key Words: *cis*-eQTL, RNA-seq, PRRS

P6035 The expression of genes connected with prion protein metabolism in sheep.

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The putative infectious agent of scrapie (fatal neurodegenerative disease of sheep and goats) is prion protein (PrP), which is encoded by the host *PRNP* gene. The disease development is connected with the prion protein expression. However, other genes involved in PrP metabolism may be linked to scrapie occurrence. The aim of the project was to analyze the expression level of several genes connected to PrP metabolism, proliferation, differentiation, and apoptosis of neuronal cells, such as *PRNP*, *RPSA*, *MGRN1*, *BAX*, *PSEN1*, and *PSEN2*. The healthy animals of two sheep breeds (Romanov, Polish Merino) were divided into age groups. RNA was isolated from brain stem, cerebellum (30 animals), and some other tissues (a few animals), normalized, and reverse transcribed. The tissue expression profile was analyzed (13 tissues). The relative quantification by real-time PCR (TaqMan probes; brain stem and cerebellum) was performed. Polymorphism of the *PRNP*gene (sequencing) was also investigated. *PRNP*, *RPSA*, *MGRN1*, *BAX*, and *PSEN1* transcripts were observed in all tissue analyzed. *PSEN2* transcripts were absent in pituitary gland, heart, and skeletal muscle. The expression level of *PRNP* gene was higher in the nervous tissue than in other tissue analyzed, and the highest was in the brain cortex. Among age groups in cerebellum tissue, the *PRNP*, *MGRN1*, *BAX*, and *PSEN2* gene expression level was the highest for the youngest sheep (7 to 9 mo old). The *PRNP* expression in the brain stem was also the highest in the youngest sheep. RPSA mRNA was the most abundant in the oldest animals (123–125 mo old) for both tissues. Four alleles (ARR, ARQ, AHQ, and VRQ) and seven genotypes of the *PRNP* gene were observed in whole group studied. Two individuals had F allele in codon 141. The further data analysis is in progress. The study was financed by grant project no. N N311 406339.

Key Words: *PRNP*, gene expression level, sheep

P6036 A polymorphism of CD163 gene is significantly associated with weight gain of the pigs under persistent PRRSV infection.

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The CD163 is a crucial receptor for Porcine reproductive and respiratory syndrome virus (PRRSV) adhesion and internerization. A polymorphism (c.3534C > T) found 3′UTR of the *CD163* was previously reported its association with Immunoglobulin G (IgG) content in blood. Our previous results suggested that this polymorphism could be associated with the weight gain of pigs experimentally infected with PRRSV. Therefore, the aim of this study was to evaluate the effect of the *CD163* polymorphism in the pigs (n = 292) with persistently infected PRRSV, which were collected from two different commercial pig farms. We genotyped the WUR1000125 marker of *GBP1* and the c.3534C > T of *CD163*. Under the commercial farm conditions with persistent PRRSV infection, the effect

of the WUR genotype was not significantly on the weight gain of pigs. However, the CD163 polymorphism was significantly associated with the weight gain and the TT genotype pigs grew faster than other genotypes although they had also significantly lower birth weight. Based on these results, naturally occurring polymorphisms of CD163 might play an important immunity against PRRSV infection. More study is necessary to validate the genetic effects of immune related genes under commercial environments of persistent PRRSV infection.

Key Words: CD163, immune response, PRRSV

P6037 Effects of CAEV infection on expression of acute phase protein genes in goat milk somatic cells. D. Reczynska* , J. Jarczak, K. Rutkowska, K. Barlowska, J. Oprzadek, D. Słoniewska, K. Horbanczuk, W. Jarmuz, and E. Bagnicka (Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Jastrzebiec, Poland), M. Czopowicz, L. Witkowski, and J. Kaba (Warsaw University of Life Sciences, Faculty of Veterinary Medicine, Warsaw, Poland)

Trauma, injury, bacterial, and viral infections induce expression of acute phase proteins (APPs). Some of them, such as proteins from serum amyloid A family (SAA) and haptoglobin (Hp), are named *positive* because their concentrations increase during diseases. Their activation is essential for restoration of homeostasis. Hp irreversibly binds free hemoglobin and thus limits availability of iron necessary for bacteria growth, whereas SAA, among other things, controls and recruits immune cells to inflammatory sites. SAAs are involved in many chronic inflammatory diseases, including rheumatoid arthritis in humans. Caprine arthritis-encephalitis virus (CAEV) infection causes serious problems in goat breeding all over the world. The symptoms of the disease are arthritis, weight loss, mastitis, pneumonia, and chronic immune-mediated inflammation. Milk composition is also influenced by the disease, with lowered protein, fat, and lactose contents. The aim of study was to estimate the expressions of *SAA3* and *Hp* genes in milk somatic cells derived from naturally CAEV-infected and healthy goats. The experiment was performed on 12 Polish White Improved and 12 Polish Fawn Improved goats. The animals were equally divided with respect to breed and parity into two groups: CAEV-free (control) and CAEV-infected (experimental). The milk samples were collected five times during lactation (Days 7, 30, 60, 120, and 180). Only samples with high-quality total RNA were used in the analysis ($N = 75$). The primer sequences were designed using the Primer3 program. The transcript levels were measured using qPCR method (Light Cycler System, Roche, Switzerland) with cyclophilin A as a reference gene. There were no differences between breeds in expression levels of both studied genes. No differences in concentration of *Hp* transcripts between control and experimental groups were also observed; however, the transcripts were found only in 60% of samples in the experimental group, and in 59% of samples of the control group. Higher concentration of *SAA* transcripts was found in the experimental group. The highest expression of the *SAA* gene was observed in peak of lactation (Day 60). It means that the expression level of the *SAA* gene is likely to depend on both CAEV infection and the stage of lactation. This work was financed by National Science Centre Project No. 2013/09B/NZ6/03514.

Key Words: goat, milk somatic cells, CAEV, expression, *SAA3*, *Hp*

P6038 Tackling the itch: GWAS-based candidate genes for psoroptic mange sensitivity in Belgian Blue cattle. A. Coussé* , L. Francois, A. Stinckens, and N. Buys (KU Leuven, Leuven, Belgium), M. Elansary, R. Abos, C. Saegerman, T. Druet, B. Losson, and M. Georges (University of Liège, Liège, Belgium), C. Sarre and E. Claerebout (Ghent University, Ghent, Belgium), and X. Hubin (AWE asbl, Ciney, Belgium)

Belgian Blue cattle suffer from an extreme sensitivity for infection by the *Psoroptes ovis* mite, causing an itchy, crusty dermatitis with economical and animal welfare consequences. Interbreed differences suggest mange sensitivity is heritable, while the intrabreed differences open perspectives for genetic selection as a sustainable solution. Our study is the first attempt to unravel the genetic background of psoroptic mange sensitivity. Six-hundred-and-seventy Belgian Blue animals were phenotyped based on lesion extent, lesion appearance, and mite counts, measured at three consecutive farm visits. Collected blood samples were then genotyped with the Illumina Bovine SNP50 v2 BeadChip. A haplotype based association analysis (GLASCOW) of the animals with extreme phenotypes showed a suggestive signal at the telomere of chromosome 11, with the most prevalent haplotype cluster having a substantial impact on the phenotype. A 1.3 Mb region of interest could be delineated after imputation and consequent bootstrapping of the region around the peak. Due to the intergenic location of the associated markers, a candidate gene is presented based on its location in the specific region and its relationship with other parasitic diseases. In addition, the possible role of collagens and lipocalins is

described, illustrating the need for future research that can eventually lead to a genetic selection program for more mange resistant Belgian Blue.

Key Words: cattle, *Psoroptes ovis*, genetics

P6039 Expression of β-defensin and cathelicidin genes in milk somatic cells derived from mammary glands infected with coagulase-positive or coagulase-negative *Staphylococci*.

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The study was conducted on 40 Polish HF cows suffering from the chronic, recurrent mastitis caused by coagulase-positive (CoPS) or coagulase-negative (CoNS) *Staphyloccoci* vs. 18 healthy (H) cows in two age groups: first or second lactation (1/2) and in third or fourth lactation (3/4). The expression was examined of β-defensin (*DEFB1*, *BNBD4*, *BNBD5*, *BNBD10*, *LAP*, *TAP*) and cathelicidin genes (*CATH4* = indolicin, *CATH5* = *BMAP28*, *CATH6* = *BMAP2*) in milk somatic cells (MSCs) derived from udder quarters; one quarter from each cow. Following groups of samples were distinguished: H1/2 ($N = 9$), H3/4 ($N = 9$), CoPS1/2 ($N = 14$), CoPS3/4 ($N = 14$), CoNS1/2 ($N = 9$), and CoNS3/4 ($N = 9$). The samples were chosen on the basis of milk microbiological examination. qPCR was performed in the Light Cycler480 (Roche, Germany) with the SYBR-Green technique. Analysis of variance was conducted with the Tukey-Kramer test. The *DEFB1*, *BNBD4*, *BNBD5*, *BNBD10*, and *LAP* mRNAs, but not of *TAP* mRNA, were detected in all investigated samples regardless of the animals' age and microbiological status of the udder, but at different levels. The expression of *BNBD4* and *BNBD5* were the highest in CoPS1/2 group. The highest expression of *DEFB1* gene was found in the CoNS3/4 group, while *LAP* in the H3/4 group. The *DEF10* mRNA level was similar in all groups except for CoNS3/4, where the expression was the lowest. It may indicate a constitutive expression of *DEF10* gene in MSCs. No expression of *CATH5* was found in any sample. *CATH4* and *CATH6* showed expression patterns similar to *LAP*, which is contrasting to any other Antimicrobial Peptides (the highest expression in MSCs from bacteria-free udder quarters). Increased expression of most of genes encoding β-defensins in MSCs derived from the infected udder quarters confirms their crucial role in the defense of cow's udder against mastitis. On the other hand, the elevated cathelicidin transcripts in healthy quarters indicate their role in maintenance of healthy state of the udder. Financed by the National Science Centre of Poland No. NN311075339.

Key Words: dairy cows, milk somatic cells, mastitis, defensin, cathelicidin, expression

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P6040 Allele specific expression analysis of the porcine blood transcriptome reveals extensive *cis*-regulation in immunity-related genes.

