S0001 – S0016

Invited Speaker Abstracts

S0001 The power of comparative genetics and genomics

Kerstin Linbald-Toh.
Broad Institute, USA; Uppsala University, Sweden.

S0002 Using intra-species variation to understanding basic biology

Ewan Birney.
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Quantitative genetics based on large, outbred populations has had a long history in both animal breeding and human disease studies. It is one of the few techniques which one can apply to understand a complex phenotype when nothing else is known about the phenotype. A traditional downside of quantitative genetics has been the need for both reasonably large numbers of individuals studied and a large number of markers which needed to be typed. To reduce the required marker density often populations with reasonably long linkage disequilibrium were used, preventing the use of quantitative genetics in a number of scenarios. The logistics therefore prevented quantitative genetics being widely used outside of complex phenotypes.

However, the economics of quantitative genetics has been completely changed by the advent of ultra-high throughput sequencing. Now very higher marker density, include the ultimate full resequencing is achievable at reasonable cost. This is a huge boon to "traditional" complex phenotype quantitative genetics, but also opens up this tool for basic molecular biologists studying fundamental processes in biology. I will describe some recent success in exploring the interaction of chromatin structure and function in Humans and oogenesis in Drosophila using quantitative genetics, and explore the similarities and differences in using quantitative genetics for basic biology compared to complex phenotype exploration.

S0003 Epigenetics, Imprinting and Disease Susceptibility

Randy Jirtle.
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Human epidemiological and animal experimental data indicate that the risk of developing adult onset chronic diseases such as cardiovascular disease, diabetes, obesity, and cancer is influenced by persistent adaptations to prenatal and early postnatal nutrition. Two epigenomic targets that potentially link environmental exposures to chemical and physical agents early in development to adult disease susceptibility are imprinted genes and those with metastable epialleles. Genes with metastable epialleles have highly variable functions because of stochastic allelic changes in the epigenome rather than mutations in the genome. Genomic imprinting is an unusual epigenetic form of gene regulation that evolved 150 million years ago in mammals with the development of the placenta and the advent of viviparity. It results in monoallelic, parent-of-origin dependent gene silencing. Thus, only a single genetic or epigenetic event is required to alter the function of an imprinted gene. The potential importance of these two novel subsets of epigenetically labile genes in normal human variation, and the etiology of environmentally-induced diseases will be discussed.

S0004 Finding the causal variant in selective sweeps

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The human genome contains hundreds of regions with patterns of genetic variation that reflect recent, positive natural selection, yet for most the underlying gene and the advantageous mutation remain unknown. We have developed a method, the Composite of Multiple Signals (CMS), that, by combining multiple different tests for natural selection, increases our resolution by up to 100-fold. By applying CMS to the International Haplotype Map, we localize hundred signals, reducing the candidate region for each to just ~50-100kb. In many cases, we can identify the precise gene and polymorphism targeted by selection. This includes genes involved in infectious disease susceptibility, skin pigment, metabolism, and hair and sweat. Nearly half of the ~200 regions we localized contain no genes at all, and 13 contain long, non-coding RNAs, which can regulate nearby genes. In several regions we significantly associate variants under selection with the expression of nearby genes.

We are now applying CMS to preliminary data available from the 1000 Genomes Project, a full sequence dataset which should contain the actual functional mutation in most cases, and are identifying new, intriguing coding and regulatory variants. With the cost of sequencing falling dramatically, full sequence data for many individuals will soon be available not just for more human populations, but for many other species as well. Although the current version of CMS is tailored to the population history of humans, the remarkable power of the composite approach suggests it can help elucidate the evolutionary history of a wide range of species.

S0005 Domestication, dispersal and hybridisation: The next generation of emerging narratives

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Investigating the patterns and processes of animal domestication from a genetics perspective has thus far been carried out primarily (though though not exclusively) by analyses of mitchondrial sequences. The greater resolution offered by DNA as compared with more traditional morphological approaches to within species questions has led to a great deal of novel results including the recognition that many more discrete populations have been involved in domestication across the Old World than previously suspected. Still, the maternal inheritance pattern of mtDNA has necessarily limited the DNA perspective. With the advent of high throughput sequencing technology (including the development of large-scale SNP arrays), the nuclear genome is becoming increasingly available. This paper offers a brief summary of early results in this vein and in so doing, describes both the new narratives emerging from domestication studies, and a new hybridization hypothesis across numerous domestic animal taxa.

S0006 Genome-wide association studies (GWAS) in humans: Is the genetic revolution finally here?

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Identifying the genetic basis of complex traits is considered one of the major challenges science is facing today. Complex traits in humans include for example susceptibility to disease or drug response variation. The identification of the underlying genes to these traits has the potential to revolutionize the way medicine is practiced. On the one hand, this may enhance drug discovery and on the other hand help developing personalized medicine, "the right drug for the right patient". In recent years, the development of novel technologies has brought about large scale genome-wide association studies (GWAS). Hundreds of GWAS have been reported so far, revolutionizing the landscape of the genetics of complex traits through the identification of associated genes. Nevertheless, in most instances, the proportion of variance explained by the detected genes is relatively modest. Furthermore, most polymorphisms identified do not necessarily have a functional effect. Whole genome sequencing is yet another technology which may further close the gap to the delineation of the genetic basis of complex traits. This technology has already been successfully applied at a relatively small scale, its full utilization still requires a cost- reduction of at least an order of magnitude. Revolutions are spotted as such usually only after they have occurred. It seems too pretentious to state that the genetic revolution is here, but I find it safe to say that it has begun.

S0007 Identifying regulatory QTN in livestock

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S0008 Exploiting genomics to dissect the genetic control of complex traits in humans

Tim Frayling.
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Genome wide association (GWA) studies in humans have identified several hundred variants associated with common human traits. These traits include diseases and continuous measures, including diabetes, autoimmune diseases, bone disorders and inflammatory conditions. Many of these traits are relevant to animal studies including, height, body mass index and fat mass. Here I will give some brief background to GWA studies in humans before focusing on a "model" trait – adult height. This trait has proven to be a model trait in that genome wide genetic and phenotypic data is available from >200,000 individuals, it is strongly heritable and highly polygenic. Recent studies by the Genetic Investigation of Anthropometric Traits (GIANT) consortium, have identified 180 loci associated with human height. These loci are non-randomly distributed across the genome, being enriched for genes known to be involved in mammalian growth. I will conclude with where human genetic studies are likely to go next, a step which is likely to involve whole genome and exome sequence data as well as genotype data.

S0009 How selective sweeps in domestic animals can teach human medicine

Leif Andersson.
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Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Strong directional selection in domestic animals leads to dramatic changes in allele frequencies, selective sweeps, of genetic variants with notable effects on the trait under selection. The identification and molecular characterization of the causative mutations underlying such sweeps can generate new basic knowledge of biomedical importance. The characterization of the paternally expressed IGF2 QTL in pigs affecting muscle growth and fat deposition is a prime example of this. First we used QTL mapping and haplotype sharing analysis and showed that the causative mutation is a single nucleotide substitution at an evolutionary conserved site in intron 3 of IGF2. Now we have demonstrated that this mutation disrupts the binding of a previously unknown transcription factor that we named ZBED6. ZBED6 has evolved from a domesticated DNA transposase and is an innovation in the placental mammals. Our further characterization of ZBED6 has revealed that it is widely expressed both during development and in adult tissue. ChIP-sequencing has revealed more than a thousand potential downstream targets besides IGF2. Major topics for ongoing research include studies how ZBED6 regulates transcription of IGF2 and other target genes and whether mutations in ZBED6 or ZBED6 binding sites are associated with human disease.

S0010 The role of NK cells in health and disease

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Natural Killer cells are lymphocytes of the innate immune system that have been known for their roles in cancer and transplant immunology. During the last ten years it has been found that they contribute to the onset and shaping of adaptive cellular responses through interplay with dendritic cells and T-cells. These possible roles in resistance to infections and vaccine development have lead to new interest for NK cells in veterinary infection biology.

The activation of NK cells is regulated by the interplay of activating and inhibitory receptor signals as well as cytokine stimulation. NK cell receptors fall into two structural categories; the killer cell lectin-like receptors and the leukocyte immunoglobulin-like receptors. Both categories include activating and inhibitory variants, however different categories of genes have expanded to become gene families that bind to MHC class I ligands in different species. These NK cell receptor multigene families may show polymorphism; different individuals may have haplotypes with varying gene contents as well as allelic variants of individual genes.

The main roles of NK cells in infections and the most important NK cell receptors will be discussed with focus on the knowledge achieved in rodents and humans and will be extended to data generated in veterinary relevant species where information exists. The aim of the talk will be to raise interest about NK cells and their receptors among listeners with a genetic approach to disease susceptibility.

S0011 How manipulation of resistance and tolerance to infections can alter animal health and disease transmission

David Schneider.
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Evolution provides two main routes a host can use to reduce the costs of infection. The first route is "resistance" and is the ability to reduce pathogen levels. We know much about resistance - most of immunology focuses on these killing mechanisms. The second route is "tolerance", which is defined as the ability of the host to maintain its health in the presence of pathogens. For example, a tolerant host would not get very sick as pathogen levels increase. We have found that it is simple to manipulate both resistance and tolerance mechanisms in our model system and this provides hope for new therapies. Tolerance is not a panacea, however, as it can result in hosts carrying high levels of pathogens and therefore we need to think carefully about how resistance and tolerance can be manipulated together to increase animal health during an immune response.

S0012 Mapping resistance to chicken gut bacterial pathogens – Salmonella and Campylobacter

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Salmonella and Campylobacter are major zoonotic bacterial pathogens. Around 10,000–30,000 cases of human salmonellosis and 40,000-60,000 cases of human camylobacteriosis were reported per annum in England and Wales alone in the past decade (Health Protection Agency, 2008; http://www.hpa.org.uk). The vast majority of human cases arise from the consumption of infected poultry. One sustainable solution would therefore be to reduce contamination of poultry by identifying disease resistance genes. In contrast, intravenous challenge with *S. Typhimurium* provides a valuable model for systemic infection, often causing a typhoid-like infection, with bacterial replication resulting in the destruction of the spleen and liver of infected animals.

Resistance to systemic salmonellosis, and to colonisation with either *Salmonella or Campylobacter*, is partly genetically determined. For systemic salmonellosis, we have confirmed and refined a resistance locus, SAL1, to a region of Chromosome 5 spanning 14 genes, including two very striking functional candidates; CD27-binding protein (Siva) and the RAC-alpha serine/threonine protein kinase homolog, AKT1 (protein kinase B, PKB).

Two inbred lines of chickens, 61 and N, are respectively resistant and susceptible to colonisation with either *Salmonella or Campylobacter*. Using a backcross experimental design and a high density genome-wide SNP panel, we have identified 4 resistance QTL for each colonisation model. Interestingly and perhaps surprisingly, none of the QTL regions are in common for the two colonisation models.

S0013 Livestock improvement: how fast have we gone; how fast can we go?

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There has been a wide range among species and countries in the effectiveness with which modern livestock breeding methods have been employed. Most of the improvement has been made in quantitative traits using selection methods based on phenotype of individuals and their relatives for these traits, despite effort put into, for example, using genetic markers to identify QTL. Questions to address are: (1) What are current rates of response and are they sustainable? (2) To what extent will new concerns such as greenhouse gas emissions and pressure on limited natural resources influence opportunities, and how might these be met? (3) What opportunities are offered by new technologies to enhance rates of improvement both by utilizing genomics and other 'omics in conventional programmes and by more radical means?

S0014 Improving efficiency of animal protein production with genomic selection

Ben J. Hayes.
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Production of sufficient protein to satisfy demand given projected population growth will be a major challenge in coming decades, particularly if climate change predictions are considered. Animal breeding has met the challenge of improving efficiency of protein production in the past, and will continue to do so. However rates of genetic gain for efficiency can be improved using a new technology called genomic selection. Results from genome wide association studies in livestock, and humans, has lead to the conclusion that the effect of individual quantitative trait loci (QTL) on complex traits, such as yield, are likely to be small, and that a large number of QTL are necessary to explain the genetic variation in these traits. Genomic selection overcomes this problem by estimating breeding values as the sum of the effect of all of the dense DNA markers across the genome. In dairy cattle breeding, the accuracy of estimated breeding values which can be achieved and the fact that these are available early in life has lead to rapid adoption of the technology. Genomic selection allows for increased rates of gain for traits which have been hard to select for in the past: the example is given here of reduced sensitivity of milk production to heat stress.

S0015 Genetic modification of farmed animal species: current state of the art and opportunities for applications

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Genetic modification technologies for the major livestock species were developed in the 1980s, although methods for use in poultry have been slower in becoming available. The technologies have become more sophisticated and more efficient, with the use of nuclear transfer in mammals and the use of lentiviral vectors in birds and mammals. The advantages and limitations of these methods will be discussed. Methods that have been developed for genome modification in the chicken, for example primordial germ cell culture, will also be described. Transgenic technologies continue to be improved by adopting advances from other fields, particularly the gene therapy field. The potential for use of site-specific zinc finger nucleases for direct manipulation of the genome, by inducing site-specific mutations and with the potential for promoting high frequency homologous recombination is an exciting new area in transgenic technologies. The potential for generating ES cells from livestock species, a goal that has not been achieved by adapting the mouse ES cell derivation methods, by using the induced pluripotential stem cell approach, also has the potential to increase the efficiency and sophistication of transgenic technologies in farmed animal species.

Now that increasingly efficient and more sophisticated genome modification technologies are available, it is possible to consider the potential for their use in modification of production animals. Many potential applications have been discussed over the last 20 years but few have been extensively tested. The possible areas of applications will be discussed, with the example of avian influenza resistance by transgenesis in the chicken, and the issues of public acceptance considered.

S0016 Trypanosomiasis resistant cattle

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Bovine African Trypanosomiasis is prevalent in 36 countries of sub-Saharan Africa. It is caused by *Trypanosoma congolense*, *T. vivax*, and *T. brucei brucei* and is transmitted by tsetse flies. Humans are protected from these parasites due to an innate immune complex called trypanosome lytic factor (TLF), a subtype of high-density lipoprotein. TLF is a primate specific immune factor ONLY found in humans, some Great Apes and Old World Monkeys. Within the lipid/protein complex apoL-I is the component that is necessary for the lethal action of TLF. We have shown that expression of exogenous human APOL1 in mice, which are susceptible to trypanosomiasis, protects them against infection by *T. congolense*, *T. b. brucei* and *T. evansi*.

Two other species, *T. b. rhodesiense* and *T. b. gambiense*, are resistant to human TLF and therefore infect humans and cause sleeping sickness. Cattle are also infected by *T. b. rhodesiense* and due to their close proximity with humans act as reservoirs that facilitate the transmission of Human African Trypanosomiasis.

Some Old World Monkeys including baboons are naturally resistant to all African trypanosomes. We have recently isolated the baboon APOL1 orthologue, which is 60% similar to human APOL1. Mice transiently transfected with this gene are protected against human infective species *T. b. rhodesiense* as well as the cattle pathogens *T. congolense* and *T. b. brucei*. Due to this discovery we are developing transgenic cattle that carry baboon APOL1 and will evaluate their ability to resist infection. If successful this could lead to improved sustainability of livestock in a broad region of the developing world.

W0001 – W0011

Invited Workshop Speaker Abstracts

W0001 Exploring evidence for an imprinted QTL for muscle depth on OAR18

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We present a case study exploring evidence for an imprinted QTL for a complex trait, using variance component QTL techniques applied to both field and simulated datasets. Previously we have demonstrated the existence of a QTL for muscle depth on OAR18 in Texel sheep. This QTL mapped to a commonly imprinted region which includes the Callipyge mutation, which itself exhibits a polar overdominance mode of inheritance. However, the Callipyge mutation itself was not segregating in the population under study. Variance component QTL mapping on an enlarged population of 4376 lambs with a complex pedigree structure presented significant evidence for both paternal and maternal parent-of-origin QTL effects, with variance components for both effects being similar in magnitude to the QTL variance component obtained ignoring parent-of-origin effects. Simulation was then used to explore the expected pattern of QTL variance components obtained under various inheritance models, including additive, full and partial paternal imprinting and polar overdominance. The polar overdominance model, in which the heterozygote inheriting the mutation from the sire is the only genotype expressing the altered phenotype, was the only genetic model which produced results consistent with the results from the field data. We propose that the Texel OAR18 QTL exhibits polar dominance akin to the adjacent Callipyge mutation. Further, the magnitude of the variance components suggest that the QTL frequency may be close to 0.5, this being the frequency that the QTL is expected to move towards with phenotypic selection on muscle depth.

W0002 Multi-platform Next Generation Sequencing of the Domestic Turkey (Meleagris gallopavo): Genome Assembly and Analysis

The Turkey Genome Sequencing Consortium
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Next-generation sequencing technologies were used to rapidly and efficiently sequence the genome of the domestic turkey. The current genome assembly (1.1 billion base pairs) includes 917 million base pairs of sequence assigned to specific chromosomes. Innate heterozygosity of the sequenced bird allowed the discovery of more than 600,000 high quality single nucleotide variants. Annotation pipelines predicted nearly 16,000 genes, with 15,093 recognized as protein coding and 611 non-coding RNA genes. Comparative analysis of turkey, chicken and zebrafinch genomes, and comparisons with mammals, supports the notable stability of avian genomes and identifies genes unique to birds. Clear differences are seen in the number and variety of genes of the immune system where gene duplication events are less frequent in birds than gene losses. The turkey genome sequence provides resources to further understand the evolution of avian genomes and the reference to discover genetic variations underlying economically important quantitative traits.

W0003 The genome and transcriptome of the duck unravels codes of a host's adaptability to influenza

International Duck Genome Sequencing Consortium, presented by Ning Li, College of Biological Sciences, China Agricultural University, Beijing, China

We present a draft genome sequence of the duck, *Anas platyrhynchos*, together with the gene expression analysis of the lung tissues from two influenza A viruses (IAVs) infected individuals. The duck represents a fascinating and interesting organism because it is a principal natural IAV host and provides important evolutionary information about both a resistance to IAVs and avian divergence in general. We use sequence comparison with the galliform, passeriform and mammalian lineages to shed light on the structure and evolution of genomes and genes. Although the duck holds a contractive immune gene repertoire, specific features, such as the scarce endogenous retroviruses and the expanded β-defensin and butyrophilin genes, may improve its adaptability to remain in a state of evolutionary equilibrium with IAVs and maximize its ability to fight opportunistic infection. In addition novel potential immune genes and pathways responding to IAVs were found. This information provides an invaluable resource to unravel the interaction between host and IAVs with important consequences for human and animal health.

W0004 The chicken genome: Filling in the gaps

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The chicken was the first livestock species to have its genome sequenced. However, six years after the first chicken genome assembly became available many of the chromosomes are still not covered. Currently, sequence maps are lacking for the six smallest chromosomes (33 to 38) and chromosomes 29, 30 and 31. Despite extensive efforts, no linkage, sequence or physical maps have become available for these micro-chromosomes except for two unassigned linkage maps (E22C19W28_E50C23 and E64). It has been suggested that this might be due to the high GC content of these chromosomes or the presence of specific sequences on these chromosomes that interfere with the cloning in *E coli*, a step involved in al these mapping and sequencing efforts.

In order to obtain sequences covering these missing chromosomes and to avoid these potential biases in sequencing and cloning, a whole genome shotgun approach was used based on Roche 454 and Illumina GAII sequencing. In total 12x sequence depth was generated using the Roche 454 platform and 60x using the Illumina GAII platform. These new sequence data were used to generate a new build (UMD_2.0) of the chicken genome and we compared this to the previous assembly (Gallus_gallus-2.1). The contiguity statistics of UMD_2.0 are superior to Gallus_gallus-2.1. The genomic representation of the two assemblies is similar but the new version has collapsed polymorphic loci that are known to be present in Gallus_gallus-2.1. According to Kelley and Salzberg (Genome Biology 11:R28), the previous assembly contained 17.53 Mb of erroneously duplicated haplotype sequence which has been corrected in the new UMD assembly.

To develop linkage maps for the missing chromosomes, we also attempted to identify SNPs on the missing micro-chromosomes by aligning IIIlumina sequences derived from reduced representation libraries of 2 broiler and 2 layer lines to 454 contigs that did not align to build Gallus_gallus-2.1. A selection of these SNPs was included on the Illumina chicken 50K beadchip and genotyped on the Wageningen mapping population. Of the 1,219 unassigned SNPs on the 50K chip, 431 could be mapped on the chicken genome. Unfortunately, the majority (379) of these SNPs mapped to chromosomes that were already covered by build Gallus_gallus-2.1 and only 52 were assigned to 3 new linkage groups.

W0005 Genome-wide assessment of diversity in the pig and other suids

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The pig has a complex and long history of domestication and breed formation. It has been domesticated independently multiple times, and has likely experienced introgression of local wild boar after the spread of agriculture. Using the Illumina GA sequencing platform we have identified hundreds of thousand s of SNPs in the porcine genome and used these to develop the Illumin porcine 60K iSelect Beadchip. This chip has enabled whole-genome characterization of linkage disequilibrium and haplotype structure in commercial and local breeds, and comparison with wild boar. It has also allowed an investigation into whole-genome patterns of variation, including signatures of selection associated with domestication and breed formation as well as elucidating demographic events such as population bottlenecks, and providing insight into the complex origin of domesticated populations by examining patterns of haplotype sharing. Currently around 2400 pigs and wild boar have been genotyped with the porcine 60K beadchip, pertaining more than 60 pig breeds, both local and commercial ones, from around the world. In addition, more than 30 different wild boar populations distributed throughout Eurasia have been characterized. Museum and archeological samples were included to provide a wider insight into domestication and geographic history. Applying the 60K assay to other Old World Suidae, including the other species in the genus *Sus* as well as African warthogs, bushpigs, red river hog and the enigmatic *Babyrousa*, allowed the estimation of the ancestral allele and the origin and relative age of the porcine SNPs.

To further investigate the degree of sequence divergence and allele sharing between *Sus scrofa* and closely related species, we sequenced the complete genomes of 4 additional species: (1) The Javan warty pig (*Sus verrucosus*) occurring only on Java, (2) Celebes warty pig (*Sus celebensis*) endemic to Sulawesi, (3) Bearded pig (*Sus barbatus*) from Borneo and (4) the African warthog (*Phacochoerus africanus*). Genome-wide patterns of divergence and allele sharing between the five sequenced species revealed correlation with recombination frequency and balancing vs. directional selection, and provides a framework to further investigate patterns of selection during speciation and domestication.

W0006 From gangland murder to the wildlife trade – forensic DNA identification of individual animals

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The use of STR profiling systems to match evidence items to individual animals is of increasing interest to enforcement officers investigating crimes against humans and wildlife. The availability of validated marker panels and corresponding population data enables the forensic identification of biological material in a legal context. Here we report on two recent cases in which prosecutions have relied on forensic genetic analysis of animal DNA to secure convictions. The first case concerns a murder in London in which trained dogs were used to attack and immobilize the victim. The dogs were subsequently stabbed during the attack, leading to the deposition of both canine blood and saliva on victims and at the scene. The second case involved the use of a novel rhinoceros STR profiling system to identify the individual source of illegally traded horn seized on export from the UK.

W0007 Forensic DNA analysis of Cumbria's *Cervus elaphus* population

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Cervus elaphus is one of the two truly native deer species in Britain and they are protected by Deer Act 1991, Chapter 54. Over the last few years there has been an increase in deer poaching and there is currently no way of linking evidence collected from wildlife crime scenes with evidence collected from suspected poachers' clothing or vehicles. This project is aiming to design and develop a DNA profiling kit specific for *Cervus elaphus* species that will produce highly discriminating results. Suitable microsatellite markers were selected from published literature and database search as candidates for use in this project. Candidates were screened in single-plex reactions to determine specificity, heterozygote frequency and allele range. Extensive optimisation was then performed, to produce two multiplex PCR reactions, each containing eight STRs, including an Amelogenin marker for sex determination of the deer samples. Individual STR alleles are being sequenced to determine the microsatellite type and number of repeats at each locus. *Cervus elaphus* samples from the Grizedale forest herds have been genotyped to determine the allele frequencies for each locus. Population genetics studies will be performed to calculate match probability and the co-ancestry coefficient (θ) for the DNA profiling kit. The results obtained so far indicate that the selected markers are appropriate for the individualisation of *Cervus elaphus* in our chosen population.

W0008 Better breeding – can we paint animal genomics on a wider (ethical) canvas?

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Better breeding for what? SABRE's current animal genomics programme is targeted at characteristics which aim to deliver various ethical 'goods' – food safety and quality, animal welfare and reducing adverse environmental impact. These are all valuable in themselves. But if these innovations are viewed against a wider canvas of ethical values and global issues, what happens? What does SABRE's programme look like when viewed from different framings and different concepts of a desirable future? How can this type of analysis contribute to a wider concept of ethically sustainable breeding programmes?

W0009 Genomic tools for breeding, fisheries and evolutionary biology of European Sea Bass

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European sea bass (*Dicentrarchus labrax* L.) sustains a regional fishery and is commonly farmed in the Mediterranean basin and SE Atlantic Ocean. It has been the target of genetic improvement over the past ten years leading to a doubling in growth rate. The Sea Bass Genomics Consortium developed a suite of new molecular tools for applications in aquaculture, fisheries, evolution and conservation. Genetic markers include large numbers of allozymes, AFLPs, microsatellites and SNPs. 30,000 EST traces from 14 cDNA tissue libraries have been used to prepare a linkage map of 374 markers and a radiation hybrid map of 1440 markers. A previously constructed BAC library has been assembled to a physical map of BAC end sequences by a comparative approach. All three maps have been linked. The consortium has compared the genomes of European sea bass and several model teleosts by high-throughput BAC end sequencing and comparative mapping. A high percentage (>80%) of evolutionary conserved regions were spotted with the genome of the three-spined stickleback. Conservation of synteny dropped with phylogenetic distance as analysed in spotted green pufferfish, fugu, medaka and zebrafish. Assembly of the European sea bass genome is nearing "a good quality draft" following whole genome shotgun sequencing by a combination of Sanger, 454 and Solexa sequencing technologies. Based on experiments under commercial conditions, QTLs for growth and stress have been detected. Finally, putative genes under selection have been identified in populations across the natural range.

W0010 The interaction between innate and adaptive immunity in a species without lymph nodes

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In mammals, antigen presentation to the adaptive immune system by dendritic cells (DCs) takes place mainly in local draining lymph nodes near the sire of infection. The chicken lacks lymph nodes and, although we suspect it happens in diffuse lymphoid aggregates, again local to the site of infection, the actual site of antigen presentation in the chicken is unknown.

Using DCs grown out from bone marrow cells as a model, we have begun to characterise chicken DCs, both functionally and phenotypically in terms of the cell surface markers, chemokine receptors and co-stimulatory molecules they express, both as unstimulated immature DCs, and as mature DC stimulated under a variety of conditions. We have also begun to isolate *ex vivo* DCs and to track the migration of skin DCs. Finally, we have begun to determine the ability of DCs to drive Th1 and/or Th2-like responses in the chicken, in particular focusing on the interactions of ligand-receptor pairs such as RANK/RANKL and Tim-1/Tim-4.

W0011 Mapping complex traits in dog breeds

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The domestic dog is a remarkable model organism for finding the genes and variants underlying diseases with complex patterns of inheritance. Domesticated from wolves tens of thousands of years ago, purebred dogs passed through a second, tight population bottleneck much more recently, when the modern dog breeds were created. The reduced genetic diversity in dog breeds, as compared to human populations, limits disease heterogeneity and simplifies phenotype definition. As the mutations underlying complex diseases likely predate modern breeds, when several breeds suffer from high prevalence of the same disease, they often share the same causative mutation. However, dog population history can also confound disease mapping studies, as it limits the genetic diversity required to detect disease associations and increases the potential for spurious associations reflecting population structure. Tools designed for human association projects are not always applicable in canine studies. Here, I review the lessons learned as we work to identify genes underlying canine diseases with complex inheritance, such as diabetes and cancer.

POSTERS 1001 – 1038

Structural and Comparative Genomics

P1001 Evolution and adaptive radiation of Humboldt penguin genus (*Spheniscidae*) using *Mhc* class II *DRB*-like gene region

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The Major Histocompatibility Complex (Mhc) genomic region of many vertebrates is known to contain at least one highly polymorphic class II gene that is homologous in sequence to one or other of the human Mhc DRB1 class II genes. The diversity of the avian Mhc class II gene sequences have been extensively studied in chickens, quails, and some songbirds, but have been largely ignored in the oceanic birds, including the flightless penguins. We have previously reported that several penguin species have a high degree of polymorphism on exon 2 of the Mhc class II DRB1-like gene. In this study, we present for the first time the complete nucleotide sequences of exon 2, intron 2, and exon 3 of the DRB1-like gene of 20 Humboldt penguins, a species that is presently vulnerable to the dangers of extinction. The Humboldt DRB1-like nucleotide and amino acid sequences reveal at least eight unique alleles. Phylogenetic analysis of all the available avian DRB-like sequences showed that, of five penguin species and nine other bird species, the sequences of the Humboldt penguins grouped most closely to the Little penguin and the mallard, respectively. The present analysis confirms that the sequence variations of the Mhc class II gene, DRB1, are useful for discriminating among individuals within the same penguin population as well those within different penguin population groups and species.

P1002 Development of molecular marker as DNA barcode sequence from Manipuri pony of India

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Manipuri pony is a unique indigenous horse breed of Manipur, India, which is known for its fastness, intelligence, surefooted moves and high endurance. In the framework of breed conservation, DNA barcoding is an important step allowing preservation of the breed integrity and is a prerequisite for efficient management of genetic resources. Our lab is working on different DNA barcode sequence analyses from a variety of groups of animals and plants. Different barcode amplified primer combinations, which are available in our lab, were studied by *in silico* methods for their feasibility to generate the targeted fragment of the *COI* gene of pony breed of horse. The best combination of forward primer for bird and reverse primer for fish DNA barcoding was able to amplify *in silico* horse mtDNA, and an *in vitro* PCR amplification based on the total DNA extracted from hair samples of Manipuri pony gave an amplification product of 699 bp. After PCR amplification followed by sequencing, a similarity and homology search using GeneBank database showed a 99% similarity with the cytochrome c oxidase gene of already reported mitochondrial genome of Poland horse (*Equus caballus* Accession No.NC_001640) and it lies within the *COI* gene of the mitochondrial genome, which is considered as the universal DNA barcode region for animals. Based on the analysis of the sequence chromatogram, the five mismatches observed in the sequence of the PCR amplified partial *COI* gene of Manipuri pony were unique to this breed of horse.

P1003 Polymorphisms in genes of the somatotrophic axis are associated with milk production, fertility, survival and animal size in Holstein Friesian Dairy cattle

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The somatotropic axis consisting of pituitary derived Growth Hormone (GH) and circulating insulin-like growth factor 1 (IGF-1) have been well established as key regulators of animal health, metabolism, lactation, fertility, body composition and growth rate. The aim of this study was to identify novel polymorphisms in the *GH*, its receptor *GHR*, and *IGF-1* genes and to quantify associations with performance traits in dairy cattle. Regions of these genes encompassing both promoter and regulatory flanking regions were sequenced across a panel of 22 cattle. Multiple regression analyses regressing daughter performance on novel (n=76) and previously published SNPs (n=33) on up to 848 Holstein-Friesian sires was undertaken using mixed models. Seventeen novel and 13 published SNPs were significantly associated with at least one of the traits analysed including milk yield, milk fat and protein composition, udder health, calving interval, survival and 11 body size traits. For example, novel SNPs in the 5' non coding region of *GHR* and in the 3' region of *igf-1* were associated with effects on lactation milk yield of 41.11 Kg ($P<0.001$) and functional survival of 0.7 % ($P<0.05$) respectively. In addition another novel SNP in *GHR* was associated with calving interval and survival ($P<0.01$) while a published SNP in *GH* was associated with cull cow carcass weight ($P<0.05$). For several traits including milk fat yield, somatic cell count, survival and carcass fat, SNPs in all three genes were independently associated with performance, reinforcing the key role of each gene on animal development.

P1004 *Mc1r*, Agouti signaling protein (*ASIP*) and *CBD103* are involved in brindle coat color of Boxer and Great Dane dogs

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In most mammals, variations in skin and hair colour (brown/black eumelanin and the red/yellow phaeomelanin) are mainly achieved by the interaction between two genes: *Agouti signaling protein* (*ASIP*) and *melanocortin 1 receptor* (*Mc1r*) encoding a ligand-receptor system that controls the eumelanin/pheomelanin switching. In domestic dogs, pigment type-switching is also controlled by an unexpected allele (*CBD103*) of *K* locus encoding a secreted protein (β-defensin) previously studied for its role in immunity. Interaction studies reveal that *Mc1r* is epistatic to variation at *Agouti* and *K* loci. In this work, we examined *Mc1r*, *ASIP* and *CBD103* as candidate genes for brindle or fawn Boxer and Great Dane animals. The brindle phenotype in domestic dogs consists of an irregular pattern of red-yellow stripes alternating with black-brown. We found that all brindle dogs are heterozygous for ΔG23 mutation previously reported by Barsh's team. In addition, we report for the first time two interesting insertions in *Agouti* promoter region. The innovation of this work is the discovery of new alternative transcripts of *Agouti* that could be involved in brindle coat colour pattern in dogs. The interaction between *CBD103* mutation and *Agouti* insertions promoting the different transcripts is discussed. This can be a starting point in better understanding their involvement in pigment switching leading to the brindle pattern formation.

P1005 A Whole Genome Radiation Hybrid Panel in Duck

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A collection of duck whole-genome radiation hybrids (WGRH) is being generated by fusing 6,000 rad irradiated female duck fibroblasts to (HPRT)-deficient hamster cells and the hybrids are selected on HAT media. To determine the extent to which donor fragments are retained in the hybrids, 31 microsatellite markers were amplified by PCR from DNA obtained from each of the 125 hybrids produced so far. Among the hybrids produced, 43 were selected for their high retention frequency to be part of the final panel. They show an overall retention rate of 24% of the whole duck genome, the average retention frequencies being 19.8% and 27.7% for the macrochromosomes and the microchromosomes respectively.

A whole genome alignment of the 7205 duck sequence scaffolds larger than 10 kb produced by the duck whole genome sequence project, coordinated by CAU, with the current chicken assembly allowed to position 1787 on the chicken genome and to identify 41 mapping to at least two different chicken chromosomes. This suggests either assembly problems in the duck sequence or the first demonstration of inter-chromosomal rearrangements, as no such rearrangements have been observed to date between the chicken and duck lineages.

Eighteen of these scaffolds were selected for analysis with our 43 radiation hybrids by developing 39 PCR markers. The results showed that 17 out of 18 the scaffolds were misassembled while the last one possibly identified one inter-chromosomal rearrangement between duck and chicken. Finally, our RH analysis allowed us to build a first comprehensive map for APL22, containing 7 markers.

P1006 Expression Quantitative Trait Loci (eQTL) study in *gluteus medius* muscle of Duroc pigs

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In the last 20 years, there has been significant research toward defining the genetic basis of meat quality and lipid metabolism related traits in pigs. Nowadays, the study of the transcriptome and its regulatory mechanisms allows going far beyond in the genetic dissection of these complex traits. We have carried out an eQTL study in a Duroc half-sib population where a number of QTL had been detected. The present study was conducted to detect pig genome regions that regulate levels of skeletal muscle mRNA expression and that are associated with lipid metabolism and meat quality traits. *GeneChip Porcine Genome*® arrays (*Affymetrix*) was used to determine the gene expression levels in the *gluteus medius* of 105 pigs. The whole genome scan with a panel of 110 microsatellites allowed detection of 613 eQTL at genome-wide significance level. Target probes (478) were mapped to the pig reference genome sequence using CLCBio software. Functional classification genes within eQTL regions indicated that these regions contained genes that were related to fat deposition, lipid metabolism and muscle development. A total of 63 probes (out of 478) had a *cis*-acting eQTL, and were located in genome regions where QTL for lipid metabolism and meat quality had been described. Additionally and in order to improve the pig QTL annotation, correspondence between cM and Mb for each chromosome region was extracted using the bovine genome annotation. Finally, a comprehensive list of positional and functional candidate genes was elaborated on the basis of genome-wide significant eQTL related to lipid metabolism and pork quality.

P1007 Analysis of coat colour loci in Vicugna pacos (Alpaca) and association with coat colour variation

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Alpaca fibre is renowned for its strength, softness and lustre and is a highly valued fibre in the textile industry. Colour is an important characteristic of alpaca fibre because it helps to determine the potential applications and value of the end product. However, the inheritance patterns of colour in alpacas remain relatively unknown. This has highlighted the need for research into alpaca fibre colour genetics. Three genes, known as the Melanocortin-1 receptor, Agouti and Tyrosinase Related Protein-1 are known to be the major controllers of pigment colour in mammals. These genes control the relative amounts of dark and light pigment present in the fibre. Our research has identified several mutations that occur within these genes. These mutations, when fully examined alongside phenotype and pedigree data, have the potential to affect pigment production in alpacas. Markers for colour variants would be advantageous to the alpaca industry by allowing targeted breeding for specific colours. The industry could then tailor fibre production to the current demands of the market more effectively.

P1008 ArkMAP: the ArkDB map drawing application – integrating genomic maps across species and datasources

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The ability to align genetic and genomic maps within and between species and across datasources is critical for comparative genomic analyses. Whilst annotation, integration and exploration of genomic sequences is well supported by bioinformatics tools and resources, fewer tools are available for displaying maps for less well characterized species, or for integrating genetic maps (linkage, QTL, radiation hybrid, cytogenetic maps etc.) with the annotated reference genomes.

ArkDB curates genetic mapping data for farmed and other animals. These data are available through both the ArkDB web application (where data can be browsed or displayed graphically as aligned maps) and the ArkDB web services (for bulk data export in a simple XML format defined by our generic GMD schema).

To facilitate data integration we have developed ArkMAP, a desktop application that retrieves, draws and aligns maps from ArkDB and Ensembl web services, allowing relationships between markers on the maps to be explored within and between species. ArkMAP is freely available as a Java Webstart download from www.thearkdb.org and runs on any platform. The modular design of ArkMAP allows it to exchange information directly with any datasource which exports data in our GMD exchange format; alternatively, plug-in modules can be written to convert from other dataformats (demonstrated by our Ensembl module). An example use of ArkMAP would be to align Turkey QTL maps with regions of conserved synteny on the Ensembl annotated Chicken assembly, by traversing relationships through Chicken linkage maps sharing Turkey markers, and ePCR maps of markers on the genome assembly.

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P1009 Next generation sequencing characterizes the indel distal to the growth hormone 1 gene in cattle

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The indel distal to the *growth hormone 1* (GH1) gene was one of the first DNA variants discovered in cattle. Two locations for the indel were reported, approximately 1 kb distal to the coding sequence. Although the indel was shown to be associated with milk production traits, its assay has fallen into disuse. This assay required analysis of a restriction fragment length polymorphism (RFLP) by Southern blotting and autoradiography. Other polymorphisms in the GH1 gene, such as the Leu>Val substitution in exon 5 and the *Msp*I RFLP in intron 3, replaced this assay partly because they were direct PCR tests. As part of our next generation sequencing of the bovine genome, we generated at least 16 Gb of genomic sequence each for a Brahman, an Africander (grade) and a Tuli using the Illumina GAII. These sequences were visualized against the UMD3.0 assembly of the Hereford reference sequence using the SAMtools suite of utilities and Gbrowse. We examined the distal region of GH1 to determine the location of the indel so as to develop automatic methods of finding indels. Relative to the Hereford sequence, the Brahman sequence was homozygous for a 0.9 kb deletion and the other animals were heterozygotes. Using the primers GH1DELAU2 5'- CCATTAGGGAAGCAGAGTGTG-3' and GH1DELAD1 5'-AAGTGCAGAGGGAGAGAAAAT G-3' we confirmed these genotypes and resequenced the region. These primers generate clear bands of 0.9 and 1.8 kb in a PCR at an annealing temperature of 62°C and these bands can be separated on a 1.5% agarose gel yielding easily scored genotypes for the GH1 indel.

P1010 Residual feed intake candidate genes

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Residual feed intake (RFI) is a measure of net feed efficiency, an economically important trait in livestock. RFI is affected by many factors including both diet and genetics. RFI of an animal depends on the ability of the animal to consume less feed than expected based on their weight gain and weight maintained during the feed testing period. Recent work has implicated mitochondrial function as being involved in the feed efficiency of livestock. The objective of this study is to identify genes involved in mitochondrial function that may affect feed efficiency in cattle. Several quantitative trait loci (QTL) affecting feed efficiency were mapped in Jersey x Limousin double backcross progeny in 3 sire families. Based on the QTL mapping results, ten candidate genes related to mitochondrial function were identified. These genes have been sequenced in the 3 sires in order to find DNA variants for association studies. Twenty two DNA variants were found, and among the DNA variants discovered, there were 3 potentially functional single nucleotide polymorphisms (SNPs) in *HADHB, ALDOB* and *NDUFA8*. DNA variants have been genotyped in the Jersey x Limousin progeny in order to determine if the variants are associated with net feed efficiency. At least two of the variants in genes likely to impact mitochondrial function, *AMPK* and *ATP2B*, were significantly associated with RFI.

P1011 Transcriptional profile of bovine Y chromosome

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The bovine genome has been sequenced. As the sequenced DNA was isolated from a cow, the bovine Y chromosome (BTAY) sequence, gene content, transcriptional profile and gene function are still not available. To study the transcriptional profile of BTAY, we have conducted a direct cDNA selection by hybridizing the bovine testis cDNA with the entire BTAY DNA as a probe. The selected cDNA was sequenced using Illumina GA2, resulting in ~13 million (36 bp) reads. Approximately 5 million reads matched to the existing BTAY BACs. Assembly of these Y-related reads yielded ~4500 contigs (≥100 bp). After annotation, we identified 16 known genes that have not been analyzed in cattle and over 100 bovid lineage-specific novel Y genes/transcripts. Thirteen of the BTAY genes (PRKY, EIF1AY, EIF2S3Y, ZFY, USP9Y, DDX3Y, UTY, UBE1Y, RBMY, OFD1Y, TSPY, HSFY, UBE2D3Y) have orthologs in other mammalian Y chromosomes. The remaining three genes (ZNF280AY, ZNF280BY, PRAMEY) are bovid-specific, derived from an autosome-to-Y gene transposition and acquired functional advantages for male spermatogenesis during evolution. TSPY, HSFY, UBE2D3Y, ZNF280AY, ZNF280BY, and PRAMEY are all multi-copy gene families with active and/or pseudogenized copies on BTAY, which fits well with the "birth and death" evolutionary model. ZNF280BY and HSFY are the most abundant gene families with >100 loci, spreading over ~2/3 of BTAY as the main elements of the ampliconic region. We found that the majority of the novel Y genes/transcripts are non-coding RNAs. Like those known BTAY genes, the novel genes/transcripts are expressed predominantly or exclusively in testis.

P1012 Identification of species in Buffalo's meat products analysis by microsatellite

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In recent times, there is growing attention of consumers to foods that are well characterized with regard to their nutritional, organoleptic, healthy, as well as transparency of the production chain. The objective of this study was to develop a genetic test traceability muscle buffalo based on microsatellites. This method of genotyping may help to ensure the traceability of meat along the transformation process. The buffalo species is of considerable importance in the food sector in Italy and given the vast landscape of genetic testing that is performed on it, it becomes necessary to develop a highly automated method to perform simultaneously: investigation of kinship, and the analysis of polymorphisms of genes candidates involved in the quality of food products, identification of genetic mutations responsible for diseases and genetic identification using microsatellites. The scope of work was to determine whether the microsatellite analysis allows us to detect minimal concentrations of buffalo meat in meat products. The scope of work was to determine whether microsatellite analysis allows us to detect the minimum concentrations of buffalo meat products in meat. The samples, totaling 30, were prepared by mixing the muscles of bovine and buffalo in concentrations of 0.1%, 1%, 5%, 30% and 50%. The analysis by PCR, has allowed to determine the relative quantity of buffalo meat and the mixture of beef in detection limit of 1%. In contrast, the microsatellite analysis has enabled, however, to determine the presence of buffalo and bovine species to small concentrations.

P1013 You Can Teach an Old Robot New Tricks

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ARK Genomics is a high-throughput technology laboratory focused on studies of genome structure and genetic variation, gene expression and gene function. The facility aims to provide the techniques and high throughput tools to facilitate studies in these areas. The implementation of the Illumina Infinium protocols within the laboratory allowed us the opportunity to develop new liquid handling techniques on our oldest equipment, the Perkin Elmer Multiprobe II.

The Infinium II Whole-Genome Genotyping assay is designed to interrogate a large number of SNPs at high levels of loci multiplexing. The assay uses a single bead type, dual colour channel approach and scales genotyping from ten thousand to hundreds of thousands of SNPs. The protocol involves hybridisation of amplified/fragmented DNA to an Illumina Infinium BeadChip and subsequent enzymatic base extension and staining.

The enzymatic base extension and staining steps are very labour intensive and take approximately three hours to complete. Here we describe the development of an automated protocol on the Perkin Elmer Multiprobe II and the results generated.