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The blood appears as an interesting surrogate tissue to phenotype animals for health and various physiological traits, and as such, contains a reservoir of potential biomarkers to be explored. In pigs, we have already shown that blood transcriptome is informative to quantify immunity parameters measured in the blood (e.g., cell counts) or after in vitro blood stimulation (e.g., phagocytic activity, production of cytokines). In this study, our aim was to identify genetic markers associated with gene expression variations in the blood, based on Allele Specific Expression (ASE) analysis from RNaseq transcriptome sequencing. Whole blood RNAs of 38 Large White pigs sampled at 60 d of age were sequenced in 100 pb single-end reads (Illumina HiSeq2000). After masking ~70000 RNaseq SNPs detected with the Genome Analyzer Toolkit (GATK) and remapping the RNaseq reads with STAR, the ASEReadCount tool in GATK was used to determine allelic read count variants. Significant ASE SNPs were identified with a binomial test. After correction by false discovery rate (FDR < 0.05), 2286 SNPs with a significant ASE were detected, and 63% of them were referenced in dbSNP. Thus, the other 37% of these SNPs need to be further validated as new SNPs (or RNA editing) or discarded as artifacts. ASE SNPs overlapped 1132 transcripts from 989 genes, which represent 10% of all genes expressed in blood and having at least one heterozygous SNP. Overall, the transcription variation of 8% of the genes expressed in the blood was found to be regulated by at least one variant in *cis*. ASE genes were found significantly enriched in different biological processes by using Ingenuity Pathway Analysis (IPA) and ClueGO (Cytoscape). Enriched pathways and processes included metabolism, cell death, signalization, and immune biological processes. Remarkably, 39 genes were found involved

in the leukocyte activation ($P = 1 \times 10^{-10}$) and 45 genes were linked to immune system development ($P = 5 \times 10^{-6}$). Overall, our results reveal that the genetic control of blood transcription variation of numerous genes, and notably immunity-related genes, is subjected to extensive *cis*-regulation. This study is a step toward the identification of genetic factors that may influence candidate relevant biomarkers of individual immune capacity.

Key Words: blood transcriptome, allelic specific expression, pig immunity, RNaseq

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P6041 Microarray analysis of genomic aberrations of horse sarcoids. K. Pawlina* , A. Gurgul, and M. Bugno-Poniewierska (National Research Institute of Animal Production, Balice, Poland), J. Klukowska-Rötzler and C. Koch (University of Bern, Bern, Switzerland), and K. Mählmann (University of Veterinary Medicine Hannover, Hannover, Germany)

Equine sarcoid is the most common skin neoplasm occurring in horses. It is classified as a locally malignant, fibroblast benign tumor of fibrous tissue. Moreover, it occurs in six types differing in phenotype and aggressiveness. In spite of the fact that it does not metastasize, it may recur if treatment is not performed properly. When it comes to its causes, Bovine papilloma virus (BPV) is considered to be the main etiologic agent of this cancer. The aim of our study was to give insight into aberrations, such as CNV (copy number variation) and cnLOH (copy neutral loss of heterozygosity), occurring at the genome level in horse sarcoids. To this end, we applied a high throughput method enabling genome-wide analyses that is Equine64K SNP microarray (Illumina, NEOGEN). The research material consisted of DNA isolated from 16 samples of horse sarcoid tissue as well as healthy, control skin tissue. The whole procedure encompassed, in short, DNA amplification, hybridization, labeling, and washing, and was performed according to the Infinium Ultra protocol (Illumina). The microarrays were scanned with the use of HiScanSQ scanner (Illumina). The obtained data were analyzed using OncoSNP (2.0) software. The analysis showed great diversity between single tumors, which manifested not only as the number and type of aberrations, but also their size or presence of genes. Moreover, we also observed substantial variability between chromosomes in the number of the identified aberrations and the percentage of the genome which they span. The analysis of pathways of genes localized in the aberrant regions resulted in the identification of pathways connected with cancer and the immune system, such as: microRNAs in cancer, cytokine–cytokine interaction, PI3K-Akt pathway, herpes simplex infection. The functional analysis of single genes showed their engagement in neoplastic transformation of a wide variety of human tumors. Moreover, many of those gene have been reported to occur in CNV and/or cnLOH regions in the human cancer genomes. In conclusion, horse sarcoids appear as a nonuniform group, which may stem from various causes, like heterogeneity of a tumor or different clinical types of the examined tumors. In general, they are characterized with the increased number of aberrations in comparison to healthy tissue, which suggests the increased instability of their genome and potential engagement of CNV and cnLOH in the carcinogenesis of this tumor.

Key Words: CNV, horse, sarcoid

P6042 Transcriptome characteristic of horse sarcoids. E. Semik* , A. Gurgul, K. Ropka-Molik, T. Zabek, and M. Bugno-Poniewierska (National Research Institute of Animal Production, Balice, Poland), C. Koch (University of Bern, Bern, Switzerland), and K. Mählmann (University of Veterinary Medicine Hannover, Hannover, Germany)

The aim of this study was to identify changes in transcriptome of horse sarcoids, a locally invasive skin tumors of equids, which are considered to be the most common equine skin neoplasm. The global expression of genes was investigated in four tumor samples and in the samples of healthy skin, obtained from the same individual. To describe alterations arising in the transcriptome of the equine skin during sarcoids' progression, the Horse GE Two-Color Gene Expression Microarray (4×44, Agilent) was used. The analysis of the microarray data showed that, of 901 significantly ($p < 0.05$) differentially expressed genes with a fold-change greater than 2.0, the expression of 273 genes was up-regulated, and the expression of 628 was down-regulated in the sarcoid tissues. To validate cDNA microarray data, nine genes with a fold change greater than two were chosen for qPCR. The selected genes are presumed to be involved in processes associated with malignant transformation (*SFN*, *S100A14*, *POU2F3*, *KLK8*, *TSG-6*, *EFEMP2*, *SPARC*, and *ABL1*) as well as with response to virus infection (*GOLM1*). All changes in gene expression determined by the cDNA microarray were confirmed by qPCR. The list of differentially expressed genes was next uploaded into KEGG (Kyoto Encyclopedia of Genes and Genomes) to evaluate the contribution of genes in common biological pathways, identifying 222 associated pathways. The largest group consisted of genes involved in metabolic pathways (ecb01100) responsible for the changes in the supply of nutrients, such as glucose or lipids, as well as regulating energy metabolism of the

cell (47 genes) and in the PI3K-Akt signaling pathway (ecb04151), associated with important functions in life processes and prevention of programmed cell death (14 genes). Among selected DEGs, there were also genes involved in regulation of pathways associated with response to the HTLV-I virus infection and viral carcinogenesis, which may result from participation of the Bovine papillomavirus in the pathogenesis of equine sarcoids. In the analyzed tumor tissues, we also found changes in the activity of genes associated with the Wnt, p53, and RAS signal pathways. It is believed that the disorders in the functioning of these biological pathways are a serious threat to the proper functioning of cells and can lead to various pathological processes, including carcinogenesis. These results may be helpful to identify genes associated with sarcoid progression and can be useful in the development of new strategies for the treatment of this disease.

Key Words: DEGs, horse, sarcoid

P6043 Evaluation of chromosome rearrangements of an intersex horse applying molecular cytogenetic techniques. M. Bugno-Poniewierska*, T. Zabek, A. Gurgul, and K. Pawlina (National Research Institute of Animal Production, Balice, Poland)

In this study, we performed molecular cytogenetic and genetic studies of an intersex horse. The investigated animal had overall female body conformation, however, its external genitalia consisted of incompletely developed vulva and penis. The 6-mo-old mare showed a karyotype containing a Y isochromosome in mosaic form: 63,X/64,XY(qi). The chromosome aberration was confirmed by CBG and GTG banding and fluorescence in situ hybridization with chromosome painting X and Y probes, as well as the Y-specific region USP9Y gene. We analyzed 1262 metaphases in total, in which 1230 showed 63,X karyotype and the remaining 32 metaphases presented 64,XYqi karyotype (97,46 and 2,54% respectively). Twenty-nine Y-specific loci, and another three located on ECAY having their homologs on X chromosome (AMELX/Y, ZFX/Y, and DDX3Y) were amplified. All investigated loci located on ECAY spanned the whole euchromatin region. Amplification results showed the presence of most of the Y-specific regions in the examined horse, except for four loci located on ECAY (CLY046, CLY052, CLY057, and AMELY) and ZFY, which were negative. These results indicate the occurrence of microdeletions in fragments of the Y chromosomal region present in this mare. DNA of the mare was then subjected to a high throughput analysis with the use of array CGH technique. The resulting data were analyzed using Genomic Workbench software and the following parameter settings: minimal number of probes, 3; average log R ratio, 0,25. In total, 102 aberrations were identified, which were localized on 22 chromosomes. The average aberration size amounted to 67 kbp. The results allowed for characterizing genes and pathways which were altered in the genome of the investigated mare. Among genes colocalized with the identified aberrations were genes engaged in reproduction, such as *STS* (steroid sulfatase) or *NR0B1* (Nuclear Receptor Subfamily 0, Group B, Member 1). The aCGH results were validated with the use of qPCR technique which showed 80% concordance. The work was financed by project No. 297267 "Directions of use and protection of genetic resources of livestock in conditions of sustainable development" from the National Research and Development Center, Warsaw, Poland.

Key Words: horse, karyotype, aCGH

P6044 Genomics-assisted introgression of viral resistance in commercial common carp strains. R. Tadmor-Levi, E. Asulin, and L. David* (The Hebrew University of Jerusalem, Rehovot, Israel), and G. Hulata (Agricultural Research Organization, Beit-Dagan, Israel)

Viral fish diseases pose a major global threat to aquaculture. Breeding resistant fish strains is the most sustainable solution to this problem. Cyprinid herpes virus-3 (CyHV-3/KHV) is a dsDNA virus that inflicts massive mortalities on commercial common carp (*Cyprinus carpio* L.) and Koi strains around the world. CyHV-3 resistance was identified in a feral carp strain that does not fit commercial aquaculture. Using a CyHV-3 disease model we established in the lab, we screened families of different strains and found considerable variation in susceptibility levels between families and strains. We progressed in family-based selection trials to continuously produce resistant and susceptible families for research and breeding purposes. Furthermore, we found good correspondence between the level of resistance in the family of the parent and that in its progeny, indicating resistance has good heritability. By analyzing viral loads, we found that resistant fish restrain the viral spread in their bodies better than susceptible fish, suggesting that in this case, resistance reflects a difference in the function of their immune response. To enhance the selection program and to understand the genetic basis of resistance, we identified quantitative trait loci (QTLs) associated with resistance. We adopted the genotyping-by-sequencing (GBS) approach to carp and genotyped individuals from two semiresistant families. From over 25,000 markers with Mendelian segregation, almost

9,000 were shared by both families. We constructed a high-density genetic map consisting of 48 linkage groups and spanning 5,142 cM, and mapped on it 192 resistance associated markers that were grouped into three QTLs. Two QTLs were shared by both families, and we are confirming these associations in several other families. Finding multiple QTLs validated our phenotypic results, implying that CyHV-3 resistance is a complex trait. Markers in validated QTLs will therefore assist future breeding. Due to the preliminary state of the common carp genome, we are using the GBS tag sequences for mapping the QTL regions to the scaffolds of the duplicated carp genome. Additionally, we are using comparative genomics to the closely related zebrafish to identify candidate genes in these QTLs. Our research will support breeding of resistant common carp strains and enhance our understanding of fish immunogenetics.

Key Words: common carp, fish disease, disease resistance, QTLs, breeding, genotyping by sequencing, immunogenetics, viral disease

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P6045 Dine mapping a sheep genomic locus involved in viral restriction of ovine lentivirus.

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Ovine lentivirus (OvLV) affects half of all sheep flocks in the United States, causing high economic and animal welfare costs. Since there are no effective vaccines and no feasible treatments for ovine progressive pneumonia, breeding for improved control through marker assisted selection is a desirable way to combat OvLV. Proviral concentration in white blood cells correlates with disease severity, therefore we used a genome-wide association study (GWAS) followed by marker validation in diverse populations to identify a small insertion mutation. This validated marker is associated with consistently lower proviral concentration; on average, insertion homozygotes have less than half the proviral concentration of other genotypes. Analysis of the region surrounding this insertion identified four zinc-finger transcription factor genes located within sixty-five kilobases, none of which are currently described as viral restriction factors in any mammalian system. We resequenced eight sheep from phenotypic extremes in Rambouillet and Suffolk breeds to identify all predicted amino acid substitution and splice site variants. A mapping population of Rambouillet sheep that had the strongest association from the original GWAS was used to genotype these variants and test for association. Based on initial analysis of 15 out of 26 variants across all four genes, there is no improved or equivalent association with proviral concentration phenotype among our current marker set. Furthermore, joint modeling does not suggest synthetic association where the insertion marker might be a representation of multiple functional mutations already in our data set. We hypothesize that one or more causal mutations lie within a gene regulatory element and not within a protein coding gene. We will test our hypothesis with gene expression studies currently underway to help identify one or more novel viral restriction factors for OvLV.

Key Words: ovine lentivirus, ovine progressive pneumonia, viral restriction

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P6046 Precision medicine and the 99 Lives Cat Genome Sequencing Initiative.

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Precision medicine is an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle. Precision medicine gives clinicians tools to better understand the complex mechanisms underlying a patient's health, disease, or condition, and to better predict which treatments will be most effective. Overall, an individual's specific genetic makeup will become an intricate part of their standard health care. The 99 Lives Cat Genome Sequencing Initiative is developing the cat genome DNA variant database to apply precision medicine to cats. To date, the 20 to 30× whole genome sequences (WGS) of 73 domestic cats from different breeds and populations and nine wild felids including, three black-footed cats (*Felis nigripes*) and one Pallas cat (*Otocolobus manul*) are included. Maverix Biomics (San Mateo, CA) provides the variant calling and a web-based interface for filtering for specific cats and variants types in a given analysis. Over two dozen researchers and veterinarians have participated worldwide. Several successes have been demonstrated for the project in the past 2 yr. The causal genes and variants for three inherited retinal degenerations have been identified in Persian, Bengal, and black-footed cats. A new variant in *PKD1* has been identified in the Pallas cat with renal disease, further demonstrating the power of cross-species analyses. Sequencing of an affected sibpair led to the discovery of the variant causing an immuno-lymphoproliferative disease in British Shorthair cats. A

single cat with GWAS localization of disease was sequenced, identifying the variant in *COLQ* for congenital Myasthenic syndrome in Devon rex. Recently, a patient was presented to the University of Missouri, Veterinary Health Center. The cat was diagnosed with an undefined but potential lysosomal storage disease. WGS of this individual patient identified a homozygous and unique variant in the gene for Niemann-Pick Type 1 (*NPC1*). NPC1 has been previously identified in cats; however, the variant in this singleton cat is novel. Although NPC1 has no effective treatment, if additional cats are identified, they could further develop gene therapies and treatments for the disease. The variants identified for disease in the wild felids suggest DNA testing is required for proper management in their captive breeding, species survival plans. The 99 Lives project has proven successful within and across felid species, including analyses of diseases with and without a priori genetic localization.

Key Words: *Felis catus silvestris*, Niemann-Pick Type I, progressive retinal atrophy, immune-lymphoproliferative disease, wild felids

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P6047 Expression of TLR2 pattern recognition receptor on mononuclear cells cultured with ligands among cattle ranked by estimated breeding values for adaptive immune response traits. L. Wagter-Lesperance* , H. Atalla, M. Emam, N. Gallo, D. Hodgins, M. McLean, L. Read, and B. Mallard (Department of Pathobiology, University of Guelph, Guelph, ON, Canada)

Toll-like receptor 2 (TLR2) is a pattern recognition receptor expressed on epithelial cells and some leukocytes. Binding of bacterial ligands to TLR2 induces proinflammatory innate responses and contributes to the development of adaptive immune responses. A study was conducted to investigate differences in expression of TLR2 on blood mononuclear cells (BMC) ex vivo, and in vitro following an 18-h culture with TLR2 ligands. Dairy cattle (N = 18) were classified into five phenotypic groups based on their estimated breeding values (EBV) for antibody-mediated (AMIR) and cell-mediated (CMIR) immune responses. The association of EBVs for AMIR and CMIR (as categorical variables) and TLR2 expression on BMC was evaluated. Cattle that were greater than one standard deviation above the mean EBVs for AMIR or CMIR were classified as High (H), those more than one standard deviation below were classified as Low (L), and responses in between were classified as Average (A). Phenotypic groups studied were as follows: L-AMIR/L-CMIR, L-AMIR/H-CMIR, A-AMIR/A-CMIR, H-AMIR/L-CMIR and H-AMIR/H-CMIR. Ligands tested included lipotechoic acid from *S. aureus* (LTA), mannose-capped lipoarabinomannan from H37Rv *M. tuberculosis* (Man-LAM), and PAM3CSK4 (PAM, a tri-acylated synthetic lipoprotein). Mononuclear cells were stained using monoclonal antibodies (clones HCA152F, MM61A, BAQ155A, MM1A specific for TLR2, CD14, B cells, and CD3, respectively), and characterized using flow cytometry. Ex vivo, CD14+ mononuclear cells had the highest percentage of cells expressing TLR2 among all BMC. Among groups, H-AMIR/H-CMIR cows had a significantly higher median fluorescent intensity (MFI) of TLR2 expression compared with A-AMIR/A-CMIR cows on all BMC. In vitro, on all BMC, H-AMIR/H-CMIR had a significantly higher MFI of TLR2 expression compared with H-AMIR/L-CMIR following culture with PAM or LTA. For the CD14+ cell subset, MFI was highest for H-AMIR/H-CMIR group compared with L-AMIR/H-CMIR, A-AMIR/A-CMIR, and H-AMIR/L-CMIR cows following culture with PAM only. Although Man-LAM did influence the percentage of cell subsets expressing TLR2, no significant differences were noted among EBV groups. The higher expression of TLR2 on BMC among cattle that rank high for both AMIR and CMIR may contribute to enhanced adaptive immune responses and disease resistance.

Key Words: Toll-like receptor 2, blood mononuclear cells, estimated breeding values of immune response

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P6048 Investigating a single nucleotide polymorphism in DDB2 as a risk factor for squamous cell carcinomas of the nictitans in the Haflinger and Belgian horse breeds. R. Bellone* , J. Liu, S. Vig, C. M. Reilly, and M. Lassaline (University of California-Davis, Davis, CA), T. M. Michau (BluePearl, Tampa, FL), E. Bentley (University of Wisconsin-Madison, Madison, WI), J. L. Petersen (University of Nebraska, Lincoln, NE)

Squamous cell carcinoma (SCC) is the most common cancer of the equine eye, and the second most common tumor of the horse overall. Ocular SCC can originate on the limbus, the nictitans, and upper and lower lids. Several risk factors have been implicated for SCC, including exposure to ultraviolet (UV) radiation and genetics. The Haflinger breed is overrepresented in terms of incidence for SCC of the limbus. A genetic investigation in this breed identified a polymorphism in damage-specific DNA binding protein 2 (DDB2, p.Thr338Met) as the potential link between UV damage and the genetic risk for this cancer. DDB2 recognizes and binds to UV damaged DNA to begin the nucleotide excision repair process. The Haflinger

and Belgian breeds, which recently were shown to be genetically closely related, also have documented cases of SCC of the nictitans and have similar *DDB2* allelic frequencies. Therefore, we propose that the identified *DDB2* mutation also increases risk of SCC of the nictitans in these closely related breeds. Genotyping for this polymorphism in SCC affected horses with confirmed pathology (N = 11) and clinically confirmed unaffected horses (N = 47) showed a strong but not perfect association in these two breeds (Fisher's Exact Test, $P = 4.98 \times 10^{-8}$). Therefore, further work is needed to determine if this is a causative variant for SCC of the nictitans. Specifically, genotyping additional horses will determine the relative risk associated with this variant in each breed. Furthermore, since threonine at position 338 is conserved across 95 vertebrates and nearby highly conserved residues Phe334, Gln335, and His336 make contact with the bases on the opposite strand of the UV-caused lesion, we predict that this substitution (p.Thr338Met) affects proper formation of the protein and thus interferes with DDB2 binding to damaged DNA; this remains to be investigated.