P1014 Identification and Characterization of the Pig ABIN-1 Gene and Investigation of its Association with Reproduction Traits

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A20-binding inhibitor of NF-κB activation (ABIN-1) is a novel protein believed to mediate inhibitory effects of A20 on nuclear factor-kappa B (NF-κB) and have important regulatory roles in many cellular activities and embryo development. In this study, two transcripts of pig ABIN-1 cDNA, ABIN-1L and ABIN-1S, were identified by RT-PCR. The former longer transcript, ABIN-1L, was 2248 bp in length and contained an additional exon of 81 bp which encodes an extra 27-amino acid stretch in comparison to the shorter transcript ABIN-1S. The ABIN-1 gene, in a 'GPX3/ABIN-1/ANXA6' linkage group of chromosome 16, is over 60 kb in length and contains more than 16 exons. Quantitative real-time analysis indicated that ABIN-1L was abundantly expressed in pig kidney and ovary, while ABIN-1S was mainly expressed in the spleen. Differential expression of ABIN-1L and ABIN-1S was also observed in abdominal fat, kidney and stomach. Moreover, a total of 10 SNPs were identified in ABIN-1 genes and five of them were missense mutations. Association analyses showed that the SNP A+2078C was related to average birth weight of piglets ($P=0.0429$) and surviving litter size ($0.05<P<0.10$). Female pigs with genotype CC gave birth to piglets with the highest birth weight, while pigs with genotype AC seemed to produce more piglets. In conclusion, in this study, we cloned and characterized the pig ABIN-1 gene and investigated the association of an SNP (A+2078C) with pig reproduction traits.

P1015 γδ T cell receptor in *Camelus dromedarius*: gene organization and repertoire

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γδ T cells represent a link between adaptive and innate immune system. Their similarities and differences relative to the more populous αβ T cells have been well studied in human and mouse. However comparative studies in other living species provide insight on the molecular evolution of the cellular immune system. As a matter of fact the frequency and physiological distribution of γδ T cells vary greatly between species, some (i.e. artyodactils and chicken) showing higher percentage of circulating γδ T cells with respect to human and mouse (γδ low species). Arrangement and expression of TR γδ genes in γδ high species such as ruminants and pig have been so far characterized, showing the potential of larger germline repertoire. Here, we report for dromedary, an artiodactyl species, an extensive analysis of the TR γ/δ chains repertoire. A representative database of both germline and cDNA sequences reveals that at least three TRDV gene subgroups and five TRDJ genes are implicated in the generation of the δ chain variable region; whereas the γ chain repertoire is generated by two single member TRGV gene subgroups, four TRGJ and two TRGC genes arranged in two V-J-C recombinational unit. Moreover, our data suggest that the potential diversity of the dromedary TR γ/δ chains repertoire is generated not only by the conventional V(D)J recombinational mechanism but also by mutation in the V regions. Such finding would have functional implications for γδ T cells in Artyodactyls and phylogenetic significance for understanding the evolution of mechanism generating diversity in the vertebrate immune system.

P1016 Expression and genomic organization of dog TRB locus

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The recent high-quality draft genome sequence of the domestic dog (*Canis lupus familiaris*) allowed the inference of the TRB locus genomic structure. In most mammals, TRB locus features a library of TRBV genes positioned at the 5' end of two in tandem aligned D-J-C gene clusters, each composed of 1 TRBD, 6-7 TRBJ and one TRBC genes, followed by a single TRBV gene located at the 3' end with an inverted transcriptional orientation. Such genomic organization is retained in human, mouse, rat, chimpanzee, rhesus monkey, and horse, the only exception is in artiodactyls (ruminant species and pig) where three D-J-C clusters have been reported.

The IMGT annotated canine TRB sequences reveal the presence of 34 V, 1 D, 6 J and 1 C genes, followed by one V gene in opposite orientation, suggesting only one D-J-C cluster in dog genome. 5'RACE using constant gene first exon primers on total RNA from peripheral blood allowed us to identify the T cell receptor beta chain expressed repertoire composed by 14 TRBV and 6 TRBJ. In addition, five TRBJ genes, not found in the dog annotated sequences, showed high level of identity to the human TRBJ2 genes. With the intent to better define the structure of TRB locus in an organism belonging to Carnivora order, long PCR experiments and sequence analyses on canine genomic DNA were carried out. Preliminary data indicate that the five TRBJ genes are a portion of the second D-J-C cluster that contains 1 D and 6 J genes.

P1017 Polymorphisms in regulatory regions of lipid metabolism related genes in ruminants

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The objective of this study was to identify mutations in regulatory regions of five genes that code for proteins involved in fat synthesis and lipid metabolism in *Bos taurus* (Asturiana de los Valles, Parda de Montaña, Pirenaica and Holstein-Friesian), *Ovis aries* (Rasa Aragonesa, Mallorquina and Assaf) and *Bos indicus* (Cuban Zebu) species. The analysed genes are *fatty acid synthase* (*FASN*), *glycerol-3-phosphate acyltransferase mitochondrial* (*GPAM*), *melanocortin-4 receptor* (*MC4R*), *stearoyl-CoA desaturase* (*SCD*) and *perilipin* (*PLIN*). Several polymorphisms in regulatory regions of these genes have previously been described and associated to fat related traits in bovine and ovine breeds. Our results showed that the most polymorphic gene in *Bos taurus* was *GPAM*, since 6 mutations were identified; the mutations g.543T>C and g.802-803AG>C displayed the most high frequencies within the four analysed breeds. In Cuban Zebu the promoter of *MC4R* showed the major variability across species, displaying a total of 16 polymorphic changes. Four of them were previously described in Chinese cattle. *SCD* and *PLIN* of Zebu sequence also presented a high variation. The new variants in both genes showed elevated frequencies. The G deletion at g.1011 in the *SCD* promoter exhibited remarkable frequencies in Zebu and in Holstein-Friesian breed. In *Ovis aries* the promoter of *GPAM* presented the major variability, showing 5 unreported SNPs. The TCT deletion in *MC4R* promoter appears to be the most frequent variant among the found novel alleles. The g.1627C>A mutation in *SCD* promoter was previously identified in other sheep breeds, and due to its localization in the bovine sequence it may be a possible marker for fat content and fatty acid composition.

P1018 Transcriptomic profiling of bovine oocytes and early stage embryos using a high density Combimatrix Customarray® 90K

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Early embryonic loss is a large contributor to infertility in cattle. The initial stages of embryo development depend on mRNA and proteins accumulated in the oocyte which are essential for fertilization, first cleavage and embryonic genome activation until the onset of transcription in the embryo. Global RNA gene expression analysis of bovine oocytes and early stage embryos (8, 16 cells up to blastocyst), was carried out using a custom high density microarray, to investigate stage-specific gene expression and the effect of genotype on the embryo viability.

The array was created using information on bovine transcript sequences obtained from Ensembl release 50, and the bovine UniGene and dbEST databases. A pipeline was written in Perl to align all these sequences and select a unique set of minimally redundant bovine transcripts. A set of 43,768 unique probes, each representing a single bovine transcript, was designed using this dataset. Analysis of the transcriptome of embryos at different developmental stages, and of different genetic type, was performed by hybridising amplified embryonic RNA to this array. The aRNA was produced by two successive rounds of *in vitro* T7 RNA transcription using ampULSe amplification and labelling kit (Kreatech biotechnology) to produce typically ~100 ug RNA.

Preliminary results have identified genes whose expression is activated between the MII oocytes and early stage embryos. Data on gene expression patterns in early development will be presented.

P1019 Molecular characterization and expression analysis of *calreticulin* and *ERp57* induced by hyperthermal stress of the giant tiger shrimp (*Penaeus monodon*)

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Under stress conditions, homeostasis and protein folding in the endoplasmic reticulum (ER) are disrupted, causing protein unfolding and misfolding. These should promote expression of calreticulin (CRT) and ERp57, which are chaperones coordinately function in the ER. Here, the full-length cDNAs and genomic structures of *CRT* and *ERp57* in *P. monodon* (*PmCRT* and *PmERp57*) were identified. The full-length cDNA of *PmCRT*, characterized by RACE-PCR, was 1682 bp long containing an open reading frame (ORF) of 1221 bp, corresponding to a deduced protein of 406 amino acids. Genome walking analysis showed that the *PmCRT* gene spanned 3006 bp containing 4 exons and 3 introns. *PmERp57* possessed two transcript isoforms of 2055 and 2224 bp with an identical ORF of 1458 bp (a 485-amino acid residue protein) but a length polymorphism of the 3´ UTR. The *PmERp57* gene spanned 8918 bp, composed of 10 exons and 9 introns. Quantitative real-time PCR revealed that expression of these genes in hemocytes of the juvenile shrimp was upregulated by hyperthermal stress (35°C, 3 h). The *PmCRT* and *PmERp57* transcript levels were increased approximately 25 and 39 fold, respectively, at 0 hour post treatment (hpt), and returned to the baseline level at 24 hpt ($N = 5$; $P < 0.05$). Additionally, recombinant PmCRT and PmERp57 were expressed *in vitro* and exhibited an ability to form a complex as analyzed by a gel-shift assay. Results from this study suggested the potential of both genes to be biomarkers for temperature stress responses in *P. monodon*.

P1020 Differential expression of genes during differentiation of myogenic satellite cells into adipocyte-like cells

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Myogenic satellite cells (MSCs) are the adult skeletal muscle stem cells located at the endomysium region of skeletal muscle. Like the other stem cells, MSCs differentiate into myofibers during muscle injuries. Additionally, MSCs have also been reported to differentiate into adipocyte-like cells (ALC), osteocytes and nerve fibers. To understand the molecular mechanism of the differentiation of MSCs into ALCs, bovine MSCs were cultured in the differentiation specific environment and gene microarray analysis was performed between MSCs and ALC. The differentiation specific environment was provided by switching the media into adipogenic media once the cultured MSCs reach 70% confluence. Altogether, 363 genes were up-regulated and 217 genes were down-regulated during differentiation of MSCs into ALCs. The genes which were identified to be differentially expressed during differentiation were confirmed by real time RT-PCR. Real time RT-PCR revealed that 80% of the genes above and below four fold difference agree with the expression pattern of gene microarray data. The expression of the genes identified to be up-regulated above 5-fold were analyzed at different time point (3 days, 7 days, 14 days and 21 days in adipogenic media). Interestingly, two previously unknown genes showed time point dependent up-regulation. Similarly, fatty acid transporter CD36 and MYL2 were continuously up-regulated as the time lapses. Taken together, the study added novel information in the way of understanding the molecular mechanism of differentiation of MSCs into ALCs. Moreover, further study may identify the marker genes related to differentiation of MSCs into ALCs.

P1021 Analysis of genes expression during differentiation of myogenic satellite cell

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Myogenic satellite cells (MSCs) are mononuclear progenitors of adult skeletal muscle possessing multipotential capacity of forming adipocyte-like cells (ALC) in addition to myocytes. To identify the skeletal muscle type-specific myogenic and adipogenic genes during bovine MSCs differentiation, total RNA was extracted from bovine MSCs, myotube-formed cell (MFC), and ALC from each of *Beef shank, Longissimus Dorsi, Deep pectoral*, and *Semitendinosus*. DNA microarray analysis (29,903 oligo chip) was performed considering MSCs as control compared with MFC and ALC, respectively. In total 138 genes were differentially expressed in each of four regions (> 4 fold) and expression of 30 genes was confirmed by real-time PCR. Furthermore, expression of 30 genes were checked with whole tissues RNA from four skeletal muscle by real-time PCR and 6 genes were found to be differentially expressed in *Beef shank*. Among which, 1 gene in MSCs, 4 in MFC, and 1 in ALCs were highly expressed. This study will provide an insight for better understanding the molecular mechanism of differentiation of skeletal muscle type-specific MSCs. The identified genes may be used as a marker to distinguish different skeletal muscle types.

P1022 Molecular cloning and characterization of the porcine apolipoprotein C-II gene

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Apolipoprotein (apo) C-II gene has been suggested to associate predominantly with the circulating triglyceriderich lipoproteins, chylomicrons, and very low density lipoproteins. In *vitro* and in *vivo*, apo C-II could activate lipoprotein lipase to regulate the lipoprotein-lipid metabolism. The cDNA of porcine apo C-II gene was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) based on the information of the human and cow. It had been determined that the open reading frame (ORF) of the porcine apo C-II gene consists of 306 bp, which encodes a predicted protein composed of 101 amino acids. The gene region included four exons and three introns. The phylogenetic tree analysis revealed that the porcine apo C-II has a closer genetic relationship with the apo C-II of bovine. The RT-PCR expression indicated that the porcine apo C-II gene was mainly expressed in hypophysis, hypothalamus and liver. The different expression levels of apo C-II mRNA on days 90 and 120 between Jinhua and Large white pig breeds suggest that apo C-II plays an important role in regulation of lipid metabolism. Our study established the primary foundation for further research on porcine apo C-II gene.

P1023 Variation in the coneodesmosin gene within the sheep Major Histocompatibility Complex (MHC) Class I region

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Corneodesmosin (CDSN) is a protein component of the transmembrane corneodesmosomal complex found in cornified epithelia and the hair follicle inner root sheath. In humans, corneodesmosin is associated with psoriasis, hyperproliferative skin disease and the autosomal dominant disorder hypothricosis simplex of the scalp. Deletion of *CDSN* in mice causes desmosomal disintegration, resulting in a chronic defect in the epidermal permeability barrier. This study aimed to characterise the sheep corneodesmosin gene and identify genetic markers within. A Bacterial Artificial Chromosome (BAC) identified in previous hybridisation studies to contain the sheep *CDSN* was amplified using *CSDN* primers based upon cattle sequences and the resulting products sequenced. Sheep-specific primers were then designed to amplify and sequence the 4579 bp of sheep genomic DNA. Sheep *CDSN* is 3638 bp in length and encodes a predicted protein of 546 amino acids. Two exons, 85 bp and 1553 bp respectively are separated by a 2045 bp intron. Comparison of genomic sequence from 12 unrelated merino sheep and data from the International Sheep Genome Consortium (ISGC) identified 58 Single Nucleotide Polymorphisms (SNPs) within the 4579 bp fragment. This gave a frequency of 1 SNP in every 80 bp. Sixteen SNPs in the coding sequence resulted in 8 synonymous and 8 non-synonymous changes. Phylogenetic comparison of the predicted amino acid sequence with other species showed significant identity and confidence levels between sheep and cattle. This work will contribute to the further understanding of this gene and the role of the MHC in skin related infectious diseases.

P1024 Speaking the Same Language: A Common Nomenclature For Vertebrate Genes

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It is widely acknowledged that the analysis of novel genomes, and scientific communication in general, is greatly aided by applying the same gene name to orthologous genes in different species. At the 22nd International Society for Animal Genetics conference in 1990 it was agreed that animal gene names should 'follow the rules for human gene nomenclature, including the use of identical symbols for homologous genes and the reservation of human symbols for yet unidentified animal genes'. In the intervening 20 years little formalised progress has been made in this direction, but only in recent years have large amounts of data from vertebrate genomes appeared in the genome databases. These data now necessitate the implementation of standardised naming across vertebrates in an organised manner. In October 2009 the HUGO Gene Nomenclature Committee (HGNC), the group responsible for naming human genes, organised a meeting to bring together invited experts from the fields of gene nomenclature, phylogenetics and genome assembly and annotation to discuss the issues of coordinating gene naming across vertebrates. Here we present the key conclusions from this meeting, and summarise how a common gene nomenclature does currently, and can in future, apply to all vertebrates.

P1025 Next generation sequencing at ARK-Genomics

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The high throughput, next generation sequencing offered by ARK-Genomics, using the Illumina Genome Analyzer II system, offers the simplest and fastest workflow for a broad range of high-throughput sequencing applications. Sample libraries are prepared and clonal clusters are generated on Illumina sequencing flow cells using the cluster generation system. By using Illumina's sequencing by synthesis technology, hundreds of millions of clusters are sequenced in parallel, generating more than 50Gbps information in a long, paired end sequencing run.

We currently have proven extensive experience performing whole-genome or *de novo* sequencing, resequencing comparing to a reference genome and are developing targeted resequencing, SNP discovery, and identification of copy number variations and chromosomal rearrangements. ARK-Genomics have the ability to multiplex up to 12 libraries in one flow cell lane, reducing the cost of this service for small genomic libraries. The Genome analyser II system gives flexibility of insert size and different length (36bp-101bp reads), single or paired-end protocols, enabling the broadest range of genomic sequencing applications.

The techniques that we offer are genomic DNA sequencing, Mate pair sequencing, mRNA Sequencing (Transcriptome Analysis), microRNA sequencing and we are developing DGE and targeted resequencing techniques.

P1026 Differential expression of imprinted genes in in-vitro produced cattle embryos

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Genomic imprinting is a phenomena causing monoallelic parent-of-origin expression for a subset of genes shown to have critical roles in early embryonic, placental, and neonatal development. Disruption or knockouts of some murine imprinted genes have been reported to result in low postnatal survival rates and embryonic lethality. However, little is known on the role of cattle imprinted genes in the early embryo and how they could impact embryonic loss. This study aims to profile the expression of imprinted genes in blastocyst and degenerate (abnormal or growth retarded) embryos providing possible candidates involved in the cellular pathways for early embryonic development. Using *in-vitro* fertilization, three independent pools of degenerate embryos and three pools of blastocysts (n=20 embryos per pool) were constructed using two different sires. The expression profiles of the four imprinted genes *NDN, MAGEL2, UBE3A*, and *MKRN3* were evaluated in the blastocyst and degenerate populations using quantitative real-time PCR. Our results revealed upregulation of *NDN, UBE3A*, and *MKRN3* in the degenerate embryos. The *MAGEL2* gene showed upregulation in blastocysts. Functionally, these genes seem to have roles critical for success of a dividing cell including cell-cycle regulation, RNA transcription, and polyubiquitination. As such, these genes may serve as possible candidates for successful early embryonic development.

P1027 Whole genome sequencing of Hanwoo (Korean Cattle) using next generation DNA sequencing methods

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The bovine genome sequence will allow us to better understand of biology and evolution of ruminants. Up to now, two different whole-genome assembly versions, Btau4.2 and umd3, based on the sequences of whole genome shotgun data of Dominette and BAC shotgun data. The genome of a Hanwoo proven bull, Korean cattle, was sequenced and assembled with 33.85-fold redundancy using the SOLiD 3 paired-end sequencing method. The genome sequences of Hanwoo covered 99.9% of the umd3 reference genome assembly. We identified 6,026,242 SNPs (2,420,020 homozygous SNPs and 3,606,222 heterozygous SNPs) and 237,026 small indels, which was 2.3% of the reference genome of 2.6 Gb. Also, we found approximately 1,880 inversions of over 10 kb between Btau4.2 and umd3. Among them, 5 large inversion regions of over 75 kb were compared to the BAC end sequences of Hanwoo. The orientation of Hanwoo genome was agreed to 3 of umd4.2 and 2 of Btau3.

P1028 Partial structural analysis and mapping of porcine *MEF2A, MEF2B, MEF2C* and *BMP2* genes

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The myocyte enhancer factors 2A, 2B and 2C (MEF2A, MEF2B, MEF2C) are members of MADS/MEF2 family of DNA binding proteins and the bone morphogenetic factor 2 (BMP2) belongs to transforming growth factor-β (TGF-β) superfamily. They are involved in regulation of myogenesis. Specific PCR-primers were designed for amplification and comparative sequencing (Meishan and Pietrain breeds) of the genes in pigs. Many polymorphisms were identified and some of them were used for linkage mapping in the Hohenheim Meishan x Pietrain F_2 family: *MEF2A* gene was mapped to SSC1 (*SW2130*—26.8—*MEF2A*—0.0—*EEF1A1*-0.5-*IGFR*), *MEF2B* and *MEF2C* genes to SSC2 (*MYOD*—7.4—*MEF2B*—2.3—*RETN*—2.3—*SW395*—6.4—*MEF2C*—6.2—*S0010*) and *BMP2* gene to SSC17 (*S0296*—3.4—*BMP2*—26.2—*SW1920*). The whole structure of the *MEF2B* gene was completed using primer-walking approach applied on a porcine genome BAC clone and the gene was mapped using radiation hybrid panel (IMpRH) on chromosome 2 with *S0091* as the closest marker (25 cR; LOD=12.26). Significant associations with carcass and meat-quality traits were found in the Hohenheim Meishan x Pietrain F_2 family for *MEF2A* gene (average area, average diameter and number of white, intermediate and red muscle fibres), *MEF2B* gene (half carcass weight, back fat depth, lean cuts percentage), *MEF2C* (soluble protein content from subcutaneous inner backfat layer, lean cuts percentage, back fat weight) and *BMP2* gene (birth weight). The analysed genes are considered relevant candidate genes for meat production in pigs. (Supported by the Grant Agency of the ASCR, project no. KJB500450801).

P1029 Havana group and pig communities combining efforts to improve the annotation of the porcine genome

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It has been one year since the first release of both the high-coverage assembly of the pig genome and the Ensembl gene build 57.9a, which predicted 17,493 protein-coding genes. The Havana group at the WTSI provides high quality manual annotation of finished vertebrate genomes using protein and transcriptional homology, and is helping to improve the Ensembl pig gene annotation in two different ways. Firstly, we are enabling the pig research communities to annotate their genes of interest (e.g. immune response genes) using the publicly available otterlace annotation tool developed in-house. Through a series of workshops, researchers are trained in using the otterlace tools and advised in annotation guidelines used by Havana to produce standardised consistent annotation. This community annotation can be viewed in Ensembl via a DAS source and eventually will be merged into the ensembl gene build. In addition, Havana is starting whole finished clone annotation of the porcine X and Y chromosomes, with 66 loci manually curated so far. This will provide further insight into the organisation and evolution of mammalian sex chromosomes previously drawn from studies in human and mouse. These finished quality sequences will be available alongside the annotation in Vega and then will be incorporated into the subsequent pig assembly and Ensembl gene build. Besides adding value by growing the list of annotated pig genes, our analysis has also identified assembly errors, thus helping improve both the current assembly and the upcoming Sscrofa10, due to be released in early summer this year.

P1030 Comparative analysis of porcine *EEF1A1* and *EEF1A2* genes: structure, polymorphism, mapping and expression

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Eukaryotic translation elongation factor 1 alpha (EEF1A) plays a key role in protein synthesis, as it is responsible for transport of aminoacylated tRNAs to the A site of the ribosome. In higher vertebrates EEF1A occurs in two forms, EEF1A1 and EEF1A2, encoded by distinct genes. We determined complete genomic sequences of porcine *EEF1A1* and *EEF1A2*, including 5´ and 3´ downstream sequences. Both genes are composed of 8 exons. *EEF1A1* transcription unit encompases 3,102 bp, while *EEF1A2* 8,588 bp. The deduced EEF1A1 and EEF1A2 proteins contain 462 and 463 amino acids, respectively, and share 92.4% identity. Polymorphisms in the two genes were detected by comparative sequencing. One polymorphism in each gene (*EEF1A1* – FM995601:g.2111A>G; *EEF1A2* – FM992107:g.6609C>G) was used for linkage assignment in the Hohenheim F_2 families and for estimation of allele frequencies in different breeds. *EEF1A1* was mapped to SSC1 (*SW2130–EEF1A1–IGF1R*) and *EEF1A2* to SSC17 (*GNAS–EEF1A2–SW2427*). The assignments were confirmed by SCH and RH mapping, respectively. mRNA expression of both genes was studied. *EEF1A1* was expressed in all studied tissues, but the level of expression varied. Very high expression was in foetal muscle (44 days of gestation), while low expression occurred in adult liver and brain. No expression of *EEF1A2* was observed in foetal muscle, but the gene was expressed in adult skeletal muscles, heart, diaphragma and brain. No expression was observed in other tissues. *EEF1A1* is located in a QTL interval for muscle fibre traits. (Supported by the Czech Science Foundation 523/06/1302 and 523/09/0844).

P1031 Bovine sex identification and parentage verification with 21plex and 20plex PCR

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For parentage testing and individual identification *multiplex* PCR amplifying DNA microsatellites are routinely used. In this study we propose a main set of 20 microsatellite markers (BM1818, BM1824, BM2113, CSRM60, ETH10, ETH152, ETH225, ETH3, ILSTS006, INRA005, INRA023, INRA063, MGTG7, SPS115, TGLA122, TGLA126, TGLA227, TGLA48, TGLA53 and TGLA57) and a sex one (AME) and an additional set of 20 markers, (AGLA293, BRR, CSSM022, CSSM066, CYP21, ETH185, HAUT24, HEL1, HEL5, HEL9, HEL13, ILSTS005, ILSTS011, INRA032, INRA035, MGTG4B, MM12, RM067, SPS113 and TGLA263) from the 2009-2010 International Society for Animal Genetics (ISAG) Comparison Test. The markers for each panel are co-amplified simultaneously. Changes in primers were required to achieve a final configuration allowing all markers to be analyzed together. Robotic procedures were implemented to minimize the risk of mistakes.

DNA from whole blood was extracted using *BioSprint 96 Blood Kit* (*Qiagen*) and markers were simultaneously amplified using the *QIAGEN Multiplex PCR kit*. PCR products were analyzed in a single run on an *ABI PRISM® 3130xl Genetic sequencer*.

The mean allele number for the microsatellites included in the proposal panels is 5.80 in the main one and 5.05 in the additional panel. The cumulative exclusion probability reached 99.9996640% using the main set and 99.9820777% using the additional one. The main panel is used for routine bovine parentage verification, and the additional one, as a complementary set of markers for more complex paternity analysis involving related sires or when exclusion for one only marker is observed in the main panel.

P1032 DNA sequence variation at the imprinted bovine *IGF2* and *IGF2R* loci and associations with performance traits in dairy cattle

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Genomic imprinting, a feature of at least 100 mammalian genes, results in monoallelic expression from one of the two parental chromosomes. To-date, most studies have been directed on imprinted genes in murine or human models; however, there is burgeoning interest in the effects of imprinted genes in domestic livestock species. In particular, attention has focused on imprinted genes that influence foetal growth and development, and which are associated with several economically important production traits. For example, previous investigations have demonstrated that the imprinted insulin-like growth factor 2 gene (*IGF2*) is responsible for a major quantitative trait locus (QTL) that affects muscle mass and fat deposition in pigs. We have systematically screened DNA sequence variation in the imprinted bovine *IGF2* and IGF2 receptor (*IGF2R*) genes to identify single nucleotide polymorphisms (SNPs) that may influence important production traits in dairy cattle. Following this sequence screen, we genotyped seven validated SNPs across both genes in 848 dairy animals for which detailed phenotypic data were available. Genotype-phenotype association analyses revealed significant associations between *IGF2* and *IGF2R* SNPs and several important performance traits. Our results support the hypothesis that imprinted genes contribute significantly to economically important complex genetic traits in cattle. Furthermore, these *IGF2/IGF2R* SNPs may be usefully incorporated into marker-assisted and genomic selection breeding schemes.

P1033 Detection of porcine miRNA in various tissues by next generation sequencing

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Micro RNAs (miRNAs) are small regulatory RNAs which play an important role in gene regulation at the post transcriptional level. Currently, there is only a limited number of miRNAs annotated in the pig genome (172; miRBase 15). Therefore, we have mapped all available pig mature miRNAs as well as all human mature miRNAs sequences to the Sus scrofa genome (SSC9 assembly) and could detect additional 252 potential miRNAs. We constructed sequencing libraries from size selected total RNA derived from small intestine mucosa, muscle, brain, liver and kidney tissue according to AB's protocol for the SOLiD-3 sequencer. In total we had between 16 and 43 million raw reads of which 27%-56% were mappable. Of these, 1.3-25% map to the known and potential miRNAs.

Ssc-mir-31_nr1 was highest expressed in small intestine (30% of potential miRNAs), kidney (21%), brain (15%). In liver the most abundant miRNA was ssc-mir-122_nr1 (64%), and in muscle ssc-mir-331_nr1 (17%).

The reads that mapped to the genome but not to known miRNAs or other non-coding RNAs were used for *de novo* miRNA prediction using the miRDeep pipeline. As miRDeep does not take color space sequences as input, we used the mapping information obtained with AB's small RNA pipeline and translated the color space into DNA space sequence according to the mapping coordinates on the genome. Currently, we are in the process of *de novo* prediction. Due to the small number of known miRNAs and the unprecedented sequencing depth, we expect to find several new miRNAs in the pig genome.

P1034 High frequency of telomeric repeats and mitochondrial DNA insertions in the horse genome

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The insertion of telomeric repeats at intra-chromosomal sites gives rise to the so called interstitial telomeric sequences (ITS), while mitochondrial DNA fragments captured at genomic DNA sites are known as *numts*. In this work, we analyzed 12 ITS and 12 *numt* loci in 20 horses and identified an unexpected high frequency of insertion polymorphism. In fact, at 4 of the ITS and 5 of the *numt* loci we detected null "empty" alleles due to the absence of the insertion. By comparing the flanking sequence of ITS- and *numt*-containing alleles with the sequence of the corresponding empty alleles we could define the mechanism of insertion at several informative loci; similarly to our previous conclusions obtained from the analysis of Primate and Rodent ITSs, these horse insertions were generated during the repair of DNA double-strand breaks via non-homologous end-joining.

The results presented here are in agreement with the notion that the horse genome is in a rapidly evolving state since in humans insertion polymorphism of ITSs was never observed and *numt* polymorphic loci are rare. The application of this type of insertion polymorphism to population genetics studies in different horse breeds and to evolutionary analyses in Perissodactyla will be presented.

P1035 Identification ABCG2 gene polymorphism in Iranian Holstein bulls

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ABCG2 (ATP binding cassette subfamily G member 2) gene have mapped in chromosome 6 which is encoded ABCG2 protein that transports various xenobiotics, cytostatic drugs across the plasma membrane and cholesterol into milk. A single nucleotide change (A→C) in base 86 of exon 14 is capable of encoding a substitution of Tyrosine-581 to Serine (Y581S) in the ABCG2 protein and increases milk yield and decreases fat and protein concentration. The aim of this research was to study polymorphism of exon 14 and partial sequences of intron 13 and intron 14 of ABCG2 gene in Iranian population of Holstein bulls. Genomic DNA of 105 bulls were extracted from semen samples using high Pure PCR template preparation kit. Primers were designed with Oligo software and utilized in PCR, whereas the PCR fragments were then sequenced. Some SNPs (Single Nucleotide Polymorphisms) detected for the first time in intron 13, exon and intron 14 as compared with NCBI sequence databases that may affect performance of ABCG2 gene. The T→G mutation is capable of encoding substitution of Valine to Glycine in base 38 of exon 14 and it has the highest frequency between all observed mutations. The frequency of alleles T and G were 0.68 and 0.32, respectively. The A→T mutation in 4139 base has the highest frequency in intron 13. The allele frequency of A and T were 0.87 and 0.13, respectively. In base number 2 of intron 14, T→C mutation has the highest frequency between mutations. The frequency of T and C were 0.82 and 0.08, respectively. The A/C mutation in base number 86 of exon 14 was observed with 0.02 frequency.

P1036 Sequencing of full-length canine platelet *GPIb*β and characterization of the promoter region

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The platelet membrane glycoprotein complex GPIb/GPV/GPIX is composed of four membrane-spanning polypeptide subunits: GPIbα, GP Ibβ, GP IX and GP V in 2:2:2:1 stoichiometry. The complex plays a key role in the initial step of haemostatic process mediating adhesion of platelets to the exposed subendothelium by binding von Willebrand factor under the condition of high shear stress. All four GP subunits are encoded by different genes. The predicted canine *GPIb*β locus is not annotated in the Ensembl database. Using 5' RACE PCR and sequencing of the mRNA purified from canine platelets, the entire gene was identified on the chromosome 26 syntenic with the human chromosome 22. Homologies with the human ortholog are 87% and 84% at the nucleotide and amino acid level, respectively. The gene codes for a 205 aminoacids protein with many leucine-rich repeat motifs and an overall GC content of 80%. The gene is composed by two exons: the leader exon includes 5'UTR not exceeding 40 nt with respect with the 600 nt of the human counterpart. Two different transcription start sites were found. The putative promoter region was predicted by Proscan software v1.7 in the 300 nt upstream of the 5' cap site. The core promoter harbours different regulatory motifs: many SP1 and AP-2 but also T-Ag, GCF, CREEB, SIF and GATA. Notably, two GATA motifs are highly conserved when compared with humans.

The cloning of the mRNA could eventually confirm the two different transcription start sites found with direct sequencing.

P1037 Identification association of ABCG2 gene Polymorphism with reproductive traits in Iranian Holstein bulls

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ABCG2 (ATP binding cassette subfamily G member 2) gene have mapped in chromosome 6 of cattle. A single nucleotide change (A→C) in base 86 of exon 14 is capable of encoding a substitution of Tyrosine to Serine in the ABCG2 protein and effects milk components. The aim of this research was to study polymorphism of exon 14 of ABCG2 gene in Iranian population of Holstein bulls and their association with reproductive traits.

Genomic DNA of 105 bulls were extracted from semen samples using high Pure PCR template preparation kit and utilized in PCR, whereas the PCR fragments were then sequenced. Statistical analysis of association between new observed mutations in exon 14 and Breeding Value of Reproductive traits such as First Service To Conception(FSTC), Interval between calving to First Insemination(CFI), Calving Ease(CE), Calving Interval(CI), Age at First Calving(AFC) indicated, mutation in base number 81(C→G) causing the substitution Tyrosine to Stop code, have significant effects on AFC ($P<0.05$).

P1038 Comparative cDNA-AFLP analysis of male and female PersianSturgeon (*Acipencer persicus*) gonads

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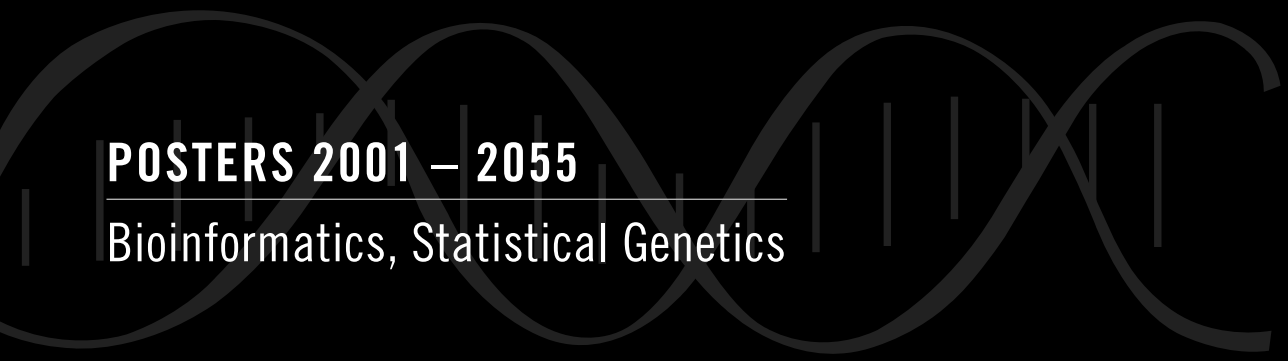
In sturgeon aquaculture, where the main purpose is caviar production, a reliable method is needed to separate the fish according to gender. Regarding the failure of PCR methodologies, using of alternative approaches focusing on the expression patterns is recommended. In the present study we searched for sex markers in mature male and female *Acipencer persicus* gonads by cDNA- Amplified fragment Length polymorphism (AFLP) molecular marker. The sex of Persian sturgeon specimens was determined by gonad observation. When comparing cDNA-AFLP patterns of the testis and ovary, via 68 primer combinations (*Eco*+3/ *Mse*+4), produced a total of 3000 cDNA fragments. Two differentially accumulated TDFs (Transcript- Derived Fragments) where identified, cloned, characterized and confirmed on individual samples of cDNA and genomic DNA. Two markers (TDF1, TDF2) derived from gonads were detected in mature A. persicus ovary cDNA, while were not detected in tailfin genomic DNA (therefore, the designed specific primers could not be used in quick detection of sex in Persian sturgeon. The results of BLAST indicated that two identified TDFs were not directly linked to a sex- determining gene. The provided cDNA-AFLP data could be considered as a starting base for subsequent studies focusing on expression patterns involved in sex determination and differentiation at different stages of gonadal maturation.



POSTERS 2001 – 2055

Bioinformatics, Statistical Genetics

P2001 Alteration of gene expression in mammary gland tissue of dairy cows in response to dietary unsaturated fatty acids

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The aim of this study was to determine the effects of unprotected dietary unsaturated fatty acids (UFA) from different plant oils on gene expression in the mammary gland of grazing dairy cows. A total of twenty-eight Holstein-Friesian dairy cows were randomly assigned to four concentrated UFA-sources for 23 days after which all 28 cows were switched to a non-UFA-supplemented concentrate for an additional 28 days. On the last day of both periods, mammary gland biopsies were taken to study genome-wide differences in gene expression on Affymettrix GeneChip® Bovine Genome Arrays. Supplementation with UFA increased milk yield and decreased milk fat and protein content. Furthermore, the proportion of *de novo* fatty acids in the milk was reduced whereas that of long-chain fatty acids increased. A total of 3,490 genes were found to be significantly affected by UFA supplementation. Gene sets related to cell growth, proliferation and development, apoptosis, cell cycle, signalling, nutrient metabolic process, as well as immune system response were predominantly down-regulated by UFA supplementation. In contrast, gene sets associated with electron transport chain, and oxidative phosphorylation processes were up-regulated. Therefore, supplementing grazing dairy cows with unprotected dietary UFA can improve the health and nutrition quality aspects of dairy milk, but may also affect mammary gland integrity and health.

P2002 Structural variation in the pig genome using next-generation sequencing

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Several recent studies have described copy number variation (CNV) and insertions/deletions (indels) as common and frequent structural variation present in mammalian genomes. Although the detection of such variation has been traditionally accomplished with SNP or CGH arrays, next-generation sequencing has recently proven to achieve better resolution and accuracy.

In this study, we report the detection of structural variation in the pig genome by analyzing whole-genome Illumina paired-end sequencing data of 4 Duroc boars at 15 fold coverage each. We describe genome-wide maps of CNV and segmental duplication based on read depth of coverage, while indels are detected in intra-reads and in inter-reads using paired-end information. Functional analysis provides genes and pathways putatively associated with important known QTLs.

This study provides the highest-resolution map of genomic structural variation throughout the porcine genome to date and reveals interesting targets for follow-up studies.

These results are obtained through the EC-funded FP6 Project "**SABRE**".

P2003 A factor model to analyze heterogeneity in gene expression in a context of QTL characterization

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Microarray technology allows the simultaneous analysis of thousands of genes within a single experiment. Classical approaches to analyze transcriptomic data ignore the gene dependence structure. This leads to correlation among test statistics which affects a strong control of the false discovery proportion.

We focus our study on a method called FAMT (Friguet et al, 2009) which captures the components of expression heterogeneity into factors. The relevance of factor modeling is first shown on illustrative gene expression data sets in simple situations of heterogeneity. We also use a real expression data set, primarily generated to map QTL for abdominal fatness in chickens (Le Mignon et al, 2009). The genelist found through FAMT was more related to the fatness trait that the one found by the classical way. Indeed, a PCA generated with the FAMT genelist discriminates much more fat and lean chickens. FAMT provides also functional information about a QTL region through a gene related to the fatness trait and controlled by this region (DHCR7) not observed by a classical approach. Then we interpret the independent factors extracted from this biological data set using known information about both experimental design and genes. We show that some factors may have different and complex origins, which can be related to particular metabolisms.

As we extract biological information from what was before simply considered as statistical noise, analyzing heterogeneity in gene expression yields a new point of view on transcriptomic data (Blum et al, submitted).

P2004 GenotypeChecker: an interactive tool for checking the inheritance consistency of genotyped pedigrees

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The raw data for performing genetic analyses consists of pedigree information about related individuals together with genotype data for any number of detectable polymorphic markers. The pedigrees analyzed may be large in size and complex in structure. Furthermore, the emergence of techniques such as microarray SNP-genotyping has led to an explosion in the scale of data, where 1000s of markers may be genotyped across the population.

Downstream processing of such data, for example to analyze linkage associations between markers, is highly sensitive to errors in the data. Errors in the recorded pedigree structure or incorrect genotypes generate inconsistencies with the rules of Mendelian inheritance, which invalidate or skew genetic analysis algorithms.

Identifying and cleaning data errors prior to downstream processing is complex for such large datasets and, because genotype or identification errors have consequences on the apparent inheritance pattern of relatives, it is difficult to unambiguously identify exact source errors.

'GenotypeChecker' allows users to load and analyze large pedigree/genotype datasets and check the data for inheritance inconsistencies. The tool combines a genotype checking algorithm which applies the rules of Mendelian inheritance across all markers for the pedigree, with an interactive exploratory interface that visualizes the inheritance of markers in the context of the pedigree structure. The data are presented to the user in colour-coded tables which can be sorted and filtered to explore the reported errors. The user can 'mask' individual suspect genotypes prior to rechecking inheritance, providing verification of erroneous datapoints that should be cleaned from the data.

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P2005 From The Genome of Giant Panda to Three Extreme-Environment Animals

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Using next-generation sequencing technology alone, BGI have successfully generated and assembled a draft sequence of the giant panda genome. The assembled contigs (2.25 gigabases (Gb)) cover approximately 94% of the whole genome, and the remaining gaps (0.05 Gb) seem to contain carnivore-specific repeats and tandem repeats. The assessment of panda genes potentially underlying some of its unique traits indicated that its bamboo diet might be more dependent on its gut microbiome than its own genetic composition. We also identified more than 2.7 million heterozygous single nucleotide polymorphisms in the diploid genome. After the successful assembly of the genome of giant panda, BGI have sequenced the genomes of three extreme-environment animals (polar bear, penguin and Tibetan antelope) subsequently and each sequencing depth is about 60x. We are going to sequence the genome of *Panthera tigris altaica* and African lion in the next project. We study these animals at a genome level, including identifying the functional elements and decrypting the genetic information in the genome, in an attempt to understand their evolution and adaptation to the harsh environment, particularly to protect the animals at high risk of extinction. These genome projects are important part to accomplish the 1000 plant and animal reference genomes project launched by BGI.

P2006 Accuracy of a family-based genotype imputation algorithm

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One way to reduce genotyping costs is to genotype a reference population with a high-density marker panel and to impute the untyped genotypes of selection candidates typed with an evenly spaced low-density panel. Given the small number of expected recombinations in the recorded pedigree, family information can greatly help to impute large haplotype segments. The objective of this study was to evaluate the accuracy of a family-based imputation algorithm over several generations. Imputation was done in three steps: 1) genotypes that could be inferred with high certainty from family information were filled in, 2) haplotypes were reconstructed and 3) haplotypes of progeny were matched to those of the parents and untyped loci were filled in. The accuracy of the algorithm was evaluated using simulated data, with a base population consisting of 100 sires and 300 dams, each producing 2 offspring. Fifteen discrete generations of random mating and selection were considered. T wo chromosomes were simulated with a SNP density corresponding to the 50K bovine SNP chip (45K after edition). The first three generations were treated as the reference population. In later generations, densities corresponding to panels of 1k, 3k, 5k and 10k equidistant SNPs were created. The haplotyping accuracy for all generations was > 0.99. The algorithm resulted in very high imputation accuracy for all low density panels. In generation 3, accuracy was above 0.995 for 3k or denser panels. The accuracy slightly decreased over generations reaching 0.97 for the 3k panel in generation 10.

P2007 resSpecies: a data-handling system for genotype-phenotype mapping and association studies

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It is a sad fact of scientific life that each separate analysis package – almost without exception – requires data to be presented to it in specific and unique ways such that no two input files are alike. The consequences of this are that biologists must spend many hours modifying their data for each package with the associated risks of errors being introduced and changes being made in one file not being replicated in the alternative copies of the data, either those held locally or those being used by collaborators elsewhere in the world.

To address these problems, we developed a system called 'resSpecies'.

Written in modular Java code and delivered primarily as a web application (at www.resspecies.org), the resSpecies system operates on top of a freely available, open-source relational database system (postgres). It provides a central, shareable repository for data relating to linkage mapping, QTL mapping or other phenotype-to-genotype association studies. It permits full access control to enable granular sharing of sensitive pre-publication data and has the facility to render copies of that data publicly available post-publication. Using series of web-based forms, the user can easily select the data to be exported and then have that data automatically formatted as required by the chosen analysis package. The formatted files can either be saved directly from the web browser or be emailed directly to registered users.

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P2008 Fine mapping of a QTL affecting meat quality in the chicken using genetical genomics

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Many genetic studies have successfully identified QTLs controlling complex phenotypes. However, very few have led to the identification of genes and causative mutations, in livestock as well as in model organisms. Variations in these complex traits are believed to be under the genetic control of regulatory elements. It has now become possible to screen all genes for differences in expression via different approaches including Microarrays. The present study applied a targeted genetical genomic approach to a QTL controlling meat pH on the chicken chromosome 1. Meat pH is one of the most important indicators of chicken meat technological quality, which can lead to changes in meat colour and drip loss. To identify the genes and gene networks contributing to variation in the trait, 16 Agilent 44k chicken arrays were used in a dye-balanced design to compare the gene expression levels of the alternative homozygous genotypes at the QTL (QQ vs. qq). The first top candidates, in the list of differentially expressed genes, include several highly significant genes at the QTL position. The gene list constituted some relevant gene networks which potentially contribute to the variation in meat pH. Identification of the causative genes and mutations is under investigation. The results clearly suggest that this cost-effective approach is promising to dissect the genetic architecture of complex traits, and to identify naturally occurring genetic polymorphisms controlling economically important phenotypes.

P2009 GridQTL: A Grid Portal for QTL Mapping of Compute Intensive Datasets

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QTL mapping is an essential tool for understanding the genetic basis of complex traits, including product yield and quality in agricultural species and risk factors for disease in animal and human populations. *QTL Express* has provided a user-friendly and web-accessible analysis tool that has seen wide use for the analysis of experimental data, particularly from studies of outbred species. The availability of dense SNP marker maps for thousands of individuals has increased the dimensionality and complexity of QTL analyses requiring computationally intensive and more advanced QTL mapping tools. The *GridQTL* project aims to provide an expanded and improved QTL analysis tool, in a user friendly environment, harnessing Grid computing technologies to deal with these increased computational demands. In addition to the existing analyses for QTL mapping in structured outbred populations (e.g. whole genome scan for additive and, where appropriate, dominance QTL effects in backcross and F2 crosses and half-sib families), *GridQTL* can offer an automated analysis to search exhaustively for pairs of interacting QTL. For general pedigrees, variance component analyses can be performed fitting a QTL along with a polygenic component. This analysis includes the option of incorporating linkage disequilibrium through the specification and modelling of population history enabling a more precise QTL location to be obtained.