Key Words: equine, ocular cancer, DNA repair

P6049 Challenges in the investigation of eight inherited diseases in ruminants—An Australian perspective. I. Tammen* , S. A. Woolley, E. R. Tsimnadis, N. Nowak, R. L. Tulloch, and M. S. Khatkar (Faculty of Veterinary Sciences, School of Life and Environmental Sciences, The University of Sydney, Camden, Australia), and B. A. O'Rourke (The Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle, Australia)

Modern animal breeding technologies have accelerated the genetic gain in cattle and sheep for many desirable traits. However, at times this has resulted in decreases in effective population sizes and elevated rates of inbreeding, which can increase the risk of deleterious recessive alleles to be observed in homozygous form; as well as an increased risk for deleterious alleles to be dispersed widely. This can manifest in an increased occurrence of inherited diseases, leading to production losses and animal welfare concerns. We have investigated eight diseases with a predicted recessive mode of inheritance in Australian sheep and cattle using 80 k SNPchip based homozygosity mapping and/or candidate gene analysis, with the ultimate aim to identify disease causing genes and mutations. We confirmed that the mutation known to cause congenital contractural arachnodactyly in Angus cattle caused several cases of congenital contractural arachnodactyly in Murray Gray cattle. Congenital mandibular prognathia has been reported in several Droughtmaster cattle, and was mapped to a 3.1 MB region on BTA26. A positional candidate gene has been partially sequenced. Homozygosity mapping analysis for two suspected cases of Niemann-Pick disease in Angus cattle identified a region of homozygosity on BTA24 that contains the candidate gene *NPC1*. Sequencing identified a possible causative mutation which is currently being validated. Incomplete sequencing of the bovine candidate gene for pulmonary hypoplasia with anasarca in Persian sheep has so far not identified a disease associated mutation. Two suspected cases of cardiomyopathy and woolly haircoat syndrome in Poll Hereford calves tested negative for the mutation in *PPP1R13L* known to cause this disease in this breed. Partial sequencing of the gene did not identify a disease-causing mutation and homozygosity mapping revealed that neither animal was homozygous in the region of the candidate gene. The mutation in *ABCA12* known to cause congenital ichthyosis in Chianina cattle was excluded as the disease causing mutation in a single Hereford calf that presented with ichthyosis. Homozygosity mapping confirmed *ABCA12* as a strong candidate gene. Homozygosity mapping for congenital blindness or oculocutaneous albinism in two White Shorthorn cattle identified four regions of interest for further analysis. Samples for cervicothoracic vertebral sublaxation in Merino sheep have been collected and are awaiting homozygosity mapping. Despite limitations relating to funding, sample sizes, pedigree information, and at times, poor phenotype description, some progress toward the understanding of these suggested cases of inherited disease has been achieved.

Key Words: ruminant recessive disease

P6050 Extended scrapie incubation time in goats singly heterozygous for PRNP S146 or K222: An update after seven years. S. White*, and D. A. Schneider (USDA-ARS Animal Disease Research, Pullman, WA), S. White, J. O. Reynolds, D. A. Schneider, and K. I. O'Rourke (Washington State University, Pullman), and D. F. Waldron (Texas A&M AgriLife Research, San Angelo, TX)

Scrapie is the transmissible spongiform encephalopathy of sheep and goats, and efforts to eradicate scrapie are underway in many countries worldwide. Goats may serve as a reservoir for scrapie and, to date, there has been no experimental inoculation confirming strong, lifelong genetic resistance in goats. Goats bearing the S146 or K222 amino acid substitution in the goat prion protein have been present in scrapie-exposed herds, but significantly underrepresented in disease cases. Furthermore, both of these variant

proteins give low cell-free protein conversion efficiency to the disease form, PrP^{Sc}. To ensure consistent exposure among test subjects, we performed an oral scrapie challenge of goats singly heterozygous for either S146 or K222. All common haplotype homozygous controls became clinically scrapie positive by an average of 24 mo postinoculation; in stark contrast, none of the S146 and K222 heterozygotes have scrapie-positive lymphoid biopsy tests or confirmed scrapie at incubation times now approaching 7 yr or longer ($P < 0.0001$). Recent reports indicate detection of natural scrapie in less than five S146 and K222 heterozygotes, suggesting heterozygotes will not have complete resistance. However, the scrapie incubation times are now as long as or longer than many commercial operations keep goats for production purposes. This suggests breeding goats singly heterozygous for S146 or K222 may reduce the probability of clinical scrapie during the productive life spans of many commercial goats. These results also suggest much longer relevant trace-back histories for goats of these genotypes in scrapie-eradication programs. Finally, these data support additional consideration of the potential scrapie-resistance present in homozygotes for these genotypes, since, to our knowledge, there have never been natural scrapie positives in homozygotes for either allele.

Key Words: scrapie, resistance, goat

P6051 Incidence of SOD1 mutation in a sample of pure breed German Shepherd in Colombia.

M. A. Novoa* and E. Bernal (Genetica Animal de Colombia Ltda., Bogota, Colombia)

The degenerative myelopathy in dogs is caused by SOD1 mutation *c.118G>A*, described previously. The penetrance of this genetic disease is not well understood, considering animals can express symptoms being carriers. In Colombia, its incidence and risk for pure breed German shepherd dogs is unknown. We extracted DNA from sampled hair roots and buccal swabs of 36 breeding animals of the Colombian German Shepherd Dogs Association (APPA). We did a monoplex PCR and sequencing to determinate the mutation in the SOD1 gene. We found seven carriers in 36 animals, with an allele frequency of 0.097. We estimate that the entire population frequency can be about 0.1, which is higher than what is reported in other German Shepherd populations. It is the first time that this genetic test was used in Colombia, so the implementation of it can be useful to take down the prevalence of this disease, to avoid breeding between carriers.

Key Words: degenerative myelopathy, dogs

P6052 Evaluating the accuracy of imputation in the highly polymorphic MHC region of genome.

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The major histocompatability complex (MHC) region encodes several proteins with crucial function in mounting immune responses and protecting the host against pathogens and cancer. MHC in cattle are known as bovine leukocyte antigens (BoLA). BoLA is scattered over three million base pairs of *Bos taurus* autosomal chromosome 23. Within BoLA region, DRB3 is the most polymorphic locus with >130 alleles. The association of this region with immune responses was previously shown by our group in three GWAS studies. As health traits are gaining more attention from industry, and sires with high immune response indexes are being used by farmers, it is important to investigate and monitor the polymorphism within BoLA. Maintaining the polymorphism of BoLA is considered critical to allow appropriate recognition of a wide range of pathogen epitopes and to reduce the impact of emerging or reemerging disease outbreak at the population level. However, monitoring the BoLA region is not possible due to a gap in the current genotyping platforms (chip arrays) over the BoLA region. Alternatively, imputation to full sequence can be applied to predict the alleles of BoLA. In this study, 27 cows from seven herds in Ontario with no common sire, dam, or maternal and paternal grandsire were selected to investigate the accuracy of imputation in this region. The sample population was genotyped by Illumina BovineSNP50 chip, and the results were imputed to 777 k genotypes in a first step using 2387 reference group and then to full sequence using FImpute software. In parallel, the second exon of BoLA DRB3 was sequenced by Sanger method. Imputation resulted in 50 polymorphic nucleotides within the MHC region, which were compared with their corresponding nucleotide in Sanger sequencing results. Our preliminary results indicated a wide range of concordance rate over 50 single nucleotide polymorphisms (SNPs) in DRB3.2 locus with the average of 75.4% (Min: 48.1%, Max: 100% between SNPs, and Min: 62%, Max: 84% between individuals). These results showed low prediction rate of imputation for the most

polymorphic region of the genome relative to other loci in the bovine genome. Given this low prediction, we will now further investigate novel methods, such as genotype-by-sequencing that are not limited to biallelic SNPs, as well as increasing the sample size and using a more accurate imputation method. In addition, the results of imputation will be combined with other software that are designed to predict BoLA DRB3.2 alleles to increase the accuracy of prediction.

Key Words: BoLA, imputation, DRB3.2, MHC

P6053 Domestic animal forensics at the UC Davis Veterinary Genetics Laboratory. R. A. Grahn* and C. D. Lindquist (University of California Davis, Veterinary Genetics Laboratory Forensics Unit, Davis, CA), and C. Penedo (Veterinary Genetics Laboratory, School of Veterinary Medicine, UC Davis, Davis, CA)

Over the last 15 yr, the Veterinary Genetics Laboratory Forensic Unit (VGL-Forensics) at the UC Davis School of Veterinary Medicine has participated in a wide range of forensic cases from all over the world, with sample types and species diversity unlike those encountered by its human counterparts. As the only crime laboratory in the United States accredited for analysis of DNA from domesticated species, VGL-Forensics serves a large and diverse clientele from human-on-human crimes where dog or cat biological evidence links a suspect to the crime, to large-scale dog fighting investigations, species identification, and animal cruelty cases. The laboratory has assisted in several high-profile cases and cold cases, examples of which will be presented. Additionally, VGL-Forensics actively participates both in the Society for Wildlife Forensic Science (SWFS) and the national Organization of Scientific Area Committees (OSAC) Wildlife Forensics Subcommittee, promoting wildlife forensics while developing and maintaining unified quality standards, proficiency testing, and certification programs. An update on the work of the SWFS and OSAC Wildlife Forensics Subcommittee will be presented. Finally, the laboratory is committed to extending forensic resources for both domestic and wild animal species. Current research increasing the discriminatory power of the VGL-Forensics canine mitochondrial database will be presented.