Further developments are underway and additional functionality will be offered in the future.

P2010 Identification of divergently selected regions between Japanese Black and Holstein cattle based on a bovine 50K SNP array

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Domesticated cattle have been under strong artificial selection for various economic traits. Hayes *et al.* (2009) demonstrated that sliding window approach is a useful method to identify genomic regions that have been differentially selected for production types between two breeds. The aim of this study was to identify divergently selected regions between the production types between Japanese Black (JB) and Japanese Holstein cattle (JH) based on the sliding window approach. Genotype information on 54,001 SNPs were obtained from Illumina BovineSNP50 BeadChip using a total of 100 samples including JB (n=50) and JH (n=50). The 40,635 SNPs on bovine autosomes were polymorphic. As for these SNPs, the absolute value of the difference between JB and JH allele frequencies were calculated. For each set of 10 adjacent SNPs, the average of the absolute value of the difference in allele frequencies was calculated. These windows were termed sliding window averages. At the $P<0.001$ threshold, there were 39 significant sliding window average differences in the allele frequency differences of more than 0.435 between JB and JH. These 39 windows were distributed in 11 regions on 8 chromosomes. These results would provide information on location of selective signatures and further investigation of these regions may lead to identification of the genes and polymorphisms associated with economic traits.

P2011 Inference of individual ancestry in cattle breeds using DNA markers

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Identification of the breed source of livestock individuals and their products is vital for consumer confidence in food authenticity. In the food industry, breed names may be fraudulently adopted to boost prices. Genetic markers can be used to identify and verify the breed origin of individuals. The Illumina BovineSNP50 BeadChip featuring ~54,000 Single Nucleotide Polymorphism (SNP) markers can be exploited to create a reduced set of markers to determine food authenticity. The objective of this study was to determine how many markers from the BovineSNP50 BeadChip are required to verify the origin of individuals in European cattle breeds. The data consisted of allele frequencies for 16 reference cattle breeds and 384 individual animal genotypes of known ancestry for 40,483 markers. Breeds of interest were commercial beef and dairy breeds and traditional British breeds. Level of informativeness of each SNP was estimated from the breed-specific allele frequencies. The likelihood that an individual genotype belongs to each reference breed was estimated and assigned to the breed with the highest likelihood. Stringency levels were applied by log-likelihood ratio to assess the confidence of the assignments. Some breeds required fewer markers (<100) to achieve 100% assignment success. In contrast, closely related breeds require more markers (~200) to achieve >95% assignment success. The power of assignment success is dependent on the levels of genetic heterogeneity and pool of samples considered. We demonstrate that a substantially reduced set of SNP markers can be taken from the BovineSNP50 assay to effectively assign individuals to breed of origin.

P2012 The Diversity of MHC Class II Genes in Sheep

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Nematodes adversely affect animal health and cause serious economic losses; one option for control is the use of genetically resistant stock. There is an association between the MHC class II DRB1 locus and resistance to nematode infections of sheep. Our long-term aim is to fine-map the causative mutations but in order to design efficient and powerful experiments, we need to determine the extent of diversity at the different loci. We have therefore sequenced the second exons of all the alleles at the DQA1, DQA2, DQB1, DQB2 and DRB1 loci in 900 naturally infected lambs.from the Scottish Blackface breed and texel breeds. The MHC of sheep contains highly variable DR and DQ regions. In the Blackface breed, there were at least 7 alleles at DQA1, 14 alleles at DQA2, 13 alleles at DQB1, 10 alleles at DQB2 gene and 34 alleles at DRB1. Over 20 of the alleles had not been previously described. In comparison, the GenBank database, which contains sequences from all breeds of sheep, contains 14, 18, 19, 16 and 92 alleles at DQA1, DQA2, DQB1, DQB2 and DRB1 respectively. There are various methods for assessing diversity but they do not always agree. A composite measure, based on Rényi entropies, which differentially weights contrasting aspects of diversity was able to capture differences and similarities in diversity between and within breeds.

P2013 Screening and 454 sequencing of genes important for the mink industry

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The newly produced CHORI 231 American mink BAC library (Anistoroaei *et al*, in prep) was screened using heterologous probes for genes potentially involved in various phenotypes in mink. These included *coat coloring* (Black Cross, Blue/silver, Cinnamon, Stardust, Palomino, Pastel and Redish phenotypes), *hair growth* and *length, coarseness* and some *receptors potentially involved in viral diseases* (such Aleutian disease virus and influenza) in mink. The extensive screening yielded positive results for 22 different genes (*KIT, KITL, MLPH, LYST, TYRP1, MC1R, TYR, PMEL, DEFB1, ITGB1, HLA-DRB1, DFNA17, TMIE, AGRP, MITF, MSH, 5SLC24A5, MC2R, MC3R, RSPO2, FGF5* and *KRT71*). In the first stage, clones containing candidate genes for six of the important colour phenotypes (*KIT and KITL*- candidates for: Blackcorss, Sturdust, Cinnemon phenotypes; *MLPH* – linked to Silver phenotype; *LYST* – candidate for Aleutian color (associated with Chediak-Higashi syndrome); *TYR* described as being responsible for albino and Himalaya phenotypes and *PMEL* – candidate for various blue/silver phenotypes) were commercially sequenced by 454 Roche technology. Large contigs have been analyzed and assembled. The complete sequences of the candidate genes enable first the association to the candidate genes in established families by means of microsatellite markers and thereafter the finding of causative mutations for the targeted phenotypes.

Fourteen other loci are currently in the sequencing process at the moment using the same 454 technology and will soon be available for investigation.

P2014 Factors affecting endometrium receptivity in lactating dairy cows

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The aim of the study was to evaluate factors affecting endometrial receptivity in lactating dairy cows. After calving cows were subjected to endometrial biopsy on days 0, 7 and 14 (day 0 = standing heat) of the oestrous cycle (the monitoring cycle) followed by transfer of a biopsied blastocyst on day 7 of the subsequent cycle (the ET cycle). The both cycles were monitored by milk progesterone measurements to ensure normal cyclicity. The collected endometrial and embryo biopsy specimens were analysed in pools of three with the Affymetrix oligo arrays and Blue Chip cDNA arrays, respectively. According to phenotypic data, recipient cows that conceived and delivered a calf (the calf delivery group) had higher overall breeding values and FatKg indices compared to cows that did not get pregnant (the non-pregnant group). Comparison of gene expression in the endometrial biopsy specimens indicated significant differences between the calf delivery and non-pregnant groups on the day 0 of the monitoring cycle as well as between the first week (days 0-7) and second week (days 7-14) of the oestrus cycle. Based on the phenotypic and gene expression data two gene sets, PPAR signalling pathway and arachidonic acid metabolism were selected for further validations. According to *in silico* analysis the PPARalpha transcription factor has 8 variations, whereas transcription factors PPARdelta and PPARgamma have 18 and 717 variations, respectively. Additionally AT-rich scaffold/matrix attachment regions were analysed.

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P2015 Identification Of Protein Complexes Implicated In Bovine Mastitis

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Disease prediction methods exist for generating distinct prioritizations of candidate genes for human diseases. These methods are typically based on the similarity of disease gene characteristics (e.g. sequence features, functional annotations, expression patterns and literature descriptions). Disease prediction methods based on protein-protein interaction networks have recently been proposed based on the assumption that functionally related genes or gene products may be involved in the same or similar phenotypes. The central idea in these network based methods is that mutations in different members of a gene/protein complex may lead to similar diseases. Thus once a complex having members involved in one disease has been identified, the rest of the members become candidates for having a biological relationship with the same disease. We reasoned that functional network based inference is also suitable for genome-wide expression studies of bovine mastitis. In this study we present an approach to predict and rank genes involved in the acute phase response to mastitis in the mammary gland of dairy cattle through the integration of protein-protein interactions and genome-wide expression profiles.

These results are obtained through the EC-funded FP6 Project "**SABRE**"

P2016 RGN, ISCU and Nell2 are differently expressed in two rainbow trout strains after thermal stress

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A first comparison of gene expression profiles in liver tissue of healthy rainbow trout from the local selection strain BORN and a STEELHEAD control strain (TCO STEELHEAD II-WA) via microarray technology revealed among others three differentially expressed genes: Regucalcin (RGN), iron-sulfur cluster scaffold protein (ISCU) and neural epidermal growth factor-like-like protein 2 (Nell2). We isolated and characterized the respective cDNA sequences and examined their tissue-specific expression. Furthermore, we investigated in the first instance their gene expression in brain tissue during thermal stress (exposure to 8°C, 15°C and 23°C).

RGN is a Ca^{2+}-binding protein involved in regulation of calcium homeostasis in cells. RGN mRNA expression was significantly elevated in liver (1.84-fold), head kidney (1.73-fold) and heart (1.7-fold) of long-time selected BORN trout in comparison to STEELHEAD trout. Exposure to higher temperature caused a significant ($p<0.05$) decrease of gene expression in both BORN (8°C to 23°C: 0.62-fold) and STEELHEAD strain (8°C to 15°C: 0.53-fold; 8°C to 23°C: 0.47-fold).

ISCU is an iron-binding protein, which is involved in the assembly or repair of the [Fe-S] clusters. Its expression level was significantly elevated in liver (1.37-fold) and depressed in spleen (0.61-fold) of BORN trout compared to STEELHEAD. Thermal stress reduced ISCU copy number, which was significantly different between both rainbow trout strains (8°C to 23°C: 1.42-fold).

The extracellular glycoprotein Nell2 regulates cell growth and differentiation. NELL2 mRNA abundance is significantly enhanced in trunk kidney (2.7-fold), muscle (2.4-fold) and intestine (2.4-fold) whereas it is significantly decreased in spleen (0.43-fold) compared to STEELHEAD trout. Temperature increase leads to a significant higher Nell2 expression in STEELHEAD compared to BORN trout (8°C to 23°C: 1.96-fold).

Further investigations regarding the role of these three genes in both rainbow trout strains are planed.

P2017 Avian genome annotation in Ensembl

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Ensembl provides integrated gene annotation, variation, comparative and functional genomic resources for 50, predominantly vertebrate, species. Four avian genome assemblies have been annotated using the Ensembl gene annotation pipeline, including draft assemblies for chicken (*Gallus gallus*) and zebra finch (*Taeniopygia guttata*), and recent preliminary assemblies based on next-generation sequencing for duck (*Anas platyrhynchos*) and turkey (*Meleagris gallopavo*).

High-coverage genome assemblies in Ensembl are annotated by combining a set of standard analyses with the alignment of sequence data to produce a set of predicted gene models.

Due to the relatively low levels of protein and cDNA evidence available for avian species, a wider variety of evidence was used in constructing gene models than is typically the case, with species-specific EST-based models being included in the chicken and finch gene sets.

The turkey and duck assemblies are among the first produced using next-generation sequencing technology to be annotated using the pipeline. Compared to the traditionally sequenced finch and chicken, their assemblies are fragmented. An investigation of how the next-generation sequencing technology assemblies affected the gene annotation was carried out. Reduced coverage thresholds and orthologue projection were used in addressing the challenges presented by these genomes. The numbers of protein coding genes predicted for each species is as follows: chicken 16,736, zebra finch 17,475, turkey 15,295 and duck 15,634. Non-coding RNAs were also annotated for all species.

The annotation and additional resources are available at www.ensembl.org and http://pre.ensembl.org.

P2018 Estimating the genetic variance captured by dense SNP chips

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The fraction (q^2) of additive genetic variance (V_A) explained by SNP chips (i) determines the accuracy of genomic selection and so is relevant to all such breeding schemes, (ii) sets targets for technology development for species, and (iii) places into perspective q^2 derived from GWAS – 'missing heritability'. Squared accuracy (r^2) of genomic evaluation is given by the ratio of (i) the product of the heritability h^2 and the number of records per locus affecting the trait, and (ii) the same term plus 1. This can be extended to the number of independent segments, a property of the species genome, and q^2<1. Then, for a series of evaluations, the reciprocal of r^2 is linearly related to the reciprocal of the number of records. In the regression, the intercept is $1/q^2$, and the slope depends on the species genome, the trait, and why the variation remains untagged. In testing with USDA cattle data, it was estimated that for the Illumina Bovine SNP50 BeadChip q^2=0.8, leading to a maximum accuracy ~0.9. q^2 is an expectation and attribute of the chip. For particular traits the ultimate r^2 using a chip may be more or less than q^2, e.g. if a SNP is included in the chip and explains all V_A, then r^2 will ultimately be 1. However for most traits this will not be the case and the theory provides a prediction of the adequacy of the chip and a realistic assessment of the impact of increasing the number of records on achieved accuracy.

P2019 Genome-wide Copy Number Variation and Temporal Genes Expression Analysis in Marek's Disease-Resistant or -Susceptible Inbred Lines of Chickens

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Viruses that cause cancers are a great threat to human and animal health. Marek's disease (MD) in chickens is a lymphoproliferative disease caused by Marek's disease virus (MDV). The over-expression of Hodgkin's disease antigen in MD makes it an ideal model to study the progression mechanism of Hodgkin's disease *in vivo*. Three inbred chicken lines ($L6_3$, $L7_2$ and LM) with different susceptibility to MD were examined by array-based comparative genomic hybridization (CGH) and expressional microarray analysis using samples taken at different time points (5dpi, 10dpi and 21dpi) post MDV infection. A total of 43 CNVs were identified in the three chicken lines with total sizes ranging from 1.4 Mb in $L7_2$ to 1.6 Mb in LM. While only 22% of the sequence found within CNV regions are unique for $L6_3$ (MD-resistant) and LM (intermediate in MD-resistance), 62% are unique for $L7_2$ (MD-susceptible). Several anti-viral pathways, and particularly the NF-κB pathway, were found activated in the early cytolytic stage (5dpi) in $L6_3$ chickens. The array-CGH and gene expression microarray results revealed a CNV loss located on chromosome 4 present in both $L6_3$ and LM chickens but absent in $L7_2$ chickens that is associated with the expression of a *CD8α* homologue before and after MDV infection. To our knowledge, this is the first report of a CNV loss that may be related to MD-resistance in chickens.

P2020 QTL mapping for performance and carcass traits on chicken chromosome 4 using composite interval mapping with mixed models approach

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We used composite interval mapping (CIM) combined with mixed model to map QTL for performance and carcass traits on chromosome 4 of a Brazilian chicken F_2 population (layer x broiler). We compared our results with those from interval mapping (IM) using the QTL Express. Ten microsatellite markers were used to genotype 360 F_2. Phenotypes were adjusted using family and sex as fixed effects and hatch as a random effect. Conditional probabilities of QTL genotypes were obtained from QTL Express and cofactors were selected using stepwise regression with a window size of 10 cM. Codes were developed in the R software and a significant threshold was defined as LOD>1.64 using a permutation test. CIM with mixed model mapped nine QTL: three for BW35 (110, 174 and 253 cM), two for BW41 (173 and 253 cM), one for weight gain 35-41 days (WG3541) (241 cM), for feed efficiency 35-41 days (FE3541) (241 cM), for back (163 cM) and for legs (241 cM) weights with R^2 ranging from 1.95% to 7.03%. IM mapped only four QTL: one for BW35 and BW41, both at 174 cM, and one for WG3541 and FE3541, both at 241 cM. Most QTL presented negative additive effects, indicating that the favourable alleles came from the broiler line. According to literature, two new QTL regions were found: one associated with back weight (*LEI0076-MCW0240*) and another with WG3541, FE3541 and legs weight (*LEI0085-MCW0174*). In these regions, two putative candidate genes were evidenced: *FGF2* and *PPARGC1A*, which need to be further investigated.

P2021 Increased DNA Methylation Level of CD4 gene in Clinical Mastitis Dairy Cattle

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Cow mastitis is the most significant disease affecting dairy production in the world wide. As a result of the low heritability of mastitis, it is hard to improve the resistance of mastitis in dairy herds using traditional genetic improvement. DNA methylation of immune related genes is a mainly epigenetic regulatory factor for lower heritability trait especially for disease resistance. $CD4^+$T cells participate in immune response of bovine mastitis. Here we detected and compared CpG methylation levels on the promoter region of *CD4* gene in blood cells between eight clinical mastitis (SCC > 1000,000) and eight healthy (SCC < 100,000) Chinese Holstein cows. Using bisulfite pyrosequenceing assays, significantly different DNA methylated *CD4* promoter were measured in the mastitis (72.0%±3.8%) and healthy cows (63.4%±5.1%) ($P<0.01$). The results were further confirmed by cloning sequencing. We found that a novel C→T SNP in *CD4* gene was significantly associated with the EBV of SCS in Chinese Holsteins. In the present study all of the samples were CT genotype for the SNP. The results suggest that under the same genetic background of *CD4*, increased DNA methylation level in *CD4* promoter may contribute to the development of clinical mastitis in Chinese Holsteins.

P2022 A comparative genomic approach to identify placenta-specific genes

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The placenta is a crucial organ, facilitating nutrient and gas exchange between the mother and foetus, altering maternal physiological conditions to sustain pregnancy, while also protecting the foetus from the maternal immune system. Abnormalities in placental development are associated with early pregnancy failures in humans and animals. There is likely a genetic basis for many of these failures, but few candidate genes have thus far been identified. Knowledge of the genes involved in placenta formation and function would provide useful targets for further study of the genetic basis of variability in placentation and the growth and survival of the foetus. Using a comparative genomic framework and new genomic resources from the platypus, opossum, wallaby and a previously published core mammalian gene sets, we have rapidly identified a suite of candidate genes that we are investigating to determine their involvement in placental development and function in eutherian mammals. We are currently investigating these in an ovine model to confirm specificity and timing of expression.

P2023 PermuteNGS: The significance testing of transcriptome profiling for RNA-sequencing data

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RNA-Seq uses recently developed deep-sequencing technologies. Compared with microarray data, it has several advantages; i) it is not limited to detecting transcripts that correspond to existing genomic sequence. ii) it has a large dynamic range of expression level over which transcripts can be detected. Considering these revolutionary advantages for transcriptomics with cost-effective sequencing technologies, it is speculated that tons of thousand RNA-Seq data will be generated to discover dynamic nature of transcriptome in the near future. Although significance test statistics have been developed for the previous digital gene expression data such as EST data (Audic and Claverie 1997; Susko and Roger 2004), there is no rigorous statistical method for RNA-Seq data. Here we present a random permutation method to success the previous test statistics in the era of next generation sequencing technologies. Real data showed higher statistical power in our method compared with the previously known methods. We used 25 combination of human 5 liver and 5 kidney RNA-seq data from (Marioni, Mason et al. 2008) and compared with their results. Using our program showed higher empirical power than using their method. The method is implemented both in user friendly web programming and R package and freely available for academic users.

P2024 An alternative approach to detect accelerated molecular evolution for genome-wide comparative data

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The nonsynonymous/synonymous rate ratio indicates the selective pressure on the protein. In many proteins, a high proportion of amino acids may be largely invariable due to strong functional constraints, the criterion of the ratio be > 1 is very stringent one for detecting positive selection. Therefore, branch-site model was developed to detect positive selection, however it need a predefined phylogenetic tree. Here we propose an alternative approach to identify differentially evolved genes in each gene level as detecting accelerated molecular evolution between species which is less stringent and phylogenetic topology free. We applied the method to identify Devogs between two birds versus four mammals; chicken, zebra finch, opossum, dogs, mouse and human. Surprisingly, the result indicates that using 6,023 of 1:1 orthologous gene sets of the six species, about 3,631 genes (60.3 %) may have been differentially evolved between the birds and mammals. We also performed simulation study to validate the method.

P2025 Estimation of variance components for carcass traits in Japanese Black cattle using 50K SNP genotype data

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Genomic selection method with high-density SNP genotype data may accelerate genetic improvement in livestock animals. In this study, we attempted to estimate variance components of 6 carcass traits in Japanese Black cattle using fattened steers' SNP genotype data. The 673 steers were genotyped using Illumina BovineSNP50 BeadChip and phenotyped for cold carcass weight, rib eye area, rib thickness, subcutaneous fat thickness, estimated yield percentage and beef marbling score (BMS). The samples used were from animals with high or low BMS values for the purpose of QTL mapping. The 39,422 SNPs were chosen, where minor allele frequencies were >0.01 and genotype call rates were >0.95, and which were in Hardy-Weinberg equilibrium (p>0.001). Additive polygenic variance and the variance attributable to set of SNPs having statistically significant effects on the trait were estimated by Bayesian analyses via Gibbs sampling using the models with genomic relationship matrix and with the SNPs effects fitted, respectively. As the number of SNPs chosen increased, the proportion of the estimated variance attributable to the SNPs became higher, while the polygenic variance decreased in the estimated total variance for each trait. The selection of SNP did not largely affect the estimates of residual and total genetic variances, which were nearly equal to those estimated by the model including only random polygenic effect. For each trait, no fraction of total genetic variance was explained by the set of SNPs that had no significant association with the trait (p>0.1). For more precise estimation, we are planning to add more samples.

P2026 The placenta-specific promoter of the bovine *Cyp19* gene is epigenetically repressed in caruncles and activated by the upstream stimulating factors 1 and 2 in trophoblasts

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Placenta-derived oestrogens are important for the growth and differentiation of the tropho-blast, and are involved in processes initiating and facilitating birth. The enzyme that converts androgens into oestrogens, aromatase cytochrome P450, is encoded by the *Cyp19* gene. In the placenta of the cow, expression of *Cyp19* relies on promoter 1.1 (P1.1). The aim of this study was to find out, how the expression of *Cyp19* is regulated in the bovine placenta. We examined if epigenetic mechanisms might be involved. To this end we measured the DNA methylation of P1.1 in maternal caruncles and fetal cotyledons by bisulfite genomic sequencing, and also transcription of *Cyp19* by quantitative RT PCR. We found that P1.1 is differentially methylated in caruncles and cotyledons, and that in caruncles, methylation is involved in *Cyp19* rexpression. We further analysed P1.1 in *vitro* and in cultured trophoblast cells. We found, that binding of placental nuclear proteins to an E-box element is required for full promoter activity. We enriched the E-box-binding proteins from placental nuclear extracts and showed by Western blot analysis and supershift EMSA experiments that the proteins were the transcription factors USF1 and USF2. Depletion of the USFs by RNAi and expression of a dominant-negative USF mutant, were both associated with a significant decrease in P1.1-dependent reporter gene expression. Furthermore, scatter plot analysis of P1.1 activity *vs.* USF binding to the E-box revealed a strong positive correlation between the two parameters. These results strongly suggest that USF1 and USF2 are activators of P1.1-dependent transcription in the bovine placenta.

P2027 Web based services for cooperative DNA programs in breed management

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DNA based investigation has been recognized for many years as the standard for verification of identity and parentage assignment in pedigrees. The work associated with that task requires not only a high level of responsibility and accuracy from every project participant but also suitable genetic markers as well as reliable platforms of hard- and software. Today, most projects are cooperative with various partners contributing samples and genotypes sometimes from worldwide. We present a new level of networking using an internet based platform (Realpact-Mago) that supports creation and worldwide secured management of a shared data pool equipped with a palette of tools for generation, evaluation and analysis of genetic data. Realpact-Mago comprises: I) state of the art calculations for judgement of marker and marker panel characteristics (e.g. polymorphism information content, effective number of alleles, power of discrimination, power of exclusion) with consideration of inbreeding and population stratification; II) applications that help build up and maintain an outstanding quality in genotypes as well as individual data; III) analysis of individual marker data and parentage assignment cases with consideration of mutation rates; and IV) analysis of populations for individual relationships. Functions are demonstrated by: a) resolving some of the parentage assignments as requested by the ISAG comparison tests, and b) examples from several years of managing the rise of a joint project data pool with up to 50,000 individuals.

P2028 Identification of canine DNA from mixed DNA sample in traces

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The aim of the study was to identify the canine DNA on the object from the crime scene. DNA was isolated from the swab of the object using QIAamp DNA Investigator (QIAGEN). The obtained DNA was amplified for 18 small tandem repeat (STR) loci and sex determination amelogenin marker using Canine Genotypes, Panel 1.1. kit (Finzymes Diagnostics). Repeatability of the reaction was estimated with three repetitions of polymerase chain reaction amplifications. Specificity of reaction was examined by amplification of DNA samples isolated from different animal species. Only in few STRs some unspecific amplifications were obtained after amplification of cattle, human, horse and sheep DNA samples. Amelogenin gene was amplified in all tested species. Sensitivity of amplification was assessed by amplifying varying amounts of control canine DNA (from 2 ng to 7.5 pg of DNA). Complete STR profiles were produced from 250 pg of DNA, while some STR peaks were lost after amplification from 125pg of DNA. Multilocus profile obtained from DNA on the object indicated mixed STR profiles of different dogs by the presence of more than two peaks at some loci. The STR profiles in the sample from the object were in agreement with STR profiles of at least two different dogs. Our data show that DNA recovered from the object was of sufficient quality and quantity to perform multiplex STR analysis.

P2029 Optimising power and robustness in Genome Wide Association scans

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Genome Wide Association (GWA) analyses test many Single Nucleotide Polymorphisms (SNPs) to find Quantitative Trait Nucleotide (QTN). One problem with GWA studies is their elevated false positive error rate (FPER) caused by Spurious Disequilibrium (SD), i.e. the statistical association between unlinked loci. Tests robusts to SD are less powerful than tests that are not robust. We propose a new solution to optimise power and robustness when family trios are sampled: everyone genotyped and progeny phenotyped. Two equivalent models arise naturally: $y=b_0+b_Ax_A+b_Sx_S$ and $y=b_0+b_Bx_B+b_Wx_W$. Here, y is the phenotype, b_0 an intercept, x_A the progeny genotype score, x_B the average parental genotype, x_W a deviation of progeny genotype from x_B, $x_S=x_B-x_W$ and b_i the regression coefficient (i=A,S,W,B) with standard error seb_i. Analyses with both models are performed and results saved. SD is tested with $t_S=b_S/seb_S$. The number of significant t_S tests in a genome scan is z. If z is significantly greater than the expected value under no SD then SD is detected. Given m unlinked SNP and an error rate α per SNP then $z\sim Poisson(\alpha m)$ under no SD. Linkage changes the parameter of the Poisson. Although an empirical distribution can be constructed using permutation, we have shown it is unnecessary. If there is no SD then $t_A=b_A/sebA$ is reported as it is a powerful (not robust) test, otherwise $t_W=b_W/seb_W$ is reported as it is a robust (not powerful) test. Hence, this way of accounting for FPER in GWA studies may be the best compromise between power and robustness.

P2030 Equine microsatellites analysis of challenging samples

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Horse identification and parentage control are established in all countries where equestrian sports are developed. As in human forensics, the horse identity can be confirmed by DNA typing (STRs). Blood, saliva, hair roots and tissue fragments provide a reliable source of DNA, but, when it is impossible to recover those biological samples, you can extract the DNA from challenging samples, such as bone, tooth and urine samples. In cases of extreme degradation of equine remains or fossil, bones or teeth may be the only material available for successful genetic typing. DNA extraction from bone and teeth includes a pulverization step, decalcification and phenol/chloroform extraction. This organic method yielded amounts of DNA with higher levels of inhibition: to remove substances that could inhibit PCR, it could be useful performing an additional extraction protocol by PrepFiler™Forensic DNA Extraction Kit (Applied Biosystems), adapting the manufacturer's protocol to these challenging samples, or purifying by FAM PCR&GEL Purification kit (FAMBiotech s.r.l.). Our results show that a purification protocol increases the success rate of PCR amplification and STR analysis. DNA profiling from urine has consistently problematic and results unpredictable, depending on some factors such the small amount of DNA contained in urine samples, the presence of inhibitors and rapid degradation of the DNA. DNA was extracted by PrepFiler™Forensic DNA Extraction Kit, obtaining good amplification results. DNA typing from urines could be useful during anti-doping tests, to remove any question of sample substitution and could give unequivocal identification of the horse that provided the sample.

P2031 Analysis of 16 microsatellites markers and parentage testing in Italian Trotter horse

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Accurate determination of relatedness is of great importance in horse breeding industry. In the past decade, the DNA forensic field was primarily focused in humane genome typing, for identification and parentage determination purposes. The discovery of polymorphism in microsatellites loci (STRs) and the introduction of polymerase chain reaction technique has led to the establishment of extremely powerful method for identification and for parentage control also in animals. Microsatellites have been in fact identified in all eukaryotic species studied so far and modern tools of molecular biology provided means for fast and accurate way for animal genotyping. Currently the majority of horse breed registries adopted DNA technology in order to verify pedigree records and to solve queries of parentage.

In this study we verified the genealogy of 14,110 Italian Trotter foals with 16 microsatellites markers. Among these, 358 non related individuals were selected in order to evaluate the feasibility of the STRs panel to verify parentage and individual identification. Number of alleles, heterozygosities, polymorphic information content (PIC) and exclusion probabilities of each system were calculated. A total of 113 alleles was found, with a significantly high mean number of alleles. The mean polymorphic information content (PIC) was 0,67 and the exclusion probability (PE) of one false parent of the system was 0,998. Mutational events observed were at an acceptable level for parentage testing.

P2032 On simulating single nucleotide polimorphism (SNP) data

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Single Nucleotide Polimorphism (SNP) have been used as markers in Qtl mapping and genomic selection studies. Because SNPs can be found in large amount in the genomes, Qtls can be located with improved resolution and its effects can be better estimated than using microssatelites markers. For some livestock species 60K SNP panels are easily found while in humans 500K SNPs panels are already available. However, genotyping is still expensive, thus, simulation studies can be useful. SNP data simulation is not a trivial task, since lots of information must be generated and stored, which means a high demand for computer memory and processing capabilities. Usually, simulate a SNP as one would do with a single locus is not efficient since some information as alleles effects must be created and stored as a zero, being a waste of critical memory. Besides that, SNPs simulated in this way are stored themselves with other loci, making some processes less efficient. Thus we are trying to develop a SNP simulation data algorithm which consider each SNP as a special entity, which contains only the fundamental information in order to save memory. The SNPs simulated are also stored in different containers within the chromosomes in order to improve the performance of some algorithms like sort and search, minimizing the processing demand. These results are obtained through the EC-funded FP6 Project "**SABRE**", Fapesp and CNPq funding agencies.

P2033 Canine STRs analysis in a forensic casework

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Dog-bite related injuries and fatalities are increasing in incidence and represent an important public health concern, as dogs are intensely integrated in human social life. Many cases of dog attacks have been reported and sometimes the seriousness of the wounds can lead to death. Forensic investigations in dog attack usually involve the examination of bite marks and tooth-prints. Generally, it was possible to obtain a canine specific STR profile from the dog's saliva left on the wound area, even when high background of human DNA was present (blood). The reported case concern a 6 years old child found dead in a private abandoned ground, with a lethal injury on his neck clearly due to a dog-bite. The investigation was carried out on four dogs found on the crime scene, blood samples of the dogs have been collected and analysed using STR techniques. As no biological samples were collected from the bite-marks zone on the victim's body the only available evidence was the victim's blood soaked T-shirt. Analyses were performed on the T-shirt scratches areas using several DNA extraction protocols and additional extractions to the purpose to avoid the large amount of inhibitors and human DNA. A dog DNA profile was isolated and it was compared to the four suspected dogs profiles but no matches could be found.

P2034 Generating markers from an existing pedigree data

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A simulation data software would be more useful if it is capable to generate phenotypic and marker data from an existing population with a complex pedigree. This procedure allows the user to study some parameters and others characteristics of the population for several generations, even if the real population is not evolving anymore. Federal University of Viçosa has data from an outbred cross population which evolved up to 3 generations (F2). This population is the base to implement our simulation procedures. In order to achieve success three reading routines have being implemented to read phenotypic data, genotypic data and map data into the program respectively. As the data are read, the pedigree structure is simultaneously stored. To the base generation (parental generation) the polygenes and Qtls also needed to be simulated, since the simulation method is the one based on gene-level. Using the pedigree data read from the offspring a gametogenesys processes is carried out in the parents in order to generate the diploid genome of the offspring. This sequence is repeated for each new generation, making possible to evolve a population for several generations according to a existing pedigree by simulation. These results are obtained through the EC-funded FP6 Project "**SABRE**", Fapesp and CNPq funding agencies.

P2035 LZ5 – a free-software for data simulation

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The scenarios needed for research vary largely, thus, simulators are build as small pieces of program which intends to accomplish one or few specific tasks. As a result, data simulator softwares have a short life and it is not suitable to be shared by others groups but the one who develop it. LZ5 is a free data simulation software intended to be more flexible and more longer-life than most of the simulation softwares in animal breeding area. LZ5 development is based on collaborative work in order to keep it as much updated as possible. The software is developed for the Linux plataform using C++ as the programming language. The binary and its corresponding sources files can be obtained from the site: www.dracena.unesp.br/luccaz/lz5. The software is intended to perform the basic tasks of any simulation data software as well as to perform some more specialized ones, e.g., generate populations from an existing pedigree structure and simulate SNP panels data. The development of the program is organized in four modules: 1. Simulation, 2. Selection, 3. Genetic Evaluation and 4. User Interface. These results are obtained through the EC-funded FP6 Project "**SABRE**", Fapesp and CNPq funding agencies.

P2036 Epistasis adds to the complexity of genetic networks influencing economically important traits in pigs

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Most QTL mapping methods have focussed on identifying individual QTL effects in the absence of interactions (epistasis). In order to gain a more accurate understanding of the genetic background of complex traits, epistasis needs to be investigated. In the present study a genomic scan for epistatic QTL was conducted on animals from a three generation full-sib population (Pietrain sires × crossbred dam line). 386 animals were genotyped for 88 molecular markers covering 10 autosomes. Phenotypic data was available for up to 315 F_2 animals for traits associated with physical and chemical body composition, daily gain, feed intake and meat quality. The methodology applied followed a two stage approach: (i) a 5 cM scan across all positions to pre-select potential candidate regions with epistatic effects and (ii) a 1 cM scan around the pre-selected positions using a complete epistatic model. In total, 65 significant epistatic QTL pairs were identified explaining between 5.0 and 10.9% of the phenotypic variance. Epistatic QTL were identified on all considered chromosomes with the additive-by-additive effect being the most prevalent. The epistatic QTL pairs with the highest effect were identified for daily gain and protein accretion rate from 90-120 kg body weight (between SSC1 and SSC2), entire loin weight (between two locations of SSC7), and pH at 45 minutes post-mortem (between SSC10 and SSC13), explaining 10.3, 10.2, 10.2 and 10.9% of the phenotypic variance, respectively. This study indicates that economically important traits are influenced by complex genetic networks involving interactions between QTL located throughout the genome.

P2037 eGIFT: A text-mining and knowledge extraction tool for curators

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eGIFT (extracting genic information from text) is a software tool designed to extract informative terms (iTERMS) from PubMed extracts. The motivation for eGIFT was to provide a rapid means for curators to identify specific terms that are informative about gene functions. iTERMS are identified by collecting and analyzing the PubMed abstracts that are relevant to a specific gene. The salient iTERMs are defined by identifying those words that are found in the abstracts about the target gene at a much higher frequency than the words are found in a background set of over 600,000 abstracts. Further processing removes redundancy and categorizes iTERMs into grouping such as Processes and Functions or Cellular Components. We have processed abstracts describing over 4900 genes and have set up a Web server allowing users access to the extracted information. The iTERMs for each gene are presented to the user through a Web interface and each iTERM is linked to those sentences in the abstracts that contain the term. The sentences link to the appropriate abstracts in PubMed. Currently, we are working closely with curators identifying Gene Ontology terms for the chicken to provide a workflow that quickly identifies candidate terms and the relevant literature for the curator to review. eGIFT should be generally useful to others pursuing curation efforts for a variety of targets such as GO terms, anatomy or protein-protein interactions. eGIFT can be accessed at: http://biotm.cis.udel.edu/eGIFT/

P2038 Population studies for 6 cattle breeds by 11 microsatellite loci

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Prior to the introduction of DNA marker as a tool for paternity test in cattle, a population analysis was carried out. Bovine genotypes™ PCR typing kit (Finnzymes) was used. At first an effective database was established and connected with the Central Database of Slovenian Cattle in which pedigree data is stored. We developed procedures which are verifying crosswise accordance of pedigrees by microsatellite information. Population analysis of 436 animals and 6 cattle breeds (Brown, Limousine, Charolais, Simmental, Holstein and autochthonous Cika breed) showed that all populations are in HWE (99.9%). Observed heterozygosity was highest in Cika breed (0.80) compared to 0.62-0.73 in other breeds. Pairwise Fst values (Weir & Cockerham) ranged between 0.05 and 0.14 ($p<0.01$). Holstein breed turned out to be the most distinct. Combined power of exclusion (CPE) 99.99 was reached if all 11 markers, recommended by the International Society for Animal Genetics (ISAG) were included. If 8 markers were included CPE was lower than 99.95. Assignment and Migrants Detection test was carried out using GeneClass2. Results from assignment test revealed that 92.0 % animals (401) were assigned to populations they were designated as. Detection of first generation migrants (Paetkau, D. et al.) pointed out 9 individuals.

P2039 Angiotensin I converting enzyme (ACE) inhibitory peptides derived by different bovine β-casein variants: molecular dynamics simulation

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Current attention focuses on mechanisms of controlling blood pressure through the inhibition of Angiotensin I Converting Enzyme (ACE). Bioactive antihypertensive peptides of food origin are increasingly gaining importance as cooperate with synthetic drugs in hypertension therapy. The ACE inhibitory properties of peptides derived from bovine protein milk have been demonstrated *in vitro* and *in vivo* studies and only recently bioinformatics tools were carried out but without considering the genetic milk protein variants. Molecular dynamics (MD) allows the understanding of the interaction between biological molecules at molecular and atomic level.

In this work, peptides derived from the different genetic variants A1, A2, A3 and B of bovine β-casein (CSN2) were investigated for their interaction with ACE and were evaluated for their influence on ACE structure. To choose ACE inhibitory peptides a simulated gastrointestinal digestion was carried out on the CSN2 variants. Six exclusive peptides were identified and we started investigating their possible interaction mode with ACE, using molecular dynamics simulations. The bioactive peptide Val-Pro-Pro (VPP), known for its high efficiency inhibition of ACE, was also simulated via MD and the results compared with the other peptides.

We run multiple simulations (50 ns each) and analyzed the flexibility of different peptides in complex with ACE. The conformational changes of ACE and changes of the thermodynamic parameters were also observed and compared with the different peptides.

The application of molecular dynamic to study the interaction between ACE and biopeptides is very helpful as initial screening tool to look for ACE inhibitory peptides.

P2040 We Need a Livestock Gene Nomenclature Committee

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The tremendous advances in DNA sequencing technology have opened new opportunities for livestock genomics. Sequencing projects are underway or completed for cat, cattle, chicken, dog, duck, horse, goat, sheep, swine, turkey, and water buffalo. Ready access to genomic sequence information provides an important tool for comparative genomics. However, genomic studies both within and across evolutionary clades are hampered due to the difficulty of identifying phylogenetic homologs. Furthermore, the expansion/contraction of gene families can cause problems in assigning orthology, but they are not the only issues associated with definitive gene nomenclature. For example, one can encounter population level variation in gene models that span the small, medium and large scale. Compounding these issues are the draft assemblies themselves in that 10-15% of the gene models are inadequate. These problems can be compounded over time as new databases arise and the names assigned to genes proliferate. Thus, standardized gene nomenclature is absolutely necessary to facilitate effective communication between scientists. Furthermore, gene nomenclature must be based on existing nomenclature whenever possible to truly facilitate data analyses across species. Therefore, we highly recommend the formation of the Livestock Gene Nomenclature Committee (LGNC), an international and centralized effort to provide standardized gene nomenclature for livestock species. Under the LGNC umbrella, we suggest that farmed mammalian, avian, and farmed aquatic species form working groups to address unique genes within a species or closely related species. Furthermore, we suggest that the LGNC work in close conjunction with the other vertebrate nomenclature committees to promote standardized gene nomenclature.

P2041 Genome-wide QTL detection for growth, body composition and quality related traits in chicken

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During the past decades, genetic improvement for growth, body composition and quality related traits in chicken was obtained through a strong selection based on the phenotype. Since 20 years, the genetic variability has been studied through quantitative trait loci (QTL) detection in many different breeds or selected lines. This approach has led to the identification of many QTLs but with localisation intervals too large to allow marker assisted selection. Since the sequencing of complete genomes, thousands of single nucleotide polymorphisms (SNPs) were identified. Therefore, regions unexplored before can be studied and QTL locations refined. The aim of this study was to fine map QTLs affecting 26 economically important traits by genotyping 1536 SNPs and 127 microsatellites on 579 F2 animals obtained by crossing divergently selected fat and lean lines. QTL interval mapping was performed with QTLMap software which was developed for populations containing a mixture of full and half-sib families. In addition to single QTL mapping, hypotheses such as the presence of two linked QTLs influencing the same trait were tested. A total of 57 QTLs was detected at the 5% chromosome wide level and 28 QTLs were suggested at the 10% chromosome wide level. A further list of 13 QTLs was detected by multi-QTL approach. Our results confirmed some QTLs previously identified with the set of microsatellite markers and refined their position. Interestingly, additional QTLs were identified mostly because this study provided a better coverage of the chicken genome (28 chromosomes), including chromosomal regions which had never been thoroughly studied.

P2042 Bovine Genome Database: Update and Plan for Gene Nomenclature System

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The Bovine Genome Database (BGD; http://bovinegenome.org) facilitates the integration of bovine genomic data. BGD is largely based on GMOD software, and includes the Chado database schema, GBrowse, the Apollo Annotation Editor, BLAST services and gene pages. Genome browsers, available for both scaffold and chromosome coordinate systems, display the bovine Official Gene Set (OGS), RefSeq and Ensembl genes, non-coding RNA, repeats, pseudogenes, SNPs, markers, QTL and alignments to cDNAs, ESTs, and protein homologs. Gene pages, created using Ruby on Rails, display information for individual OGS gene models, including transcript variants, functional descriptions, gene symbols, gene ontology, annotator comments and references to external databases. We have been developing new controlled vocabulary terms and data converters to improve standardization of data representation. The system now supports the exportation of computational analysis and annotation data in formats like GFF3, chado-XML and BSML. The next phase of data-sharing will include RDF/OWL to describe the annotation as well as the characteristics of the workflows which generated the results of the computational analyses. This will provide richer information for consumption by the community as well as semi-automated discoveries by autonomous expert systems. In addition to bovine specific resources, we plan to implement a system for standardizing livestock gene nomenclature, in collaboration with the Livestock Gene Nomenclature Committee (LGNC). The system will maintain a database of existing gene names and symbols, allow users to query and request new gene symbols, enforce standards recommended by the LGNC, and facilitate transferring nomenclature data to NCBI.

P2043 Buffalo (Bubalus bubalis) Whole genome sequence initiative

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Buffalo is an integral part of agriculture in Asia. It plays a pivotal role in livestock industry by contributing milk, meat and draft power. India has 57% of the world buffalo population that constitutes 35.6% of the total bovines, but contributes 56% of the total milk production of India. The buffalo population in India has steadily increased 1.93% per annum for the last two decades and is now more than 100 million. India possesses the best and most diverse buffalo genetic resources comprising 10 registered breeds and several local populations adapted to different ecological niches. Buffaloes are known for higher butter fat content, lean muscle mass, higher muscle to bone ratio, and decreased susceptibility to diseases. The genetic bases for all these attributes have not been worked out due to scanty information and poor genomic resources. The Buffalo Genome Sequence project will enhance buffalo genomic resources and provide insights into production, reproduction, disease resistance and adaptive traits. Whole genome sequencing of one farm bred female Murrah buffalo was undertaken using the Illumina sequencing platform. A total of 65 GB of genomic DNA sequence data has been generated providing ~ 22X genome coverage. The genome sequence data has been submitted to NCBI in SRA database available under accession # SRR032564.1; SRR034148.7; SRR034232.2 and SRR035526.1. A draft assembly of Buffalo genome was made with cattle as reference and all the 24 autosomes and the X chromosome sequence have been assembled as pseudo molecules based on buffalo radiation hybrid (RH) map. A total of 1,263,193 contigs were generated with an average length of 2098 bases. The draft bubaline genome assembly has a total length of 2630.58 Mb excluding 256.1 Mb unassigned sequence. The first draft Buffalo genome assembly is expected to be available on NCBI database by September 2010.

P2044 Polymorphirms of genes related to meat traits in crossbred zebu cattle

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The present research aimed associate the BM1500 (chromosome 4), CSSM66 (chromosome 1) and BM8215 (chromosome 14) markers with carcass traits such as weight of hot carcass, weight of forequarter, weight of hindquarter, weight of spareribs in F2 terminal crossbreeding zebu cattle. After PCR, amplified products were analyzed by a vertical electrophoresis with 8% non-denaturing polyacrylamide gel. The allele frequencies were estimate by direct counting the electrophoresis fragments. Statistical analysis was realized using the SAS program associating quantitative and molecular data. Significant differences were found for weight of hot carcass, weight of forequarter, weight of hindquarter and weight of spareribs traits among genetics groups. It was concluded that studies with large populations are needed to associate productive traits with markers loci. Further studies with more microsatelite markers on chromosome 14 are necessary, especially in the region with 45-51 cM, due to strong evidence of the localization of a QTL with effects on weight of carcass in this region.

P2045 Impact of a Leptin SNP and Zilpaterol Hydrochloride on Growth and Carcass Characteristics of Finishing Steers

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A functional SNP (C/T) in the *ob* gene (Leptin R25C) resulting in an arginine to cysteine amino acid substitution affects carcass characteristics of cattle. Zilpaterol Hydrochloride (ZH), a beta-adrenergic agonist fed to cattle during the final phase of cattle feeding, increases live and carcass weight gain, leanness, and reduces marbling. This study determined if leptin genotype interacts with ZH. A total of 4,179 steers were used in a 3x2 factorial RCBD consisting of three leptin genotypes and two levels of ZH (0 or 21 days of ZH feeding). Regardless of ZH treatment, TT steers were fatter than CC steers at slaughter evidenced by a greater percentage of YG 4 ($P < 0.02$) and a lower percentage of YG 1 carcasses ($P < 0.01$). Regardless of leptin genotype, feeding ZH reduced the percentage of YG 4 ($P < 0.01$) and increased the percentage of YG 1 carcasses ($P < 0.01$). Leptin genotype x ZH interactions were detected for marbling score ($P < 0.02$) and percentage of USDA Choice carcasses ($P < 0.01$). Tendencies for interactions ($P < 0.14$) between leptin genotype and ZH were observed for carcass weight gain and ribeye area. The carcass weight interaction involved a tendency for CC steers to gain more carcass weight than TT steers in response to ZH ($P < 0.10$). Increases in ribeye area in response to ZH tended to differ by genotype with TT steers showing a greater increase than CC steers ($P < 0.12$). These data indicate that leptin genotype, ZH, and their interactions are all significant factors affecting carcass phenotypes.

P2046 Accuracies of Direct Genomic Breeding Values for calving ease estimated on Italian Piedmontese bulls with a principal component approach

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Genomic selection programmes are characterised by a marked asymmetry between number of markers and phenotypes. A possible solution could be the use of multivariate techniques to reduce the dimensionality of predictors when calculating direct genomic breeding values (DGV). In the present work, number of predictors for calculating direct genomic breeding values for calving ease (DGV) in cattle is reduced by using principal component (PC) analysis. A total of 323 Piedmontese bulls were genotyped with the 54K Illumina beadchip. SNPs retained after edits were 41,878. The number of PC extracted was 2,536, explaining about 70% of the original variance of the system. Effect of PC on polygenic EBV for direct calving ease was estimated in the reference population with a BLUP model. Three ratio reference:prediction population were considered (70:30, 80:20, 90:10). Accuracy was calculated as correlation between DGV and polygenic EBV. DGV were also calculated by using all 41,878 edited SNP. DGV accuracies obtained using all markers for the three scenarios reference:prediction were 0.32, 0.30 and 0.33. Better values were obtained when PC were used as predictors: 0.38, 0.37 and 0.44. Results obtained in the present work suggest that PC analysis could be used to reduce the number of predictors in the estimation of DGV, allowing for easier calculations and keeping or improving DGV accuracy.

P2047 Genome-Wide Association Analysis to Identify Loci for Milk Yield in Gyr Breed

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A genome scan was conducted to identify QTL affecting milk yield in a Brazilian Gyr population of progeny test bulls (N=319). Data used in this study were derived from traditional genetic evaluation records computed by the Embrapa Dairy Cattle and released in May/2009 (http://www.cnpgl.embrapa.br/nova/informacoes/melhoramento/Gir/artigos/classificacao_geral_2009.pdf/). Genotyping was performed using Illumina's BovineSNP50 BeadChip with approximately 54,000 SNPs. For analysis, any SNP with low call rate (<90%), departure from Hardy-Weinberg equilibrium (exact test p<0.01), and minor allele frequency below 5 percent, were excluded from the final analysis (24,532 markers retained). The Bonferroni correction threshold was 0.01. ITSNBN software, which uses a linear model and pedigree informationwas used in order to detect associations. For all traits, significant SNP were found on BTA 3, 4, 5, 6, 10, 14, 23, and 25. Most significant SNP were localized to specific chromosomal regions and often within a single linkage disequilibrium block. Results suggest that marker solutions from genomic evaluations may be useful for identifying genomic regions that merit further study to identify causal mutations.

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P2048 Estimation of genetic parameters for body weight at different ages for Mehraban sheep

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This research was performed to estimate genetic parameters of growth traits in Mehraban sheeps such as Birth Weight (BW), Weaning Weight (WW), Six Month Weight (6MW), Nine Month Weight (9MW) and Yearling Weight (WY). Data and pedigree files were fixed and made from data during 1997 to 2008 using FOXPRO software. A set of univariate animal models were used to estimate genetic parameters by restricted maximum likelihood procedure using Powel algorithm applied in ASReml software. Direct Heritabilities of BW, WW, 6MW, 9MW, WY were 0.284±0.045, 0.368±0.043, 0.323±0.046, 0.339±0.043 and 0.375±0.043 respectively it was increase with increase of age. BW and 6MW had highest and lowest Maternal Heritability respectively. BW and WY had lowest and highest Genetic, Phenotype and environment Variances respectively. Correlation between direct and maternal genetic effect were negative for all traits.

P2049 Shiny future for genetic using Artificial Neural Network

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Artificial neural networks (ANNs) are biologically inspired computer programs designed to simulate the structure and/or functional aspects of biological neural networks in which the human brain processes information. An ANN is formed from hundreds of single units, artificial neurons or processing elements (PE), connected with coefficients (weights), which constitute the neural structure and are organized in layers. The power of neural computations comes from connecting neurons in a network. In most cases an ANN is an adaptive system that changes its structure based on external or internal information that flows through the network during the learning phase. Once the network is trained and tested it can be given new input information to predict the output. Many types of neural networks have been designed already and new ones are invented every week but all can be described by the transfer functions of their neurons, by the learning rule, and by the connection formula. In addition, ANNs can combine and incorporate both literature-based and experimental data to solve problems. Scientists far removed from direct research in the field of Artificial Neural Network are now investigating applications to their own areas. Recently application of ANN increased in genetic. As a technique for computational analysis, neural network technology is very well suited for the analysis of molecular sequence data. It has been applied successfully to a variety of problems, ranging from gene identification, to DNA/RNA and protein sequence analysis.

Also ANN was used as a model of the dynamics of gene expression. The process of gene expression is described by a single network and by two linked networks where transcription and translation are modeled independently. Each of these processes is described by different network controlled by different weight matrices. The combination of ANNs and PyMS was able quantitatively to detect the component indole when a single straing of *E. coli*, containing the tryptophanase gene, was grown on a minimal supplemented salts medium incorporating various amount of tryptophan, in the range 0–253 mg. In contrast, supervised methods, such as ANNs, are able to recognize subtly different biological entities. Furthermore ANN was used to diagnostic and predict genotypes according to their traits informations. A basic description of artificial neural networks is given and applications of neural nets to problems in human gene mapping are discussed. The application of Artificial Neural Network to the prediction of structural and functional features of protein and nucleic acid sequences are reviewed.

P2050 Genetic analysis of milk production traits for Iranian Holstein dairy cattle

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Genetic parameters were estimated for milk production traits (milk yield, fat yield, fat percent, protein yield and protein percent traits) in Iranian Holstein cattle, records of 235,816 during years between 1964 to 2009 were estimated with restricted maximum likelihood method under univariate Animal model for the first lactation using ASremel software. Total number of animals was 343,059 individuals in pedigree and breeding values were predicted with BLUP procedure. Estimates of heritability for milk yield, fat yield, fat percent, protein yield and protein percent were 0.28 ± 0.0063, 0.22 ± 0.0062, 0.32 ± 0.0063, 0.22 ± 0.0087, 0.34 ± 0.0096 respectively.

P2051 Development of high-throughput high-density SNP genotyping array for bovine

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Simultaneous genotyping of many single nucleotide polymorphisms (SNPs) has been made possible by the development of array-based hybridization platforms. High-density genotyping empowered the success of genome-wide association studies for determination of genetic variation affecting complex traits. Medium-density studies in agricultural organisms have been beneficial in marker-assisted breeding, mapping quantitative trait loci, and other applications. Now there is interest in expanding to higher-density options to further refine and develop these techniques. The bovine research community has undertaken a massive genome sequencing and SNP screening effort to develop a comprehensive solution for a versatile, high-throughput high-density bovine SNP Genotyping Array. A consortium of academic thought leaders and breeding associations has combined efforts to sequence genomes of 15 bovine breeds from *Bos indicus* and *Bos taurus* to produce an extensive collection of SNPs and their genotypes. A vast subset of this collection has been screened across populations representing taurine, indicine, tropical taurine, and Asian breeds on the high-throughput Affymetrix Axiom Genotyping platform to produce a library of high-quality, validated SNPs. The screening arrays have been developed by a method to optimize physical and genetic coverage across the sequenced genomes. A multi-breed relevant subset of these SNPs will be selected for the high-density bovine genotyping array. The full library of validated SNPs can be utilized for customized breed-specific array development. An effective approach for the development of a high-density genotyping assay to support marker assisted breeding and other applications in cattle is described.

P2052 Genetic variability and population structure of the Chilean Terrier

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The Chilean Terrier is a recently characterized dog breed. The breed standard defines this breed as brave, tamed and energetic. This relatively small dog (5-8 kg) has fur coat white, short and dense. The head has a distinctive color pattern, ranging from black to red and mixtures. Nowadays, the pedigree of this breed comprise more than 3 generations, however little is known about the actual structure of the breed and the genetic variability. This is an important issue when assessing how closely the founders of the breed related are, for assessing the rates inbreeding of the population and for paternity testing. Genomic DNA was extracted from buccal swabs from 50 dogs known to be unrelated, based on known pedigree of the breed. A total of 10 microsatellites were genotyped, but one of them failed to yield a PCR product (AHTk253). The genotypes were binned using software TANDEM. The number of microsatellite alleles ranged from 11 to 4 in accordance to what has been observed in other dog breeds. The mean expected heterozygosity was equal to 0.84. Except from 2 loci (FH2175 and FH2132) all loci were in Hardy-Weinberg equilibrium. There was little differentiation between the known geographical subgroups. This information provides the first step for assessing the variability of the founders of the breed and for managing the breed constraining matting's between related individuals.

P2053 GeneBook: analysing genomes using AJAX and web-services

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Elucidating the genes, pathways and markers involved in any biological system remains a complex and challenging problem. However, due to the emergence of genetic and genomics techniques, a huge body of data now exists, both in the public domain and privately. Accurate analysis and visualisation of biological systems requires that the most up-to-date annotation of the genome is integrated with genetic markers and quantitative data from functional genomics experiments, allowing a systems biology approach to be adopted. We present GeneBook, a lightweight system for genomic and genetic analysis that pulls data from remote sources and displays it, live, to users in a web-browser. GeneBook uses web technology commonly implemented in social networking sites, enabling users to personalise their environment. They can upload and integrate their own data with the remote data and display it in the context of the genome.

P2054 Web Tools For Integrating Genomic And Post-genomics Data In The Chicken

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Modern biological research involves experiments at different levels of the system under study, which generate many different datasets and data types. All of these need to be integrated with each other and the public data to provide researchers with a view of the system under study. This integrative biology is an essential component of systems biology. We present web tools for the chicken genome, including a genome browser and a SNP database, which use open source, published software to present a front-end to many different datasets, allowing researchers to visualise and query across them. Specifically, we have mapped all chicken microarray datasets in the NCBI Gene Expression Omnibus (GEO) to the genome, so that scientists can now not only visualise the structure and location of genes, but also their expression in any number of published microarray experiments. We have also included SNPs from Illumina experiments, and copy-number-variation data. Researchers are able to see at a glance the behaviour of biological entities in a number of different experiments, alongside genetic and genomic data, allowing them to judge whether each entity is worthy of further investigation. The systems are extensible and can display any type of quantitative data.

P2055 A mulitplex PCR based assay for determining fragment length and quality control of Canine DNA samples

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The determination of the quality of canine DNA samples prior to genotyping analysis, is increasingly necessary in order to reduce the number of samples lost due to poor genotyping efficiency and the subsequent costs incurred in repeating or re-sampling the individual. The ultimate test of a sample is the ability to produce quality results in the application of interest and genotyping applications predominantly involve PCR amplification. We present a multiplex PCR based assay that can be scored cheaply and efficiently on an agarose gel to determine the quality of canine and related canid samples. The assay amplifies reference gene fragments of specific sizes and includes an internal reference to control for the presence of PCR inhibitors. Poor quality is determined by lack of amplification of the larger sized fragments. The assay was shown to be effective in assessing DNA quality in multiple breeds of canine and several canid samples indicating the flexibility of the use of this assay, and could determine amplifiable fragment lengths of samples extracted from formalin fixed paraffin embedded tissue. This assay can be amplified from as little as 1ng of DNA, determined by a sensitivity test, and can indicate the best use of samples for different genotyping platforms.

POSTERS 3001 – 3098

Genetic Diversity, Selective Sweeps and Domestication

P3001 Domestication centers, migration routes and admixture patterns of domestic cattle – is Central Asia a genetic melting pot?

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The phylogeographic patterns of domestic cattle have been a study topic since the beginning of molecular genetics. The regions corresponding to the main domestication centers – Fertile Crescent, Northeast Africa and Indian subcontinent – have been assessed in previous studies resulting in acquaintance of the genetic pool of some important regions. Central Asia was also pointed out as a possible domestication center for cattle, however the data on this region was scarce. Our aim was to test whether central Asia is another domestication center for cattle or if it is a mixture of other populations. To achieve our goal we compared the genetic diversity of Near East and central Asia regions for *Bos taurus* and *Bos indicus*, recurring to the characterization of 16 populations, in a total of 514 samples belonging to 31 breeds, including 44 new sequences from Near East and 128 from central Asia. We examined the patterns of genetic variability of mtDNA D-loop fragment in our populations with statistical, phylogenetic and geographical analyses. Near East contains a genetic uniqueness, while central Asia emerges as a blend of diversity, although possessing its own genetic distinction concerning T4 haplogroup. Central Asia acted as a pathway of migrations and trades, and at the same time, it was the scenario of one of the domestication centers for several animal and plant species. Our data support central Asia as the most likely domestication center for T4 haplogroup and as melting pot of cattle genetic diversity.

P3002 Estimation and comparison of phenotypic value changes in silkworm pure lines during consecutive generations under selection pressure

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This experiment was conducted in order to investigat the phenotypic value changes of silkworm economic traits including cocoon weight, cocoon shell weight and cocoon shell percentage under individual selection based on cocoon weight. All stages of rearing and data record were performed over four rearing periods. Each pure line contained two groups as selected and random groups. The effect of selection methods, the effect of pure line, and generation effect on the phenotypic values were compared. Main effects of pure line, generation, sex and group on cocoon weight, cocoon shell weight and cocoon shell percentage were significant ($P<0.01$). Interactions of the pure line × generation, pure line × group (except for cocoon shell percentage), generation × group, pure line × generation × group, pure line × sex, generation × sex, pure line × generation × sex, group × sex (except for cocoon shell percentage), pure line × group × sex (except for cocoon weight and cocoon shell weight), generation × group × sex (except for cocoon shell percentage) and the pure line ×generation × group × sex have been significant on the cocoon traits characteristics ($P<0.01$). Obtained results indicated cocoon weight and cocoon shell weight in selected group are higher than control or non-selected group. Both selected and non-selected groups had the the lowest cocoon weight, cocoon shell weight and cocoon shell percentage in the fourth generation due to unfavorable environmental conditions.

P3003 Characterization of three SINEs in intron 1 of porcine GDF11 gene

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Growth/differentiation factor 11 (*GDF11*), also known as bone morphogenetic protein 11 (BMP11), is a secreted signaling molecule in transforming growth factor-β (TGF-β) superfamily and acts as a negative regulator of thoracic and lumbar vertebrae formation in mice. In this study, the intron 1 sequence of porcine GDF11 gene with a size of 5.7 kb was obtained and three SINEs (PRE1e, PRE1c and PRE_ss) belonging to Porcine Repeat Elements (PRE) family were characterized in different pig breeds including Yorkshire, Landrace, Duroc, Laiwu, Dapulian and wild boars. PRE1e lies in the opposite orientations to GDF11 gene. The three PRE elements share similar secondary structure and are flanked by a direct repeat sequence. PRE1e has a C insertion and an A/T transition and PRE1c has A/T transition in their flanking repeat sequences. PRE1_ss has RNA polymerase III promoter motifs, A box and B box. Phylogenetical and molecular evolutionary analyses conducted with MEGA 4 suggest that PRE1e and PRE1c are closer and PRE1_ss is younger in evolution. The distribution of the three PREs is not breed-specifc, and in porcine genome, they are located on chromosomes 1, 4, 5, 7, 11, 13, 14, 15, 17 and X in the same way, with copy numbers of 560, 309 and 107 respectively.

P3004 Association analysis between Prolactin SNPs and milk production traits in Spanish Churra Sheep: preliminary results

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In a previous study a QTL affecting milk fat percentage was detected in ovine chromosome 20. Looking for candidate genes mapping in the confidence interval of this QTL, the Prolactin Precursor gene (*PRL*) was selected as a positional and functional candidate. This gene maps in the central region of OAR20 and encodes an essential hormone for mammary gland development, lactogenesis and milk protein gene expression. Based on this, we studied the genetic variability of the ovine *PRL* gene in 15 rams of Spanish Churra sheep. Hence, the complete coding region of the gene (5 exons), the fourth intron of the gene and the 5´UTR region, which includes the gene promoter, were sequenced. A total of 33 single nucleotide polymorphisms (SNPs) were identified. Four of the SNPs identified were located within exons (exons 2 and 3), three of which were silent mutations whereas the other one caused an aminoacid substitution (Pro>Gln). Five of the SNPs identified were genotyped across a commercial population of Spanish Churra sheep. For these SNPs a preliminary association analysis with production traits was performed by using phenotypic measurements (milk yield, protein percentage and fat percentage) obtained from 600 lactating ewes. The statistical results showed significant associations for three of the analyzed SNPs and milk yield. However, the analysis did not reveal any significant association for milk fat percentage. Further analyses, including larger sheep populations, are needed to understand the possible relationship between the *PRL* gene and milk production traits in Spanish Churra sheep.

P3005 The occurrence and expression of silver dilution gene in the horse coat colour

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MC1R allele T in homozygous state leads to producing pheomelanine (chestnut coat colours) and is responsible for inhibition of the silver dilution gene (*PMEL17* T allele) expression. Horse's coat colour is one of the traits the breeders are selecting for. A total of 133 horses representing Estonian Native, Estonian Heavy Draught and Tori breed were genotyped. Key polymorphisms at C901T in *MC1R*, 11 bp deletion in *ASIP* and C1457T in *PMEL17* were considered to determine variation and selection possibilities to increase silver dilution in horse coat colour. Our genotyping results showed T allele frequency in *MC1R* gene as follows: Estonian Native 66.7, Estonian Heavy Draught 77.5 and Tori 87.8%, and T allele in *PMEL17* 5.2, 6.3 and 0%, respectively. According to the stud book 10% of Estonian Native horses were registered with silver dilution. Considering single *PMEL17* gene, silver coat colour could be revealed phenotypically at 12% of Estonian Heavy Draught horses, however due to unfavourable covariation with *MC1R* T allele it occurred in two per cent of horses. Besides similar level of silver dilution occurrence in Estonian Native and Estonian Heavy Draught horses, similar allelic variations in *MC1R* and *ASIP* genes were found. Moderate level of C allele frequency at *MC1R* in Estonian Native and Estonian Heavy Draught horses (33.3 and 22.5%, respectively) and low of T allele in *PMEL17*, indicates feasibility of producing silver foals by pair mating of parents with T allele in *PMEL17* and C allele in *MC1R* gene.

P3006 Mitochondrial DNA haplogroup R in modern cattle: a contribution of Italian aurochsen?

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The debate on the genetic contribution of European aurochsen to taurine cattle gene pool after the generally agreed Neolithic domestication that occurred in the Fertile Crescent from local *Bos primigenius*, is still open. In our study we have sequenced the D-loop of over a 1,000 taurine cattle from 26 European breeds confirming the overall clustering within haplogroups of Near Eastern ancestry (T1, T2 and T3), but also identifying 24 mtDNAs (1.8%) not clustering within haplogroup T. Complete mtDNA sequencing of non-T samples revealed 10 subjects belonging to the novel haplogroup R, which represents a very early split (~139 ky) in the mtDNA phylogeny of *B. primigenius*. The remaining 14 samples clustered within the recently discovered haplogroup Q. Phylogeographic data indicate that R mtDNAs might derive from female aurochsen of the Italian Peninsula sporadically included in domestic herds, whereas Q and T subclades were most likely involved in the same event of Neolithic domestication in the Near East.

P3007 Study of Insertion and Deletion (Indel) Polymorphism as Genetic Markers in Myanmar Native Chickens

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The genetic diversity of Myanmar native chickens was evaluated with 101 Indel markers. Indel, as defined as either an insertion or deletion of one or more nucleotide base pairs into a DNA sequence. The chicken genome includes 446,696 Indel polymorphisms (2010, January). The present study was focused on Indel polymorphism as a new genetic marker for studying genetic diversity.

Eighty blood samples of Myanmar native chickens were collected from three areas, Yangon (n= 27), Mandalay (n= 40) and Pegu (n= 13). The Indel sequences of 20-30 bp around every 10 Mb of chromosome length were targeted for primer design. The information of Indel polymorphism and primer setting were conducted by using NCBI, Ensembel website, BLAST and Primer3 software. Totally 101 Indels markers were designed from chromosome No. 1 to 22.

Out of 101 Indel loci, 93 showed polymorphism and 68% of the polymorphic loci had a minor allele frequency (MAF) of >10% and 47% of > 20% MAF. The average observed heterozygosity (not include monomorphic) was 0.245±0.017. The *P poly* and $\overline{H}$ values of three locations were, 0.87, 0.87, 0.71 and 0.266 0.263, 0.239 respectively. A phylogenetic tree was constructed using Nei's genetic distance and UPGMA method. Its topology reflects Yangon and Mandalay population was genetically closer to each other (0.048) than Pegu (0.073 to Mandalay and 0.077 to Yangon). Myanmar native chickens exhibited high polymorphism on Indel loci and they are not much isolated to each other as they showed small genetic distance between them.

P3008 Characteristic of seven Japanese native chicken breeds based on egg white protein polymorphisms

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In this study, to evaluate genetic variability within a population/breed and genetic relationships between populations/breeds, we genotyped a total of 606 birds from seven Japanese native chicken breeds using seven egg white protein loci and compared those with Asian native chicken populations and commercial breeds. Genotyping of the Japanese native chickens showed that four loci (ovalbumin (*Ov*), two ovoglobulins (G_3 & G_2) and ovotransferrin (Tf_{EW})) were polymorphic and three loci (ovomacroglobulin (*Omg*), ovoflavoprotein (*Rd*) and lysozyme (G_1)) were monomorphic. The proportion of polymorphic loci (P_{poly}) and the average heterozygosity ($\overline{H}$) were ranged from 0.286 to 0.429 and from 0.085 to 0.158, respectively. The coefficient of gene differentiation (G_{ST}) was 0.250 in Japanese native chicken breeds. This estimate was higher than those of Asian native chicken populations (G_{ST} =0.083) and of commercial breeds (G_{ST} =0.169). Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendrogram and principal component analysis (PCA) plot showed that Satsuma-dori, Jitokko, Amakusa-daio and Hinai-dori were closely related to each other and grouped into Asian native chickens and that Tsushima-jidori, Nagoya and Chan were ramified far from other Japanese native chicken breeds. The egg white protein polymorphisms demonstrated that the population differentiation of the seven Japanese native chicken breeds was relatively large.

P3009 Genome-wide Differential DNA Methylation in the Wild and Domestic Chicken

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Cytosine DNA methylation is an important epigenetic modification termed as the fifth base that functions in many biological processes. Epigenetic modifications may exert their influence on phenotype during the process of selective adaptation. Here we show the genome-wide DNA methylation map of birds, using the chicken as a model organism and an immunocapturing approach followed by high-throughput sequencing. In both of the ancestral red jungle fowl and the domestic chicken, the avian broiler, DNA methylation and gene expression profiles were described separately for the liver and muscle. Compared with the red jungle fowl, DNA methylation in muscle tissue of the avian broiler, showed dramatically decline on a genome-wide scale. Furthermore, the length of the highly methylated regions (HMRs) has become shorter in the avian broiler, which has suffered intense artificial selection. In addition to the global changes in DNA methylation, transcriptome-wide analysis of the two breeds of chicken revealed that the patterns of gene expression in the domestic chicken have undergone a specific bias towards a pattern that is more suited to human-made environments with variable expression in certain gene functions, such as immune response and fatty acid metabolism. Our results demonstrated a potential role of epigenetic modification in animal domestication besides the genetic variations.

P3010 Meat Quality and Carcass Traits Related to HGD Single Nucleotide Polymorphism in Thai Established Beef Cattle

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The effect of homogentisate 1, 2 dioxygenase (HGD) gene on meat quality and carcass traits was investigated in 196 Thai established beef cattle (Kabinburi and Tak cattle). Single nucleotide polymorphism of the HGD gene in intron1 was used to identify polymorphism. Two polymorphisms of restriction sites for endonuclease *HGD-Bst*XI and *HGD-Hae*III were detected. The *HGD-Bst*XI genotypes showed highly significant effects on cooking loss (1, 7, 14, and 21 days), drip loss (7, 14, and 21 days), Warner-Bratzler shear force (WBSF) (1, 7, 14, and 21 days), carcass percentage, and loin eye area ($P<0.01$) in both breeds, Tak and Kabinburi cattle. The *HGD-Hae*III genotypes also showed highly significant effects on cooking loss (1, 7, 14, and 21 days), drip loss (7, 14, and 21 days), Warner-Bratzler shear force (WBSF) (1, 7, 14, and 21 days), carcass percentage, and loin eye area ($P<0.01$). The AA, AC, CC genotypes of *HGD-Bst*XI had WBSF at 21 day, 7.83, 4.82, and 2.81 kg, on average respectively. The AA, AC, CC genotypes had cooking loss at 21 day, 32.56, 17.27, and 11.69 percents, on average respectively. The AA, AG, GG genotypes of *HGD-Hae*III had WBSF at 21 day, 4.84, 7.43, and 2.74 kg, on average respectively. The AA, AG, GG genotypes had cooking loss at 21 day, 17.34, 30.91, and 11.41 percents, on average respectively. Finally, the SNP of the HGD gene can be developed to use for marker-assisted selection (MAS) to improve genetics of meat quality traits of Thai beef cattle.

P3011 Y chromosome and mtDNA polymorphism in Eastern Adriatic sheep populations

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The spread of farming into the Northern Mediterranean was frequently analysed through maternal and paternal DNA lineages of humans and domestic animals, as an important topic for understanding the Neolitic agricultural revolution. We analyzed mitochondrial DNA (partly control region and 12S rRNA coding region and fully tRNAPhe) and specific Y chromosome (microsatellite SRYM18 and SNP AY604734.2:g.67A>G) polymorphisms of 159 domestic sheep (9 breeds) and 21 mouflon (*Ovis musimon*) rams sampled from Eastern Adriatic, all along Croatian cost. Maternal and paternal lineages for, both, sheep and mouflon rams indicated ancestral homogeneity, with extensive variation around most frequent haplotypes. We indentified 70 mtDNA haplotypes, including one new present only in mouflons, and five Y chromosome haplotypes, including H18 here described for the first time (SRYM18 with 135). The large number of sheep (96.9%) and mouflon (100%) rams had haplotypes classified in mtDNA haplogroup B. More precisely, there was 26.4% of sheep rams with H3 haplotype while 37.1%, 20.8% and 7.6% of rams had haplotypes that were one, two and three mutations remote, respectively. The remaining five sheep rams had haplotypes classified in mtDNA haplogroup A. Similarly, 89.3% of sheep and 100% of mouflon rams had Y chromosome haplotype H6 while seven, five, three and two rams had haplotypes H8, H18, H7 and H5, respectively. Our study shows that present sheep populations of Eastern Adriatic, with exception of several rams that are indicating remote origin, are homogenous and belong to the lineages characteristic for European sheep populations.

P3012 Analysis of genetic variability among Indonesian local chickens using single nucleotide polymorphism (SNP) markers

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Indonesian local chickens have been considered as an important genetic resource since they produce an excellent meat and egg. However, the studies of their molecular genetics are limited. This study was conducted to analyze the genetic variability and relationships of four Indonesian local chicken populations i.e. Black Kedu (BK), Kedu, Kampung and Arab. Genomic DNAs from 192 individuals were collected in Central and West Java. The DNAs were genotyped using 73 autosomal SNP markers in which 63 were found polymorphic. The proportion of polymorphic loci and average heterozygosity of each population were 0.75-0.88 and 0.23-0.27, respectively. STRUCTURE analysis suggests that the probable number of genetic clusters (K) was calculated to be four ($K = 4$) with maximum likelihood. The clustering with maximum likelihood shows that each of Arab and BK populations was assigned to a distinct cluster, while Kedu and Kampung were admixed populations with a similar ancestry proportion. Principal component analysis reveals that PC-1 and PC-2 separated Arab and BK from the others, respectively. Neighbor-joining tree constructed by pairwise F_{ST} estimates confirms that Kedu and Kampung were closely related, while BK and Arab were diverged at a distance from the two.

P3013 Genetic diversity among major ovine populations registered in Slovakia using microsatellite markers.

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Microsatellites are used as molecular markers in genetics for parentage testing and population studies. The aim of this study was analyse the polymorphism of 9 microsatellite markers and their usefulness for parentage verification and genetic diversity in ovine populations registered in Slovakia. DNA polymorphism of 9 microsatellite markers (*INRA063, FCB11, FCB20, CSRD247, CP49, FCB304, MAF214, AE129, FCB128*) was investigated in four purebred (Tsigai, Improved Valachian, Laucaune, Muton Charollais) and one hybrid (Tsigai x Laucaune). Genomic DNA was prepared from blood samples using Wizard kit (Promega). DNA was amplified in two multiplexes for amplifying these microsatellites. The automatic analysis was performed using an ABI 310 sequencer and size of analyzed DNA fragments was determined in base pairs using computer package GeneScan v.3.7 (AB). Statistical parameters were calculated using Powermarker v.3.25 software and Popgene v.1.31 software. Allele frequencies, expected and observed heterozygosities, polymorphic information content and exclusion probabilities (PE) and F-statistics were calculated. The number of allele per each locus ranged from 2 (*MAF214, AE129, FCB128*) to 19 (*CP49*). The average values of H_e was 0.5864 (Charollais) to 0,8556 (Improved Valachian) and H_o was 0,6049 (Charollais) to 0,7407 (hybrid Tsigai x Laucaune). Mean PIC values ranged from 0.5478 (Charollais) to 0.8397 (Improved Valachian). The combined exclusion probability was (PE>0.998) in all breeds. The values of PE confirmed the usefulness of this set of microsatellites in parentage testing of sheep in Slovakia.

P3014 Investigation on genetic variability of Podolica breed through microsatellite analysis

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The Podolica cattle is an ancient breed, mainly reared in rural areas of Southern Italy. In the present study, the genetic variability of the Podolica has been investigated, using 25 microsatellite markers. A total of 106 individuals from twenty farms located on the Gargano promontory, in Apulia region were analysed. Number of alleles, allele frequencies, deviations from Hardy-Weinberg proportions, linkage *disequilibrium* among loci and Wright's F-statistics were calculated in the population involved. The average number of alleles per locus was high (9.52), evidencing a great genetic variability. Estimates of Wright's *F*-statistics revealed a moderate homozygote excess in the whole sample ($F_{IT} = 10.8\%$), partly due to the variation of allelic frequencies among subpopulations ($F_{ST} = 7.4\%$) and to the homozygote excess within population ($F_{IS} = 3.4\%$). Analysis of homozygote excess performed at population level, revealed a significant excess at eight loci ($P < 0.05$). However, the population was in Hardy-Weinberg equilibrium, showing an observed mean of heterozygosis of 0.7292, whereas the expected mean was 0.75531. The assignment test indicated that 97.94% of subjects can be assigned to the appropriate herd of origin. The significant linkage *disequilibrium* among loci ($P < 0.05$) observed could be likely ascribed to the contraction of number of Podolian head intervened in the last decades. The results obtained suggested that the population of Podolica cattle breed investigated preserved a high genetic variability. This was ascribed to the clusterization in subpopulations due to both different selective strategies and low exchange of genetic material among herds.

P3015 Interpretation of mtDNA region sequence diversity in Iranian Domestic sheep

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New insights for the systematic and evolution of the domestic sheep are provided by molecular phylogenies inferred from Maximum parsimony, Bayesian, Maximum likelihood, and Neighbor-Joining methods. Among the genetic markers mtDNA sequencing is one of the most useful and commonly employed methods for inferring phylogenetic relationships among closely related species and population. The mtDNA control region contain variable block that evolve four to five time faster than the remainder of the mtDNA molecule. The phylogeny of the domestic sheep was based on D-loop sequences of 28 samples representative of most of the sub-species described in the genus Ovis. In total of 28 unrelated sheep blood and meat samples were collected (Baluchi breed N=14 and Moghani breed N=14). The DNA was Extracted and the HVR1 region was amplified with specific primers using PCR. The HVR1 segment was sequenced, aligned and compared with other breeds from all over the world. Finally the sequences were registered in NCBI under accession number EU308497, FJ531545. Results showed that these breed belongs to haplotype group A and were nearest Turkish breed which were not unpredictable because of geographical vicinity. The branch style tree was drawn for analyzed samples in A haplotype group and results suggest that the Ovis oarial is the origin of Asian breeds.

P3016 CONBIAND network: A cooperative consortium for Latin American sheep breeds biodiversity studies

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The CONBIAND network (www.biovis.jimdo.com) is a consortium integrated by researchers involved in biodiversity, sustainability and conservation biology. Within this network, a study group for sheep breeds in Latin America has been constituted in order to clarify the sheep colonization history. Creole sheep were originated from animals brought to America by Spanish and Portuguese explorers more than 500 years ago. They are the result of the genetic drift followed by artificial and natural selection in parallel with subsequent migrations. The main goal of this project is to collate a critical mass of scientific information in the Latin American area with the purpose of increase the knowledge about the Creole breeds and their genetic relationships with Iberian breeds. Several international breeds have been added in order to determine their influence on the Creole breeds. A panel of twenty microsatellite markers has been utilised, based on results of previous studies developed by the participants. At this moment we have analyzed 60 traditional or rare breeds from different countries of the American continent, Caribbean Islands and the Iberian Peninsula. Our results have shown the genetic diversity levels in Iberian and Creole breeds and the genetic relationships among them. The results derived from this project will have a great social impact, as may lead to the official registration of those populations, which are not yet officially recognized as breeds due to a lack of genetic supporting studies, but have an unquestionable productive rule in marginal areas and in a subsistence agriculture context.

P3017 A Single Assay for Genotyping Recessive Red and Dominant Black Alleles in Cattle.

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Some cattle breeders require their animals to be genotyped for coat colour characteristics, the two tests known as “recessive red” and “dominant black”. Reasons for genotyping include conforming to breed standards for registration purposes and the belief that the red coat colour is more heat tolerant than black coat colour and is also less attractive to Buffalo Fly. Three alleles of the Melanocortin 1 Receptor (MC1R) gene are involved in the red/black phenotype. Genotyping was previously performed using two separate RFLP tests (one for the recessive red allele and another for the dominant black allele). A test specific for the wildtype allele is generally not performed and is deduced from the results of both the recessive red and dominant black tests. Below we describe a simple, quick and cost effective assay consisting of a single PCR reaction followed by capillary electrophoresis (CEP) to detect all red and black MC1R alleles.

P3018 Genetic diversity and structure in *Bos taurus* and *Bos indicus* populations analyzed by SNP markers

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The purpose of this study was to assess genetic diversity, phylogenetic relationship and population structure among nine Eurasian cattle populations using 58 single nucleotide polymorphism (SNP) markers. The calculated distribution of minor allele frequencies and heterozygosities suggested that the genetic diversity of *Bos indicus* populations was lower than that of *Bos taurus* populations. Phylogenetic analyses revealed the main divergence between the *Bos taurus* and *Bos indicus* populations, and subsequently between Asian and European populations. By principal components analysis, the *Bos taurus* and *Bos indicus* populations were clearly distinguished with PC1 (61.1%), however, six *Bos taurus* populations clustered loosely and the partial separation between European and Asian groups was observed by PC2 (12.5%). By analysis using STRUCTURE program, distinct separation between *Bos taurus* and *Bos indicus* was shown at K=2, and that between European and Asian populations at K=3. At K=4, 5 and 6, Mongolian population showed an admixture pattern with different ancestry of Asian and European cattle. At K=7, all *Bos taurus* populations showed each cluster with little proportion of admixture. The 58 SNP markers in this study could sufficiently estimate the genetic diversity, relationship and structure for nine Eurasian cattle populations.

P3019 Porcine Endogenous Retrovirus (PERV) Integration in the Pig Genome.

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Porcine endogenous retroviruses (PERV) inherited as proviruses in the pig genome can infect human cells, posing a potential risk of zoonosis in pig-to-human xenotransplantation. Introduction of PERV into the pig genome is relatively recent and integration sites are highly polymorphic. It is predicted that only a limited number of PERV loci are active and can direct production of infectious viruses. Identification of such active PERV loci and determination of their distribution in the pig population would help identify, breed or genetically engineer pigs free from problematic PERV.

We have investigated PERV integration sites in 8 animals by two methods of flanking sequence cloning: splinkerette and LAM-PCR. Examination of about 5,500 clones yielded 544 potential integration sites, full-length provirus and solo LTR inclusive. Distribution of these integrations in different individual pigs and their correlation to PERV infectivity will be discussed.

In parallel, we performed a preliminary search for PERV integration in the pig genome sequence using the Sscrofa9 assembly by the RetroTector program (Sperber et al. NAR 2007;35:4064) as well as manual BLAT/BLAST analyses. About 20 loci were identified to harbour PERV proviruses of more than 7kb length in this Duroc sow genome. The fact that only 4 out of these 20 loci have been found to be shared by a Large White BAC library (Rogel-Gaillard et al. Cytogenet Cell Genet. 1999;85:205-11) highlights the highly polymorphic nature of PERV integration. A more accurate picture of PERV integration is hoped to be realised by analysing the upcoming version 10 assembly.

P3020 CONBIAND network: A cooperative consortium for Latin American bovine breeds biodiversity studies

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The CONBIAND network is a consortium integrated by researchers involved in biodiversity, sustainability and conservation biology. A research group was constituted within this network to investigate the genetic relationships among Iberian and Creole cattle in relation to the colonization history. The main objective is studying the genetic signatures of the Iberian breeds primarily taken to the Americas following the dispersion routes of cattle throughout the territory from the Caribbean Islands and South-Western United States to the Patagonia. European selected and Zebu breeds have also been included here in order to identify their influence in Creole cattle. Nineteen microsatellite markers were analysed in 3333 samples belonging to 81 cattle populations from Spain, Portugal and related Creole populations. The genetic differentiation between populations was moderate with a mean F_{ST} value of 0.11. The AMOVA, Factorial Correspondence Analysis, Neighbor-Net and Structure results showed that the proximity of most of the Creoles to their Iberian ancestors is stronger than their relation to the Zebuines although some Creoles appear to be more admixed with zebu but also commercial breeds. Despite the time frame since the end of the colonization period, the genetic influence of Iberian breeds in Creole cattle are evident. Creole cattle differentiated and became locally adapted and today represent a valuable genetic resource with their own identity.

P3021 DNA barcoding for the identification of freshwater fishes from the São Francisco River basin, Brazil

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Fisheries have evidently declined in recent decades manly because of over fishing, habitat degradation and introduction of non-native species. In the 1970s, landings in the São Francisco River, the fourth largest river system in South America, were around 25 kg/fisherman/day, while in the 1980s they were reduced to about 11 kg/fisherman/day. The São Francisco River fish fauna is composed of at least 200 known neotropical species. Among these, there are several threatened species, some of them endemic from this river basin. Accurate and unambiguous identification of fishes, from eggs to adults and fish products, is important in many areas. It will enable retail substitutions of species to be detected, assist in managing fisheries for long-term sustainability, and improve ecosystem research and conservation. Therefore, we have undertaken an effort to barcode the ichthyofauna of this system as a contribution to FISH-BOL, the campaign to barcode all fishes. We have already collected and identified specimens from one hundred seven species. So far, over four hundred BARCODE sequences have been obtained, representing eight-one species. With a few exceptions, all species could be differentiated by their COI sequence, demonstrating that barcoding can achieve unambiguous species recognition for the species analyzed herein. We expect that this data will be an invaluable tool for fisheries managers and fisheries ecologists.

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P3022 Evaluation of the panel of 14 microsatellites in testing individual identity and parentage verification in Murrah buffaloes raised in Brazil

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The buffaloes were introduced in Brazil in the beginning of the XIX century. Despite of this fact genealogical control is still one of the weaknesses in the Brazilian selection and breeding programs. Considering the importance to develop a system that allows to certify animal genealogy as well as its unequivocal identification, a panel of 14 microsatellite markers (BMC1013, BM1706, BM922, CSSM19, CSSM38, CSSM42, CSSM47, CSSM60, CYP21, INRA26, INRA6, MAF65 and RM4 and D5S2) was used to test 100 Murrah buffaloes. A total of 92 alleles were detected in the whole sample and the number of alleles varied from 1 (locus D5S2) to 13 (locus CSSM47). The PIC values ranged from 0.0 (locus D5S2) to 0.84 (locus BM1706). The probability of exclusion (PE) for the set of 14 microsatellites reached 99.9998%. The performance of this multiplex panel of markers suggests that it will be useful in parentage tests of Brazilian Murrah buffaloes. Considering that this panel is already used in Italy for the Mediterranean buffaloes, one of the four breeds raised in Brazil, the next step will be to check these markers also for Jafarabadi and Carabao.

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P3023 Genetic diversity in Mongolian native horses: Polymorphism of coat-color genes (Extension, Agouti and Brown) in Mongolian native white horses

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Many Mongolian native horses with various coat-colors inhabit the grasslands in Inner Mongolia. Among these semi-wild horses, about 100 heads of white coat horse live as a herd in West-Wuzhumuqin. It is known that the white coat-color of horse is the result of several gene mutations that inhibit the development of melanocytes containing pigment granules. In this study, the 3 basic coat-color genes (Extension [MC1R], Agouti [ASIP], and Brown [TYRP1]) were analyzed to determine their frequency and polymorphisms in Mongolian native white horses.

The horses were classified into 3 groups: dominant white horses (4%); grey horses (88%); and sabino horses (8%). A single nucleotide polymorphism (SNP) (C901T) was found in the second domain region of the MC1R gene and was classified into EE, Ee and ee phenotypes. The gene frequency of *E*-allele was 0.55. A deletion of 11bp was detected in exon 2 of the ASIP gene. The gene frequency of *A*-allele was 0.88. A SNP (C189T) was also found in exon 2 of the TYRP1 gene and was classified into BB and Bb phenotypes. No bb phenotype was found. The gene frequency of *B*-allele was 0.98. The deviation in the Hardy-Weinberg equilibrium was not found between the observed value and the expected value of three coat-color genotypes.

We consider that the Mongolian native white horse has the major coat-color genes, but that their expression is inhibited by the white coat color-related genes.

P3024 Population structure of Japanese Black, a major cattle breed for meat production in Japan

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Assessment of genetic diversity in livestock is important for retaining a sufficient genetic pool for future improvements and to avoid inbreeding depression. Although the inference of population structure is essential for the assessment of genetic diversity, the population structure of Japanese Black, a major cattle breed for meat production in Japan, has not undergone investigations utilizing molecular markers. Thus, we inferred the population structure of 427 bulls of the breed that had been genotyped with 31 microsatellite markers. A Bayesian clustering approach with a Dirichlet process prior was used to infer the number of subpopulations and the assignment of individuals into the subpopulations simultaneously. This method has been developed based on the algorithms previously proposed by Pella and Masuda (2006) and Huelsenbeck and Andolfatto (2007). As a result, five subpopulations were detected from the bulls. The number of individuals and the mean observed heterozygosity in each subpopulation ranged from 9 to 179 and from 0.568 to 0.723. The pairwise Fst values between each subpopulation ranged from 0.032 to 0.179. For a half of the combinations, the values were above 0.1. These results show that Japanese Black is divided into subpopulations that are well differentiated from each other in spite of its relatively small population size. This fact implies the possibility of a loss of genetic diversity within each subpopulation due to genetic drift.

P3025 Genetic relationships of North East Argentina Creole pig populations with other American Creole, Iberian and exotic pig breeds

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A set of 24 STR markers was analyzed in 892 pigs belonging to 2 Creole pig populations from the Wet and Dry areas within the North East Argentinean Region. American Creoles, Iberian, Chato Murciano, Negro Canario, Duroc, Large White and Landrace breeds were introduced in the study in order to establish the genetic relationships with the Argentinean Creoles. The NJ tree constructed with D_A distance and the Multiple Correspondence Analysis demonstrated that the six Creole populations grouped in the same cluster, Iberian occupied a second cluster and the last one separated the rest of the breeds. The proportion of mixed ancestry in the populations was further evaluated with the Bayesian clustering algorithm implemented by the Structure v.2.1 software. Creole populations, including the Argentinean ones, grouped in a unique cluster when K =5. From K=6 to K=16 the Creoles grouped in different clusters, although they did not share cluster with no Creole breeds. These results suggest that Creole pigs constitute a different group do not necessarily composed of a genotype with a greater proportion of any of the supposed ancestral breeds. This work has been developed within the CONBIAND network, a consortium integrated by researchers involved in biodiversity, sustainability and conservation biology.

P3026 Study on frequency and expression of coat colour genes of Mongolian horse

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The Mongolian horse with an individual origin is one of the most ancient breeds in the world and it has various colours which make it an ideal model animal for the research of equine coat colour genetics.