Key Words: forensics

P6054 Limited MHC diversity and an exotic virus may have contributed to the decline of red squirrels in the United Kingdom. K. Ballingall* (Moredun Research Institute, Edinburgh, UK), A. McIntyre, Z. Lin, and C. J. McInnes (Moredun Research Institute, Penicuik, UK)

The red squirrel (*Sciurus vulgaris*) population in the United Kingdom has declined dramatically over the last century although not in the rest of its Pan-Eurasian range. This is the result of the presence of the eastern gray squirrel (*S. carolinensis*) which was introduced from North America in the 19th century. The principal factors that underlie the rapid decline of the red squirrel and replacement by gray squirrels in the UK include competition from the gray squirrel and disease caused by infection with the squirrelpox virus (SQPV). SQPV, a member of the *Poxviridae* family, is thought to be transmitted by asymptomatic gray squirrels to highly susceptible red squirrels. In areas where red and gray squirrels coexist, the decline of red squirrels is up to 25 times faster if the gray squirrels are carrying SQPV than if they are free from the virus. Population genetic diversity provides some resilience to rapidly evolving or exotic pathogens. Such diversity is highest at loci involved in the immune response, including genes clustered within the major histocompatibility complex (MHC) where any reduction in diversity is predicted to leave a population vulnerable to exotic pathogens. Using the class II *DRB* locus as a marker for diversity across the MHC region, we genotyped 110 red squirrels from locations in the United Kingdom and continental Europe. While high levels of diversity were identified at two functional *DRB* loci in the continental populations, only limited diversity was observed in the UK population. While a direct link between MHC diversity and disease susceptibility remains to be determined, the UK squirrel population lacks the extensive MHC diversity present within continental populations, a feature which may have contributed to their decline.

Key Words: diversity, MHC class II, red squirrels

POSTERS: GENOME EDITING AND TRANSGENIC ANIMALS

P7000 Growth performance and meat characteristics of Awassi sheep that holds the Callipyge gene. K. I. Jawasreh, A. H. Al-Amareen* , and A. Y. Abdullah (Jordan University of Science and Technology, Irbid, Jordan)

For improving meat quality and quantity of Awassi sheep, frozen semen of four Rambouillit rams (R, homozygous for the mutation of the Callipyge gene, CLPG) was imported from the United States (Utah

University). The introgression of the CLPG into Awassi was started by producing the F1 by crossing R with Awassi (AW) ewes, while the first backcross (FBC; 75% AW and 25% R) was formed by using the F1 Callipyge carriers with AW ewes. To examine the performance of the FBC (heterozygous for CLPG) compared with the AW, a fattening trial was designed using 16 weaned male lambs (eight from each of AW and FBC lambs) kept in individual pens for 98 d. A well-balanced ration was offered, feed intake was recorded on a daily basis, and weights were monitored weekly. At the end of the fattening period, lambs were slaughtered to investigate the carcass cuts and meat characteristics. Callipyge carrier lambs (CAW) exceeded AW lambs in their slaughter weight ($P = 0.0002$) and the average daily gain for AW and CAW were 0.189 and 0.332 kg/d, respectively ($P < 0.0001$). Awassi lambs consumed 1.217 kg feed/kg live weight more than CAW ($P = 0.0254$). The slaughter weight of the CAW was higher than the AW ($P = 0.0001$); CAW weighed 50.9 kg at slaughter age (176.5 ± 1.34 d), while AW weighed 37.07 kg. The hot and cold carcass weights, dressing percentage, and shoulders, legs, rack, and loin weights were significantly higher in the CAW. Fat tail weight was nonsignificantly affected by the genotype. The heart, liver, kidney, and kidney fat weights were significantly higher in CAW. Longissimus, total leg muscle weight, intermuscular and subcutaneous fat, and total bone weights were higher in CAW compared with AW. The ratio of total muscle to leg weight and total bone to leg weight were higher in CAW, while the intermuscular fat/leg weight subcutaneous fat/leg weight were nonsignificantly affected by the genotype. Eye muscle area was 14.8099 in AW, while 25.3521 ± 1.4086 in CAW ($P < 0.0001$). CAW was higher than AW of about 0.168 kg in the eye muscle weight ($P = 0.0003$). Shearforce values were 7.2844 in CAW compared with 3.2187 in AW ($P < 0.0002$). Meat color nonsignificantly differs between CAW and AW. CLPG can be used for improving meat quantity and quality of AW.

Key Words: sheep, introgression, Callipyge gene

P7001 Heritable gene disruption in goats with CRISPR/Cas9 results in expected phenotypes.

X. Wang* and Y. Chen (Northwest A&F University, Yangling, China)

Precision genetic engineering will accelerate the genetic improvement of livestock for agriculture and biomedicine. Recent advances in the study of the CRISPR/Cas9 system have provided a precise and versatile approach for genome editing in various species. However, the applicability and efficiency of this method in large animal models, such as the goat, have not been extensively studied. Here, we successfully generated gene-modified goats with either one or both genes disrupted through co-injection of one-cell-stage embryos with Cas9 mRNA and sgRNAs targeting two genes (*MSTN* and *FGF5*) with well-known function. The targeting efficiency of *MSTN* and *FGF5* in cultured primary fibroblasts was as high as 60%, while the efficiency of disrupting *MSTN* and *FGF5* in 98 tested animals was 15 and 21%, respectively, and targeting two genes simultaneously was 10%. The on- and off-target mutations of the target genes in fibroblasts, as well as in somatic tissues and testis of founder animals, were carefully analyzed, indicating that simultaneous editing of several sites can be achieved in large animals. We further show that the utility of the CRISPR/Cas9 system by disrupting *MSTN* and *FGF5*, resulting in expected phenotypes; for instance, higher body weight in *MSTN*-disrupted animals and increased fiber length in *FGF5*-disurpted animals. We provided adequate evidence to illustrate that the gene modifications induced by the disruption of *FGF5* did occur at both morphological and genetic levels. In addition, the knockout alleles were capable of germline transmission. Together, with studies performed in other gene-modified livestock species such as pigs, our results demonstrate that the CRISPR/Cas9 system has the potential to become a robust and efficient gene engineering tool in farm animals, and therefore will be critically important and applicable to breeding purposes.

Key Words: CRISPR/Cas9, MSTN, FGF5, goat, genome editing, animal breeding

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P7002 Skin-specific transgenic expression of ovine β-catenin in mice.

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β-catenin is an evolutionarily conserved molecule that functions as a crucial effector in the development of hair follicles. The primary goal of the present study was to investigate the effect of ovine β-catenin on the hair follicle. Transgenic mice were produced by pronuclear microinjection with skin specific promoter of human keratin14 (k14). Transgenic mice were identified by PCR and Southern blot analysis and the β-catenin gene could be stably inherited was validated. Furthermore, qRT-PCR and Western blot analysis indicated that high level of β-catenin was expressed

specifically in the skin tissue. The β-catenin was visualized strongly expressed in the inner root sheath and dermal papilla of the hair follicle based on immunohistochemical analysis. Also, the results of the frozen sections analysis showed different phenotypes in follicle position of transgenic individuals relative to their negative full-sibs or wild mice, that every follicle contained two or three hairs in 70% of the random sites of the transgenic skin sections, other than one hair in wild mice. These results suggested that the transgenic mice model with overexpression of ovine β-catenin was produced successfully, provides a material for deciphering the molecular mechanism involved in hair follicle growth and development, and provides a foundation for further producing β-catenin overexpression in transgenic sheep.

Key Words: β-catenin, transgenic mice, overexpression, ovine

P7003 Heritable multiplex gene editing via CRISPR/Cas9 exhibits no detectable genome-wide off-target effects in sheep. X. Wang*, and Y. Chen (Northwest A&F University, Yangling, China)

The CRISPR/Cas9 system provides an innovative and flexible approach for genome engineering of loci that control economically important traits in livestock. However, off-target mutations induced by CRISPR/Cas9 nucleases may lead to unintended negative and undesirable consequences, and are the major obstacle in using this technology in agriculture. The document and evaluation of genome-wide off-target mutations are crucial to animal health and the biosafety of animal products. We successfully achieved precise gene targeting in sheep by coinjection of one-cell-stage embryos with Cas9 mRNA and RNA guides targeting three genes (*MSTN*, *ASIP*, and *BCO2*) with well-known functions. We carefully examined the sgRNAs:Cas9-mediated targeting effects in injected embryos, somatic tissues, as well as gonads via a traditional cloning and sequencing approach, which demonstrated that microinjection of zygotes is an efficient approach for generating gene-modified sheep. Furthermore, we performed whole-genome sequencing of three trios (~10.8× coverage) to identify potential off-target mutations (indels and structure variations) that may have been introduced by Cas9 manipulation. No detectable off-target modifications were attributable to nucleases in all three trios. We observed that disruption of *MSTN* resulted in an increase in body weight, and provided evidence to support that gene modifications had occurred at the genetic and morphological levels. The detection of germline transmission further indicated that the phenotypes may be potentially transmitted to the offspring. We describe the successful generation of gene-modified sheep via the CRISPR/Cas9 approach. The results indicate that favorable phenotypes were acquired in the *MSTN*-disrupted sheep with high specificity. This approach represents a versatile and robust method to produce genetically modified sheep with biological safety.

Key Words: genome editing, CRISPR/Cas9, sheep, MSTN, off-target, germline transmission, animal breeding, whole-genome sequencing

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P7004 Screen of transgenic integration sites and construction of site-specific transgenic pig by CRISPR-Cas9. L. Ma* , X. Hu, and N. Li (China Agricultural University, Beijing, China), and Y. Xing (The State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing, China)

Transgenic technology is widely used in biological research. However, random integration has several limitations. Because of the uncertain integration site, transgenes are susceptible to the local chromatin environment and can cause endogenous gene disruptions. Moreover, transgenic DNA concatemerized into a large array is subject to repeat-induced gene silencing. Site-specific transgenesis may overcome these hurdles by targeting the transgene to a specific chromosomal locus. However, there are very few studies on site-specific integration in livestock. Here, our aim is to achieve site-specific integration in pig. First, we seek out three candidate transgenic integration loci from open and actively transcribed chromatin area through genome-wide screening. Second, we successfully construct targeting vectors for three candidate sites, and expect to achieve site-specific integration on candidate loci in pig with the help of the CRISPR-Cas9 system. Third, we conduct the verification of whether candidate sites are suitable for expression of exogenous genes on cell and individual level. All three candidate sites can support sustained and stable expression of exogenous gene. Exogenous genes integrated on candidate sites show typical histone marks of open and transcribed chromatin by chromatin immunoprecipitation analysis. Then, we obtain a site-specific transgenic pig on a candidate site which stably expresses the exogenous gene in different tissues. Finally, we establish a master cell line on the candidate site, which is convenient for subsequent site-specific integration.