In this research, partial sequences of melanocortin-1-receptor (*MC1R*), Agouti-signaling-protein (*ASIP*) and tyrosinase related protein 1 (*TYRP1*) have been cloned and sequenced with techniques of molecular biology. 334 horses (non-gray, non white) of 3 breeds which included the Mongolian horse (5 groups), Sanhe horse and Thoroughbred were selected. One mutation in exon of *MC1R* (C901T) of the chestnut horse was detected by PCR-RFLP, and there is no difference in the sequence from that of the reported chestnut allele. The 11 bp deletion in exon2 of *ASIP* was detected by PCR-AFLP. The deletion was found homozygous and completely associated with recessive black of the horse. A substitution was detected in exon2 (C189T) in *TYRP1*, which causes a change of threonine to methionine and the function of which is yet unknown. In addition, to quantitatively detect the expression level of *MC1R*, *ASIP* and *TYRP1* in various coat colours with real-time fluorescence quantitative PCR(RTQ-PCR), skin, liver, muscle, hypophysis and some other related tissues were collected from different coat colour horses and total RNA from these tissues were extracted. In this result, the relationship between the *MC1R*, *ASIP*, *TYRP1*genes expressions and the phenotype of equine coat colour was observed. Thus, a systematical and complete comprehension of coat colour genetics can be obtained.

P3027 Mapping Mendelian inheritance in traditional chicken breeds by whole genome SNP genotyping

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Centuries of human driven selection of domestic animals have led to a large number of diverse phenotypes. With hundreds of breeds and varieties that are defined by morphological and production traits, the domestic chicken represents a unique resource for mapping genetic traits in birds. The availability of the chicken genome, high throughput genotyping and sequencing technologies offers the opportunity to unravel the genetic control of quantitative and Mendelian traits within the species. Here, we evaluate the 60K Illumina iSelect™ chicken BeadChip as a mapping tool for Mendelian traits across traditional chicken breeds. We confirm the yellow skin (Eriksson *et al.* 2008) and the hyperpigmentation (Dorshorst *et al.* 2010) chromosomal regions using a small number (n = 30) of unrelated birds of known phenotypes. Some current limitations of the 60K Illumina iSelect™ chicken BeadChip are identified. The results highlight the usefulness of this approach for the mapping of new traits, and the unique value of the phenotypic diversity of traditional breeds and their breeding history for the understanding of the genetic control of Mendelian traits in chickens.

P3028 Complete mitochondrial genome sequence of the dromedary camel (*Camelus dromedarius*)

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Here we report the complete mitochondrial sequences of dromedary camel. The length of mt-DNA sequence was determined as 16,643 bp and the number and order of genes were found to be the same as the common vertebrates. This, however, is not absolute due to the presence of pronounced heteroplasmy caused by variable numbers of the tandem repeat units CACGTA, which was detected between CSB-I and II in the 3' portion of the control region. On the whole, the structure of dromedary mitogenome is similar to that of the alpaca and Bactrian camel. However, comparing the 13 peptide-coding genes of alpaca and Bactrian with that of dromedary suggests that it is the GTG not the ATG that serves as the *ATPase6* gene start codon in dromedary. The tandem repeats ranged from 42 to 60. Interestingly, *ATPase6* and *NADH* genes showed more polymorphism than the D-loop suggesting a better region for phylogenetic studies in camelidae. The dromedary mitogenome information should shed more light on evolutionary history of camelidea.

P3029 Characterization and validation of bovine Gonadotripin releasing hormone receptor (GnRHr) polymorphisms

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Gonadotropin releasing hormone and its receptor (GnRHr) play a critical role in sexual differentiation and reproduction. Available evidence shows a strong genetic component in the timing of puberty. In bovines, there are significant differences within and among beef breeds in the time when bulls reach puberty. Despite its economic importance, there are not many SNPs or genetic markers associated with this characteristic. The aims of the study were to identify DNA polymorphism in the bovine GnRHr by re-sequencing analysis, determine haplotype phases, and perform a population study in a selected tag SNP in 6 breeds. Eight SNPs were detected, including: one in the URR, five in the coding regions, and two in non-coding regions. This polymorphism level corresponds to one variant every 249.4 bp and a global nucleotide diversity of 0.385. Two haplogroups comprising nine haplotypes and two linkage blocks were detected. Despite 5 tag SNPs were required to capture all variability, just one SNP allowed to define both haplogroups, and only two SNPs were needed to differentiate the most common haplotypes. An additional taq SNP was necessary to identify both URR variants. Allele-frequency analysis of a selected taq SNP among breeds showed a geographical cline. European *Bos taurus* breeds had lower frequencies of the C allele than *B. indicus* type cattle, while Creole cattle and Wagyu breeds had intermediate frequency. There was a significant correlation between frequency profile and timing of puberty among the studied breeds, which seems to suggest that genetic variation within bovine GnRHr gene could explain at least part of the reported variability.

P3030 SNP detection and prediction of variability between chicken lines using genome resequencing of DNA pools

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The next-generation sequencing technologies are widely used for detection of millions of SNPs, which provide means for prediction of their variability in order to compose subsets of highly informative SNPs for region-specific or genome-wide analysis. In this study, we investigated the SNP identification sensitivity and the possibilities to predict SNP-informativity based on ~5X genome resequencing using ABI Solid technology and DNA pools from two chicken lines divergently selected for growth. Therefore, we compared resequencing results with allele frequency data obtained by 60K SNP chip genotyping with 31,363 SNPs, which showed variation within or between the two chicken lines. With three non-reference reads as detection threshold the resequencing detected 48% of these SNPs including ~67% of the SNPs with a non-reference allele frequency > 0.9. The variation between chicken lines based on 60K chip analysis was positively correlated with the informativity between lines computed from all SNP detection read scorings in flanking regions. This was tested for different sizes of flanking regions and the genome-wide correlation was strongest (Pearson $r = 0.51$; $P < 10^{-15}$) when their total size was 62 kb. However, for the microchromosomes this interval size was considerably shorter (38 kb) and the correlation weaker (Pearson $r = 0.45$; $P < 10^{-15}$), probably due to higher recombination rate. We suggest that the interval size showing maximum correlation approximately corresponds to the average LD block size and that the described computational analysis can provide a valuable indication of the expected SNP-variability between resequenced populations.

P3031 Genome-wide Molecular Characterisation of Indigenous Zebu Cattle of Western Kenya

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Indigenous zebu cattle in Kenya are predominant across the country owing to their arid-adapted physiology and disease tolerance traits. These breeds have been embraced by most small-scale farmers, who are the drivers of the low-input sustainable agricultural sector within the country. Previous studies using autosomal microsatellite markers have described indigenous East African zebu as admixed and genetically distinct from pure taurine and pure indicine breeds. There is however a growing concern that the genetic erosion of these indigenous populations through unregulated breed improvement programmes, introduction of exotic germplasm via indiscriminate crossbreeding and increased inbreeding may result in the loss of alleles of economic importance. The aim of the study was to establish the population genetic structure of indigenous East African shorthorn zebu in Western Kenya. The study is part of the Wellcome Trust funded multidisciplinary and multi-institutional "Infectious Diseases of East African Livestock (IDEAL)" project. The study area represents a unique juxtaposition of 4 different agro-ecological zones within which 20 sub-locations were randomly selected and a total of 552 calf blood samples collected. Extracted DNA from all the calf samples were genotyped using the Illumina® bovineSNP50 beadchip. Population structure including genetic diversity and relationships within and amongst animals, sub-locations and agro-ecological zones will be presented as well as the level of zebu, African and European taurine admixture across the study population.

P3032 Allele frequencies of gene polymorphisms related to economic traits in *Bos taurus* and *Bos indicus* cattle breeds

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In the last decade, the advance of molecular biology techniques has innovated animal breeding and selection schemes with genomic data utilizable for marker/gene-assisted selection. In cattle, several gene polymorphisms have been reported to be associated with beef traits in *EDG1, GH, RORC, AKIRIN2, FASN, SREBP1, SCD* and *CAPN1* and to be related to lactation traits in *DGAT1, OLR1, ABCG-2, GHR* and *Pit1*. The aim of present study was to investigate allele frequencies of these 13 gene polymorphisms using a total of 240 animals from *Bos taurus* and *Bos indicus* breeds including two Japanese groups (Japanese Black and Japanese Brown), two East Asian groups (Korean and Mongolian), three European groups (Holstein, Angus and Hereford) and a *Bos indicus* group in South Asia (Myanmar, Laos and Cambodia). In current study, the alleles with favourable effects on commercial traits in each polymorphism were shown with superscript f. For example, the favourable allele of *FASN* polymorphism was described as FASN^{f}. Genotyping results revealed that EDG1^{f} frequency was relatively high in Japanese cattle (0.383-0.483), intermediate in East Asian cattle (0.250-0.133) and low in European cattle (0.000-0.017). High GH^{f} frequency was observed in Japanese Black (0.517), while the other indicated low level frequency (0.00-0.200). *Bos taurus* groups showed higher frequency of SCD^{f} (0.583-0.833) than *Bos indicus* group (0.267). In the DGAT-1^{f} associated with increase in milk fat content, *Bos taurus* groups had a wide range of frequencies (0.000-0.717), while *Bos indicus* group showed the highest frequency (0.950).

P3033 DNA sequences of milk protein genes in *Equus asinus*

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During the last few years growing attention has been given to donkey breeding addressed to milk production. In popular tradition, as well as in many recent clinical trials, donkey milk was proved to be a valid alternative to cow milk in both IgE-mediated and non IgE-mediated cow's milk protein allergy. These properties still need to be demonstrated at a scientific level. The research is focusing on milk protein fraction composition. In donkey milk, nitrogen content and casein/whey proteins ratio are very low (1.56-1,80% and 0.79-1.45 respectively) and quite different from those reported in cow milk. Recently, an interesting phenotypic variability has been reported in individual milk samples belonging to Ragusano breed and resulting in four different Isoelectrophoretic patterns. In particular, two rare defective patterns, characterized by the apparent absence of the α_{s1} casein and of β-lg II, respectively, were identified. This heterogeneity might affect milk protein composition and, potentially, the allergenic properties of donkey milk. In Italy within a national project called "SELMOL", funded by the Italian Ministry of Agriculture (2007-2010) a research sub-unit is carrying out the sequence analysis of the main milk protein genes. DNA sequences are now available for donkey CSN1S1, CSN1S2, CSN2 and BLKG II genes (GenBank accession numbers: FN386610, FM946022, FN598778 and HM012799-800 respectively).

The aim of this study is to identify molecular markers to be exploited in breeding programmes, in order to improve and qualify donkey milk production for human health.

P3034 Population Structure and Genetic Diversity of Himalayan and Sri Lankan Pigs

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Indigenous pigs are important to poor communities in Sri Lanka, Bhutan and Nepal. Their hardiness and disease resistance suit low input environments. However, their rapidly declining populations require conservation for sustainable utilization. Their genetic structure and diversity were investigated using 21 FAO/ISAG microsatellites markers, revealing four domestic and one wild boar populations in Bhutan, two domestic pig populations in Nepal, and clearly segregated populations of village pigs and wild boar in Sri Lanka. All populations showed equal or higher expected heterozygosities than Australian composite breed commercial pigs. The average F_{ST} across all loci among Bhutanese, Nepalese, and Sri Lanka pig populations were 0.09 (SE 0.01), 0.07 (SE 01), and 0.14 (SE 0.02) respectively. There was negligible genetic differentiation between one Bhutanese and one Nepalese population, consistent with illegal cross-border live-pig trade, although overall Bhutanese and Nepalese domestic pigs are closely related. Surprisingly, the Sri Lankan village pigs clustered with Australian commercial pigs implying substantial contamination by European pig breeds. Mitochondrial DNA sequences are currently being analyzed from these regions to further analyse their biodiversity and contribute to their conservation.

P3035 Genetic characterization of the Sobrarbe chicken breed from Spain

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The Sobrarbe chicken is a traditional poultry population from Aragon (Northern Spain). The Breeders Association (AGASOB) is devoted to the recovery and to the characterization of these birds. Here, we report the first molecular genetics study on these chickens. We studied 96 individuals from several farms for 16 microsatellite markers, chosen from the FAO/MODAD set (MCW0069, LEI0094, MCW0295, MCW0216, MCW0034, MCW0111, MCW0016, MCW0330, MCW0037, MCW0078, MCW0067, MCW0098, MCW0123, MCW0183, MCW0165, and ADL0112). The number of alleles ranged 3 (MCW0216, MCW0165) to 8 (MCW0034). The mean number of alleles per locus was 4.940. PIC values ranged 0.135 (MCW0098) to 0.595 (MCW0069). The mean PIC value was 0.453.Total exclusionary powers were 0.931 (first parent) and 0.996 (second parent). From multilocus estimations, the expected heterozygosity was 0.508 ± 0.169 and the observed heterozygosity was 0.435 ± 0.168. We found a global significant F_{IS} value (0.144; $p<0.01$; IC 95%: 0.096-0.184). Closed reproduction systems based up on different isolated farms could explain for this result. We did not find any signs of genetic bottleneck.

P3036 Information content in genome-wide scans: concordance between diversity data and phenotypic association patterns

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Scanning the genome with high density SNP markers has become a standard approach for identifying regions of the genome showing substantial between-population genetic differentiation, and thus evidence of diversifying selection. Such regions may contain genes of large phenotypic effect. However, few studies have attempted to address the power or efficacy of such an approach. In this study, the patterns of allele frequency differences between two cattle breeds based on the Bovine HapMap study were compared with statistical evidence for QTL based on a linkage mapping study of an experimental population formed by a cross between the same breeds. Concordance between the two datasets was seen for chromosomes carrying QTL with strong statistical support, such as BTA5 and BTA18, which carry genes associated with coat color. For these chromosomes, there was a correspondence between the strength of the QTL signal along the chromosome and the degree of genetic differentiation between breeds. However, such an association was not seen in a broader comparison that also included chromosomes carrying QTL with lower significance levels. In addition, other chromosomal regions with substantial QTL effects did not include markers showing strong between-breed genetic differentiation. Furthermore, the overall consistency between the two studies was weak, with low genome-wide correlation between the statistical values obtained in the linkage mapping study and between-breed genetic differentiation from the HapMap study. These results suggest that genomic diversity scans may be limited in their power to detect regions that are associated with quantitative phenotypic differences between populations.

P3037 Polymorphism of the porcine *Tribbles 1 Drosophila homolog* (*TRIB1*) gene and its association with lipid metabolism traits

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The *Tribbles 1 drosophila homolog* (*TRIB 1*) gene belongs to the Tribbles genetic family that is mainly involved in the regulation of diverse cell signalling pathways. In pigs, this gene is located within the confidence interval of a SSC4 QTL affecting serum triglycerides concentration in an outbred Duroc population. Interestingly, performance of a whole-genome scan in human evidenced that variability at the human *TRIB1* locus is associated with plasma triglyceride concentration. In the present work, we have undertaken the molecular characterization of the pig *TRIB1* gene. With this aim, we have amplified 2.2 kb of the pig *TRIB1* locus in eight Duroc pigs. This fragment encompassed 75% of the coding region as well as a fragment of the 3´UTR. Analysis of the inferred amino acid sequence with Scan Prosite allowed us to detect a protein kinase domain. Moreover, we have identified a 2-bp indel polymorphism in the 3´UTR region. This insertion has been genotyped by primer extension analysis in the aforementioned Duroc population. A preliminary association analysis revealed that the *TRIB1* genotype is associated with serum triglyceride, total cholesterol and HDL-cholesterol concentrations. Additionally, a significant association between *TRIB1* genotype and saturated fatty acid content of the *gluteus medius* was observed. Least squared means revealed that homozygote individuals carrying two copies of the insertion showed increased levels of both serum lipids and muscle saturated fatty acids percentage. Sequencing of the *TRIB1* promoter region to uncover additional polymorphisms is currently underway.

P3038 Evolutionary history of indigenous Arabian Peninsula camel *Camelus dromedarius* populations

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Domestic one-humped or Arabian camels *Camelus dromedarius* are a major livestock genetic resource for the Arabian Peninsula. At least ten breeds are recognized, although the genetic status of these populations remains largely unknown. Five hundred and seventy-one blood and hair samples were collected from unrelated camels representing all common camel types in the Arabian Peninsula. The aim of this study is to (i) unravel the genetic history of domestication of dromedary camels and subsequent dispersion across the Arabian Peninsula, (ii) unravel the breed/population history through the study of their genetic relationships and diversity, and (iii) understand the local adaptation of dromedary to their environments. In order to achieve these objectives analysis of sequences variation of mitochondrial DNA control region, genotype data of microsatellite loci and polymorphism at candidate coat colour genes will be performed. Software for population genetic analyses of molecular marker data will be used to estimate various diversity measures and genetic distances, and infer population structure and clustering patterns. The results obtained will provide evolutionary insights on the history and local adaptation of the Arabian Peninsula dromedary and contribute to the design of breeding strategies for the conservation of dromedary genetic diversity and the improvement of their productivities.

P3039 Genetic structure and phylogeography of European sheep

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Sheep has played a fundamental role in the production of meat, milk, hides and wool since the Neolithic period. Domestication of wild sheep occurred about 11,000 years ago in a restricted area of south-western Asia, corresponding to modern day Iran, Turkey, and Cyprus. Domestic sheep quickly spread west following human migrations. Diffusion routes, during the following millennia, ran from Middle East through the Balkans, across the Danube valley and along the Mediterranean coast. For both geographical and historical reasons Italy, being a migration route from the Neolithic to the Middle Age, has been a crucial zone for the distribution and selection of several sheep breeds.

The aim of our research was to use mitochondrial DNA sequences to shed light on management and migrations that led to the formation of modern breeds. We sequenced a total of 1.65×10^6 bp of D-loop from 10 European mouflon (*Ovis musimon*) and 1198 sheep (*Ovis aries*) samples belonging to 57 breeds from the Mediterranean and eastern Europe, including domestication and migration areas. Since phylogenetic studies on Italian sheep are scarce, particular attention was paid to Italian breeds.

More than 270 haplotypes were identified and clustered into maternal haplogroups constructing a phylogenetic tree. Most individuals were clustered in three principal haplogroups. Samples originating from Middle East and Turkey showed a higher genetic diversity as compared to European ones, which were mostly grouped in a single haplogroup. Preliminary results suggest that haplotype diversity is mainly distributed along ancient migration routes.

P3040 Microsatellite DNA polymorphism in red deer (*Cervus elaphus*) in Poland

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DNA microsatellites play a major role in population genetics, linkage mapping, and parentage studies of mammals. In addition, they may be used for forensic purposes, if an individual identification of a specific animal is necessary.

We attempted to develop a multiplex of microsatellite markers for the analysis of genetic diversity of red deer (*Cervus elaphus*) and use it for individual identification.

We tested 45 microsatellites specific for cattle (23 loci), sheep (14 loci), reindeer (4 loci) and wapiti (4 loci) for polymorphism in red deer. DNA amplification products were obtained from 32 loci of which 26 were polymorphic. Twelve of these highly polymorphic microsatellites were selected for further analysis. The following STR were determined: BM1818, OarAE129, OarFCB5, OarFCB304, RM188, RT1, RT13, T26, T156, T193, T501 and TGLA53.

We used DNA extracted from 412 tissue samples taken from wild red deer from different location in Southern Poland. DNA extracts were amplified by PCR for the 12 microsatellites and AMELgene (FJ946990) 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*).

The number of alleles ranged from 10 (OarFCB304) to 22 (TGLA53), the average expected heterozygosity (He) and observed (Ho) were He=0.8583, Ho=0.7406 and average positive inbreeding coefficients Fis=0,1366. The average polymorphism information content (PIC) and power of discrimination (PD) were 0.8435 and 0.9581, respectively. The combined power of discrimination values for all 12 loci reached value high as 0.9999999. The cumulative probabilities of parentage exclusion, with no know parents and one known parent (PEc_1 and PEc_2 respectively) were 0.999976 and 0.999999.

Our results show the possibility to use microsatellite polymorphism in the identification of red deer in forensic applications like poaching.

P3041 Breed Discrimination in chicken using Mitochondrial DNA sequence and MHC polymorphisms

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Korean native chicken has been developed to the meat type chicken by crossing with other chicken breeds. In order to determine the genetic diversity and breed discrimination among chicken breeds, mitochondrial (mt) DNA D-loop sequences and polymorphisms in major histocompatibility complex (MHC) region were investigated. A total of 693 birds were considered for this study, 336 mtDNA sequences were obtained. Two of the commercial Korean native chicken breeds (357 birds) were used for investigation of genetic relationships and breed differentiations. The sequence data indicated that 19 haplotypes were observed and the largest number of birds was represented in haplotype 1. Analysis of haplotypes in Neighbor-joining phylogenetic tree indicates that the genetic diversity and relation among the breeds. The three mtDNA polymorphisms were also used for breed identification. The discrimination rate of the two Korean native chicken populations using mtDNA TAT haplotype gives more than 70 % indicating that these markers can be used for the breed identification. While, the investigation of MHC polymorphism gives 13 different alleles which can also be used for breed identification markers. The results obtained in this study can be used for designing breeding and conservation strategies and also development of breed identification markers.

P3042 Development of a tetranucleotide microsatellite-based parentage test in sheep.

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In many domestic animal species, dinucleotide microsatellites have been used for the routine determination of parentage. In humans, the use of dinucleotide microsatellites in parentage testing was discontinued largely due to the interpretational difficulties often associated with amplification artefacts such as “stuttering” and nontemplated additions. In humans, tetranucleotide and pentanucleotide microsatellites were identified as an alternative with a reduced predisposition for “stuttering” and alleles that could be reliably and reproducibly determined. These types of microsatellites currently form the international standard for human parentage and forensic analyses. The identification and use of these tetranucleotide microsatellites in sheep has been hampered by their relative rarity in the ungulate genome. Despite this, we have identified tetranucleotide microsatellites in sheep, initially through the selective capture of segments of DNA containing the tetranucleotide repeat motifs but subsequently using the BAC end sequences deposited in Genbank. We have identified 24 polymorphic loci, of which twelve were developed into two six-plex PCR reactions. An analysis of 229 dams and sires from a Dohne sheep population showed that the number of alleles for each locus varied from 4 to19 with a PIC ranging from 0.53 to 0.87. The combined power of exclusion was calculated at 0.00018 and a typical PI of 25,346. It is anticipated that a 12-plex will eventually be developed. Currently, an additional 4-plex plus a Y chromosome marker is under development. These systems will form a cost effective means of parentage testing in sheep.

P3043 FAO Guidelines on Molecular Genetic Characterization of Animal Genetic Resources

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Member countries of the FAO adopted the Global Plan of Action (GPA) for Animal Genetics Resources (AnGR) in September 2007. The GPA outlines priorities and actions to be taken to improve the global management of AnGR globally. The GPA stresses the role of FAO in facilitating the implementation of the GPA. Therefore, FAO is producing a revised and expanded set of guidelines for the management of AnGR, including guidelines for molecular genetic characterization. Characterization is one of the first activities to be undertaken in the management of AnGR, as it allows one to understand the diversity and value of a particular AnGR. Such knowledge is critical for decision-making in breed development, utilization and conservation. FAO collaborates closely with ISAG in order to develop and update guidelines for molecular genetic characterization with additional contributions of the participants of the EU GLOBALDIV project. The guidelines from 2004 included panels of microsatellite markers for ten livestock species. However, technologies for genomic analysis have advanced dramatically in the past six years. The new guidelines reaffirm the utility of the existing microsatellite panels, particularly for species of secondary importance in industrial agricultural and for meta-analysis of existing data, but also call for the adoption of the most advanced and informative technologies. The need for research on new standards and protocols for using high-throughput and next-generation sequencing technologies is strongly recommended. Finally, the guidelines describe how results from molecular characterization may be considered in breed conservation and utilization decisions.

P3044 Mitochondrial D-loop DNA sequence variation among Arabian horses

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Specific insight into maternal lineages within breeds is provided by two particular properties of the mitochondrial DNA (mtDNA): The maternal inheritance of the mtDNA molecule and the hypervariability of the mtDNA D-loop region. In this study the mtDNA D-loop of 41 purebred Arabian horses including a group of 26 Straight Egyptians is analyzed. By studbook information these 41 horses are related to 15 maternal lineages. Aim of this study is to check the reliability of pedigree data in dam lines of purebred Arabian horses as well as similarities and differences between purebred Arabian horses and Straight Egyptians. Sequence analysis of a 446 bp mtDNA D-loop fragment revealed 13 different haplotypes. Compared to the 446 bp mtDNA sequence (GenBank X79547, positions 15402 – 15847) in total 26 variable sites are observed (positions 15485, 15495, 15538, 15542, 15585, 15597, 15601, 15602, 15604, 15635, 15650, 15666, 15667, 15703, 15709, 15720, 15726, 15740, 15771, 15777, 15806, 15809, 15811, 15826, 15827 and 15838). The most frequent haplotype is observed in 11 animals. Interestingly this is the only haplotype appearing in both purebred Arabians as well as the subgroup of the Straight Egyptians reflecting a common maternal ancestry in at least one case. From the remaining 12 haplotypes 6 belong to the Straight Egyptians and the other 6 to the purebred Arabians. In most cases the mtDNA haplotypes are in agreement with stud book information. Nevertheless the mtDNA haplotypes from four animals differ from their maternal lineages.

P3045 Epistatic genetic interaction of QTL pairs related to growth traits in a porcine Duroc x Pietrain population

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In this study, epistatic QTL analysis was performed to investigate the impact of epistasis on growth traits. Genotypic data of a 3 generation full-sib design of a Duroc x Pietrain cross comprising 127 markers over all 18 autosomes were analysed. Phenotypic information of 9 different growth traits, recorded in different fattening stages of 330 F2 animals were available. The QTL analysis based on comparisons of different models suggested by Estelle et al. (2008) was performed with the software package Qxpak. The QTL and epistatic QTL detections revealed 16 single QTL and 21 significant interactions between different chromosomes. Each pair accounted 4 to 9% of the phenotypic variance. All types of genetic epistatic effects were identified. However, the dominant x dominant effect and additive x dominant effect were most prevalent. For the trait average daily gain from 60 to 90 kg live weight 7 significant epistatic QTL pairs were detected. Among these five interacting QTL pairs belong to SSC2 with SSC4, SSC7, SSC8, SSC10 and SSC17. In this region, on SSC2, the INSR gene is located, which plays a role in the inhibition of the IGF1. Another interesting epistatic interaction was found between SSC5 and SSC15 for average daily gain from birth to weaning, where 9% of the phenotypic variance was explained. The findings of this study demonstrate the importance of considering epistatic relationships of chromosomal regions.

P3046 Investigation and validation of SNP in swine candidate genes for meat quality

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Seven swine candidate genes for meat quality were investigated in order to identify informative SNPs. Molecular analyses were performed on twenty-two animals representing the extreme tails of the Gaussian distribution for three selected phenotypes (muscle compactness, fat thickness and the principal component) of 240 Large White and Landrace individuals.

Among the 23 identified SNPs, 2 SNPs in the CRADD gene, 2 SNPs in the PTPRD gene and 1 SNP in the PIK3R2 gene were validated in the whole population and association analysis between genotypes and selected phenotypes was performed.

These SNPs were subsequently tested in another group of 560 Italian Large White animals with extreme EBVs (Estimated Breeding Value) for fat thickness and four of them were validated in this population. Because of the important function of PIK3 in metabolic pathways involved in muscle growth, the SNP found in PIK3R2 gene was tested on 600 samples of three different Italian breeds (Large White, Duroc, Landrace) obtained from the National Association of Pig Breeders of Italy. Within each breed, the 100 individuals with the highest and the 100 individuals with the lowest values for average daily gain were analyzed and this SNP resulted polymorphic in each breed. The further step will be to verify the association between these SNPs and fat related phenotypes, thigh weight and feed conversion index.

P3047 Genetic structure of two pony horse breeds based on microsatellite DNA analysis

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The study is monitoring the genetic structure of different pony population. Genetic research is aimed at the two ponies breed: Welsh Pony (WBP, n=32) and Shetland Pony (SHP, n=31). Hair samples were collected from 63 horses. The animals were genotyped for 17 microsatellites markers (*AHT4, AHT5, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, VHL20, ASB2, ASB17, ASB23, CA425* and *LEX3*) recommended by International Society of Animal Genetics. The number of allele per each locus ranged from 4 (*HTG7* and *HMS3*) to 12 (*ASB17*) with a mean of 7.5 alleles for WBP and 6.4 for SHP. The allele frequencies, observed and expected heterozygosity, polymorphism information content, exclusion probabilities and combined exclusion probabilities were calculated. The alleles found with the highest frequency across both tested ponies breeds were as follows: *HMS1* – allele *M, HMS2* – allele *K, HMS7* – allele *L, HTG4* – allele *M, HTG10* – allele *O* and *HTG6* – allele *O*. The highest heterozygosity was observed for locus *AHT5, HTG10, ASB2, ASB17, ASB23, CA425* and *LEX3* – over 0.75 in all breeds. The lowest value was determined for locus *HTG4* in SHP (0.50) and WBP (0.66). The probabilities of paternity exclusion/one parental genotype unavailable/and parentage exclusion were in Shetland Pony 99.99%/99.92%/99.99% and Welsh Pony 99.99%/99.99%/99.99%. The results have revealed that all pony breeds have quite a high genetic variability, as shown by the allele number and heterozygosity level. *This work was supported by the Ministry of Agriculture of the Czech Republic – project no. QH92277.*

P3048 Mitochondrial DNA D-loop sequence variation among clusters of diverse chicken populations

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Since domestication, chickens have been distributed to various countries, continents and cultures. As a result of many generations of adaptation and selection, a wide range of chicken breeds exists today. Numerous studies, using microsatellites, have shed light on the extent of diversity of chicken breeds worldwide. In this study, microsatellite data generated in different projects were jointly analyzed within the framework of the EC project GLOBALDIV (AGRI GEN RES067 under Council Regulation (EC) No 870/2004). The genetic structure of 85 chicken populations, originating from various continents and management systems, was assessed by cluster analysis as implemented in the software STRUCTURE. The results suggested a grouping of populations into three main clusters: cluster C1 – Asian populations, cluster C2 – South-East European and African populations, and cluster C3 – North and Central European populations. From these clusters we selected a subset 640 individuals (C1 – 17 populations including 3 Red Jungle fowl populations; C2 – 20 populations; C3 – 25 populations) to analyze genetic relationships based on mitochondrial DNA sequence polymorphism. As a reference, a skeleton of haplotypes most frequently reported in the literature was constructed. This skeleton was used to assign clades in our study to possible regions of domestication. A median joining network was constructed using both mtDNA sequences taken from Genbank (reference sequences) and sequences generated in this project. Analysis of mtDNA polymorphism revealed that most of the populations of C3 (European cluster) belonged to a single mtDNA clade which may have its roots in India.

P3049 Fixed deletions in divergent chicken lines revealed through whole-genome resequencing

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We examined the whole genomes of two lines of meat-type chickens, divergently selected for high (HWS) and low (LWS) body weight at eight weeks of age. This weight selection has also impacted on the variant lines' feed consumption, reproduction, metabolism, endocrine and immunological responses. After aligning the massively parallel sequencing data to the reference red junglefowl sequence, we scanned for deletions fixed within or between lines. Deletions were defined as a region within a line which had no read coverage for at least 100bp, but which did have coverage in the reference bird. One of the largest events spans 18kb and removes the majority of *SH3RF2* (SH3 domain containing ring finger 2). This deletion was fixed in HWS, occurred at low frequency in LWS, and lies within a QTL for body weight previously identified in an intercross between these lines. The role of *SH3RF2* in humans and animals is unknown, however analysis of the deletion in 400 HWS x LWS F8 birds showed a highly significant association between the presence of the deletion and increased growth (Del/Del = 600g, Wt/Wt = 500g at 70 days; $p<0.001$). We showed that *SH3RF2* was ubiquitously expressed in chicken, but with 10 fold increased expression in the brain. Notably, hypothalamic expression of *SH3RF2* observed in LWS was absent in the deleted HWS, congruent with a known genetic defect in hypothalamic appetite regulation displayed by this line. The exploration of *SH3RF2* is ongoing and inroads into expression and possible functional significance of this gene will be discussed.

P3050 Investigation of genetic distance and heterosis effect in rabbit crossing using microsatellite markers

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In animal breeding there is a demand to raise specific lines and found commercial populations. One way of specialization would be to breed for good combining ability, which is based on heterosis effect. The prognosis of heterosis effect comes from cross breeding of closed, specially selected populations can be important in rabbit breeding too. Hypothetical basic is, that the heterozygote state and sufficient allele combination are more advantageous to express quantitative traits than the homozygozity on the same locus. Degree of expected heterozygosity can be closely related to the genetic distance, which is directly proportional to different alleles on the same loci.

The aim of this study was to assess whether the heterosis effect is predictable or not. We expected that the high genetic distance between rabbit parents meaning big differentiation in the gene pool causes higher level of heterosis effect in their progenies. 101 individuals were genotyped using 13 microsatellite markers to get information about genetic distance. At each mating we correlated the genetic distances to some traits related to reproductivity. Our results showed that genetic distances of the parents based on 54 alleles do not have any correlation to the monitored traits of the progenies.

In conclusion, genetic distance on individual level can not prognose the heterosis effect. It may be hypothesized, that there are some areas of the genome which are particularly important in a quantitative trait, or the heterosis effect is not the result of allele combinations, but other interactions between genes, or epigenetic effects.

P3051 Positive selection and gene conversion following gene duplication in the vertebrate TLR1-gene family

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TLR1-family plays a crucial role in innate immunity by recognizing pathogen associated molecular patterns (PAMPs). In mammals, TLR1/2, TLR2/6 and TLR2/10 heterodimers or homodimers of TLR10 perform this function. In birds, TLR1-family consists of TLR1A/B and TLR2A/B. We analyzed the evolution of TLR1-family in vertebrates using sequences from 4 birds, 28 mammals, 1 amphibian and 1 fish. Detailed analysis resolved a complex evolutionary history of gene duplication and gene conversion events. Gene conversion in C-terminus of TLR1A/B and TLR1/6 produced paralogs more similar than their orthologs. Similarly, gene conversion was found in both the N and C termini of TLR2A/B. The phylogenetic tree of the TLR1-subfamily suggested gene duplication 359 MY, which gave rise to TLR1A and TLR10, and their paralogs, TLR1B and an ancestor of TLR1 and 6. Gene duplication in mammals 270-282 MY produced the paralogs TLR1 and 6. Similarly, phylogenetic analysis shows TLR2A to be orthologous to TLR2 in mammals. These genes shared a common ancestor with TLR2B, 431-356MY. In mammals, a putative pseudogene was found orthologous to avian TLR2B. A series of likelihood ratio tests suggested positively selected sites were distributed throughout the molecules of TLR1-family proteins in mammalian. In birds, sufficient data was only available for TLR2A/B, where positively selected sites clustered in the central domain defined as the ligand binding region that recognizes PAMPs. Therefore combining phylogenetic and structural analyses of vertebrate TLR1-family genes has provided clues to the function of these molecules and how they provide defence against invading pathogens.

P3052 Genetic Diversity and Selection within Rambouillet and Gulf Coast Native Breeds

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Animal husbandry combined with genetic selection has been used to breed domestic animals which display specialised production characteristics. The Rambouillet is a sheep breed selected for the production of wool and its maternal ability, while the Gulf Coast Native is adapted to tropical conditions and has demonstrated resistance to intestinal parasites. In this study, approximately 100 animals from each of these two breeds were genotyped using the ovine SNP50 BeadChip in order to examine genetic diversity and the impact of selection on genome variation. Analysis of 49,034 SNP revealed very high levels of diversity within the Rambouillet and Gulf Coast Native samples, with the proportion of SNP displaying both alleles exceeding 95% within each population. Using cluster-based analyses, individuals were positioned into two non-overlapping groups according to breed membership. Significant population substructure was detected within the Gulf Coast Native breed, and subtypes (Florida Natives and Louisiana Natives) could be discriminated on the basis of SNP genotype alone. To search for signatures of positive selection, population-specific F_{ST} was calculated for each SNP before outliers were identified using a smoothing algorithm. Analysis of the Gulf Coast Native animals identified regions on chromosomes 2, 6, 13 and 18 that displayed evidence of positive selection. Analysis of the Rambouillet animals identified five different selection signatures (on chromosomes 3, 6, 7 and 16) and one signature on chromosome 6 in common with the Gulf Coast Native. The regions identified were often large (average 3.7 Mb) and contained multiple transcripts. Two genes (*ABCG2* and *GHR*) previously shown to be under selection are located in signature regions identified in this study. In summary, this genome scan has identified genetic regions that may impact production in two U.S. sheep breeds.

P3053 Analysis of mitochondrial D-loop diversity in Bolivian alpacas (*Lama pacos*)

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The origin of the domestic species alpaca (*Lama pacos*) and llama (*Lama glama*) has been a matter of debate. After genetic analysis it seems that alpacas descended from vicuñas and llamas from guanacos. However, large-scale hybridization between llamas and alpacas in the Andes has been reported in previous genetic studies. In the present work, we investigated the genetic diversity of Bolivian alpacas based on the sequence variation in 511 bp of the control region of mitochondrial DNA in 37 animals of the Suri and Huacaya breeds from the North and Central Regions of Bolivia (Ulla-Ulla and Sajama). Furthermore, four animals belonging to each of the wild populations of South American camelids, guanaco (*Lama guanicoe*) and vicuña (*Vicugna vicugna*) and one llama, were used as references. A total of 41 polymorphic sites were detected, giving 26 different haplotypes from which 11 were singletons. Nucleotide diversity ($\pi = 0.02153$) indicated the great genetic variability observed in Bolivian alpacas. The median joining analysis displayed two different haplogroups, the first (group V) containing the vicuñas and 35 percent of the alpacas analysed, and the second (group G) including the llamas, the guanacos and 65 percent of the alpacas included in the study. Regarding the breed allocation between groups, Suris were evenly distributed and Huacayas were mainly gathered (70%) in group G. These preliminary results may indicate that hybridization using male alpacas to breed with females llamas has been more frequent in Huacayas that in Suris.

P3054 Evaluation of the porcine *acyl-CoA synthetase long-chain 4* gene (*ACSL4*) as candidate gene for meat quality traits in pigs

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Long-chain acyl-CoA synthetase (ACSL) family consists of five isoforms involved in the metabolism of fatty acids. Members of this family catalyse the formation of long-chain acyl-CoA from fatty acid, ATP and CoA, thus, playing an important role in both *de novo* lipid synthesis and fatty acid catabolism. Previous studies in our group, evaluated the *ACSL4* as a positional candidate gene for a quantitative trait loci located in chromosome X in an Iberian x Landrace cross. A c.2645G>A SNP located in the 3'UTR (DQ144454) was associated with the percentages of oleic fatty acid and monounsaturated fatty acid. The aim of the present work was to evaluate the functional implication of this genetic variant. An expression analysis was performed in 49 individuals with different genotypes for the c.2645G>A polymorphism using the real-time quantitative PCR (SYBR green) method. Differences between genotypes were identified in liver, being the ACSL4 mRNA expression levels higher in animals with the A allele than in animals with the G allele. Furthermore, the *ACSL4* promoter was sequenced and analyzed, but polymorphisms were not found in this region. An *in silico* analysis showed differential attached microRNAs in the 3'-UTR between both alleles, suggesting a role of the microRNAs in determining the different expression observed between ACSL4 alleles.

P3055 Findings from the ISGC HapMap Exeriment: Phylogeny of domestic and wild sheep

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The phylogeny of 74 breeds from all continents was reconstructed on the basis of genotypes for 49034 SNPs. The high density of markers allows an estimation of divergence times of breeds on the basis of LD as an alternative to classical genetic distances. A NeighborNetwork phylogenetic graph of divergence times displayed for most breeds similar branch lengths, which supports the validity of the estimates. The network separated breeds according to geographic origin and also showed the intermediate positions of crossbred populations. In a graph of Reynolds' distances branch lengths are uneven as the consequence of variable genetic drift. The shortest branch lengths were found in Spanish, Italian and Iranian breeds. The tree topology was robust with respect to selection of different subsets of SNPs and showed a remarkable demarcation of geographic breed clusters. Labelling breeds according to the Y-chromosomal haplotype suggests a paternal founder effect in England or North Europe. Genotypes of non-domestic populations revealed that the feral European mouflon most closely resembles Northern European sheep. Sardinian mouflon is most related to the Sardinian Ancestral Black as the plausible consequence of mutual introgression. Surprisingly, Asian mouflon, which is the wild ancestor species, is more related to Sicilian, Greek and Turkish sheep than to sheep from the Southwest Asian region of domestication. This localizes the phylogenetic root of current domestic sheep in the region around the Ionean Sea. Combination with an ongoing metaanalysis conducted by the EU GlobalDiv project will result in a more comprehensive coverage of the worlds' sheep population.

P3056 Conservation Genetics of U.S. Federal Bison Herds

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American bison (*Bison bison*) were reduced from tens of millions at the time of European colonization to a few hundred by the mid-1880s. There were remnant herds of both North American subspecies, plains bison and wood bison; crosses between the bison subspecies and between plains bison and domestic cattle were widespread. The 12 plains bison herds that occur in U.S. national parks and wildlife refuges are an important resource for the long-term conservation of American bison, and have been analysed at mtDNA and microsatellite loci. While most of the herds show low levels of introgression (<1% cattle alleles) dating from the time when they were saved from extirpation, no historical hybridization has been detected in bison from Yellowstone or Wind Cave National Parks. Despite the fact that most of the U.S. federal herds had few founders and have been maintained for many generations at relatively low population sizes, they do not show effects of inbreeding. They have retained significant amounts of genetic variation when measured by average heterozygosity and allelic diversity. There are presently few conservation herds that are large enough to maintain genetic variation over centuries. It is recommended that the federal herds be expanded or established as metapopulations. Analysis using single nucleotide polymorphism (Bovine SNP50) is currently underway in the federal herds to further evaluate the variation in individual bison.

P3057 Findings from the ISGC HapMap Experiment: Genetic Diversity and Global Patterns of Genetic Structure

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Human mediated breeding has generated a spectrum of phenotypically diverse sheep breeds adapted to the production of meat, milk and wool. In order to understand breed history, genetic diversity and domestication the International Sheep Genomics Consortium (ISGC) assembled and genotyped over 2,800 sheep from 74 diverse breeds using the *ovine* SNP50 BeadChip. The level of genetic diversity present within breeds, measured as observed heterozygosity (*He*), was evaluated as a function of geographic origin and population size. In order to account for ascertainment bias, LD based SNP pruning was performed which reduced the over estimation of diversity within European breeds which were used heavily during SNP discovery. Following this correction, breeds from the Middle East and Southern Europe displayed the highest heterozygosity of any group. High diversity in sheep from the Middle East (Turkey and Iran) supports existing molecular evidence indicating this was the center of sheep domestication. Similarly, high diversity within breeds from Southern Europe likely reflects the first migrations of Neolithic communities and their animals who used the Mediterranean. Further, a gradient of decreasing heterozygosity was evident with increasing physical distance radiating outwards from the Middle East, consistent with a serial founder effect involving progressively smaller subsets of animals participating in migration. Global patterns of genetic structure were inferred by principal component (PC) analysis of relatedness. This revealed clustering according to geographic origin and the identification of breeds with mixed origin. Together, the results provide the first in depth view of genetic diversity for this livestock species.

P3058 Phylogenetic analysis of African-derived cattle mitochondrial genomes

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African-derived mitochondrial lineages have been found in Creole cattle of the Americas, which originated from Iberian or African animals introduced during colonization. These matrilines were defined based on the hypervariable control-region but their relationship to other African lineages that have been fully sequenced is unknown. Recent studies have shown that complete mtDNA sequences are needed for accurate phylogenetic inferences about the origins and relationships among haplogroups. The objective of this study was to investigate the positioning of the African-derived haplotypes of Creoles (AA-haplogroup) in the phylogenetic tree of cattle mtDNA genomes. Complete sequences (16,348 bp) were obtained for 8 animals with African matrilines and 2 with the ancestral southern European Q-lineage. Based on control-region motifs, 4 Creoles had the AA-haplogroup, 2 Iberians and 1 Creole were presumably of the African T1a-haplogroup but possibly related to AA-lineages, and 1 Creole had a distinct African T1-lineage. Haplotypes were obtained by comparison with the *Bos taurus* reference sequence (Genbank acc. V00654). The phylogenetic analysis included reference sequences representative of cattle mtDNA haplogroups. The best-fitting evolution model determined with Modeltest v3.7 was the GTR+I+G with the shape parameter of the gamma distribution α=0.9402 and the proportion of invariable sites I=0.8577. Phylogenies constructed with maximum parsimony, maximum likelihood and Bayesian methods were mostly consistent. The results showed that AA-lineages formed a cluster within the African T1a-haplogroup. Two Iberian animals and 2 sequences (EU177847-8) from Italy and Iraq previously classified as T1a belong to the AA-haplogroup. Presence of a Q-haplotype in Iberian cattle was also confirmed.

P3059 Selective breeding provides an approach to increase resistance of olive flounder (*Paralichthys olivaceus*) to bacterial and viral diseases

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Our study was conducted in order to assess microsatellite DNA markers previously developed for *P. olivaceus* in their ability to assign correct parentage to flounder progeny, and to improve the olive flounder through selective breeding. In order to establish a broad genetic base for long term of selective breeding, the broodstock from different geographical locations was included in the base population and have been genotyped using microsatellite DNA markers.

In 2005, F1 family was produced based on mating design, and then phenotypic and genetic analysis of F1 offspring were performed. A total of 7,192 offspring were selected randomly and tagged with PIT tag, and individual phenotypic characteristics such as body weight, total length, body depth were recorded. Parentage assignment using eight microsatellite markers was also performed, and all 218 families existed in 7,192 offspring. Genetic variance, residual variance and heritability at 330 days of age were estimated. Heritabilities for weight, body length, body depth fall in the 0.7–0.8 range, and heritabilities for body shape, and condition factor fall in the 0.3–0.5 range. The genetic correlation between length and weight was very high (0.98), and negative genetic correlations were observed between body shape and length (0.38) or weight (0.17).

In 2007, the broodstock for F2 family production was selected based on their phenotypic and genetic estimates. Mating procedure and rearing fry were same as F1 production. About 4 month later, challenge tests for F2 family were conducted to detect between-family variation in survival after infection with specific pathogens. Preliminary values of heritability for number of days survived after challenge were estimated. These results indicate that survival data can successfully be used in selecting more disease- resistant olive flounder within the breeding programme.

P3060 Genetic variability comparison of wild populations and cultured stocks of flounder *Paralichthys olivaceus* based on microsatellite DNA markers

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Six microsatellite DNA markers were used to investigate the genetic variability between wild populations and cultured stocks of olive flounder *Paralichthys olivaceus*. The average of observed (Ho) and expected heterozygosity (He) ranged from 0.722 to 0.959, and from 0.735 to 0.937, respectively. There was no distinguishable difference between the wild populations and cultured stocks in terms of the observed and expected heterozygosities. However, number of alleles per locus differed markedly between the two fish groups: 19.7 to 21.8 for the wild populations and 12.0 to 14.7 for the cultured stocks. This result gives important information concerning the production of seedling for the improvement of genetic diversity in this species.

P3061 Genetic comparison of Slovenian and World horse breeds using microsatellites

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In the present study, genetic diversity and distance between three Slovenian autochtonous horse breeds and Arabian, Gidran and Standard breed trotter was compared using genotypic information from 17 microsatellite loci. The Slovenian horse breeds included Ljutomer trotter, Posavje horse and Slovenian coldbloded horse. Of especial interest was comparison of Ljutomer trotter to Standard breed trotter with the aim to investigate genetic relationship between these two breeds. The effective number of alleles ranged from 4.79 in the Posavje horse to 5.72 in the Ljutomer trotter. The average observed heterozygosity (Ho) differed little between horse breeds (0.63-0.69), but was considerably higher in Ljutomer trotter (0.72). The average level of inbreeding within breeds, as estimated by F_{IS} was 7.3%, but was higher (13.4%) in Ljutomer trotter. Phylogenetic analysis showed the existence of clusters supported by high bootstrap values: trotter cluster (Ljutomer trotter, Gidran, Standard breed trotter), the Draft cluster and the Arabian breed as a separate cluster.

In conclusion, the study demonstrated a clear distinction between different Slovenian horse breeds. However, Ljutomer trotter was found to be genetically very close to both Standard breed trotter and to Gidran breed, suggesting that there is little justification to consider Ljutomer trotter breed as separate breed.

P3062 The use of pedigree analysis in studies of mtDNA variation in dogs and horses

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Pedigree analysis is an indispensable source of information useful in the study of mtDNA variation in populations of dogs and horses. Two breeds of dogs (Hovawart and Polish hounds) and Arabian horses was used as examples of the application of pedigree data in the analysis of mtDNA variation. Analysis of the pedigree information allows for finding the rare sequences, occurring in the lines represented only by single individuals. It also make possible to specify the dynamics of frequency changes of individual sequences over many years of breeding. Comparison of the results of mtDNA analysis and pedigree data allows for verification of pedigrees, as well as facilitate the dating of the occurrence of any errors. It is also an important economic aspect, because the precise selection of animals for research allows to reduce the scale of laboratory work to an absolute minimum and thus to minimize cost analysis.

P3063 Genetic variability of the Bracco Italiano dog breed based on microsatellite polimorphysm

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The Bracco Italiano is one of the oldest pointing dog breed, used for hunting ever since the Renaissance time. Today it has increasing importance as to be the winner of the "Eukanuba World Challenge 2009", one of the most prestigious events of dog shows in USA.

In this work we illustrate an assessment of the genetic variability for 21 STRs typed in a sample of 72 unrelated Italian hounds ("Bracchi" - BI) and a sample of 43 dogs from other 23 different breeds ("Other dogs" - OD). The aim of the present study was to estimate the genetic variability of the BI dog breed using microsatellite markers, in order to provide information useful in conservation purposes. Three multiplexes were worked out, which allowed analyzing 21 STR markers from the panels recommended for the 2006 and 2008 ISAG canine comparison test. Allele size in bp was determined using the comparison-test reference samples as anchor values. Number of alleles, allele frequencies, deviations from Hardy-Weinberg proportions, linkage *disequilibrium* among loci, genetic similarity, genetic distances and molecular coancestry-based parameters were calculated.

The number of different alleles ranged 3 to 9 (mean 6.43) in the BI, compared with 6-11 (mean 8.52) in the OD, whereas the expected heterozygosity ranged 0.44-0.81 (mean 0.64) compared with 0.51-0.89 (mean 0.81).

In BI the genetic similarity within the whole population was high (0.455±0.018); this parameter reveals the great homogeneity of the sampled animals as confirmed also by the small kinship distance (0.336) and by the high values of the self molecular coancestry (0.703) and of the inbreeding coefficient (0.406).

P3064 Findings from the ISGC HapMap Experiment: Signatures of Selection in Domestic Sheep

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Animal breeding or selection leaves evidence of population genetic selection on the genome. Searching for evidence of such selection is a central objective for the experiments of the International Sheep Genomics Consortium (ISGC) HapMap. Using genotypes collected from 49,034 SNP across many breeds of sheep, two broad approaches were taken. First, patterns of F_{ST} across the genome were used on breeds grouped according to phenotype. A strong and broad signature of selection was detected surrounding *myostatin*, while a single gene was identified underlying the Horns (*Ho*) locus. Second, patterns of F_{ST} across the genome were evaluated in breeds not grouped by phenotype, but peaks of F_{ST} were smoothed by F_{ST} values of adjacent loci. Comparison across breeds identified regions that consistently showed evidence for both positive and balancing selection. Using this second approach other breeds than the Texel were identified as having a F_{ST} peak including the *myostatin* region. It is unclear at this point if this peak represents selection process for the same mutation present in Texel or if it represents new mutations affecting myostatin. The most important new region identified as harbouring a signature of selection is located on OAR13 and evidence for it was found in thirty-nine of the seventy-four breeds. This region does not include any immediate candidate gene to be under selection. Finally, one of the most common peaks associated with balancing selection were located in a broad region at OAR20 that include *MHC-II* components.

P3065 Genetic and demographic parameters of Bergamasco Shepherd dog (*Canis familiaris*) world population

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The aim of this work was to investigate the genetic variability of the endangered population of Bergamasco Shepherd dog (BS) and its evolution across generations to supply effective and objective tools for breed conservation and selection strategies. BS is a small population of old Italian long haired herding dog, part of FCI group 1: Sheepdogs and Cattle Dogs (except Swiss Cattle Dogs). Worldwide available official data of 8870 subjects were analysed (4127 females, 4743 males), dates of birth ranged from 1903 to 2009. All the analysis were carried out using ENDOG V 4.6 software. Mean maximum number of traced generations was 19.46, mean complete generations were 3.63 and mean equivalent generations 8.02.

Average inbreeding coefficient (F) was 0.16 and average relatedness coefficient (AR) was 0.20. The maximum number of puppies entered in the official studbooks was recorded in 1989 with 412 new born BS. A clear increasing in the population size was recorded in the eighties, in the same period low F values were calculated. In the last ten years even though a reduction in population size was recorded, F was slightly decreasing compared to the peaks recorded in the last decades of the XX century, this could be considered a good result in the genetic management of this rare ancient Italian canine breed. In this study it is also shown how a worldwide genealogical records database can supply powerful tools for genetic population analysis and breeding planning.

P3066 A population genetics approach to explore the molecular basis for athletic performance traits in North Swedish trotters

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Athletic performance is a key trait in Swedish racing trotters. The power to detect genes underpinning this phenotype will be influenced by the genetic diversity within and between the breeds studied. We performed two genome scans on three breeds (North Swedish trotters, NST; North Swedish horses, NS and Standardbreds, S), first with the Illumina EquineSNP50 BeadChip (12 individuals/breed) and then also with 144 microsatellite markers (10 individuals/breed). The total genotyping rate in individuals from the BeadChip was 0.98, where 17,887 SNPs had a MAF < 0.1 and 1331 SNPs failed the missingness test (>0.1), leaving 36,415 SNPs after frequency and genotype pruning. Identity by state cluster analysis (PLINK) revealed the three breeds separated into three distinct clusters. Measures of population diversity were made with the microsatellite data set. NS was revealed to be the most genetically diverse with the highest average number of alleles per locus ($A_N = 4.75$) and highest expected heterozygosity ($H_e = 0.666$). This was followed by NST ($A_N = 4.53$, $H_e = 0.649$), whilst S was the least diverse ($A_N = 4.40$, $H_e = 0.627$). This trend was mirrored with rarefied allelic richness (NS > NST > S; $A_R = 3.23 > 3.17 > 3.02$) however S was the most distinct when private allelic richness was considered (S, $pA_R = 0.96$; NS, $pA_R = 0.89$; NST, $pA_R = 0.86$). Genetic distance between NST and S (0.503) was smaller than NS and S (0.524) supporting geneflow between the North Swedish trotters and Standardbreds.

P3067 Variation of cattle major histocompatibility complex (BoLA) *DRB3* allele frequencies within different farm, breed and countries in South America

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The cattle major histocompatibility complex (BoLA)-*DRB3* gene is the most polymorphic class II locus in cattle, and these alleles are associated with susceptibility to several infectious diseases. To investigate *BoLA-DRB3* allele of various cattle breeds in South American, we genotyped 2343 head of cattle containing 369 Japanese, 385 Argentina, 139 Paraguayan, 328 Peruvian, 499 Bolivian and 623 Chilean using new PCR-sequence based typing method. Collected breeds were Wagyu, Japanese Shorthorn, Jersey, Holstein, Gir, Zebu, Nelore, Ovelo Colorado, Ovelo Negro, Black and Red Angus, and mixed. 72 alleles out of known 118 alleles and three unknown new alleles are detected. All three new alleles were detected from Bolivian mix-breed and one of three was also detected from Peruvian and Paraguayan Holstein. Number of detected alleles in each breeds were from 14 (Japanese Jersey) to 35 (Peruvian Zebu and Bolivian Mix breed) and Expected Heterozygosity were from 0.858 (Peruvian Zebu) to 0.952 (Bolivian Mix breed). To overview of allele frequencies, we constructed phylogenetic tree which clearly showed that intrinsic allele frequencies were neither depending on countries nor on farm but instead on breed. Especially, Holstein has intrinsic allele frequencies in all farms except one in Argentine. Meanwhile, some Ovelo Colorado breed were very near to Holstein but from some other farm it is most far from Holstein within the *Bos Taurus* breed. Moreover, Japanese Wagyu and Chilean Wagyu are resembled but Chilean Wagyu also had the characteristic of Angus. These frequency data provide infrastructure for understating the host factor of disease susceptibility.

P3068 Effects of SNPs in the myostatin gene and its promoter on young bulls at ANABIC (Italian Beef Cattle Breeders Association) performance test station

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In the Italian "Marchigiana" beef cattle breed, the economically important "double muscling" trait is caused by a G-T transversion within the third exon of the myostatin gene (GDF8). Moreover, in several beef cattle breeds, two SNPs, [T/A] at -371 and [G/C] at -805, were found within the promoter region of the myostatin gene. The aims of this study were to assess the effects of the double muscling putative mutation on some productive traits (genetic indices, linear evaluation, weight at 365 days, etc.) and to investigate the possible relationships between the two SNPs at the promoter region and the same traits. 286 "Marchigiani" performance evaluated sires were tested for their genotypes at the myostatin locus and at the two promoter SNPs, trying then to establish the potential effect of such mutations on the productive data assessed during the test. Two statistical approaches were used: the MTDFREML software and the General Linear Model procedure of SAS statistic package. The results highlighted the important effects of the genotype at the myostatin locus on the muscular and size traits of the mutated animals. This mutation might play a fundamental role in the skeletal development as well. Bulls T/A at one of the two analyzed SNPs seemed to have the best muscular traits; this mutation could be useful for future improved selection schemes. This also suggests that the studied SNPs may modulate the expression of muscular traits.

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P3069 Molecular and phylogenetic analyses of mitochondrial DNA in three Italian sheep breeds

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Genetic variation of Appenninica, Sopravissana and Merinizzata Italiana sheep breeds was investigated using mitochondrial DNA (mtDNA) sequencing. The first breed is autochthonous of central Italy, while the others are two Merinos derived breeds widely spread across the south of Italy. This study was carried out on a long segment of the mtDNA control region (nps: 15437-16616), running from base 15,394 to base 16,200, which includes the hyper-variable region of the mitochondrial genome. A total of 90 animals (30 individuals per breed) were analysed and phylogenetically compared. The preliminary results showed the existence at least of three groups of haplotypes, with a differential breed distribution. Taking advantage of this information, we have deepened the knowledge of the relationships between breeds. Intriguingly, the closest phylogenetic similarity was between Appenninica and Sopravissana, in spite of the well-known common origin of Sopravissana and Merinizzata Italiana from Spanish Merinos. A possible explanation is that these breeds arose from Merinos rams.

In conclusion, these preliminary results indicate a common female ancestry for Sopravissana and Appenninica, but not for the Merinizzata Italiana breed, which, similarly to Gentile di Puglia, could have a different genetic ascendant, at least from a female perspective. The final outcomes of this study will certainly give not only the chance to understand the molecular variations of the mtDNA of these breeds, but also to clarify their phylogenetic history and genetic relationships.

P3070 Mitochondrial DNA analysis of the autochthonous Sardinian dog breed "Cane Fonnese"

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The "Cane Fonnese" (CFO) is an autochthonous old dog population bred in Italy, in Sardinia region. The origins of this population are uncertain and actually these dogs are used as guard and herding dog. The genetic variability at mitochondrial D-loop DNA (mtDNA) of 22 samples of CFO was evaluated. To better define the origin and the phylogenetic relationships of this population, mtDNA variation at 31 dogs of 5 breeds (Schnauzer SCH, Maremma Sheepdog MAS, Bergamasco Shepherd dog BES, Italian Corso dog ICD and Italian Hound ITH) and 11 sequences from on-line database were analysed. A 694 bp fragment of dog mtDNA was amplified and sequenced. Sequences obtained were aligned with the complete canine mtDNA sequence (GenBank#NC_002008) and sequences from on-line database using CLUSTALX software. Twenty-four haplotypes were identified in the six populations. Overall and per breed haplotype diversity (Hd), nucleotide diversity (π) and average number of nucleotide differences (k) were calculated using DnaSP 4.2 software. Hd ranged from 0,643 (ICD) to 1,000 (ITH), overall 0,931; π ranged from 0,004 (ITH) to 0,012 (MAS), overall 0,011; k ranged from 2,607 (ICD) to 8,107 (MAS) overall 7,901. These results indicated moderate haplotype diversity. Genetic distances were estimated by the Kimura-two parameter method and a phylogenetic tree was constructed using the UPGMA algorithm by MEGA 4.0 software package. Median-joining network and mismatch analysis were calculated to investigate haplotypes relationship using NETWORK 4.5.0.1. Molecular investigation of CFO is a basic objective and effective step in population conservation and in the planning of breeding strategies.

P3071 Genetic characterization and relationship between two local donkey breeds (*Equus asinus*) and an African wild ass population (*Equus africanus*)

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The donkey (*Equus asinus*) has played, across the times, an important role on human societies in the transportation of people and goods. But presently the donkey is the most threatened livestock species in European Union countries. The genetic characterization of donkey breeds is an essential step in order to develop an appropriate management and conservation program. In this work we aim to do the genetic characterization of two endangered autochthonous donkey breeds from Portugal: "Burro de Miranda" and "Burro da Graciosa". Fifteen horse microsatellite markers were used to genotype 87 donkeys belonging to both breeds. Additionally we have genotyped 22 captive African wild asses (*Equus africanus*), which is believed to be the wild ancestor of the domestic donkey, for a subset of 10 microsatellite loci. Unbiased expected heterozigosity (H_e) varied between 0.60, for the African wild ass population and 0.46 for the "Burro da Graciosa" breed. Allele frequency distributions for the three populations showed that in 4 out of 10 loci it was possible to identify private alleles for the African wild ass population. Comparing obtained results for the two native-Portuguese breeds and published results for 5 Spanish donkey breeds, we can conclude that the "Burro da Graciosa" breed shows the lowest values for mean number of alleles, expected and observed heterozigosity, between all Iberian donkey breeds, confirming this breed fragile conservation status.

P3072 Molecular fingerprinting as a multi-faceted tool to implement selective breeding in farmed gilthead seabream (*Sparus aurata*) illustrated by a case study on natural spawning

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Six commercial seabream broodstocks and a progeny derived from a single-day mass-spawning event were analysed with regards to the populations genetic structure, parentage inference and genetic parameters using panels of 6 to 9 microsatellites. The *F*st analysis revealed a difference between only two broodstocks caused by a unique locus departure from HWE. This low genetic divergence was attributed to the domestication process in place within the hatchery. The parent and progeny populations showed very diverse departures from HWE, *F*is values and number of alleles. These results were explained by the differential fertility of breeders and the Robertson's effect and highlighted the risks of genetic drift in the next generations. Unique error mismatches resulting from the parentage allocation analysis were mostly attributed to mutations rather than to null alleles. The high rate of unambiguously assigned offspring, equivalent to 95.4% of the total progeny (1692 sibs), was obtained using the exclusion and likelihood-based parentage inference methods. Three new parental genotypes were identified, bringing the number of contributing parents to 37. The low effective population size (N_e=15.3) and high rate of inbreeding (ΔF=3.27%) were attributed to parents of both sexes failing to contribute to the progeny and to the elevated variance of dams and sires contributions. The heritability for body weight and GxE effects resulted low to moderate according to the age. We discuss the incidence of reproduction features on breeding programmes and highlight current limits and future potentials of molecular markers to obtain pedigree data.

P3073 Genetic diversity of village dogs from North Kenya

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Infectious agents, as one of the forces driving evolution and interactions between hosts and parasites, contribute to genetic diversity of specific populations. In this study, genetic diversity of a model population of African village dogs exposed to direct selection pressure of complex pathogens without any prophylactic and/or therapeutic interventions was investigated and compared to a group of domestic dogs. Besides neutral loci, like microsatellites, immunity-related (IR) loci, characterized by features reflecting their role in defense reactions and related to evolutionary mechanisms including polymorphisms and association with disease, are suitable markers for genetic diversity studies. 85 unrelated dogs from Mt. Kulal area in the Laisamis/Samburu Districts in the North of Kenya and owned by semi-nomadic Samburu pastoralists were analyzed for this purpose. 10 microsatellites and 19 newly developed single nucleotide polymorphism (SNP) markers within 12 IR genes (*NOS3, IL6, TLR1, TLR2, TLR3, TLR4, TLR7, TLR9, MD2, MyD88, CD14 and TLR3*) were genotyped in this group and in a group of 68 unrelated European domestic dogs (23 pure breeds and 6 mongrel dogs). Numbers of alleles, allelic frequencies, observed and unbiased expected heterozygosities were computed as parameters of genetic diversity of both groups compared. The results showed differences in the distribution of microsatellite allelic frequencies but not in genetic diversity expressed by unbiased expected heterozygosity. In the IR gene SNP markers, for some loci, like *IL6*, not only allelic frequencies, but also parameters of genetic diversity differed between the two groups.

P3074 Analysis of global variation in pig: Discovery of novel SNPs by whole genome re-sequencing

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The current release of the porcine genome draft provides a high quality reference for the analysis of genetic variation in pig. The number of single nucleotide polymorphisms (SNPs) available for *Sus scrofa*, however, is still lower compared other sequenced mammalian species: While dbSNP contains about 540,000 submissions for pig SNPs (compared to nearly 5 million cattle SNPs) and a 60K SNP chip for genotyping is available, only 8,000 SNPs are available in Ensembl Pig Sscrofa.9. Our goal was to investigate genetic variation among different individuals at whole genome level and to this end we re-sequenced the genomes of three boars at high coverage. Single- and paired-end reads uniquely mapped to Ensembl Sscrof.9 at high sequence depth for each of the sequenced animals allowed us to identify millions of novel SNPs. The predicted SNP were then compared with results from genotyping Illumina porcine 60K SNP bead chip array. In addition, we performed functional annotation of the predicted SNPs based on Ensembl Sscrofa.9 feature information. Prediction of functional effects of nonsynonymous SNPs was performed using SIFT and PolyPhen identifying thousands of SNP candidates as putative deleterious variants. The re-sequencing of individual pig genomes allows us obtain a global view of sequence variation in the porcine genome. Millions of SNPs, mostly novel polymorphisms, were identified as a valuable resource to be used as high density genetic markers for genotyping and investigation of phenotypic variations.

P3075 Relationship between inbreeding and heterozygocity in the Belgian Draught Horse breed

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The Belgian Draught Horse experienced a considerable reduction in population size after World War II and numbers stabilized only since the 80's, with approximately 900 foals born/year. This limited population size combined with unequal usage of stallions raised questions about the remaining genetic diversity of the breed. Although the studbook was founded in 1885 with recording of the pedigree since then, the "computerized" pedigree information is limited to the 1960's. In order to assess the current genetic diversity of the breed, a combined analysis of pedigree information and microsatellite marker data seemed advocated.

Fourteen microsatellites from the ISAG panel were genotyped in 127 horses and used to compute heterozygosities (0.657 and 0.663 for observed and unbiased respectively). Pedigree analysis shows that the breed has intermediate genetic diversity with an effective size just below 100. The individual index of heterozygosity was significantly correlated to the level of inbreeding computed from the pedigree.

P3076 Polymorphisms in the *KIT* gene of Holstein and Japanese Black cattle

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In bovine, the spotted coat colour locus has been mapped to the position of the *KIT* gene on the chromosome 6. The SNP of intron 3 of *KIT* gene has also been reported for the linkage disequilibrium with spotted locus of cattle. However, the causative mutation of *KIT* gene to the spotted locus has not yet been elucidated. In Japan, the spotted breed of Holstein and the solid colour of Japanese Black cattle are a popular breeds of cattle, and F1 of these breeds are widely used for beef production. The aim of this study was conducted to develop identification method of these breeds by using the variation of *KIT* gene associated with spotted coat colours. The 21 exons and both of the flanking regions of them are sequenced in 60 cattle of the random population of each breed. The two novel polymorphisms, insertion (TTCTC) and deletion (TATC) were detected in the region of intron 3. Three haplotypes, A (no-insertion and no-deletion), B (insertion and no-deletion) and C (no-insertion and deletion) were found. Japanese Black cattle have high frequencies of Haplotype B and C, and have a few frequencies of Haplotype A, but Holstein breeds have only Haplotype A. These loci are not enough to utilize as perfect genetic markers, but it may be available for partial determination of these breeds. Namely, it may be predicted that B- and C- genotypes are not Holstein, and BB, BC and CC genotypes are Japanese Black cattle. Further analyses to search the new other polymorphisms are under way.

P3077 Establishing a Stud Book for the Native Skyrian Pony Herd

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The Greek Island of Skyros has been home to a unique breed of pony since the middle ages. The majority live wild in the mountainous southern part of the island, although increasing numbers are now living on the farms. The small size of the population and the presence of wild mules has given rise to problems with inbreeding and crossbreeding. This has led to the breed being considered endangered. To address this, various bodies in an attempt to improve the management of the herd and ensure the purity of the breed provided funding to establish a stub book for the breed. Hair samples were collected and sent to ARK-Genomics for analysis. DNA was extracted from the follicles and genotyped using ABI equine stockmark kits. The data were then analysed using the software "Cervus" to generate a list of likely parents for each pony. These data and local knowledge were pooled to generate an embryonic stud book. Samples have subsequently been collected from new born foals over the last year and added to the information gained so far.

P3078 Genetic relationships among chicken and junglefowl populations using 77 autosomal SNPs by DigiTag2 assay

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To date, the genetic relationships of chickens and junglefowls were conducted by means of short tandem repeat (STR) analysis or sequence comparison of the specific DNA regions. Recently, single nucleotide polymorphisms (SNPs) have been used to identify genes or genomic regions responsible for economic traits including genetic diseases in domestic animals. In this study, we carried out genotyping of 10 chicken and four junglefowl populations using 77 autosomal SNPs of the DigiTag2 assay to understand the genetic relationships of these 14 populations. These results revealed the following: 1) Of the 77 autosomal SNPs, 75 were polymorphic and the remaining two were not. 2) A minor allele frequency (MAF) of ≥2% was observed for 72 SNPs (72/77 = 93.6 %). 3) Average heterozygosity within a population ranged from 0.246 (broiler) to 0.165 (Ingie) in the chicken populations and from 0.279 (red junglefowls) to 0.025 (green junglefowls) in the junglefowls. 4) Phylogenetic analysis using the concatenated 77 autosomal SNPs formed clusters of chickens belonging to the respective populations. In addition, the red junglefowls were closely positioned to chickens, while the Ceylon, grey and green junglefowls were not.

P3079 Genetic variability and inbreeding evaluation in Brown hares populations from two different protected areas of northern Italy

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The brown hare (*Lepus europaeus*) is widely distributed throughout Europe where it constitutes an important game species. However, the conservation of the brown hare is also a concern, because of a marked decline in populations recorded since the 1960s. During the past decades, restocking programs with the introduction of allochthonous individuals have been carried out in several European countries. Here we analyzed hares from two protected areas located in Northern Italy where no animals were released in the last ten years and absence of gene flow could have led to allelic fixation and inbreeding with reduction of population reproduction, disease resistance and fitness performance. The aim of the study was to assess genetic variability and inbreeding status of the two populations for management programs. Blood samples from 109 hares (35 male, 74 female) captured in Alessandria (n=56) and Tortona (n=53) areas were tested using eight known polymorphic microsatellites: SAT5, SAT12, SAT13, SOL8, SOL33; LSA1, LSA2, LSA6. Primers for each locus were labeled at 5' with fluorescent dyes (FAM; VIC; NED) and two multiplex PCR reactions were optimized. Fragment analysis was run on 3130 Genetic Analyzer with ROX 500 size standard. Number of alleles per locus, allelic frequencies and observed and expected heterozygosity were calculated. The inbreeding coefficients were: 0.098 (95% Confidence Interval: 0.03582 – 0.13948) for the Alessandria population and 0.046 (95% Confidence Interval: 0.03387 – 0.10608) for the Tortona population.

P3080 Genetic traceability of four Italian native sheep breeds using ISAG panels of microsatellites markers

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Food traceability is a growing concern for EU's food safety policy, helping to identify and address risks and to protect public health. Molecular traceability can be an effective tool for the protection from food frauds and for the valorisation of traditional products and of high quality certified food like Protected Designation of Origin and Geographical Indication (PDO, PGI). Recently, Italian native breeds, reduced in number during the past decades, have experienced an increased interest due to their good adaptive traits, their value as genetic resources and because of their use for the production of typical foods linked to old traditions. In this study, four Italian sheep breeds from the Piedmont region were analyzed using the panels of markers suggested by the ISAG Standing Committee on "Applied Genetics in Sheep and Goats". The aim was to assess the effectiveness of microsatellites markers for the identification of breed-characteristic genetic profiles in order to trace animals and derived products back to their breed of origin. A total of 195 sheep blood samples from four breeds (Biellese, Frabosana, Sambucana and Delle Langhe) were tested using 14 polymorphic microsatellites (CSRD0247; HSC; INRA0063; MAF0214; OarAE0129; OarCP0049; OarFCB0011; OarFCB0304; D5S2; INRA0005; INRA0023; MAF0065; McM0527; OarFCB0020) combined into two multiplex PCRs. Number of alleles per locus, allelic frequencies and observed and expected heterozygosity were calculated; preliminary statistical analysis showed discrete grouping of individuals into four breed-related clusters. Based on the genetic variability detected, an assignment test for these sheep breeds may be feasible.

P3081 Development of a DNA analysis method for species identification to control illegal trade of dog and cat furs

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Regulation (EC) 1523/2007 bans the placing on the market and the import/export of cat and dog fur, and Member States must inform the European Commission of the analytical methods they use to identify the species of origin in fur. DNA analysis can be a suitable method, but processing treatments damage DNA and dyes can inhibit PCR reaction. In this work, a fur DNA analysis was standardized, studying all the variables influencing the efficiency of the method. Species specific primers (three pairs amplifying DNA fragments of 50-70bp, 100-130 bp, 150-180bp) were drawn for each of the following species: dog, cat, raccoon dog, rabbit, fox, mink. All the PCRs were set up on blood or other tissues of these species apart raccoon dog that was not available. Then fur samples from all the species, except cat that was not present, were submitted to DNA extraction with six different protocols, based on spin columns, magnetic beads, and DNA precipitation. Presence of inhibitors was tested for each sample. The best performances were obtained carrying out an extraction with precipitation and a two-round PCR with primers amplifying 100-130 bp: nearly all the samples gave a positive and correct result. All the PCR products resulted to be species specific and no products of the expected size were obtained combining one species with the primers specific for another one. In conclusion, this method resulted efficient, easy to perform, applicable by most of the laboratories and thus suitable for this control activity that can also have legal implications.

P3082 SNP panels and the risk of ascertainment bias in diversity studies

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GLOBALDIV "A global view of livestock biodiversity and conservation – www.globaldiv.eu" is a project funded by the European Commission in the framework of the AGRI GEN RES initiative. It reviews and disseminates current advanced and integrated methodologies for the characterization, evaluation prioritization and conservation of livestock genetic resources. One of the tasks of the project is the review of new genomic. In fact SNP panels may be subject to ascertainment bias when used in estimates of diversity. In the discovery breeds, rare SNPs are missed or discarded, leading to an overestimation of the within-breed diversity. In breeds not comprised in the discovery panel, highly polymorphic SNPs may be missed while SNPs that are rare or even monomorphic included, leading to an underestimation of within breed diversity parameters.

We calculated the observed heterozygosity in a set of taurine, indicine and crossbred breeds using three SNP subsets extracted from the 35K panel of the Bovine HapMap Consortium: 14K and 0.4K panels discovered in the taurine Holstein and Limousin breeds respectively, and a 6.5K panel discovered in the indicine Brahman. In all cases heterozygosity values identified the breed in which SNPs were discovered as the most variable. Ascertainment bias can better be minimized at the source, by including in the SNP discovery panel carefully selected breeds/individuals to represent most of the variation existing in a species. In some case it can be corrected ex-post, by applying algorithms that adjust the frequency spectrum in the whole dataset by using allele frequencies in the ascertainment samples.

P3083 Genetic variability and population structure of Southern Italy sheep breeds

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Different native sheep breeds are raised in the southern regions of Italy. These breeds display interesting traits such as a complete adaptation to the local Mediterranean environment, a peculiar morphology and history and a particular taste of their products. The aim of the study was to characterize sheep biodiversity in Southern Italy at DNA level in order to allow efficient conservation strategies and to develop protocols of genetic traceability for sheep products. A total of 743 individuals from seven autochthonous breeds (Altamurana, Bagnolese, Comisana, Gentile di Puglia, Laticauda, Leccese and Sarda) were sampled in different flocks avoiding relatives. Genotypes were assessed for 19 microsatellites and for 104 SNP loci. Allele richness at STR loci ranged from 8.8 (Sarda) to 11.6 (Gentile di Puglia), while private allele richness was higher in Leccese (0.53) and lower in Sarda (0.32). A moderate level of inbreeding (F_{is} ranging from 0.147 in Gentile to 0.05 in Sarda) was observed with STR markes while F_{is} values were lower when considering SNP loci ; this discrepancy suggests the existence of null allele at STR loci. Overall F_{ST} values were similar for SNP and STR markers (0.04916 and 0.4894, respectively) and indicate a moderate level of breed differentiation. Considering pair-wise F_{ST} values, Altamurana and Sarda resulted to be the most distant and differentiated breeds. Structure analysis highlighted (at k 10), the the partitioning of genetic variability not only among breeds but also notably among flocks. The findings of this study can be exploited to implement effective policies to protect sheep biodiversity as well as protocols to authenticate sheep products.

P3084 The deposition of cuticle on the eggshell of laying hens is a moderately heritable trait which directly influences microbiological egg safety

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The cuticle is the proteinaceous layer deposited onto the eggshell just prior to oviposition. The cuticle is part of the eggs natural defenses to bacterial penetration but it was not known if this occurred over the range of natural genetic variation observed. In this study we investigated the role of genetics in determining the amount of cuticle deposition and examined how this influences bacterial ingress through the shell.

Two eggs from 878 Rhode Island Red hens from 38 sire families were used. to the cuticle was quantified using the % reflectance at 650nm before and after staining with a cuticle dye. Heritability was estimated by REML using a model including fixed effect of housing and the random effects of sires and dams within sires. *Escherichia coli* (*E. coli*) expressing green fluorescent protein (GFP) was used to investigate the relationship between cuticle deposition and bacterial ingress through the shell of eggs at the tails of the normal population distribution. Eggs were pre-warmed then immersed in an ice bath containing GFP (*E. coli*) for 15 minutes. Penetration was confirmed by the presence of fluorescent colonies on the inner surface of the eggshell after incubating at 37.5°C for 24hours.

Heritability estimates for the cuticle were moderate (0.27) and there was a significant difference in cuticle coverage between eggs exhibiting high or low levels of GFP *E.coli* penetration. Cuticle deposition shows considerable promise for use as a measurement for inclusion in genetic selection programs to improve egg safety.

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P3085 Progress in populating the sheep MHC immuno-polymorphism database

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The major histocompatibility complex (MHC) contains the most polymorphic loci within the mammalian genome. MHC class II genes encode glycoproteins which bind and present pathogen fragments to circulating T cells. The extensive allelic diversity at MHC loci provides a valuable source of genetic markers for examining the complex relationships between host genotype and disease resistance. Such studies in sheep have focused on exon 2 of the *DRB1* gene. Advances in sequencing technology has resulted in an increase in sheep MHC alleles submitted to public databases. The consequence of this is a confused array of sequences with a range of different nomenclature systems. This necessitated the development of a single standardised nomenclature with associated databases. The Comparative MHC Nomenclature Committee of International Society for Animal Genetics has developed guidelines for MHC nomenclature in sheep species. The requirements for accepting new alleles at the polymorphic class II MHC locus *DRB1* includes sequence of the entire second exon from two independent PCR reactions. To meet these requirements we have developed a direct sequence based genotyping method for analysis of allelic diversity at the *DRB1* locus in sheep populations. The method has been validated sheep breeds from the UK, France, Italy, and Kenya as well as *Ovis dalli, Ovis ammon* and *Ovis canadensis*. These studies have identified 75 *DRB1* alleles of which the complete exon 2 sequences of 57 within 23 allelic families are recorded within the database. The allele databases are available at http://www.ebi.ac.uk/ipd/mhc/ovar/index.html which also includes targeted alignment and allele submission tools.

P3086 Homozygosity mapping detects a selective sweep region of 10-Mb in the Boxer genome

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The domestic dog exhibits great phenotypic diversity across breeds mainly due to artificial selection, with the resulting footprint in the genomes of the breeds. The Boxer genome was screened for signatures of selective sweeps by homozygosity mapping. A first dataset of 22,000 single nucleotide polymorphisms (SNPs) distributed throughout the genome genotyped in 25 Boxers was analyzed. Within-individual segments of homozygosity were searched genome-wide and Boxer regions of homozygosis were defined by the overlapping of these segments. We found 41 regions of homozygosity shared across all boxers and with allelic matching. These regions sum up 58 Mb (~2.4% of the dog genome) with a median size per region of 1 Mb and involved at least 5 and 20 SNPs in 33 and 6 regions, respectively. The longest match (100 SNPs, avg. spacing 87 Kb/SNP) was a region of total loss of heterozygosity (LOH) of 8.7 Mb on chromosome 26 (CanFam 2.0 Chr26: 3.1 – 11.7 Mb), representing ~20% of the chromosome. This region did not show LOH in 6 dog breeds (n=120) genotyped for the same set of markers. A second dataset of higher density (3,023 SNPs, *cfa* 26) genotyped for 119 Boxers sampled from a different geographic area replicated the region (704 SNPs, avg. spacing 12 Kb/SNP, average MAF <0.01). This LOH region encompasses 113 annotated elements which include 97 associated genes. Also, a second region of homozygosity of 3.8 Mb on chromosome 1 was detected, where a finer region has been recently associated to brachycephaly in various breeds including boxer.

P3087 Genetic diversity of six Anatolian native cattle breeds

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As part of a national project titled "*In vitro* Conservation and Preliminary Molecular Identification of Some Turkish Domestic Animal Genetic Resources-I (TURKHAYGEN-I)", genetic structure, evolutionary relationships and genetic diversities were investigated among six local cattle breeds in Turkey. A total of 246 blood samples were collected from Anatolian Black (YK), Anatolian Grey (BI), South Anatolian Red (GAK), Native Southern Anatolian Yellow (YGS), East Anatolian Red (DAK) and Zavot (ZAV) cattle and DNA samples were isolated. A total of twenty microsatellite loci were selected from the FAO and International Society of Genetics (ISAG) panel and used for genotyping. A variety of statistical methods were used to asses the data for investigating diversity within and between the breeds. Phylogenetic analyses and observed genetic diversities were in agree with evolutionary history and geographical origins of these breeds. Findings of this study may have rational application possibilities in development of conservation strategies of native cattle breeds in Turkey.

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P3088 Is the native Australian dingo a domestic dog?

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The dingo is an Australian native animal that arrived on the island continent 4000 years ago. MtDNA and microsatellites show there is limited genetic variation in dingoes. Dingoes are under threat of extinction from interbreeding with European domestic dogs and markers are needed to differentiate them. We typed 6 dingoes from different localities using the Affymetrix-Canine-SNP-Chip V2. Analysis confirms that the dingo is related to domestic dogs but groups it with ancient breeds as the genetically least variable, most distinct of the dog breeds (vonHoldt et al, Nature 464:898, 2010). A comparison of dingoes at ~50,000 SNP loci with 500 dogs of 15 breeds shows a particular excess of homozygosity in the dingo on CFA15 at 32-56 Mb, which contains the IGF1 gene, haplotypes for which are associated with size in dog breeds. As a medium size breed, the dingo is expected to carry the wild-type IGF1 allele. The homozygosity could be a result from selection at other genes, from adaptation to the Australian environment, or a remnant of selection during the early stages of domestication. 454 sequencing of 3% of dingo genome identified 10,000 SNPs that could be used to differentiate dingo from European domestic dog. Genotyping of 1000 of these SNPs in animals from the wild would have better power to detect hybrids than currently used microsatellites. Most importantly, these approaches could reveal genetic changes selected for early in the domestication process, as well as show dog or dingo genes under selection in hybrids in the wild.

P3089 A sustainable breeding programme for the Suffolk Horse

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The Suffolk Horse is a British draught horse native to East Anglia where it was traditionally used for agricultural labouring until the mechanization of the industry led to its demise as a working horse in the mid-twentieth century. There was a sharp decline in numbers in the 1960s and today there are about 460 living horses in the UK. Careful genetic management and the use of appropriate breeding strategies are very important as the Suffolk Horse is now classified as an endangered breed. Analysis of the studbook records shows that approximately 20% of males and 55% of females are used for breeding. There is significant variation in the numbers of offspring per sire and dam, with some individuals contributing more heavily than others. The minimum inbreeding coefficient among living horses is 0.003, maximum is 0.289 and the average is 0.083. The average coancestry coefficient among all living horses is 0.096 (±0.04). The observed rate of inbreeding over the last 50 years does not exceed the expected rate, indicating that matings between close relatives are being avoided. However, the rate of inbreeding per generation was 1.12% giving an estimated effective population size (N_e) of 42. This is lower than the recommended N_e considered to give a sustainable rate of inbreeding. Alternative breeding strategies, aimed at increasing Ne, are evaluated using computer simulation to determine their potential benefits for improving population sustainability and health.

P3090 Genome sequencing of the extinct wild aurochs (*Bos primigenius*)

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Archaezoological investigations have confirmed the now extinct aurochs (*Bos primigenius*) – a large, formidable ox that ranged throughout much of Eurasia during the late Pleistocene – as the wild ancestor of modern domesticated cattle. Although these studies point towards the Near East as being the primary centre of domestication for modern European *B. taurus*, the wide geographical range of the aurochs has prompted suggestions for the existence of a secondary domestication centre within Europe. More recently, surveys of partial mitochondrial DNA (mtDNA) control region sequences generated from modern *B. taurus* and European aurochs samples have supported a Near Eastern origin for European *B. taurus* with little or no genetic contributions from local aurochsen populations. Here, we present preliminary nuclear genomic DNA sequence analysis generated from an exceptionally well-preserved Mesolithic British aurochs sample. An Illumina® Genome Analyzer was used to generate the sequence reads from this aurochs sample. These genome sequence data can be used to assess pre- and post-domestic patterns of genetic variation of a major domestic livestock species and will add a novel layer of genetic information pertaining to the ancestry of European *B. taurus*.

P3091 Impact of collection, stabilization and isolation of bovine ear punches on high and low density genotyping arrays

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Genetic-based bovine parentage identification is seeing rapid uptake, driven by increasingly affordable and comprehensive approaches. To increase adoption, the processes of tagging, collection, transport, storage and genotyping of bovine samples must be integrated, more robust, and more cost-effective. Prionics has devised a simple method of tagging and sample collection (Typifix™ sample collector). Biomatrica has developed a novel approach to long-term, room temperature sample storage (DNAgard™). Illumina now offers a comprehensive suite of bovine genotyping arrays (GoldenGate Bovine 3K, Infinium Bovine SNP50 DNA Analysis BeadChip and Infinium Bovine HD BeadChips). When combined, these three elements converge to produce a desirable system from sample collection to genetic information.

The current study evaluates elements of the desired system. Specifically, the study measures the effect of shipping and storage temperature, duration of storage and DNA extraction method on the ability to generate high quality genotypes. Genotyping results are compared among the various conditions using Illumina's GoldenGate Bovine 3K, Infinium BovineHD BeadChip, as well as Infinium Bovine SNP50 DNA Analysis BeadChip.

P3092 Sequence analysis for a de novo genome assembly of *Bos indicus* (Nelore) cattle

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A second draft sequence assembly of the bovine genome based on the sub-species, *Bos indicus*, is essential to better evaluate the genetic variation underlying the prototypical beef and dairy cattle in tropical and sub-tropical production environments. A linebred bull (Futuro), two generations removed from the 1962 Brazilian importation of Nelore, was selected for genome sequencing based on validation of indicine lineage from mtDNA sequence analysis, and a relatively low BovineSNP50 cumulative heterozygosity index of 0.16 (max = 0.40). Sequence data were produced from both Roche FLX454 and Illumina GAIIx platforms using paired end reads from long (5 and 20 kb) and short (300 and 500 bp) insert libraries and single end shotgun reads. Total sequence produced was greater than 120 Gb. After trimming for redundancy and chimerism, long insert mate pair reads yielded 175X clone coverage, while short libraries yielded 27X sequence coverage. An additional 1X and 3X sequence coverage was generated 350 and 80 bp reads shotgun reads, respectively. Sequence data are being assembled *de novo* using Celera Assembler, and the final assembly will be submitted to NCBI for annotation. Two different SNP discovery analyses of Futuro's GA-derived sequence, alone or combined with that of ten additional Nelore bulls (1X sequence depth per animal), each produced approximately 15 million SNP. For draft sequence alone, SNP were uniformly distributed across Futuro's genome suggesting heterogeneity. These SNP resources were used to develop high and low density SNP assays that will be used to better characterize all breeds.

P3093 Genetic characterization of mitochondrial DNA D-loop region in Turkish native cattle breeds

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Mitochondrial DNA D-loop region is well known as the most evolving segment. Sequence polymorphism of mtDNA D-loop region is widely used for dissemination of genetic diversity in various animal populations. The objective of this study is to determine genetic diversity and phylogenetic relationship of six native cattle breeds of Turkey as part of TUBITAK sponsored project "*In vitro* Conservation and Preliminary Molecular Identification of Some Turkish Domestic Animal Genetic Resources-I (TURKHAYGEN-I)". Total DNA samples were isolated from Anatolian Black, Anatolian Grey, South Anatolian Red, Native Southern Anatolian Yellow, East Anatolian Red and Zavot breeds. The mtDNA D-loop region was amplified and the sequences obtained were aligned with D-loop reference sequence. Haplotypes were determined and phylogenetic tree was constructed using MEGA 4.0 software. The findings indicate high genetic variation within and between the populations. The results were generally in accordance with prior knowledge about history and localization of these Anatolian cattle breeds.

P3094 Distribution of growth hormone gene polymorphisms in tench, *Tinca tinca* L, populations: preliminary results

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Various tench breeds/populations exhibit different growth performance. Hence, the attention has been focused to the growth hormone (GH) gene. Based on the previously detected polymorphism, five polymorphic sites (PSs) were selected in order to determine distribution of sequence varieties across tench populations. For a preliminary study, sixteen populations from Europe and Asia, feral as well as cultured, were taken and 15-32 individuals per population were screened using RFLP for SNPs (three PSs) and fragment length analysis for indels (two PSs). Polymorphism in the five selected PSs allowed distinguishing five different haplotypes (alleles); three of them corresponded to the western and two of them to the eastern phylogroup. Individual populations bore one to four different alleles. In three populations originating from Turkey, China and Spain, only alleles of the eastern phylogroup were observed. The others displayed alleles of both phylogroups with various allelic and genotypic frequencies. The highest degree of differentiation (F_{ST}) at the level of 0.794 was observed between blue coloured tench and a Spanish cultured population, and 67.5 % of all pairwise F_{ST} values were significantly different from zero. A Neighbour-Joining tree based on Nei's standard genetic distances divided investigated populations into two major groups, which corresponded mostly with frequencies of alleles of the eastern and western phylogroup. Preliminary results indicate that the GH gene is suitable genetic marker for more complex population studies in tench. Knowledge on the distribution of GH gene polymorphism across populations will serve for testing of effect of GH gene genotypes on growth performance.

P3095 The genetic variants effecting polled/horn status in sheep

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Undomesticated sheep typically have horns. The frequency of hornless (polled) sheep originally increased during domestication 7000-9000 yrs bp, and dramatically in English breeds after 1500 AD. This trait reflects a selection sweep where the majority of variation is controlled by a single gene and the width of the region is related to the variants origin and selection history.