Key Words: site-specific, integration site, CRISPR-Cas9

P7005 Characterization of RNA editing on porcine NR3C1, COG3, and ACSM2B genes. A. I. Fernandez* (Departamento de Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria [INIA], Madrid, Spain), R. Benítez, Y. Nuñez, C. Garcia-Contreras, and Á. M. Martínez-Montes (INIA, Madrid, Spain), and J. M. Folch (Plant and Animal Genomics, Centre de Recerca en Agrigenòmica [CRAG], Consorci CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, Spain)

The RNA-Seq technology is largely used in quantitative gene expression studies, but the use of RNA-Seq technology allows also for detecting differential allelic expression and posttranscriptional modifications such as RNA-editing. RNA-editing is a posttranscriptional mechanism that generates new transcripts from a limited gene number in the genome, and consists in the chemical alteration of nucleotide bases of RNA molecules. In the present study, we have characterized RNA-editing patterns in six porcine tissues: hypothalamus, liver, diaphragm, *Longissimus dorsi*, cardiac muscles, and backfat of three genes suffering of these posttranscriptional modifications. In a previous RNA-Seq assay conducted on hepatic and hypothalamus tissues from nine Iberian × Landrace backcrossed pigs, over 90,000 SNPs were discovered and annotated. Among them, three SNPs on *NR3C1* (ENSSSCG00000014401g.102797T > C), *COG3* (ENSSSCG00000027815g.4525A > G) and *ACSM2B* (ENSSSCG00000007858g.13374T > A) genes revealed different genotype conditional on analyzed tissue. The validation through Sanger sequencing on gDNA and cDNA revealed unexpected genotypes. Some transcripts showed alleles that are not present in the corresponding gDNA sequence, supporting the hypothesis of RNA-editing modifications. These results were also validated by pyrosequencing on gDNA and cDNA and on different set of samples and tissues from Iberian and Iberian × Large White pigs. The *COG3*g.4525A > G modification has been previously described in several species, proving that at least some RNA-editing conservation happens across vertebrate species. The modification on the *COG3* transcript, key molecule in protein metabolism, changes the Isoleucine amino acid to Valine at protein position 83. Besides, it lies in an exon enhancer region involved in the promotion of alternative splicing, which is likely linked to different biological functions conditional on tissue. The *NR3C1*g.102797T > C and *ACSM2B*g.13374A > T modifications have not been previously described. The modification on *NR3C1* lies in the 3′UTR region, likely located in a potential miRNA target site. Finally, the modification on *ACSM2B* produces an amino acid change from Serine to Treonine at position 272. This change lies in a splicing site, and more importantly, it can be considered of major interest because the different hepatic transcripts showed interindividual variations that could be functionally related to the phenotypic traits. The RNA-editing patterns observed among the different tissues analyzed here can help in understanding the biological role of the genes and their modifications. The current study has revealed the existence and potential impact of naturally occurring RNA-editing, and highlight that RNA-editing should be taken into account for future genetic analysis.

Key Words: posttranscriptional modifications, RNA-editing, RNA-Seq, pig

P7006 Generation of a porcine model of obesity and complications by leptin knockout. T. Tan*, and Y. Xing (The State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing, China), X. Hu, and N. Li (China Agricultural University, Beijing, China)

The studies of human obesity disease and related metabolic complications are mainly based on rodent models. However, there are significant differences in physiology between humans and rodents. In contrast, pigs show many similarities not only in the physiological structure and function, but also in the metabolic characteristics and genetic background. Therefore, in recent years, pigs have become the best resource to research energy metabolism and obesity. Previous studies showed that leptin plays an important role in fat metabolism and in the appetite regulation pathway. In this research, we generated a pig model of obesity via zinc finger nuclease (ZFN)-mediated knockout system. The ZFN mutation rate reached 8.3%. Off-target effects have not been detected in *Leptin*$^{-/-}$ individuals. *Leptin*$^{-/-}$ pigs showed largely increased obesity related phenotypes corresponding to human phenotypes. *Leptin*$^{-/-}$ pigs have significantly improved appetite and body index, especially weight. Multislice computer tomography examination showed a significant increase of body fat rate in mutant pigs, which is reflected in the subcutaneous fat and visceral fat. It also has a bigger adipocyte cell size, which is a typical phenotype of obesity in humans. Consistent with these observations, the expressions of fat synthesis related genes have been up-regulated in *Leptin*$^{-/-}$ pig. The *Leptin*$^{-/-}$ showed a significant increase in blood glucose concentration in the age of 12 mo, and developed severe insulin resistance. By IVGTT assay, the blood glucose regulatory function is disordered in *Leptin*$^{-/-}$ pigs. Moreover, by

HE and IHC analysis, we found that the *Leptin*$^{-/-}$ pigs show serious liver lesions, such as fatty liver, hepatitis, and particularly hepatic fibrosis, which is different from the results previously reported based on the mouse models. This may imply that leptin plays different roles in the development of liver fibrosis in pigs, which suggests the similar responses to intrinsic and extrinsic influences in humans.

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

P7007 Targeted IGF1 promoter modification in mice using small intestine-specific regulatory element binding sites. Y. Zheng* , R. Zhang, Z. Yu, and N. Li (China Agricultural University, Beijing, China)

The small intestine, a digestive organ, supports the body's immune system. Hence, improving the digestion, absorption, and immunological disease resistance levels of the small intestine will directly contribute to the development of the livestock industry. Previous research has demonstrated that IGF1 could promote development of the small intestine, but it faces a shortage that the phenotypes were different in IGF1 transgenic mice attributed to multiple IGF1 transcripts, splice forms, and different promoters. The modification of the IGF1 promoter using small-intestine-specific regulatory element binding sites to improve the expression of endogenous IGF1 in the small intestine will have more biosecurity and specificity than traditional transgenic models. In this study, to analyze the activity of IGF1 promoter, we performed a series of gene transfer experiments using luciferase reporter plasmids directed by five kinds of truncated forms of the mouse IGF1 5′-flanking region. Our results indicated the repressor element was presented in the region from +282 to +514 bp, and the core area of promoter activity was from –1 to +282 bp. In addition, we optimized the consensus binding sequence (A/CTTTATA/G) of small-intestine-specific regulatory elements Cdx1 and Cdx2 by adding Poly(dA:dT) sequence and then inserting to the candidate sites, which avoid the other transcription factor binding sites of mouse endogenous IGF1 promoter region. Subsequently, Cdx1 and Cdx2 expression plasmids were constructed, then respectively co-transfected with modified promoter reporter plasmids into SK-N-MC cell line. The results showed that the modified IGF1 promoter could significantly enhance the activity. We obtained similar results in 293T, HCT116, and Hepa1 to Hepa6 cell lines. According to the strategy of the highest cellular activity, we successfully obtained the IGF1 promoter site-directed modified mice by CRISPR/CAS9. The expression level of IGF1 protein in small intestine of the modified mice showed a slight increase compared with the wild-type mice; however, the significance is less than that at cellular level. In conclusion, our study solidifies the concept that using tissue-specific regulatory elements could increase the endogenous expression level, which will provide scientific evidence and novel strategy to livestock and poultry breeding.

Key Words: small intestine, IGF1, intestine-specific regulatory element

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P7008 Characterization of CD163 modification pig for PRRSV resistance. J. Chen* , N. Li, Y. Zhao, X. Hu, and Y. Xing (China Agricultural University, Beijing)

Porcine reproductive and respiratory syndrome (PRRS), caused by Porcine reproductive and respiratory syndrome virus (PRRSV), has caused large economic losses in the swine industry in recent years. Current PRRS vaccines fail to effectively prevent and control this disease. Consequently, there is a need to develop new antiviral strategies. Scavenger receptor CD163 is a key entry mediator for PRRSV. A previous research has demonstrated that scavenger receptor cysteine-rich (SRCR) domain 5 (SRCR 5) is essential for PRRSV infection. Here we report the efficient generation of CD163 biallelic-modified pigs using the CRISPR-Cas9 system combined with a donor vector. We generate pigs with the endogenous SRCR 5 encoding exon 7 replaced by the exon 10 of human CD163-L1 (hCD163-L1). Modification of the target gene was assessed by PCR, sanger sequencing, and Southern blot. Interestingly, F0 piglets were all biallelic-modified pigs. Western blot showed no significant difference was detected between the expression of target gene in CD163 modified+/+ pig and that in wild-type (WT) pig. In vitro, CD163 modified+/+ PAMs and WT PAMs were challenged with HP-PRRSV strain JXwn06 and detected by real-time PCR, Western blot and immunofluorescence assay. The results showed that modification of CD163 could induce a significant inhibition of viral RNA and protein levels in PAMs infected with HP-PRRSV strain JXwn06. These findings suggest that modification of CD163 may provide a potential strategy for anti-PRRSV therapies.

Key Words: PRRS, CRISPR-Cas9, CD163

P7009 Precancerous molecular features committing development of colonic polyps revealed by studies on the porcine model of

human familial adenomatous polyposis.
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Colorectal cancer (CRC) has variable clinical behavior, with only a small proportion of colon polyps progressing to cancer. Molecular classification of polyps could improve the discrimination of those polyps destined to become cancerous from those that are not, and thus aid strategies for early CRC therapy. Using our familial adenomatous polyposis (FAP) pig model based on the *APC*^{1311} mutation, we attempted to identify molecular features associated with early transformation and the severity of colon polyposis. Four generations of *APC*$^{1311/+}$ pigs were produced and examined by regular endoscopic examination. We performed global gene and miRNA expression analyses on biopsied samples of normal colonic mucosa, and low- (LG-IEN) and high-grade (HG-IEN) intraepithelial neoplastic polyps. We also measured the expression of selected miRNAs in the general blood circulation. These data were integrated and analyzed for association with clinical data. As in humans, a single heterozygous *APC*^{1311} mutation resulted in polyps in the large intestine. Analysis of our pig herd revealed that genetic background influences the severity of polyposis, resulting in some full siblings with markedly different numbers of polyps. The transformation of polyps was rapid, with some young pigs showing HG-IEN. Unsupervised clustering of RNA-sequencing data revealed transcriptome changes associated with early polyp development, including molecular pathways involved in metabolic processes and immune response. We also observed that the severity of polyposis is associated with differential expression of miRNAs in normal mucosa and may be estimated using the expression profiles of circulating miRNAs. Our study identified molecular features involved in cancerous early polyp transformation that might be used for transcriptome-based classification. This work was supported by the following grants: National Science Centre in Poland, Grant No. 2013/10/M/NZ2/00284, and Mildred Scheel Stiftung für Krebsforschung in Germany. The authors are members of COST Action BM1308 'Sharing Advances on Large Animal Models' (SALAAM).