The horns locus has been mapped to a 200 Kb region in sheep using a segregating (Merino x Romney) x Merino resource. We identified a candidate gene in this region. Maximal LODs exceeded 110 for the best location. Thirty-two SNPs spanning 400 Kb were subsequently genotyped over 1000 sheep from a wide variety of polled and horned breeds. A fourteen SNP haplotype was associated with the polled locus which had >97% concordance based on breed. Sequencing the gene revealed a significant structural variant in the last exon of the candidate gene in the 3' UTR that was homozygous in male polled animals from a variety of breeds. Initial analysis showed this variant may be one of those proposed loci that affect horns status in the domestic sheep. A separate analysis of ovine 50K SNP chip data involving 14 horned animals and a variety of polled animals identified this same region and suggests the gene has been subject to a major recent selection sweep.

P3096 First insight into genetic variation of fatty acids content in flesh of common carp, *Cyprinus carpio* L

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Essential fatty acids (FAs) in food have a positive effect on human health. Hence, high attention has been devoted to improvement of FA content in flesh of farmed animals through nutrition. Information about genetic variation and improvement of FA content is rather scarce. During an experimental breeding program in common carp conducted under semi-intensive pond management, a genetic variation of FA content in fillet of market size fish (1.4 kg) was investigated. For this purpose, 158 individuals from a full factorial mating of 7 females and 36 males were selected. Content of FAs was analysed by gas-liquid chromatography. Multi-trait animal model calculation verified moderate heritability for growth (0.57 ± 0.12). Heritability of selected FAs ranged from 0.0 to 0.34, but the values were not significantly different from zero in most cases. Heritability for monounsaturated FAs (MUFA) was 0.14, and was 0.11 for polyunsaturated FAs (PUFA). Low heritability was observed also in omega-3 and omega-6 FAs (0.09 and 0.08, respectively), and for omega-6/omega-3 FAs ratio (0.12). However, significant heritability (0.34 ± 0.13) was observed for eicosapentaenoic acid (EPA), a highly health-valued PUFA. High and positive genetic correlation (r=0.74) was observed between EPA and docosahexaenoic acid (DHA) content, although heritability of DHA content was insignificant (0.05 ± 0.06). Results show that there might be possibility for genetic improvement of some FA content in flesh of common carp, but a better understanding of the bases of individual variation in FA content is needed. A cost-benefit analysis will have to be conducted as well.

P3097 Assessment of genetic diversity and admixture in pony breeds

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Within the context of conservation of Canadian equine genetic resources, we investigated genetic diversity and admixture in the Newfoundland Pony, the Lac LaCroix Indian Pony, and the Canadian Horse. We also included the feral Sable Island horse population, a population from the St-Pierre et Miquelon archipelago, and 11 other pony breeds (Caspian, Connemara, Dale, Dartmoor, Fell, Fjord, Halfinger, Highland, Icelandic, Kerry Bog, and Welsh) several of which are on conservation priority lists. We genotyped 606 individuals at 22 microsatellite markers and 157 alleles were detected. The average number of effective alleles and the average allelic richness were lowest in the Lac LaCroix (2.9 and 4.2) and highest in the Newfoundland (5.0 and 6.3). Unbiased expected heterozygosities were relatively high and ranged from 0.63 in the Lac LaCroix to 0.77 in the Newfoundland and Welsh pony. As expected, 83% of the molecular variance came from within individuals, 5% among individuals and 12% among populations. Phylogenetic reconstructions based on standard genetic distances gave similar topologies supporting a close relationship between the Newfoundland pony, the St-Pierre et Miquelon horse, the Sable Island horse and the Welsh pony. Although 95% of the individuals could be assigned to their own population, 82.5% for the Newfoundland pony, the admixture analysis revealed the presence of 13 clusters as opposed to 16 recognized populations. This study further warrants the close monitoring of the Lac LaCroix, the St-Pierre et Miquelon and the Sable Island populations. The analysis of maternal lineages based on mtDNA sequence data is underway.

P3098 Estimation of genetic parameters of semen quality-related traits in Beijing-You chicken

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This study was conducted to estimate the genetic parameters of semen quality-related traits, using a MTDFREML procedure, in 516 purebred Beijing-You (BJY) full-sib male chickens derived from the same hatching, and subsequently to assess the feasibility of genetic selection to improve the semen quality of BJY chickens. Genetic parameters were estimated using an animal model which was considered the result of non-genetic factor analysis. The heritabilities and correlations among semen volume after ejaculation (VOL), semen pH (pH), semen color score (COL), the percentage of live sperm (LP), sperm motility score (MOT), the percentage of abnormal spermatozoa (ABN), and semen concentration (CON) were estimated. The results show that the estimated heritabilities of LP, MOT, and ABN were high (ranging from 0.52 to 0.85), whereas the heritabilities of VOL, COL, and CON were moderate (0.28, 0.19, and 0.12, respectively). In contrast, pH was shown to be a low heritability trait ($h^2 = 0.03$). Genetic correlations between ABN and LP and MOT were both negative (–0.37 and –0.27, respectively), whereas a high positive genetic correlation was found between LP and MOT (0.88). This indicates that selection for reduced ABN could produce improvements in LP and MOT. Moreover, given the significantly negative genetic correlations between pH and VOL, COL, LP, MOT, ABN, and CON (ranging from –0.36 to –0.66), semen quality could be directly improved by regulating the semen pH.

POSTERS 4001 – 4107

Exploiting Genomics to Dissect the Genetic Control of Complex Traits

P4001 Quantitative Trait Loci for Egg External Traits at 300 Days of Age in Chickens

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The purpose of the present study is to map quantitative trait loci (QTLs) with main effects and epistatic interaction effects on egg external traits, using a unique resource family of chickens. 421 F_2 hens were produced by full-sib matings of F_1 birds (4 males and 19 females) from matings of a Japanese Large Game (Oh-Shamo) male and three White Leghorn females, and they were genotyped for 147 microsatellite markers on 27 linkage groups. Egg external traits, including egg weight (EW), the length of the long axis of the egg (LE), the length of the short axis of the egg (SE), eggshell weight (SW), eggshell thickness (ST) and L*, a* and b* values of the eggshell color (SCL, SCA and SCB) were collected at 300 days of hen age. QTL analyses were performed with Map Manager QTX b20 software. Experiment-wise 5% significant thresholds were calculated by 1,000 permutations. 17 main-effect QTLs for EW, LE, SE, SW, ST, SCL, SCA and SCB were detected on chromosomes 1, 5, 8, 9, 11 and Z. Furthermore, one pair of QTLs on chromosomes 5 and 8 had an epistatic interaction effect on EW. These significant QTLs explained 3-13% of the phenotypic variances.

P4002 Genome-wide CHiP-SEQ Analysis of Histone 3 Lysine 27 Trimethylation During Ovine Skeletal Muscle Development Highlights Chromosome Organisation and Regulation of Gene Expression

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Ruminant skeletal muscle undergoes a major switch in its gene expression program and metabolism during the fetal to post-natal developmental transition. We hypothesized that epigenetic modifications of the genome underpin coordinated changes in gene expression which prepare skeletal muscle for movement, support against gravity and altered metabolism in the post-natal environment. Using Illumina GAII sequencing (65 bp reads; ~6 Gb) we performed genome-wide mapping of Histone H3 lysine 27 trimethylation modifications (H3K27me3 CHiP-SEQ) for skeletal muscle samples taken at late fetal development (100 days; n=3) and 12 weeks postpartum (n=3). The sequences were mapped onto the bovine genome and processed using CisGenome. The H3K27me3 peaks fell into two major categories, local and regional peaks, with the latter being predominant. H3K27me3 peaks were associated with genes, transcriptional start sites and CpG islands. Gene-associated H3K27me3 was negatively correlated with gene expression from autosomes. There were strong associations between genes with promoters enriched for H3K27me3 and several gene ontology terms common to both biological states. Gene-associated H3K27me3 peaks present in the lamb samples but absent in the fetal samples were associated with the TGF-β and WNT signaling pathways. Homeobox genes showed strong regional enrichments for H3K27me3 in both biological states. H3K27me3 was substantially enriched on the X chromosome of females but not males, thereby implicating this epigenetic mark in X chromosome inactivation. These analyses revealed remarkable modifications of the epigenome that comment on many aspects of chromosomal organisation and gene activity in the context of development.

P4003 Screening and identification of differentially expressed genes in Beijing fatty chicken and AA broiler adipose tissue

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Great differences exist in varieties of chicken adipose tissue. Excessive abdominal fat content affects broiler carcass quality, lowers feed conversion ratio and economic benefits. Abdominal fat content of local varieties in China is lower and carcass quality is higher than Arbor Acres Broiler (AA). Breeding of low-fat broilers by adoption of molecular breeding methods is an effective way of improving broiler production efficiency and product quality. Differential gene expression between Beijing fatty chicken and AA adipose tissue is of great significance to the mechanism of genetic basis in formation of traits and gene regulation. Differential display reverse transcription PCR(DDRT-PCR) was used to identify differential expression genes in Beijing fatty chicken and AA adipose tissue. A total of 10 ESTs were found using DDRT-PCR and reverse northern dot blot, all of 10 ESTs were compared with nucleotide sequences deposited in nr database and Gallus database. XF2, YF1, YF2 and YF4 had highly similar nucleotide sequences in nucleotide databases but with unknown functions. XF4 is similar to full-length cDNA clone CL0BA010ZF08 of Placenta of *Homo sapiens*, the identity is 83%; YF3 was found highly similar to MLL5 (LOC417712) but the function is unknown; XF5 and YF5 were highly similar to HMGN3; XF3 had no significant similarity with existing genes or ESTs and was regarded as a new EST. The new EST was submitted to GenBank (Accession number: EU594549). This lays a foundation for further study on the mechanism of differential gene expression in Beijing fatty chicken and AA adipose tissue.

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P4004 IGF1R: a Candidate Gene for Cattle Puberty

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Insulin growth factor I (IGF-1) influences gonadotrophin releasing hormone (GnRH) neurons during puberty through its receptor (IGF1R). GnRH neurons guide pubertal development. Therefore, IGF1R is a biological candidate gene for age of puberty. We examined associations of IGF1R SNP (single nucleotide polymorphisms) with age of puberty, defined by observation of first corpus luteum in cattle. Genotyped heifers were Tropical Composites (TC, n=866) or Brahmans (BR, n=843) from the Cooperative Research Centre for Beef Genetic Technologies. Genome-wide association was performed with BovineSNP50 Chip (Illumina 2008), which presents 7 SNP in or near IGF1R gene. Two of these IGF1R SNP were associated with puberty in TC ($p<0.04$) and 1 in BR ($p<0.0001$). To increase the number of tested SNP in this region, 5 new SNP from dbSNP and in-house sequencing were selected for additional genotyping on our population. Linkage disequilibrium (LD) between SNP was estimated using HaploView4.1 and LD differed between breeds. Single SNP and haploytpe associations with age of puberty were examined. Both global haplotype (using 12 SNP within 224 Kbp) and specific haplotype associations (window size of 2 SNP) were investigated using test score statistics and significance was determined by permutation tests in the Haplo.Stats package for R (version 2.10). One specific SNP haplotype was associated ($p<0.01$) with puberty in BR, but not in TC. There were 2 global haplotypes associated with puberty in BR ($p< 0.05$) and 1 in TC ($p< 0.05$). Together, these results indicate that IGF1R is both a positional and biological candidate gene for cattle puberty.

P4005 The miR-15a/16 cluster regulate the fully functioning of lung during the respiratory apparatus change in Chicken embryo Development

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The 18th to 20th days of chicken embryo incubation is an important time. It is the respiratory apparatus change time (RAC), during which the yolk sac constricts and moves into the abdominal cavity, the allantoid artery and venous degenerate, the respiratory apparatus changes from the allantoid to the lung and the embryo requirement for oxygen increases remarkably. And what is more, the RAC comes with the high death ratio of chicken embryo, not only in the plain chicken, but also in the plateau ones, like Tibet chicken. But on the plateau, under the low air pressure and the hypoxia, the Tibet chicken show notably lower death ratio then the plain chicken, while on the plain they are almost the same. Our study finds out that the miR-15a miR-16, a cluster, and miR-144 show a tissue special expression in lung during the RAC. What is more, the miR-15a/16 cluster shows an experiment peak value in the 19th day of the chicken embryo development, while the miR-144 not so obviously. Then we prove that the miR-15a/16 cluster regulates their target, BCL-2, to control the development of lung, especially through regulating the apoptosis of cells of parabronchus and air vesicles to improve the air exchange efficiency. So that, because the efficient air exchange function, and, of course, other body advantages, the plateau chicken can response to the hypoxia-stress. They can survive and live better than the plain chicken, on the plateau.

P4006 Comparison of the high-resolution hyplotype block structure in Canadian hybrid beef and Holstein cattle genome

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A total of 922 Canadian hybrid beef and 647 Holstein cattle were genotyped using the BovineSNP50 Beadchip. The linkage disequilibrium *(LD)* and haplotype block structure were characterized by using 49,750 and 50,055 *SNP* markers across the whole bovine genome, respectively. The average herterozygosity, average minor allele frequency *(MAF)* and average distance between *SNP*s were 0.31, 0.24, 54.68kb for hybrid beef and 0.29, 0.22, 53.63kb for Holstein. *LD* was assessed using D' and r^2 among all pairs of syntenic markers. The average r^2 and D' at 0.1*Mb* was 0.14, 0.66 in hybrid beef and 0.21, 0.78 in Holstein. Following recent human HapMap studies, a haplotype block was defined where 95% of combinations of *SNP*s within a region were in very high *LD* (D' >0.7). A total of 992 and 1749 haplotype blocks, consisting of ≥ 2 *SNP*s were identified, respectively. The average block length was 154.1±164.0*kb* in hybrid beef and 212.4±163.2*kb* in Holstein. These blocks comprised a total of 4,285 and 8,348 *SNP*s and covered 152,883*kb* and 371,537*kb* of the sequence map, which constitutes 5.8% and 14.1% of the length of all autosomes for hybrid beef and Holstein, respectively. These results provide background information concerning the extent of long range *LD* in the hybrid beef cattle population under study compared to the purebred Holstein. They also provide fundamental information concerning bovine genome organization, which will facilitate the design of studies to associate genetic variation with economically important traits.

P4007 Identifying rare genetic variants of milk genes

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In dairy cattle only a few specific genetic variants are known to affect the quantity and/or quality of milk and DNA-based selection for/against several of these variants has been successfully implemented. The gene variants known at present typically have large effects and are widespread across the cattle population. Rare genetic variants are likely to persist undiscovered even if they have large, either beneficial or detrimental effects and thereby affecting the variation in the technological qualities of milk. We present an approach to detect both common and rare genetic variants of milk genes by targeted re-sequencing performed on pools of bovine DNA using the next-generation sequencing technology "454" provided by Roche. Targeted re-sequencing facilitates the detection of genetic variants in numerous animals simultaneously, while still retaining information about which breeds and individuals that carries the genetic variants. An experimental design for pooling of genomic DNA from >20.000 animals of different breeds has been developed and implemented on beta-lactoglobulin (β-*LG*). Amplicons covering coding sequence and intronic sequence of β-*LG* were amplified and targeted according to a coded tag ligation protocol before sequencing on the Genome Sequencher FLX System (GS-FLX). The GS Reference Mapper (Roche) was used for SNP detection and sequence data was analysed using bovine chromosome 11 (Btau 4.0) as a reference sequence. Called SNPs were validated by Sanger sequencing on individual animals. With this design we were able to detect a number of new as well as previously detected SNPs in both the coding region and in introns of β-*LG*.

P4008 Construction of chicken genetic map using the Illumina 60K SNP Beadchip

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A F2 population produced by reciprocal crosses of Silky Fowl and White Plymouth Rock, which consisted of 279 chicken in 15 full sibling pedigrees, was genotyped using Illumina 60K SNP BeadChip. Quality control was assessed in GenomeStuido, including sample call rate(<95%), SNP call frequency(<95%), heterozygosity cluster intensity and separation value, SNPs with heritability or replication error. SNPs with minor allele frequency < 0.1 were excluded. As a result, 43,134 SNPs were applied to construct linkage map with CRI-MAP(version 2.503). The sex average genetic map spans approximately 3130 cM, which consists of 29 autosomes and Z chromosome. A high-resolution linkage map is critical for linkage analysis. The high marker density makes it comprehensive for genome-wide distribution of recombination rate and thus facilitates analysis of the relationship between certain genomic structure and high recombination rates, it will also uncover the linkage disequilibrium (LD) pattern and contribute to association study.

P4010 Polymorphisms in metabolic genes associated with variation in residual feed intake in beef cattle

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Residual feed intake (RFI) is a complex trait involving the interaction of several biological processes. Among these processes, feed digestion, fermentation and metabolism account for approximately 60% of the variation in RFI. The rest of the variation is accounted for by muscular activity, thermoregulation, heat increment of feeding and other unknown processes. Variations in the structure and function of genes within these pathways could explain a significant amount of the variation in RFI between different steers.

In this study single nucleotide polymorphisms located in exons of some metabolic genes were tested for association with residual feed intake. We defined RFI as the difference between the actual feed intake of an animal and its predicted feed intake based on its maintenance and growth. We genotyped 113 SNPs in 670 steers from the University's Kinsella Ranch. Twenty four SNPs were significantly associated with the variation in RFI at a p-value of 0.05 with an r^2 of 15%.

SIFT software was used to score the effects of the SNPs on protein function. Six SNPs were predicted to cause significant effect on protein function at a p-value of 0.05.

We modelled the protein structures using Swiss model modelling software to detect effects of SNPs on protein structure. Structures were successfully created for 20 of the genes. The protein structures with and without the SNP were then compared using DaliLite Pairwise comparison of protein structures software and 4 pairs of structures had significant differences.

The biological relationship between these SNPs and RFI is yet to be established.

P4011 Identification, characterization and expression of farnesoic-O-methyltransferase (FAMeT) gene and protein of the giant tiger shrimp *Penaeus monodon*

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Farnesoic acid O-methyltransferase (FAMeT) catalyzes the conversion of farnesoic acid (FA) to methyl farnesoate (MF), a putative crustacean hormone believed to be involved in shrimp reproduction. Here, the full length cDNAs of *FAMeT* in the giant tiger shrimp, *Penaeus monodon* were identified. They were 1296 and 1311 bp in length containing ORFs of 828 and 843 bp deducing to 276 and 281 amino acids, respectively. The deduced PmFAMeT proteins contained 2 CF domains restrictively found in crustaceans. *PmFAMeT* mRNA was significantly up-regulated at stage IV (mature) ovaries in intact wild broodstock ($P < 0.05$). In contrast, its expression level in stages II (vitellogenic II), III (early cortical rod) and IV ovaries was significantly greater than that in stage I (previtellogenic) ovaries of eyestalk-ablated wild broodstock ($P < 0.05$). In addition, *PmFAMeT* in ovaries of cultured juveniles (6-month-old) was greater than that of domesticated broodstock (14 and 18 months old, $P < 0.05$). However, its expression in 18-month-old shrimp was significantly increased approximately 50 fold following serotonin administration for 1 hours ($P < 0.05$). Western blot analysis revealed the positive signals of ovarian PmFAMeT in juveniles and stages I and II but not in stages III and IV ovaries of broodstock. Interestingly, juvenile shrimp possessed either 32 kDa, 37 kDa or both positive bands whereas only a 37 kDa band owing to posttranslational modifications of ovarian FAMeT was only observed in stages I and II ovaries of broodstock. Results suggested functionally important roles of *PmFAMeT* during ovarian development of *P. monodon*.

P4012 Polymorphism analysis of genes in a 3.2-Mb region on bovine chromosome 5 associated with milk fat percentage

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The recent availability of genome-wide SNP panels in cattle enables easier mapping of quantitative trait loci (QTL). Herein we performed a genome wide association study (GWAS) in 2,407 Holstein-Friesian bulls in order to explore QTL for milk production traits. Using a principal component analysis (PCA)-based approach, we identified a genomic region on BTA 5 associated with milk fat percentage. By inspecting the human-bovine comparative gene maps, we identified 16 coding genes (*LMO3, MGST1, DERA, STRAP, EPS8, PTPRO, RERG, ARHGDIB, PDE6H, ERP27, H2AFJ, MGP, ART4, GUCY2C, ATF7IP, GRIN2B*) within a 3.2-Mb region approximately centered on the most significantly associated SNPs ($P<10^{-9}$). The genomic structure of these genes was predicted using the GENOMETHREADER software. The coding exons, the untranslated regions (UTRs) and the promoter regions (-2,000 bp) of these genes were resequenced in a panel of 12 animals representing three cattle breeds (Holstein-Friesian, Fleckvieh and Braunvieh). Overall, we screened 160 kb genomic sequence and identified 278 SNPs in an attempt to identify the causative variation.

P4013 QTL scan in SSC12 for fatty acid composition of intramuscular fat using combined information of candidate genes, microsatellites and SNP chip

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Fatty acid (FA) composition influences fat and meat quality. The aim of this work was to perform a QTL scan on SSC12 for intramuscular FA composition of *M. longissimus* in an experimental cross between Iberian and Landrace pig lines. The genotyping combined information of six microsatellites, one *SREBF* SNP and 26 SNPs selected from PorcineSNP60 BeadChip were used. Moreover, the results here obtained for intramuscular fat (IMF) were compared with those from a previous QTL scan of FA in backfat (BF). The content of 23 different FA was measured in loin samples from 56 F3, 79 F2xF0 backcross and 148 F1xF0 backcross animals. One significant QTL affecting C14:0, C16:0, C18:2(n-6), C20:4, peroxidability index, double bond index, unsaturated index, and percentages of saturated and polyunsaturated FA was detected in position 51 cM, near to an intronic probe of *ACACA* gene. A second QTL affecting saturated/polyunsaturated proportion was mapped on 33 cM (between probe SIRI0000605 and *SW874* microsatellite). In this position a suggestive QTL was also detected for C18:1 (n-9). Otherwise, the QTLs here reported for C14:0 and C16:0 do not seem to match those reported previously for the same FA in backfat. Besides, different QTLs were detected for average chain length, C18:0, C18:1(n-7) and C18:3 content in this fat depot. These differences between both QTL SSC12 scans support the hypothesis of a different genetic control of FA content in both tissues. We propose *ACACA* as a powerful candidate gene to explain the first QTL.

P4014 Transcriptome profiling reveals interaction between two QTL for fatness in chicken

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In the "genetical genomics" field, different approaches are proposed to improve characterization of QTL regions for various traits combining QTL detection and transcriptome profiling. The most common approach is to identify genes whose eQTL colocalize with QTL of interest, providing new functional hypothesis about the QTL causative mutation. A second approach used only by Schadt et al (2003) and Le Mignon et al (2009) consists in performing linkage analysis for the trait using only some of the animal subgroups of a F2 population generated on the basis of their transcriptome profiles. Such an approach can refine some QTL and reveal other ones.

This approach was applied to hepatic transcriptome profiles for 45 offspring of a chicken known to be heterozygous for a QTL for abdominal fatness (AF) on GGA5 at 168cM. 688 gene expressions significantly correlated to the AF trait were obtained using a recent method taking into account the gene dependence independently of the trait of interest (Friguet *et al*, 2009; see ISAG2010 Blum *et al*). A hierarchical cluster analysis using these 688 genes distinguished five groups among the 45 birds. After removing a subgroup of 7 animals, linkage analysis revealed another QTL on the GGA5 at ~102cM. These 7 animals presented the same paternal haplotypes, suggesting that the two QTL are in interaction. We show by different approaches (ANOVA, linkage analysis) a significant interaction between the two QTL. These results show the power of the approach: transcriptome data allows separating a population into genetically homogenous subgroups, revealing the complexity of the genetic component of the complex traits.

P4015 High density linkage disequilibrium maps of chromosome 6 and 14 in Chinese Holstein cattle

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The extent and patterns of linkage disequilibrium (LD) determine the feasibility of association studies to map genes that underlie complex traits. In this study, fifteen sire families with 2,042 daughters were analyzed for LD map of chromosome 6 and 14 in a Chinese Holstein population. The Illumine Bovine GeneChip with 50K SNPs was used to genotype 2,208 cattle in total. A total of 1,937, 1,300 SNPs on chromosome 6,14 were chosen after filtering out SNPs not meeting quality control criteria (e.g. call rate <0.95, MAF<0.05, HWE<0.00001). HAPLOVIEW under the four gamete rule was implemented to analyze the haplotype block structure. Results show 179 and 320 haplotype blocks were identified in BTA6 and BTA14, respectively, and in which the largest one spans 460kb in BTA14. The analysis of pairwise D' value revealed that LD on BTA6 decayed more rapidly than on BTA14 and LD on BTA6 is much less extensive than those in other published researches. The research regarding LDU is also investigated in our study. The results presented here can be applied in future single or haplotype association analysis in Chinese Holstein cattle.

P4016 Whole genome single nucleotide polymorphism association with residual feed intake measured under different diet regimes

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The use of marker assisted selection will enhance the genetic progress for residual feed intake (RFI) in the cattle industry. Given that growing cattle are fed on diets with different compositions in the production cycle, limited information exists on whether genetic markers associated with variation in RFI are sensitive to diet type. Our objective was to determine whether similar SNP are associated with RFI in steers fed either a grower diet or finisher diet. A feeding trial was conducted over three years. There were two feeding periods within each year where either a grower diet or finisher diet was offered such that 402 and 419 steers were present in the grower-fed and finisher-fed groups, respectively. Feed intake was measured with the GrowSafe system, and RFI calculated by linear regression. Genotyping was done using the Illumina BovineSNP50 Beadchip while Haploview was used to select 11,257 tag-SNP from 40,653 SNP (mean r^2=0.6). Whole genome association analyses were implemented within each group using Proc Mixed in SAS with sire and dam as random factors. A total of 596 and 661 SNP were associated ($p<0.05$) with RFI in the grower- and finisher-fed groups, respectively. The majority of these SNP were diet specific while only 75 SNP were common between the two groups. For these SNP common to both groups, the Pearson and rank correlations of the allele substitution effects were 0.88 and 0.91 respectively. Our results indicate that despite the existence of diet-specific SNP, some SNP are associated with RFI regardless of diet type.

P4017 A 6-bp deletion in the *TYRP1* gene causes the brown coloration phenotype in Chinese indigenous pigs

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Brown coat color has been described in Chinese Tibetan, Kele and Dahe pigs and shows considerable phenotypic variation, ranging from silver to dark brown. The genetic basis of the brown coloration remains unknown. Here we performed a genome-wide association study on Tibetan and Kele pigs using the Illumina PorcineSNP 60K Beadchips, revealing that the brown colors in Chinese breeds are controlled by the same locus on pig chromosome 1. By using a haplotype sharing analysis, we refined the critical region to a 1.5-Mb interval that encompasses only one pigmentation gene: tyrosinase related protein 1 (*TYRP1*). Mutation screens of sequence variants in the entire coding region of *TYRP1* revealed a strong candidate causative mutation (c.1484_1489del), which results in loss of methionine and glycine at amino acids 945 and 946 in a predicted transmembrane domain and is likely to be of functional significance. The deletion showed complete association with the brown coloration across Chinese Tibetan, Kele and Dahe breeds by occurring exclusively in brown pigs (n = 121) and lacking in all non-brown coated pigs (n = 745) from 27 different breeds. The findings allow genetic testing for breeders to select for or against brown coat color and provide the compelling evidence that the brown colors in Chinese indigenous pigs are caused by the same ancestral mutation in *TYRP1*. Moreover, to our knowledge, this study gives the first description of genome-wide association study identifying causal mutation for a monogenic trait in the domestic pig.

P4018 Use of a whole genome association study to characterize gene networks for pig reproduction traits

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A whole-genome association study (WGAS) utilizing commercial sows born over a 6 month period (n = 683) identified genetic markers (SNPs) associated with pig reproduction traits. These traits included total number born (TNB), number born alive (NBA), number still-born (SB), number of mummies and gestation length. Traits were recorded on all parity1 females and those 558 and 442 sows that went on to parities 2 and 3, respectively. The association analyses used a Bayes C model averaging approach, which fitted ~250-300 SNPs per iteration to predict individual genetic marker effects and consecutive five genetic marker window effects. Gene networks were constructed with PubGene software using genes located at the associated chromosomal regions for each trait among different parities. Many different chromosomal regions (and therefore genes e.g., *MEF2C* on SSC2, *PTX3* on SSC13 and *ITG6* on SSC15 for TNB in parities 1, 2 and 3, respectively) were associated with each trait in different parities. These results provide evidence of temporal gene effect trends on reproductive traits in different parities. Gene network analyses determined that genes within the associated genomic regions in different parities were related by pathways including TNF for TNB and NBA, BMP for SB, insulin for gestation length, and MAPK1, activin and the ubiquitin for the mummified fetuses. Studies focusing on additional gene/SNP discovery, haplotypes, epistasis and gene networks using high density SNP chips are recommended for further understanding of the complex genetic architecture of pig reproduction traits.

P4019 A novel resource population for mapping QTLs focusing on the weight ratio of thigh meat to live body weight in chickens

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Generally, breast meat is more popular than thigh meat because of its healthiness and cooking. In contrast, most Japanese people prefer thigh meat to breast meat. Accordingly, we developed a resource population to detect QTLs affecting the thigh meat weight ratio (%) to live body weight (TMR), relating the breast meat weight ratio (%) to live body weight (BMR), and other carcass traits. Among the 23 pairs of Satsumadori male and Nagoya female (Japanese native fowl), 2 pairs were selected to parent the resource population regarding their utility for QTL analysis. In total, 420 F2 birds were produced from 12 F1 birds (4 males and 8 females) with 16 hatcheries. Three hundred and eleven F2 birds were slaughtered at 140 days of age and 12 carcass traits were measured. The average TMRs of male and female birds were 23.4 ± 1.1 % (20.8–26.1 %) and 21.4 ± 1.0 % (19.4–24.3 %), respectively. Now we are collecting genotype data of microsatellite markers for QTL analysis. Prior to QTL analysis, the association of promising marker alleles with the carcass traits was analyzed. Alleles of some markers were significantly associated with TMR or BMR. For example, in marker ADL0019 (122 cM of Ch.1); the A allele was associated with higher TMR (p=0.014) and higher meat yield (p=0.029), the B allele with lower BMR (p=0.011) and the C allele with lower TMR (p=0.0002) and higher BMR (p=0.008).

P4020 Identification of genomic regions associated with backfat thickness in synthetic cattle

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Canchim is a synthetic beef cattle developed in Brazil which have good growth potential and tropical adaptation but suboptimal fat deposition. There are genomic regions associated with fat deposition already described, among them the centromeric region of BTA14. The scope of this work was to identify genomic regions associated with backfat thickness in Canchim (5/8 Charolais + 3/8 zebu) and MA (offspring of Charolais bulls and 1/2 Canchim + 1/2 Zebu cows) populations and to validate the association of haplotypes of the BTA14 with backfat thickness in this population. Thirty animals with extreme phenotypes were genotyped with the 54 K SNP chip, revealing 100 significant SNPs contained in chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 17, 18, 19, 20, 21, 23, 24, 27, 28 and X. Thirty-four SNPs constituted seven chromosomal regions containing 3 or more SNPs located at intervals shorter than 9 Mb and, among these, ten SNPs in BTA 14 were selected for validation. Genotyping in the population was performed by the TaqMan method in families comprising more than 10 individuals with backfat thickness information at the age of 18 months (644 animals). Validation of the BTA14 SNPs revealed two haplotypes, one in the centromeric region and another in the middle region of BTA14, significantly associated with fat thickness, both with additive effects on backfat thickness. Genes located close to these two regions should be further studied to identify potential mutations involved in backfat deposition.

P4021 MicroRNA expression analysis in bovine blastocysts by Megaplex stem-loop primer Reverse Transcription

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Mammalian blastocyst formation is characterized by two segregation events, resulting in the formation of the trophectoderm, the epiblast and the hypoblast. These differentiation events are known to be regulated by lineage specific transcription factors, but it is more and more presumed that besides these totipotency and differentiation markers, miRNAs play an important role in the posttranscriptional regulation of differentiation events.

A stem-loop reverse transcription quantitative PCR with a human Megaplex primer pool was applied for the detection of miRNAs expressed in early bovine blastocysts (day 7 p.i.) and expanded/hatched bovine blastocysts (day 8 p.i.) and resulted in the detection of 93 different bovine miRNAs. Twelve out of the 93 miRNAs were differentially expressed between the day 7 and day 8 blastocysts. Eight Bta-miRs were significantly upregulated ($P<0.05$) in day 8 blastocysts, whereas 4 Bta-miRs were significantly downregulated in day 8 blastocysts compared to day 7 blastocysts. Target genes were predicted for these 12 miRNAs using the Targetscan 5.1 database. Next, gene functions and pathways enriched in these lists of predicted targets were calculated using the Ingenuity Pathway analysis software. Several pathways, such as the growth hormone, the TGF-β, the WNT/β-catening and the Notch signaling pathway were significantly enriched in these lists of predicted targets.

The results of this study confirm the presence and tight expression regulation of specific miRNAs in bovine blastocysts. Functional characterization of the identified miRNAs and their candidate target genes is now performed to elucidate their specific role during bovine blastocyst formation.

P4022 Molecular Genetics of Cortisol Secretion in Pigs

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The hypothalamic-pituitary adrenocortical axis exerts a large range of effects on production and robustness traits and is the primary neuroendocrine system involved in stress responses. Hormone secretion by the adrenal cortices under stimulation by ACTH is a major source of individual differences and several candidate genes have been identified by genomic studies (Hazard et al. BMC Genomics 9:101, 2008). In the present experiment, we sequenced a number of these candidate genes for molecular polymorphisms and studied their association with neuroendocrine and metabolic traits in a genetically diverse population (advanced intercross between LW and MS breeders).

Candidate genes were chosen in the pathways of cortisol production and/or among genes previously demonstrated to be differentially expressed such as ACTH receptor (MCR2), cholesterol suppliers (LDLR, SCARB1, STAR), regulatory factors (CREM), metabolic enzymes (CYP11A) and several genes with a currently unknown function in the adrenal glands (EIF1B, RNF2, PPAP2B). PCR primers were designed from the most recent pig genome database (http://www.ensembl.org/Sus_scrofa/Info/Index) and amplification products from animals with extreme phenotypes were sequenced to detect polymorphisms (SNP). The whole population was then genotyped with the best available technique (sequencing, high resolution melting) for association with available phenotypes (HPA axis parameters, metabolic and production traits; Foury et al. Animal 1:967, 2007). These studies open the way towards marker-assisted selection in farm animal species.

Part of these results was obtained through the EC-funded FP6 Project "**SABRE**".

P4023 Genomic Analysis of the Stress Response of Rainbow Trout to Crowding

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Genomic analyses have the potential to impact selective breeding programs by identifying markers as proxies for traits which are expensive or difficult to measure. One such trait is the physiological response of rainbow trout to the stresses of the aquaculture environment. Typical stressors can be categorized under handling and overcrowding, sub-optimal water quality parameters, and social interactions. These stressors negatively impact growth, feed intake, feed efficiency, disease resistance, flesh quality, and reproductive performance. In general, fish respond to stress by activating the neuro-endocrine system leading to elevated blood concentrations of cortisol, the principle corticosteroid in salmonids. Plasma cortisol concentrations following a ~3 hour confinement has been used as a measure for stress responsiveness in rainbow trout, however, only weak associations with production traits have been identified. Characterization of the stress response of NCCCWA germplasm identified a heritability >46% and a positive correlation between high cortisol response and body weight at 300 days. To identify the genes affecting stress response, we conducted a genome scan with over 400 loci from the NCCCWA genetic map on a three generation pedigree characterized for stress response. To date, three significant QTL have been observed. The fourth generation of this pedigree (F2s) has been bred to generate true breeding high and low (cortisol) responders (F3s) for use in functional genomic analyses and to facilitate fine QTL mapping. Identification and characterization of the genes affecting stress response will improve our understanding of the genetics of this trait and its impact on other production traits.

P4024 A genome-wide association scan for QTL affecting the number of vertebrae and ribs in a porcine F_2 resource population

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The number of vertebrae differs strongly between vertebrates but is essentially constant within any given species. However, notable variation in the number of presacral vertebrae and rib pairs exists in the domestic pig. We conducted a genome-wide association study applying the PorcineSNP60® Beadchip comprising 64,232 SNP markers and using 106 animals from a three-generation pig ressource population. The analyses were carried using the R package GenABEL and applying principal component correction to account for the structured population. We thereby have identified a strong QTL affecting the number of vertebrae and rib pairs located distal on porcine chromosome (SSC) 7 and a QTL affecting only the number of vertebrae on SSC17. Subsequently, we evaluated the three candidate genes *MESP2*, *PSEN1* and *GSC* located on SSC7 and known to be related to axial skeleton patterning. No significantly associated polymorphisms were identified within the three genes. Quantitative real-time PCR revealed no evidence for copy number variation of these genes. As the identified genome regions comprise no further obvious candidates, it is likely that our results might contribute to a better understanding of mechanisms involved in formation of the axial skeleton in mammals.

P4025 Studies of genes affecting bovine fat colour

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Beta-carotene dioxygenase-2 (*BCO2*) is a strong candidate gene affecting white/yellow fat colour variation in cattle, as well as beta-carotene concentration in milkfat and in serum. Recently, our research showed effects of the bovine *BCO2* SNP W80X on milk fat and adipose fat colour, but other genes may also be involved. One such candidate gene is retinal dehydrogenase-2 (*RDHE2*), as described here.

Tissue samples from slaughtered cattle of unspecified breeds and crosses were obtained at commercial New Zealand abattoirs (from 237 animals with yellow fat, and 38 contemporary control animals with white fat). Animals were genotyped for *BCO2* (A allele) yielding frequencies of 24.5 and 3.9%, respectively ($P < 0.001$), and for *RDHE2* SNP V6A (C allele), yielding frequencies of 73.4 and 38.2%, respectively ($P < 0.001$), with no significant interaction between loci.

Jerseys in New Zealand are known to have more yellow fat than Holstein-Friesians. Allele frequencies of bulls of these two dairy breeds and five beef breeds in New Zealand were obtained using stored semen samples (30 to 77 bulls per breed). The frequency of the *BCO2* A-allele was found to be similar in both dairy breeds (5 and 4%, respectively), and the beef breeds had 2, 7, 0, 0 and 6%, for Angus, Charolais, Hereford, Limousin and Simmental, respectively. In contrast, the *RDHE2* C-allele frequencies from the same samples were 89 and 59% in the dairy breeds, respectively, and 26, 24, 8, 4 and 92%, respectively in the beef breeds. Further studies on yellow colour are continuing.

P4026 Metabolomic profiling for the detection of the physiological background of major gene effects on bovine growth and lipid deposition

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Identifying trait-associated genetic variation offers new prospects to reveal novel physiological pathways modulating complex traits. Recently, targeted metabolomic analyses providing a functional read out of the physiological state of an individual combined with genotype association studies have demonstrated their potential for elucidating the physiological background of trait variation. Taking advantage of a specific bovine animal model, our study confirmed two major loci independently affecting pre- and/or postnatal body weight gain as well as lipid deposition: the non-synonymous *I442M* mutation in the *non-SMC condensin I complex, subunit G (NCAPG)* gene and the disruptive *Q204X* mutation in the *growth differentiation factor 8 (GDF8)* gene. Close phenotypic monitoring identified the onset of puberty as the key interval of genotype-modulated growth. Comprehensive targeted metabolomic profiling monitoring 201 plasma metabolites at puberty clearly discriminated between the two genetic loci. The specific metabolomic patterns associated with the *NCAPG I442M* and the *GDF8 Q204X* mutations mirror their divergent effects promoting either proportional or disproportional body growth, and can serve as biosignatures of divergent physiological pathways modulating growth. Our study, which is the first comprehensive targeted metabolomic profiling study in livestock, provides a link between well-described growth-regulating metabolic functions and the previously unknown specific physiological role of the NCAPG protein in mammalian metabolism. Owing to the confirmed effect of the *NCAPG/LCORL* locus on human height in several genome-wide association studies, the results obtained for bovine *NCAPG* can add valuable, comparative information on the physiological background of genetically determined divergent mammalian growth.

P4027 Profiling of Differentially Expressed Genes in QTL regions for Meat Quality, Fatty-Acid Composition and Plasma Metabolite traits on Porcine Chromosome 6 (SSC6)

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Improvement of pork quality traits is often affecting many biochemical and physiological mechanisms. This study was conducted to detect quantitative traits loci (QTL) and genomic mechanisms affecting meat quality, fatty-acid composition and plasma metabolite traits in an F_2 reference population of Korean native pig and Yorkshire crossbreds. The three-generation mapping population was generated with 349 progeny from 62 F_2 full-sib families and 18 genetic markers were used to produce a sex-average map of the chromosom6 (SSC6).

The data set was analyzed using least squares Mendelian and parent-of origin interval-mapping models. Lack-of-fit tests between the models were used to characterize QTL for mode of expressions. A total of 13 QTL were detected at the 5% genome (chromosome)-wide level for the 77 analyzed traits (meat quality, fatty-acid composition and plasma metabolite traits).

Next, the pig Affymetrix elements mapped to the SSC6 were analyzed, and the differentially expressed genes in three tissues (liver, backfat and loin muscle) between Korean native pig (KNP) and Yorkshire were collected, in particular those genes located in the internal between makers S0228 and LEPR where a QTL affecting the many multiple traits has been detected in an F_2 reference population of Korean native pig and Yorkshire crossbreds.

These differentially expressed genes combined with QTL location may offer useful information on the genetic network or mechanisms affecting meat quality traits on the QTL region of pig chromosome 6.

P4028 Genetics of litter size and embryo survival in pigs

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Reproductive performance is a critical component of sustainable animal production systems. The genetics of reproduction of the Chinese Meishan, one of the most prolific pig breeds known, merits further investigation to identify the valuable genes underlying this superior reproductive performance, which improvements is limited through traditional selective breeding programs.

The objective of this study is to identify QTL affecting reproduction traits and characterize candidate gene(s) underlying them to find potential loci for production improvement. A QTL, found on SSC8 in an earlier study, was fine mapped revealing two QTL- for embryo survival at 124 cM, and for litter size at 105 cM, together with a QTL for number born alive. A genome scan across the genome was completed identifying eleven further QTLs - for litter size (SSC6, SSC18); for number born alive (SSC18); for ovulation rate (SSC7, SSC13, SSC15, SSC18), and for teat number (SSC5, SSC6, SSC18).

The SPP1 (Secreted phosphoprotein 1) gene, with a key role in conceptus implantation and maintenance of pregnancy, is a strong candidate gene as it is located under the peak of the SSC8 QTL for prenatal survival. Current studies are assessing the amount and location of SPP1 mRNA and protein in tissues supplying the smallest and an average sized foetus in the same uterus from 9 sows around day 40 of pregnancy.

P4029 Evaluation of suitable reference genes for gene expression analysis in the *M. semimembranosus* and *M. longissimus thoracis* of beef cattle

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Gene expression analysis is commonly used in biological studies to help with comprehension of regulatory mechanisms of complex economically-important traits and diseases. Quantitative real-time PCR is currently the most sensitive and accurate procedure in determining small variations in mRNA levels of a specific gene. The variation that is observed in gene expression is partly due to translational changes and partly due to technical errors resulting from upstream sample processing. Normalization with reference housekeeping genes (HKGs) is the most recommended way to account for this error. However, a large body of literature indicates that reference genes can be highly regulated under various experimental and physiological conditions. Normalization with unstable reference genes as well as with a single HKG may lead to erroneous interpretations. It is a prerequisite of all quantitative gene expression analyses to conduct prior validation of a set of HKGs but information regarding the expression stability of bovine HKGs in different experimental conditions is very limited. This study was conducted to evaluate the stability of reference genes that can be used for normalization of gene expression data in the semimembranosus and longissimus muscles of beef cattle. The effects of animal gender on the expression stability of β-actin (ACTB), eukaryotic initiation factor-2B (EIF2B2), glyceraldehyde-3-phosphate dehydrogenase (GAPD), peptidylprolyl isomerase A (PPIA) and succinate dehydrogenase (SDHA) were investigated using Hereford-cross bulls, steers and heifers. The geNorm statistical program was used to determine the most stably expressed HKGs and normalization factors. Results will be presented at the conference.

P4030 Effect of the Fatty Acid Synthase Gene for Beef Quantity Traits in Korean Cattle (Hanwoo) Breeding Stock

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A previous study has shown that the g.17924G>A polymorphism of fatty acid synthase (FASN) is associated with unsaturated fatty acid composition in the Korean Native cattle (Hanwoo) beef, hence this study was conducted to evaluate the effect of single nucleotide polymorphisms (SNPs) within FASN gene on the selection phenotypes of Hanwoo breeding stock.

A total of 925 progeny test steers were used to genotype g.11280G>A, g.13125T>C, and g.17924G>A polymorphisms and significant associations were found among g.11280G>A, g.17924G>A, and carcass traits, such as carcass weight, backfat thickness, and beef quantity index. No significant association was found between g.13125T>C and carcass traits. Although the degree of linkage disequilibrium (LD) was not strong among g.11280G>A, g.13125T>C, and g.17924G>A in the LD analysis, four major haplotype classes were formed with the genotypic information within the FASN gene; the frequencies of the halpotypes were -GCG-[0.378], -ATG-[0.301], -GTA-[0.191], and -ACG-[0.063], respectively.

Phenotypic association was performed among these haplotypes, and the haplotype 2 (-ATG-) was significantly associated with higher carcass weight when compared to the other haplotypes, i.e. haplotype 1 (-GCG-) and haplotype 3 (-GTA-). A copy number of the FASN haplotype 3 (-GTA-) had also a significant association with carcass weight of subjects. In conclusion, it was observed that two polymorphisms (g.11280G>A and g.17924G>A) and their haplotypes within the FASN gene are consistently associated with carcass traits.