Key Words: colorectal cancer, APC, swine

P7010 Generation of a novel glycosylated anti-CD20 monoclonal antibody in milk of transgenic cattle.
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Anti-CD20 monoclonal antibody (mAb, Rituxan) is widely used for the treatment of autoimmune diseases such as B-cell lymphomas. However, the production capacity of Rituxan is currently very limited. Here we report the expression of recombinant anti-CD20 mAb in milk of transgenic cattle, which demonstrated high yield (6.8 mg/mL), high purity (>99%), and high recovery rate (80%). It is revealed that the recombinant mAb was structurally identical to Rituxan, with the exception of N-linked glycosylation, which was mainly the afucosylated glycans in recombinant one and high degree of fucosylation in Rituxan. Target cell binding and complement-dependent cytotoxicity were also similar between the recombinant mAb and Rituxan. Antibody-dependent cellular cytotoxicity was increased in recombinant one, which was the reflection of its afucosylated pattern. The efficiency of recombinant mAb in treating the B-lymphomas in severe combined immunodeficient mice was higher than that of Rituxan. Our study examplified the concept of using transgenic cattle for cost-competitive large-scale production of therapeutical antibodies.

Key Words: CD20, monoclonal antibody, glycosylation

COMPARATIVE GENOMICS

P8000 Allelic diversity of productive, reproductive, and fertility trait genes of buffalo and cattle.
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Buffalo production trait gene identification is very important, and is a meager genomic resource with restricted availability. The use of cattle data is an alternate to fill this gap. A comparative genomics tool for the application of a potential cross-species examination of the buffalo genome is another option. However, it depends on similarity in nucleotide sequences. Genetic diversity between buffalo and cattle was

determined with 86 homologous genes in this study. There was a dissimilarity of about 3% of all nucleotide sequences in term diversity; and 0.267 ± 0.134 amino acids indicating the likelihood of successful use of cross-species genomic research strategy. There were significantly higher nonsynonymous (dN) and synonymous (dS) substitutions in cattle and buffalo; however, dN – dS had similar values for both species (4.414 vs. 4.745), respectively. Higher nonsynonymous substitutions in buffalo and cattle expressed similar levels of positive selection pressure in both species. The results were assessed for relative rate tests and chi-square test. There were no significant differences with regard to unique genetic mutation of synonymous sites in cattle and buffalo. However, there are significant differences for nonsynonymous sites, indicating an ongoing mutagenesis process to generate substitution mutations at substantially the same speed in both species. This variable rate of molecular evolution may be the first demonstration in the bovine family.

Key Words: buffalo, cattle, gene diversity, molecular evolution

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P8001 3D nuclear positioning of IGF2 alleles and *trans* interactions with imprinted genes. Y. Lahbib-Mansais, M. Marti Marimon, V. Voillet, F. Mompart, J. Riquet, S. Foissac, D. Robelin, H. Acloque, L. Liaubet, and M. Bouissou-Matet Yerle* (INRA UMR 1388 GenPhySE, Castanet-Tolosan, France), Y. Billon (INRA UE 1372 GenESI, Surgères, France), and N. Villa-Vialaneix (INRA UR0875 MIAT, Castanet-Tolosan, France)

To explore the relationship between gene activity and nuclear position, genomic imprinting leading to parental-specific expression offers a good model. In one cell, it is possible to compare the nuclear environment of the two alleles for a given locus and search for a potential correlation between their nuclear position and expression status. Using 3D RNA-DNA fluorescence in situ hybridization (FISH) in porcine fetal liver cells, we focused on the imprinted region of *Insulin-like growth factor 2* (*IGF2*), a paternally expressed gene located on porcine chromosome 2. We investigated the interchromosomal interactions implicating *IGF2*. Through a 2D FISH screening, imprinted genes from the Imprinted Gene Network (Varrault et al., 2006) were tested for interactions in liver cells. The locus *DLK1*/*MEG3* showed the highest rate of colocalization with *IGF2*. By 3D RNA-DNA FISH combined to confocal microscopy, we demonstrated a preferential implication of the expressed paternal *IGF2* allele in a *trans* association with *DLK1*/*MEG3* region (chromosome 7). We showed that this colocalization occurs also in fetal muscle and demonstrated that it occurs preferentially between the expressed *IGF2*, *DLK1*, and *MEG3* alleles. We are extending this analysis through an interdisciplinary approach to develop large *functional mapping* studies focused on the mechanisms involved in the transcriptional regulation of genes expressed in muscle during late fetal development of pig. From a transcriptomic analysis carried on fetal muscle of two extreme genetic lines to study maturity, we identified 2000 genes differentially expressed that characterize its establishment (Voillet et al., 2014). We are now constructing, by in silico processes, networks of co-regulated genes with *IGF2* as starting point. We are also developing a Hi-C approach to construct interaction maps on a genome-wide scale. A set of key genes belonging to these networks and interaction maps will be selected to study by 3D FISH their position in the nuclear space in cells of the two genotypes, and to determine if co-regulated genes implicated in the same biological function co-localize in the nucleus. These data should allow us to determine if these interactions are genotype and expression pattern dependent. This will open interesting questions to study the possible link between nuclear architecture and control of gene expression in muscle in an animal model for which extreme genotypes for maturity at birth are available.

Key Words: *trans* interactions, imprinted genes, 3D nuclear organization

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P8002 A genomic landscape of mitochondrial DNA insertions in the nuclear pig genome. G. Schiavo, A. Ribani, V. J. Utzeri, M. C. Ghionda, S. Bovo, L. Fontanesi* (Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy), and O. I. Hoffmann (Agricultural Biotechnology Center, Godollo, Hungary)

Nuclear DNA sequences of mitochondrial origin (NUMTs) are derived by insertion of DNA fragments of the mitochondrial genome (mtDNA), from both coding and noncoding regions, into the nuclear genome. NUMTs are sequence fossils present in the nuclear genome of many eukaryotes, contributing to shape their genomic architecture and evolution. In this study we provided a first genome picture of NUMTs in the pig genome and evaluated if insertion polymorphisms could be identified in this species. *Sus scrofa* reference nuclear genome (Sscrofa10.2) was aligned with circularized and consensus mitochondrial DNA sequences using LAST software. A total of 401 NUMTs were identified in the assembled pig genome in addition to a few tens of other NUMTs identified in unassembled scaffolds. In total, NUMTs covered about 200 kbp of the assembled genome (about 0.008% of the whole genome), including all mtDNA regions. Chromosome 14 had the highest percentage of

NUMT sequences. Inserted nuclear mtDNA fragments ranged in size from 42 bp to about 5 kbp, with an outlier on chromosome 2 of about 11 kbp (the largest insertion). Before present, phylogenetic analyses of NUMTs provided an estimation of the time of the insertion. Six NUMTs were analyzed to evaluate the level of insertion polymorphisms in 10 different pig breeds (Italian Large White, Italian Duroc, Italian Landrace, Pietrain, Cinta Senese, Apulo-Calabrese, Casertana, Nero Siciliano, Mora Romagnola, and Meishan) and in European wild boars. Three of the investigated NUMTs showed insertion polymorphisms in one or more pig breeds, suggesting a significant degree of variability on inserted NUMTs in the pig species.

Key Words: genome evolution, mtDNA, NUMT

P8003 The gene duplication of β-2 microglobulin in Artiodactyla remains intact only in pigs.

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From the analysis of the pig genome assembly SSC10.2, we discovered a segmental duplication of ~48 kb in size containing the entire coding sequence of β-2 microglobulin (B2M) and the protein associated with topoisomerase II homolog 2 (PATL2) genes on pig chromosome 1. Considering B2M as a subunit of the major histocompatibility complex (MHC), based on the finding, we evaluated the functional consequence of B2M duplication in possible strengthening of immune capacity through the increased expression of MHC molecules in pig cells. As a first step, we confirmed the accuracy of the B2M duplication in the pig genome by PCR and direct sequencing of duplication boundaries. Subsequently, we confirmed the copy number of B2M in the pig genome using real-time PCR at the level of mRNA and genomic DNA. We also analyzed and compared the synteny blocks of the corresponding region among pigs and other mammals to check the evolutionary conservation. Our results showed that this duplication has occurred during the speciation of Artiodactyla, but currently remains structurally and functionally intact only in pigs. As a separate experiment, we evaluated changes of protein expression level of MHC on the cell surface after transfecting B2M cDNA to a pig cell line. However, the protein level was similar to that of before transfection in our analysis. Although the functional effect of B2M duplication in pigs is still unclear, this could provide a beneficial effect to immune reaction of pigs. Further analyses are necessary to address this interesting question.

Key Words: pig genome, MHC, gene duplication, β-2 microglobulin

P8004 A comprehensive gene catalog of the horse Y chromosome.

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Historically, the mammalian Y chromosome has been considered degenerate and gene poor, with the primary or only function to initiate male sex determination and differentiation. These views started to change after the male specific region (MSY) of the human Y chromosome was sequenced in 2003, showing that MSY carries a number of coding genes and transcriptional units with functions in spermatogenesis and development. Since then, MSY sequence data have been obtained for the chimp, macaque, cattle, pig, dog, cat, and mouse—all showing that mammalian MSY carries functional genes but is also characterized by rapid gene expansions, turnover, and dynamic molecular processes that alter gene content and organization among species. Here we present the most comprehensive gene catalog of the horse MSY. The genes were mapped by sequencing and assembling 9.36 Mb of equine MSY using a tiling path of 94 BAC clones and a combination of short and long insert libraries on several next generation sequencing platforms. Genes were annotated using MAKER software, BLAST against reference genes and cDNAs, testis RNaseq transcripts, and the SNAP program to build most likely gene models. Gene expression profiles were determined by RT-PCR in a panel of nine adult tissues and nine tissues from 49- to 51-d-old equine embryos. Altogether, we identified 52 unique MSY genes and transcripts, of which 16 were novel. Thirty genes had one copy, while the copy numbers of 24 other genes ranged from two (*TXLNGY*) to 28 (*ETY3*), resulting in a total of 221 MSY genes and transcripts. This implies that the small equine MSY, with its 23.6 genes per Mb (considering all copies), is one of most gene-dense eutherian Y chromosomes studied to date. Among MSY genes and transcripts, 10 were horse- or equid-specific and Y-borne, 14 were of autosomal origin, and 30 had a gametologue in the X chromosome. Thus, horse MSY has also retained the highest number of ancestral X-Y genes among eutherian MSYs studied so far. The annotated horse MSY sequence was aligned with sequence assemblies of related equids, showing that the vast majority of the MSY sequence is substantially more conserved among equids than among primates. The expression pattern between donkey and horse was also

surprisingly comparable. The horse MSY gene catalog establishes a basis to study the contribution of Y genes to stallion reproductive biology and provides new insight into Y chromosome evolution and function in mammals.

Key Words: Y chromosome, genes, horse, comparative

P8005 Genes responding to recent selection in Berkshire and Duroc pigs. K. D. D. Song (The Animal Molecular Genetics and Breeding Center, Chonbuk National University, Jeonju, South Korea), D. Shin* and H. K. Lee (Department of Animal Biotechnology, Chonbuk National University, Jeonju, South Korea)

Berkshire and Duroc pigs have been developed for unique pork quality during the last one or two centuries. To reveal the region under selection, selection signatures were identified by comparisons between Duroc and Berkshire pigs (N = 720) based on the analysis of extended haplotype homozygosity (EHH) using 60 k single nucleotide polymorphisms (SNPs). Moreover, the selected regions were examined further using the genomic sequences of 46 pigs. Although animals in the two pig breeds selected for common objectives, each breed showed unique signatures of selection. Using 60 K SNPs, the analyses of EHH identified 10 to 15 substantial selection signatures within a breed, or comparisons between two breeds, which may reveal the regions under recent selection. The haplotype pattern decided by SNPs were in agreement with the analyses of variations extracted from the genomic sequences. In particular, genomic regions on chromosomes 4 and 17 in Berkshires were likely to be affected by recent selection, and considerable EHH was identified on chromosomes 6 and 14 in Durocs. To refine the regions supported by the relatively long range of haplotype homozygosity (>1 Mb), the mean Fst of SNPs for each gene was calculated. In summary, more than 50 genes that are involved in fat or protein metabolism were located in the recently selected regions. Identifying the regions involved in differential selection will be useful to find causal mutations affecting unique traits that explain the meat quality in Berkshire and Duroc pigs.

Key Words: Berkshire, Duroc, pork quality, selection signatures, haplotype

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P8006 Identification and characterization of copy number variations in cattle. R. Letaief*, C. Grohs, S. Fritz, D. Rocha, and M. Boussaha (GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France), D. Esquerré and J. Barbieri (Get-PlaGe, INRA, Castanet-Tolosan, France), S. Fritz (ALLICE, Paris, France), C. Klopp (SIGENAE, INRA, Castanet Tolosan, France), R. Philippe and V. Blanquet (GMA, INRA, Université de Limoges, Limoges, France), and D. Boichard (GABI, INRA, AgroParisTech, Universite Paris Saclay, Jouy-en-Josas, France)

Copy number variations (CNVs) are an important source of genetic changes. They are defined as a gain or loss of genomic region ranging from 50 bp to several megabases. CNVs have been shown to be associated with many diseases and some phenotypic traits in several species, including cattle. We used Pindel, Delly, BreakDancer, and CNVnator to identify CNVs using whole-genome sequencing data of 200 animals from eight French dairy and beef cattle breeds. We selected only deletions and duplications predicted by at least two tools and present in at least two animals. We identified a total of 29,132 autosomal deletions and duplications which cover between 31 to 34% (784 to 865 Mb) of the autosomal genome, with an average of 6,000 events per animal. Among these deletions and duplications, 27,690 were present in at least two animals. Out of theses, 26,417 events were deletions, 674 were duplications and 599 regions were both (deletion and duplication within the same region). We defined a CNV as deletion and duplication in the same region, and we termed this region as CNV-Region (CNVR). The size of CNVRs ranged from 100 bp to 9.3 Mb with a median of 1.3 kb and a mean of 45 kb. From the identified deletions and duplications, 8,283 overlapped with 9,733 annotated genes including 290 CNVRs overlapping with 974 annotated genes, including some genes known to be implicated in some traits of economic importance. Our study provides an extensive view of the CNVRs in French dairy and beef breeds. CNVRs with an effect on some commercially interesting phenotypes could be used to improve genetic selection of these eight French breeds.

Key Words: copy number variations, whole-genome sequencing, dairy and beef breeds, genome plasticity, bioinformatics

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P8007 RefSeq and Gene—NCBI resources to support comparative genomics. K. D. Pruitt*, T. D. Murphy, F. Thibaud-Nissen, and P. A. Kitts (National Institutes of Health, NCBI, Bethesda, MD)

The National Center for Biotechnology Information (NCBI) Reference Sequence (RefSeq) project provides several resources to support the animal genetics research community. These include whole genome annotation, evidence-based transcript and protein sequence records, BLAST databases, FTP data, and entries in NCBI's Gene database resource. Gene, transcript, and protein data are provided using

a combined approach of whole genome annotation by NCBI's eukaryotic genome annotation pipeline, manual curation, and collaboration. We currently provide RefSeq genome annotation for over 56 bird genomes and 90 mammalian genomes, including the alpaca, cattle, camel, goat, horse, pig, sheep, and water buffalo genomes. These high-quality standards provide a baseline that is used for a variety of applications ranging from individual gene, transcript, or protein analysis to comparative genomics. RefSeq staff have provided extensive manual curation of human and mouse sequence and gene data in the last 15+ years; this curation results in expertly annotated and informatively named transcript and protein sequences and Gene records. This curation activity also benefits the genome annotation results provided for other mammalian genomes as curated human RefSeq records are one of many input reagents used in NCBI's eukaryotic genome annotation pipeline. Informative gene and protein names are thus propagated across species based on identified orthology to human (which considers both protein similarity and local synteny). RefSeq staff also provide some manual curation support for bovine, chicken, and other animal genomes. Manual curation focuses on both sequence and gene data and, for most animals, is initiated in response to identified putative errors, user requests, or collaboration. Manual curation is essential to correct genome annotation errors, to represent atypical biology, to add representation of known genes and their products that are not yet represented in draft genome assemblies, and to add or correct functionally relevant information. Curated RefSeq records are immediately publicly available in NCBI resources and are used to improve the genome annotated when next recalculated. RefSeq data are available online in NCBI's BLAST, Gene, Nucleotide, and Protein resources. Links to download (by FTP) annotated RefSeq genomes are presented in NCBI's Assembly resource. The presentation will present an overview of RefSeq and Gene, examples of RefSeq curation, and information on data access. For more information: Assembly, www.ncbi.nlm.nih.gov/assembly/; Gene, www.ncbi.nlm.nih.gov/gene/; RefSeq, www.ncbi.nlm.nih.gov/refseq/, verified 28 May 2016.

Key Words: RefSeq, Gene, orthologs, genome annotation, biocuration

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P8008 The NCBI Eukaryotic Genome Annotation Pipeline. F. Thibaud-Nissen* , M. DiCuccio, W. Hlavina, A. Kimchi, P. A. Kitts, T. D. Murphy, K. D. Pruitt, and A. Souvorov (National Institutes of Health, NCBI, Bethesda, MD)

The National Center for Biotechnology Information (NCBI) Eukaryotic Genome Annotation Pipeline (available at www.ncbi.nlm.nih.gov/genome/annotation_euk/, verified 26 May 2016) has been used to annotate over 280 organisms, including many animals of agricultural importance. The pipeline provides content for various NCBI resources, including Reference Sequence (RefSeq) sequence databases, Gene, BLAST databases, and the Map Viewer genome browser. The pipeline uses a modular framework for the automated execution of all annotation tasks from the fetching of raw and curated data from public repositories to the submission of the RefSeq-accessioned annotation products to public databases. The quality of the annotation is highly dependent on the availability of evidence for the species or closely related species. Alignments of RNA-Seq, traditional transcripts, expressed sequence tags, transcript assemblies, and proteins by Splign and ProSplign all contribute to the prediction of gene models by Gnomon, an alignment- and Hidden Markov Model-based gene prediction program developed at NCBI. The RefSeq group curates genes and transcripts for several plant and animal genomes, including pig, horse, and cattle. When available for the annotated species, the curated transcripts are aligned to the genome and take precedence over similar models produced by Gnomon based on other (noncurated) evidence. High-quality annotation is also achieved by producing models that compensate for assembly issues using the aligning evidence. The final annotation product can include transcripts and proteins for which the sequence has been modified relative to the draft genome assembly to correct a truncating mismatch or frameshift, or to fully represent a gene only partially present in the genome owing to sequence gaps. The final products of the pipeline include the annotated genomic sequences, the genes, and the transcript and protein products named based on orthology to model organisms or Blast hits to SwissProt/UniProtKb. We aim to reannotate organisms we maintain every 2 yr, so that the annotation incorporates recent evidence deposited in public databases, and benefits from improvements in software. We produce a summary report with each annotation, containing the evidence on which the annotation is based, and statistics on the annotated products. In the case of a reannotation, we also provide details about the genes and transcripts that changed. See all NCBI-annotated eukaryotes at http://www.ncbi.nlm.nih.gov/genome/annotation_euk/all/ (verified 28 May 2016).

Key Words: annotation, whole-genome, gene prediction, RNA-Seq, curation, genome, assembly

AUTHOR INDEX

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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

Ø

Ü