Therefore, it is desirable to use the FASN polymorphisms for pre-selection program as genetic marker with improved carcass yield and beef quality of the Hanwoo sire at the Hanwoo Improvement Center as well as for commercial Hanwoo producers, the FASN genotypic information can be used for a part of selecting Hanwoo dam for superior calf production.

P4031 Expression profile of 25 genes located in SSC7-q1.2 in regards of androstenone accumulation in backcross animals

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The accumulation of androstenone in the fat contributes to the boar taint, an urine-like odor detected in the cooked meat of a small proportion of mature boar carcasses. A QTL for accumulation of androstenone was previously identified in a Large White x Meishan cross in a region of SSC7 near the centromere. We propose to examine the expression in the testis and in the liver of adult boars (backcross animals built for this region) of 25 genes from this region to progress to the identification of the causal gene.

A new high-throughput technology (Fluidigm) allowed us the evaluation of the expression of 25 genes located in this region by only one experiment of real time PCR. Only weak differences in expression of some genes were detected between the two different genotypes of backcross animals (LW-LW and LW-MS). It is interesting to underline the over-expression of TEAD3 in the liver of LW-MS animals.TEAD3 is a gene encoding for a transcription activator which could activate the transcription of HSD3B in the liver and by this way, activate the degradation of androstenone by the liver. This over expression of TEAD3 (20%) in liver of LW-MS backcross animals is not associated with a notable variation of HSD3B expression. Nevertheless we find a good correlation between liver's expressions of HSD3B and TEAD3 in Duroc animals.

Even if the involvement of TEAD3 is incompletely demonstrated, this gene remains the best candidate gene to explain this effect on androstenone accumulation.

P4032 Fine mapping of loci controlling egg shell quality in egg layers

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Egg shell quality is important for both table egg quality and chicken reproductive performance. Losses due to poor egg shell quality can be as high as 10% throughout the production cycle. We have previously mapped quantitative trait loci (QTL) affecting egg shell traits (egg shell breaking force, egg shell deformation and shell weight) in an F2-population. The aim of this study was to fine map five of these regions on chromosomes 2, 3, 6, 14 and Z. One thousand and eight hundred hens were genotyped for 768 validated SNP markers covering the QTL regions at a density of approximately 3 SNPs / cM. We used two approaches to detect association, the R-library GenABEL and a multi-marker model that accommodates the local linkage disequilibrium structure by fitting different levels of interactions among SNPs within a sliding window. Twenty SNP markers that gave most consistent results regarding QTL location and the two association analysis methods were selected for further testing in commercial egg layer lines. These results are obtained through the EC-funded FP6 Project "**SABRE**"

P4033 The QTL landscape of swine meat and cured ham quality traits varies between muscles

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Herewith, we have explored the QTL landscape of a wide range of meat quality traits (intramuscular fat content, fatty acids profile and muscle cholesterol content) in the *gluteus medius* (GM) and *longissimus thoracis et lumborum* (LTL) muscles of Duroc pigs (N=350). Moreover, we have analysed the genetic architecture of 16 sensorial traits evaluated by a panel of specialized tasters in cured hams obtained from the *semimembranosus* (SM) and *biceps femoris* (BF) muscles of these pigs. Sensorial traits under analysis included flavour (e.g. cured, mature, bitter, sweet, metallic) and texture (e.g. pastiness, melting or firmness) attributes, along with an overall hedonic punctuation. Duroc pigs were genotyped for 116 microsatellites covering the whole genome. Statistical analyses allowed us to detect eight (SSC3, 5, 7 and 18) and five (SSC1, 6 and 12) genome-wide significant QTL for intramuscular fat content and composition traits in GM and LTL muscles, respectively. With regard to cured ham sensorial traits, three (SSC1, 3, 5) and five (SSC6, 7, 14, 16, 17) genome-wide significant QTL were detected for several BF and SM flavour attributes, respectively. Additionally, one (SSC2) and three (SSC6 and 9) genome-wide QTL for BF and SM texture properties were also found. In general, QTL showed striking differences between muscles with regard to their genomic location and significance. This spatial variability of QTL effects might complicate the implementation of marker- or gene-assisted selection schemes devoted to improve meat and cured ham quality traits.

P4034 Whole genome association with susceptibility to small ruminant lentivirus in sheep

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Ovine progressive pneumonia virus (OPPV), also known as maedi-visna, causes varying degrees of respiratory distress, body condition wasting, mastitis, arthritis, and/or encephalitis. Twenty-four percent of U.S. sheep have lifelong OPPV infection and are potential sources of transmission to naive animals. Like the human immunodeficiency virus (HIV), OPPV is a macrophage-tropic lentivirus that has eluded vaccine-based prevention. There are no known treatments for OPPV, but consistent breed differences in seroprevalence and proviral concentrations suggest a genetic basis for degree of susceptibility to OPPV. A total of 1,000 animals from the Rambouillet, Polypay, and Columbia breeds were genotyped using the Illumina OvineSNP50 marker set. Infection status was determined using 1) a competitive ELISA, which detects anti-OPPV antibodies, and 2) a quantitative real-time PCR assay, which measures OPP provirus concentration in peripheral blood leukocytes. The cELISA data yielded 28 genomewide significant or suggestive markers that accounted for 30% of the variation in cELISA status; one example is a gene with limited annotation expressed in immune cells that may play a role in regulating natural killer responses. The provirus concentration data on approximately 600 of the animals yielded 11 significant or suggestive markers accounting for 26% of the total variance in $\log_{10}$-proviral concentration; one example is an antiviral gene with activity in suppressing translation of viral transcripts. The inclusion of substantial numbers of animals from multiple breeds allowed the detection of associated regions in multiple genetic backgrounds that include genes important for susceptibility to lentiviruses such as OPPV and HIV.

P4035 Transcriptome analysis of beef cattle subcutaneous adipose tissues using 3' tag digital gene expression profiling

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Fat component and its distribution in the adipose tissues are crucial to the quality and the value of the meat. Elucidation of the molecular mechanisms of lipid formation and metabolism can be employed to improve the meat quality of beef cattle production with desirable amount of marbling fat, subcutaneous and body cavity fat. In this study, we investigated differentially expressed (DE) genes in subcutaneous adipose tissues from Hereford-Angus (n=6) and Charolais-Red Angus steers (n=6) differing in backfat thickness using next generation sequencing. Twelve 3' tag digital gene expression libraries were constructed and ~9.8 to 21.9 million tags were obtained for each library. Approximately 50%-60% of the sequence reads mapped to bovine genes. A total of 18,034 genes were identified with at least 1 tag in all analyzed animals and 11,050 genes were further analyzed using 2-way analysis of variance (2-ANOVA) with backfat thickness and breed cross as main effects. A total of 807 genes were found to be differentially expressed between high and low backfat animals ($p<0.05$), while 1,091 genes were differentially expressed between the two crossbreds. We then categorized 807 fat DE genes for molecular function and biological process using Gene Ontology (GO). Fifty-two percent of the proteins encoded by these DE genes (362) had binding activities and 12 were special for the lipid binding. In addition, 33 genes were involved in lipid metabolism. These genes may be applied as molecular markers for genetic improvement of fat related carcass traits in the beef industry.

P4036 The SLC44A5 A-326G polymorphism associated with birth weight in Holsteins

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Genetic selection has resulted in larger cows with high milk production potential but a tendency for dystocia, which is an unfavorable trait for dairy producers. Odds of dystocia increase by 13%/kg increase in birth weight. Moreover, high milk production in the dam predisposes to birth of a smaller calf and smaller birth size does not have any subsequent adverse effects on productivity. To identify genes controlling birth weight, we collected DNA from 2713 Holstein cows born and recorded their birth weight in National Livestock Breeding Center and selected 86 cows whose birth weight was more than 51 kg and 86 cows less than 35 kg. A whole-genome scan with 1151 microsatellite markers showed significant linkage to BTA3, where a linkage disequilibrium block whose χ^2 was more than 0.7 ranges from 73.955 to 73.958 Mb. The block harbors a single nucleotide polymorphism A-326G in the 5' untranslated region of solute carrier family 44, member 5 (SLC44A5). The average birth weight of 61 cows carrying A/A was 45.7 ± 0.6 kg, whereas the average birth weight of 1483 cows carrying G/G was 43.1 ± 0.1 kg. We typed this polymorphism in commercially available sires in Japan and found that the average dystocia rate of daughters derived from 21 sires carrying A/- was 0.72 ± 0.06 %, whereas the average dystocia rate of daughters derived from 62 sires carrying G/G was 0.58 ± 0.02 %. Our results suggest that the SLC44A5 A-326G polymorphism might influence birth weight in Holsteins.

P4037 Development of discrimination markers between Japanese domestic and imported beef based on a bovine 50K SNP array

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In the meat industry, correct labelling of beef origins or breeds is required to assure the quality and safety. The aim of this study was to develop discrimination markers between Japanese domestic and imported beef from the United States (US) and Australia (AUS) based on a bovine 50K SNP array using a total of 110 samples: Japanese Black (n=50), Japanese Holstein (n=50) and US cattle (n=10). Genotyping information revealed 1,081 SNPs as candidate markers that were polymorphic only in US cattle. In order to examine their applicability, PCR-RFLP was carried out in 446 Japanese cattle (300 Japanese Black and 146 Holstein). Genotyping results revealed that so called "US-specific alleles" were not detected in eleven markers, therefore these were considered to be the effective markers in terms of high specificity in US cattle. Their allelic frequencies in US cattle (n=108) ranged from 0.097 to 0.250 with an average of 0.178 and the combined identification probability of US cattle was 0.987. In addition, we also verified the applicability of these US-specific markers to AUS cattle. Their allelic frequencies in AUS cattle (n=280) ranged from 0.063 to 0.224 with an average of 0.137 and the combined identification probability of AUS cattle was 0.963. In conclusion, a set of these markers could be useful for discriminating between Japanese domestic and imported beef and would contribute to identify origins and prevent falsified labelling of beef.

P4038 Polymorphisms of fat metabolism related genes and association between genotypes and carcass traits in Japanese Black cattle

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Genes associated with fat metabolism have the potential to influence beef carcass and meat quality traits including fat deposition and fatty acid composition. In this study, we searched polymorphisms in full length CDS of lipid metabolism related genes; *diacylglycerol acyltransferase 1* (*DGAT1*), *fatty acid desaturase 2* (*FADS2*), *acetyl-Co A-carboxylase alpha* (*ACACA*) and *elongation of long chain fatty acids 6* (*ELOVL6*), and investigated the effects on beef carcass traits. Some polymorphisms were identified by sequence comparison among eight animals including five Japanese Black and three Holstein cattle. Two of them, K232A in *DGAT1* and A294V in *FADS2* were predicted to cause amino acid substitutions. We investigated associations between these genotypes and carcass traits in Japanese Black cattle (N = 438). In K232A of *DGAT1*, the effect of genotype was observed on subcutaneous fat thickness by ANOVA ($P<0.05$). Tukey-Kramer's honestly significant difference test revealed that *DGAT1* K/K type indicated 0.29 point lower subcutaneous fat thickness than A/A type ($P<0.05$). The effect of genotype A294V in *FADS2* could not be analyzed because of extremely biased genotype frequencies. In conclusion, our result suggested the effect of *DGAT1* K232A genotype on subcutaneous fat thickness and it would contribute to production of high-grade meat in beef cattle.

P4039 Identification of new quantitative trait loci affecting meat production, meat quality, and carcass traits within a Duroc purebred population

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Most quantitative trait locus (QTL) detection studies in pigs have been carried out in experimental F2 populations. However, segregation of a QTL must be confirmed within a purebred population for successful implementation of marker-assisted selection. Previously, QTLs for meat quality and carcass traits were detected on chromosome 7 in a Duroc purebred population (Uemoto et al., 2008). The objectives of the present study are to carry out a whole genome QTL analysis (except for chromosome 7) for meat production, meat quality, and carcass traits and to confirm the presence of segregating QTLs in a Duroc purebred population. One thousand four Duroc pigs were studied from base to seventh generation; the pigs comprised one closed population of a complex multigenerational pedigree such that all individuals were related. The pigs were evaluated for 6 growth traits, 7 body size traits, 8 carcass traits, 2 physiological traits, and 11 meat quality traits. A total of 119 markers were genotyped and then used for QTL analysis. We utilized a pedigree-based, multipoint variance components approach to test for linkage between QTLs and the phenotypic values using a maximum likelihood method; the logarithm of odds (LOD) score and QTL genotypic heritability were estimated. In this study, a total of 42 QTLs with suggestive linkages and 3 QTLs with significant linkages for 26 traits were detected. These included selection traits such as daily gain, backfat thickness, loin eye muscle area, and intramuscular fat content as well as correlation traits such as body size and meat quality traits.

P4040 Association of the bovine *ACSL1* gene with variation of fatty acid composition in skeletal muscle

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The intramuscular fat deposition and the fatty acid profiles of beef have high implications on meat quality. High proportions of unsaturated fatty acids are related to beef flavour and nutritional value. To screen for genetic factors affecting this trait we initially performed a microsatellite-based genome scan in a F_2 Charolais x German Holstein resource population and identified a QTL for fatty acid composition on BTA27 in a region where previously a QTL affecting marbling score had been detected in beef cattle populations. The *long-chain acyl-CoA synthetase 1 (ACSL1)* gene was recognised as the most plausible functional and positional candidate gene in the QTL interval due to its direct impact on fatty acid metabolism. ACSL1 is necessary for fatty acid degradation, phospholipid remodelling and synthesis of long acyl-CoA esters. We improved the genomic annotation of the bovine *ACSL1* gene, which is supported by *in silico* comparative sequence analysis and experimental verification. Resequencing of the complete coding and exon-flanking intronic sequences in four genomic DNA pools consisting of selected animals differing regarding intramuscular fat content and desaturase activity revealed three synonymous mutations in exons 6, 7 and 20 and five noncoding gene variants in introns 5, 6, 9, 13 and 20. An association analysis identified a SNP significantly associated with the relative content of distinct fractions of unsaturated fatty acids. The results indicate a functional role of the *ACSL1* gene in mediating the fatty acid composition in bovine skeletal muscle.

P4041 Association between Copy Number Variation and QTL for fertility on BTA7 in Israeli Holstein dairy cattle

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A QTL affecting female fertility, scored as the inverse of the number of inseminations to conception, near the centromeric end of BTA7 was detected by a daughter design of the Israeli Holstein population. Five out of 10 families analyzed were heterozygous for the QTL. The allelic substitution effect was ~1% in trait units. Seven hundred and four SNP markers on the Illumina BovineSNP50 BeadChip within the QTL confidence interval of 0 to 27 cM were preliminarily tested for "concordance". A single intergenic SNP, NGS-58879, was heterozygous for all 5 patriarchs that were heterozygous for the QTL, and homozygous for the remaining 5 bulls homozygous for the QTL. A significant effect on fertility was associated with this marker in the sample of 900 bulls genotyped ($p<10^{-5}$). Approximately 20 daughters each of the five heterozygous bulls were genotyped to determine haplotype phase between the QTL and the marker. Haplotype phase was the same for 4 bulls, and corresponded to the effect associated with the marker in the general bull population. Thus concordance was obtained in 9 out of 10 families. Copy number variation (CNV), characterized by sequencing analysis with selective primers, was found in the bovine *KIAA1683* gene in the proximity of NGS-58879. The homozygous bulls for the NGS-58879 "A" allele had two variants for the CNV, while the heterozygous bulls and the homozygous bulls for the "G" allele had three or four variants. The exceptional heterozygous bull, which showed an opposite allelic association with the QTL, had a unique combination of three variants.

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P4042 Development of Polymorphic Markers in Quail by Next Generation Sequencing

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Molecular genetic analyses in quail will benefit greatly from a higher density of genetic markers. We chose therefore to obtain high numbers of SNP (Single Nucleotide Polymorphism) by high-throughput sequencing (Titanium 454 GS-FLX, Roche) of two main types of reduced representations of the genome: restriction digested fractions of genomic DNA and EST (Expressed Sequence Tag), representing the expressed genes. The genomic fractions were generated as AFLP (Amplified Fragment Length Polymorphism) fragments and the expressed ones by preparing cDNA libraries from two tissues: embryo and brain. To optimize the information content of the SNP detected for subsequent analyses, libraries were prepared from individuals selected in the two lines involved in a QTL cross and each individual in the AFLP library was tagged. Sequencing runs produced 399,189 sequence reads from cDNA, and 1,107,451 from genomic fragments, covering over 433 Mb of sequence in total and allowing the detection of 17,400 putative SNP. Further analyses using the tags information will allow the estimation of heterozygozity in the F1 males.

Besides the interest of the production of a large number of new SNP, this technology should allow to sequence GC rich regions corresponding to the smallest microchromosomes for which there is no or few sequence in chicken. The comparison of the quail sequences with the chicken genome assembly will allow a virtual mapping of the SNP obtained, based on the high synteny conservation between these two avian species.

P4043 Progress in catfish genomics: aiming for impact at the pond bank

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Catfish (Ictalurus sp.) represents the largest segment of the modest domestic aquaculture industry in the United States. While demand continues to grow for safe, local, and sustainable food sources, catfish producers have been sidelined by lack of profitability tied to poor feed efficiency, high disease mortality, and uneven product quality. Simultaneously, competition with imported catfish and tilapia have limited market share and pricing flexibility. Molecular tools and genetic improvement of broodstock can help to address the industry's need for a high-quality, low-cost standardized product. Much of the last decade has been spent in the creation of genetic and genomic resources necessary for identification of QTL and realization of marker-assisted selection in catfish. These efforts are nearing fruition, but much work remains to accurately characterize and capture important phenotypic trait variation. Here, I will outline current progress in catfish genomics in the areas of map integration, whole-genome sequencing, transcriptome profiling, bioinformatics, and catfish species identification (market substitution). I will also highlight promising research areas and approaches that are likely to have economic impacts at the pond bank in the near-term.

P4044 Genetics of global gene expression patterns and gene networks affecting muscling in sheep

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This study investigated the genetics of gene expression traits (etraits) affecting rib eye muscle depth in sheep. Samples from 40 progeny born to high and low muscling sires (based on estimated breeding values; EBVs) were subjected to microarray based transcription profiling and genotyped using the ovine SNP 50k chip. Unsupervised clustering revealed significant genetic structure associated with the gene expression data. We next analyzed differential gene expression (DE) patterns between high and low muscling EBV groups and applied weighted gene coexpression network analyses (WGCNA) to detect hub genes unique to low and high EBV groups. Identified genes from DE profiling and WGCNA were subjected to functional annotation and GO analyses. There were distinct DE patterns between the muscling groups and distinct gene networks that were predictive of muscling. For the high vs. low EBV contrasts, 2,058 genes were statistically significant (BH-adjusted $p \leq 0.05$). The high and low EBV groups each contained 11 modules (with 64-491 genes and 130-482 genes, respectively). The biological processes most impacted by sire EBV were *miRNA processing, development* and *negative regulation of metabolic processes*. Based on several filtering criteria and GO analyses, we identified 10 and 25 genes that are unique candidate biomarkers for high and low muscling phenotypes, respectively. Finally, we targeted specific lists of etraits from these biomarkers and conducted association mapping using SNPs within cis-acting genomic regions of etraits. Preliminary results reveal genetic networks underlying muscling phenotypes. This is the first report of a 'systems genetics' approach for a muscling trait in sheep.

P4045 Genetic dissection of a complex trait: White markings in the Franches-Montagnes horse population

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A steady rise of white facial and leg markings has been observed during the domestication process of several species. In horses, the basic coat color has a significant influence on the expression of white markings with chestnut horses typically having more extended white markings than bay or black horses. We applied a whole-genome SNP association mapping approach using DNA from 53 Franches-Montagnes horses with extended white markings (cases) and 64 horses with little white markings (controls). The horse DNAs were genotyped with the equine Illumina 60k SNP genotyping microarray. The analysis identified three loci of strong association influencing the expression of white markings. One of these loci is the *MC1R* gene, which determines the basic coat color (bay vs. chestnut). The other two loci were located in a region on ECA 16 containing the *MITF* gene and a region on ECA 3 containing the *KIT* gene. The analysis revealed an epistatic interaction of the basic coat color and the quantitative expression of white markings. However, these interactions between the genotype at *MC1R* and genotypes at *MITF* and *KIT* are incomplete and not fully understood so far. For the subsequent fine-mapping the number of analyzed horses was enlarged to 384 Franches-Montagnes horses. Using the Illumina GoldenGate assay 96 polymorphisms spread over 2 Mb intervals each were genotyped for the *KIT* and the *MITF* locus, respectively. In addition the coding sequences of the *KIT* gene and the *MITF* gene were sequenced, but did not contain any obvious functional variants. We present the latest fine-mapping data regarding the *KIT* and *MITF* loci.

P4046 Functional characterisation and fine mapping of a major disease resistance QTL in Atlantic salmon

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We have previously mapped a major QTL affecting the resistance of Atlantic salmon to the viral disease infectious pancreatic necrosis (IPN) to linkage group 21. Almost all the host genetic variation in resistance is explained by this QTL, with a difference in mortality rate between homozygous resistant and homozygous susceptible animals of approximately 60% in our experiments. This unusually large effect is consistent in both the freshwater and seawater stages of the salmon life cycle, and has been applied in marker-assisted selection in a commercial breeding program. To move towards an understanding of the causal factors underlying this QTL, a microarray-based comparison of the gene expression response to IPNv challenge of alternative QTL genotypes was undertaken. The significant differentially regulated transcripts and their biological pathways provide some insight into the mode of action of the QTL. Concurrently, additional SNP and microsatellite markers were genotyped to fine map the QTL to a region of approximately 3cM, and the marker resource for further fine mapping is being generated through ongoing next generation sequencing analyses. The combination of the fine mapping and functional genomic approaches is expected to lead to candidate functional genes and ultimately the underlying causal variant for this QTL.

P4047 Feeding level influences on gene expression patterns of genes related with meat quality in pig

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The objective of this study was to investigate in Iberian pigs the alterations on gene expression patterns in muscle due to a moderate feeding level restriction. Two groups of six Iberian pigs were fed during 15 weeks with respective average daily intakes of 107 g and 86 g of feed per kg of metabolic weight. Restricted pigs showed significant lower daily gain, body weight and backfat thickness. RNA samples from diaphragm and *psoas major* muscles collected from carcasses were hybridized with Affymetrix porcine Genechip. The normalized data were analyzed using a mixed model and a FDR<0.01. A total of 151 probes were identified as differentially expressed due to the feeding treatment, which represent 135 unique genes. The analysis of these genes using DAVID database showed that the response to nutrient levels was an overrepresented GO biological process. Several of the differentially expressed probes correspond to genes previously related with pork quality such as *SCD, MSTN, SPP1, CTSD, PDK4* and *IGFBP5*. Validations of expression differences were successfully carried out for these genes by RT-qPCR. Restricted pigs showed upregulation of *MSTN, IGFBP5 and PDK4* genes and unrestricted pigs showed upregulation of *SCD, SPP1* and *CTSD* genes, in agreement with the expected response to feeding restriction in muscle, including growth inhibition and reduction of carbohydrate oxidation. In conclusion, we show that a moderate restriction (20%) of the feeding level produce alterations in the expression patterns of porcine genes implicated in muscle metabolism and growth, which would influence the meat quality.

P4048 A genome-wide association study for androstenone levels in pigs

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In many countries, male piglets are castrated shortly after birth because a proportion of un-castrated male pigs produce meat with an unpleasant flavour and odour. Main compounds of boar taint are androstenone and skatole. The aim of this high-density genome-wide association study was to identify single nucleotide polymorphisms (SNPs) associated with androstenone levels in a commercial sire line of pigs. The Illumina Porcine 60K SNP Beadchip was genotyped on 987 pigs from a commercial Duroc-based sire line, divergent for androstenone concentration. The association analysis with 47,897 SNPs using the software Plink revealed that androstenone levels in fat tissue were significantly associated with 37 SNPs. Major genetic factors on SSC1 and SSC6 showing moderate to large effects on androstenone concentration were identified in this breeding line. Among them, the 5 most significant SNPs explained together 13.7% of the genetic variance in androstenone. On SSC6, a larger region of more than 10 Mb was shown to be associated with androstenone covering several known and new candidate genes potentially involved in the synthesis and metabolism of androgens. For one of the most significant SNP variants, the difference in the proportion of animals surpassing the threshold of consumer acceptance between the two homozygous genotypes was as much as 15.6 %. The size of the effect reflects the log-normal distribution of androstenone and adds promise to the application of markers to reduce boar taint through breeding.

P4049 SuperSAGE with next generation sequencing: Gene expression effects of minerals restricted diets in rainbow trout

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One of the major goals in the fish aquaculture industry is to decrease the high incidence of skeletal malformations during the developmental stage. The aetiology of skeletal malformations is still poorly established and they emerge at diverse developmental stages and persist in adulthood. In the present study the rainbow trout (*Oncorhynchus mykiss*) was used to establish the impact of reduced mineral availability on the transcriptome of the musculoskeletal system during its development. Trout were maintained on a control or phosphorus (P)-poor or calcium (Ca)-poor diet for 12 weeks from first feeding when samples were collected. RNA was extracted from the caudal region of individual trout (n=8-10) of control and treatment groups. Three SuperSAGE (Serial Analysis Gene Expression) libraries were constructed from approx. 1µg of poly(A)$^+$-RNA and amplified ditags were directly sequenced with a 454 GS FLX sequencer. Approximately 300,000 tags (26bp) were sequenced in total representing 39,500-32,500 unique tags per SAGE library. 584 and 154 tags were differentially expressed (false discovery rate 0.2) between the control and low Ca or low P diet, respectively. Almost half of the significant tags could be assigned to known proteins (Swiss-prot) using available trout EST contig sequences. Ingenuity pathway analysis (IPA) allowed the characterization and identification of altered canonical pathways (e.g. calcium signalling), gene networks and biological functions (e.g. skeletal and muscular system development and function) affected by the treatment. Notably, similar pathway responses were observed in trout on both Ca and P deprived diets, suggesting tightly linked biological regulation for the experimental conditions.

P4050 Expression of leptin and stearoyl-CoA desaturase genes in adipose tissue, liver and *longissimus dorsi* muscle of bulls of different breeds

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Leptin (*lep*) is a protein hormone regulating the level of feed intake. In cattle it determines the lipid content in carcass. Stearoyl-CoA (*scd*) is an enzyme responsible for monounsaturated fatty acid synthesis. Its activity determines the level of monounsaturated fatty acids in adipose tissue.

Using real-time PCR method we investigated the level of expression of *lep* gene in *longissimus dorsi* muscle and adipose tissue and the expression *scd* in adipose tissue, liver and muscle. Tissue samples were taken form 100 bulls of four breeds differing in productivity traits: Holstein-Fresian, Polish Red, Hereford and Limousin. Bulls of all breeds were slaughtered at the age of 6, 9 or 12 months.

In the investigated groups, we identified statistically significant differences in stearoyl-CoA gene expression patterns between breeds and also between age groups in all examined tissues. Results show that *scd* expression in Holstein-Fresian is noticeably higher than in other examined breeds of cattle. We presume that such differences may be due to intensive milk fatty acid and fat metabolism in Holstein-Fresian cattle that came from selection for high milk yield. No significant gene expression pattern differences where found for *lep*.

P4051 Liver transcriptomic profile of Holstein-Friesian and Hereford bulls

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The gene expression profile was investigated in livers of cattle breeds distinctly different in meat and milk production capacity – Hereford (HER) and Holstein-Friesian (HF); the animals were 6, 9 and 12 months old. By real-time PCR the mRNA levels were analyzed of genes involved in the action of the somatotropic axis: growth hormone receptor (*GHR*), insulin receptor (*INSR*), insulin-like growth factor 1 and 2 (*IGF1*, *IGF2*), their receptors (*IGF1R*, *IGF2R*), and IGF-binding proteins 1, 2, 3 (*IGF-BP1*, *2*, *3*). Moreover, the 10K bovine oligo microarrays (BLO, Michigan State University, USA) were used to analyze transcriptomic profiles of 12 month old bulls from both breeds. Among all ages studied higher level of *IGF1*, *IGF-BP3* and *GHR* transcripts were found in HF bulls but *INSR* and *IGF-BP2* were up-regulated in HER bulls. Microarray analysis identified 210 genes which met the criteria of fold change ≥1.4 and $P<0.05$. Functional analyzes showed that genes differentially expressed in HER *vs.* HF were involved in Wnt and Notch signaling pathways and thereby in physiological processes of lipid metabolism, regulation of cell growth and cell death. These results provide a description of liver molecular events accompanying individual development that may underlie the phenotype differences between two cattle breeds.

P4052 The methylation profile of the *INS-IGF2* locus in the liver of cattle

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The insulin-like growth factor 2 gene (*IGF2*) encodes an essential growth factor and is imprinted in cattle. *IGF2* gene is flanked on its 5' side by the insulin (*INS*) gene. Occurrence of the common *INS-IGF* transcript confirmed the close interaction between both genes. Transcription of *INS* and *IGF2* genes results in a bicistronic mRNA. Expression of the two genes may therefore be subject to common regulation. An existence and coregulated expression of IGF2 and IGF2 antisense (*IGF2-AS*) transcripts were demonstrated in various mammalian species. In bovine we observed decreasing transcription of IGF2 from fetal to adult. The examination of methylation patterns was performed on liver tissues from bulls with two different genetic backgrounds, meat and dairy – Hereford and Holstein-Friesian. The animals were 6, 9 and 12 months old, during the intensive growth and muscle development. Bisulfite sequencing analysis of the DNA methylation patterns revealed full methylation (93%) of the *INS-IGF2* promoter independent of the age and breed. Examination of the DNA methylation patterns of *IGF-AS* differentially methylated region (DMR) revealed 16% of methylation in Hereford liver independent of the age, and 5% methylation in Holestein-Friesian liver independent of age. Different methylation patterns of *IGF-AS* could affect the transcription level of the bovine *IGF2* mRNA and may be associated with variation of developmental traits.

P4053 Fatty acid food source can affect osmoregulation in tilapia (*Oreochromis niloticus*)

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Regulation of ion and water balance is crucial for fish but little is known about the effect of different vegetable oil sources on osmoregulation. Several hormones secreted from the anterior pituitary gland regulate osmoregulation and acid–base balance and are also involved in the immune response. The expression of some candidate genes: prolactin receptor (PRLR), Natural Killer receptor (KRL) and Heat Shock Protein 70 (HSP70) was analysed in the liver and muscle of tilapia using RT-qPCR. Plasma cortisol and the concentration of different dissolved minerals in tank water were also measured. In fish, Prolactin (PRL) is known to play a role in freshwater osmoregulation by preventing both the loss of ions and the uptake of water. KLR is homologous to the mammalian NKG2/CD94 family of natural killer (NK) cell receptors and HSP70 has been related to temperature stress. Tilapia (*Oreochromis niloticus*) (n= 24) were fed for 8.5 weeks with one of four diets (n=6 fish per diet), differing in lipid source (fish oil, linseed oil, sunflower oil, or high-oleic sunflower oil) and were randomly distributed in a small-scale recirculation fresh water system in duplicate tanks (n=6 tanks per diet). PRLR, KRL and HSP70 were significantly down regulated ($p<0.05$) in individuals fed normal or high-oleic sunflower oil compared to linseed or fish oil. Plasma cortisol levels in fish and tank water Ca^{+} and Cl^{-} ions were also significantly lower in individuals fed high-oleic sunflower oil. These data suggest that osmoregulation is less of a problem in fish fed sunflower oil, which was correlated with improved welfare and immune status.

P4054 Polymorphisms of 5'-flanking regions of porcine adiponectin (*ADIPOQ*) and resistin (*RETN*) genes and their association with carcass traits

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Genes encoding adipokines, including adiponectin (*ADIPOQ*) and resistin (*RETN*), are functional candidates for carcass traits. It is known that their transcription level varies in pig breeds differing in term of fat accumulation. The aim of this study was to screen for polymorphisms in the 5'-flanking region of the porcine *ADIPOQ* and *RETN* genes in relation to selected carcass traits. Searching for polymorphism by DNA sequencing in five pig breeds: Polish Landrace – PL (n=25), Polish Large White – PLW (n=25), Duroc (n=21), Pietrain (n=12) and synthetic line L990 (n=25), revealed the presence of 5 polymorphic sites in the *ADIPOQ* and 5 in the *RETN* genes. Distribution of the polymorphism was uneven in the studied breeds. Adiponectin 5'-flanking variants were more frequent in PL and L990, whereas polymorphisms in the *RETN* gene occurred predominantly in PLW. Genotyping of three SNPs and one InDel in the *RETN* gene in PLW (n=192) showed a perfect linkage disequilibrium (LD) between them and only two haplotypes were segregating. In the *ADIPOQ* gene 4 polymorphisms were genotyped: 16bp InDel and two SNPs in PL (n=243) and one SNP in L990 (n=243). Association study showed some significant relationships, e.g. between *ADIPOQ* and loin eye area ($P<0.01$) and between *RETN* and abdominal fat weight ($P<0.05$).

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P4055 Polymorphism in 3'UTR of porcine *SCD* and *FASN* candidate genes for fatness and target sequences for miRNAs

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The porcine *SCD* and *FASN* candidate genes encode key enzymes responsible for the biosynthesis of monounsaturated fatty acids and the synthesis of palmitate from acetyl-CoA and malonyl-CoA, respectively. MiRNAs are a class of non-coding RNAs that pair to sites in the 3'UTR regions of target mRNAs to direct post-transcriptional repression. The aim of this study was to screen for polymorphisms in 3'UTR regions of the porcine *SCD* and *FASN* genes with a special focus on potential sites for miRNAs. Screening for polymorphism by DNA sequencing in four pig breeds, i.e. Polish Landrace (n=25), Polish Large White (n=25), Duroc (n=21) and Pietrain (n=12), revealed the existence of 13 polymorphisms in the *SCD* gene (the total size of the sequenced region was 3389 bp) and 2 polymorphisms in the *FASN* gene (the total size of the sequenced region was 492 bp). Two software packages (TargetScan and MicroInspector) were used to search for potential target sequences for miRNAs in 3'UTRs of both genes. The MicroInspector pointed to 1 polymorphic site (c.*1034 G>A) in the *SCD* gene that residues at a potential target for ssc-miR-185. The analysis with the use of TargetScan revealed that the human and the pig share 11 conserved target sequences in the 3'UTR of the *SCD* gene. However, none of the 13 polymorphisms occurred within these sequences. Neither TargetScan nor MicroInspector showed that the 2 polymorphisms in the *FASN* gene are located within potential target sites.

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P4056 Acute heat stress induced differential gene expression in broiler chicken

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Broilers have a very high incidence of muscle damage that is further exacerbated by heat stress. The aim of the present study was to determine the genetic basis of broiler muscle damage using microarrays. Thirty two male broilers from a male line were used in a controlled climate experiment. At 6 weeks of age, 2 birds/pen were placed in a crate in one of 4 climate chambers on each of 4 days and exposed to two treatments, Control (21°C and 50% relative humidity) or High Temperature (32°C and 75% relative humidity), for two hours. Subsequently, birds were killed and ~100 mg of tissue was sampled from the *Pectoralis major* muscle, snap frozen and stored at -80°C. RNA was extracted and back-transcribed into cDNA. cDNA was pooled on the basis of crates and hybridised to 16 Affymetrix slides. Microarray data were filtered for RAM (robust average mean) extracted expression levels > 1, which resulted in 17868/38535 probes. Following ANOVA, 29, 324 and 1627 probes were found significant at nominal 0.1%, 1% and 5% level of significance respectively. Filtered data were also visualised in BioLayout Express^{3D}. DAVID$_{6.7}$ and INGENUITY were used to study the pathway analysis. AGTR1, ANXA6, AQP2, ATP2A2, BCAR1, CACNA1B, CALD1, CAPN3, CDC2L6, EVL and HDAC4 are some of the selected genes from the list of probes at 1% level of significance. Prospective gene candidates will be identified and assessed for genetic variation in future research.

P4057 Nutritional effects on epigenetic modifications and their inheritance in pigs

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There is a growing body of evidence that environmental effects including nutrition impact on epigenetic modifications in mammals. It was further demonstrated that such induced modifications can be transgenerationally inherited implying an incomplete erasure of epimutations in primordial germ cells and early embryos. We generated a three generation pig feeding experiment to study effects of differentially fed F0 boars on gene expression and carcass traits in the next but one F2 generation. Two groups of eight F0 boars received either a control diet or a methyl-supplemented diet consisting of cofactors and methyl donors required for the one-carbon metabolism. F0 boars were mated to sows to produce the F1 male generation which was fed exclusively the control diet. These F1 boars, progeny of the F0 boars which received either a methyl-supplemented diet or a control diet, produced then 36 and 24 F2 pigs, respectively. By this means we are following the paternal transmission of potential epimutations induced by feeding. We found a significant difference in back fat thickness and differences close to statistical significance in fat thickness at 10 rib and the crop, percentage in adipose tissue and percent shoulder between the two F2 progeny groups. Furthermore, by using Agilent DNA microarray technology we performed a gene expression analysis of muscle, liver and kidney RNA from 16 F2 progeny. One half of this group were descendants from F0 boars receiving the methyl-supplemented diet and the other half originated from F0 boars fed the control diet. Results from this gene expression analysis of this three generation pig feeding experiment, their interpretation and implication will be presented.

These results are obtained through the EC-funded FP6 Project '**SABRE**'.

P4058 Dissecting the genetics of maternal behaviour in chickens

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Incubation behaviour, also known as broodiness, is a complex trait resulting from interaction of the hormonal system and environment of the bird. It is associated with increased secretion of prolactin and decreased secretion of luteinising hormone and subsequent regression of ovaries and cessation of egg production. There are differences in the propensity for incubation behaviour between chicken strains which is linked to productivity. This study was based on data from an F2 population created from the cross of White leghorn (WL, 0% incidence of incubation behaviour) and Silkie chickens (SLK, 100% incidence of incubation behaviour). Broody phenotypes (280 F2 individuals in 19 F1 families) were recorded from hens placed in pens with nest boxes and were recorded each day for broodiness onset. Blood samples were collected for DNA genotyping. Phenotypes were regressed against 90 informative microsatellite markers genotypes in 23 autosomal linkage groups and the sex chromosome using the Grid QTL implementation of the Haley and Knott QTL mapping method. Test statistics for broodiness showed that out of 276 birds phenotyped for broodiness, 45% of birds showed full broodiness, 28% birds showed partial broodiness and 28% birds showed no sign of broodiness. The evidence for a QTL affecting Broody status on chromosome 5 at 79cM was significant at the genome-wide 5% level. The 95% Confidence Interval (C.I) for broody status, however, spanned a region of around 95 cM. On the basis of these results a strategy will be pursued for fine mapping/candidate gene selection.

P4059 The FTO gene is associated with milk fat content in German Holstein cattle

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The Fat Mass and Obesity associated gene (FTO) is strongly associated with obesity in humans. Investigations in mouse and rat confirmed the influence of this gene on energy homeostasis and expenditure. Body weight regulation was suggested to arise from activity of the FTO protein in brain regions, which controls food intake. The strong conservation of this protein in vertebrates points to a similar important role of FTO in the energy and fat metabolism of the bovine species. We therefore investigated the association of polymorphisms within a 2Mb region around the bovine FTO homolog with estimated breeding values (EBV) for milk fat yield in a German Holstein bull population. Five SNPs in the candidate gene region showed significant association with the EBV of milk fat yield over the first three lactations. The most significant SNP accounts for 0.65% of the variance.

In a subsequent association study two haplotype blocks were found to be significantly associated with the EBV of milk fat yield. One of the haplotype blocks is located within the candidate gene. Paternal and maternal haplotype phases were generated by using the programme SIMWALK. The decomposition into diplotypes showed that beside additive allele effects, differences in the average fat yield depend on their parent of origin. These results suggest that different genetic effects should be considered in the explanation of the phenotypic variance.

P4060 *JARID1A* polymorphisms and their associations with body and heart weight in chickens

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The *JARID1A* gene has been related to chromatin modifications, regulation of transcription, cellular proliferation, and embryonic development. In a Brazilian chicken F_2 population important QTLs were previously mapped to the *LEI0146-LEI0174* interval on GGA1, where the *JARID1A* gene is located. We searched for polymorphisms in *JARID1A* in a broiler (TT) and in a layer (CC) line and tested their associations with body weight at 35 and 41 d (BW35 and 41), and weights of heart (HW) and lungs (LW). Six males TT and six females CC (parental generation), and ten F_1 chickens were evaluated for the identification of polymorphisms. Six regions including exons and introns were amplified and sequenced. Out of nine single nucleotide polymorphisms (SNPs) detected, one was chosen for genotyping 165 F_2 chickens by RT-PCR with TaqMan® probes. Association tests of the polymorphism with phenotypes were conducted using analysis of variance. The model included the fixed effects of hatch, sex, family, the SNP genotype, and the sex x genotype interaction. The polymorphisms found in exon regions (g.24441A>G, g.24471G>A, g.34208C>T and g.45962C>T) did not cause amino acids changes. The g.34208C>T polymorphism was associated with HW. Furthermore, the sex x genotype interaction was associated with BW41. Gene action differed between males and females for this locus: whereas an additive effect was detected on females, a dominant effect was identified for males. This was the first study to associate a *JARID1A* polymorphism with traits of interest to the poultry industry, but these results should be validated in commercial populations.

P4061 Conceptus-Endometrium Communication During Embryo Implantation and Early Placental Development in Cattle

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We have employed a systems approach to understanding the interacting gene networks in trophoblast and endometrium in ruminants during the early post-implantation period, with somatic cell nuclear transfer (SCNT) being used as a perturbation system of the process. In the present study, thirteen genes considered to be involved in the conceptus-endometrium communication on the basis of our earlier transcriptome analyses were assayed for gene expression using quantitative PCR (qPCR). A total of 67 bovine endometrial (caruncles and intercaruncular areas) and trophoblast tissue samples were biopsied at days 30 (D30) and 60 (D60) of pregnancy from females carrying conceptuses derived from artificial insemination (AI), *in vitro* fertilization (IVF) or SCNT. The 13 genes were: *BCL2L1, CSH1, CTSB, ESRRA, HDAC7A, HIF1A, IGF2BP2, KPNA1, MGAT1, PNPLA6, SIVA, STAT1,* and *TLR4*. Significant effects were found for tissue source (trophoblast, caruncle or intercaruncular areas) for 11/13 genes ($P < 0.01$), while treatment (AI, IVF or SCNT) affected expression of 6/13 genes ($P < 0.05$). Day of sampling (D30 or D60) had significant effect on the expression of 9/13 genes ($P < 0.01$), thus indicating the importance of stage of development on gene expression patterns. Two genes, *STAT1* and *BCL2L1* showed a significant tissue*treatment*day interaction. These two genes were preferentially expressed in maternal tissues, with over expression in SCNT and IVF pregnancies at D30 relative to AI. These results suggest perturbed signaling through *STAT1* and increased apoptosis in the maternal component of the developing placenta of IVF and SCNT conceptuses.

P4062 Haplotype analyses of residual feed intake based on genotypes from the PorcineSNP60 BeadChip

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Residual feed intake (RFI) measures how much feed an animal consumes compared to how much would be expected based on maintenance and growth requirements. Iowa State University used one Yorkshire population to select for reduced RFI over 6 generations while maintaining a control line randomly selected for 5 generations prior to 1 generation of selection for increased RFI. Totals of 387 select and 329 control animals with RFI data were genotyped with the PorcineSNP60 BeadChip. Single nucleotide polymorphism (SNP) effects were fitted using a Bayesian model averaging approach (Bayes-C) that simultaneously fitted various combinations of 250-300 SNPs. Windows of genomic merit from 5 consecutive (Sscrofa9 build) markers were analyzed for their contribution to genetic variance. The top 10 such regions were used to predict haplotype blocks with Haploview software, followed by phasing of genotype data with PHASE software. Haplotype effects were fit as fixed effects in a linear model in R software. All 10 regions showed at least one significant haplotype contrast at the α=0.05 level. The most significant haplotypes for RFI were located on SSC3 from 60-63 Mb, SSC3 from 54-55 Mb, and SSC2 from 31-33 Mb. More work needs to be done to validate the new significant regions, but results look promising based on the combination of previously identified genes and new candidate genes.

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P4063 Genome-wide reconstruction and population tracking of identical-by-descent haplotypes generated from whole-genome sequencing of high impact Holstein bulls

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The genomes of Holstein dairy bulls, Pawnee Arlinda Chief (Chief) and his son Walkway Chief Mark (Mark), were sequenced to ~7x and ~13x coverage, respectively, using Titanium chemistry on a 454 FLX platform. Approximately 70% of sequence reads were uniquely aligned to the reference bovine chromosome assembly (Btau 4.0). We identified ~3.2 million putative SNPs, of which ~1.0 million were useful for reconstruction of identical by descent (IBD) chromosome segments inherited by Mark from Chief. Inheritance patterns of SNP alleles were then used for whole-genome reconstruction of IBD haplotypes. The precision of SNP genotyping and phasing of alleles in Mark haplotypes were validated by high-density genotyping of Mark and 92 of his offspring. The precision of allele phase reconstruction over the whole Mark genome was up to 97%, depending on the filtering criteria used for the sequence-based SNPs. The shared IBD chromosome segments distinguishing alternative haplotypes of the two bulls were resolved to an average spacing between SNPs of ~2.5 Kb (median 400 bp). The genome sequence shows large runs of homozygosity, indicating inbreeding due to the reduced effective population size following the domestication of cattle, as well as regions of dense heterozygous sites, indicating a large effective population size pre-domestication. Using SNP genotypes, chromosomal segments in Chief and Mark were traced to their modern descendants and the genome sequence in these descendants was imputed. The approach used will facilitate the simultaneous identification of the candidate QTL regions and QTNs for economically important traits segregating among the offspring of these two champion bulls.

P4064 Gene expression profiles of bovine intramuscular preadipocytes during adipogenesis

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Beef marbling of intramuscular fat deposition is an economically important trait in the beef industry. To investigate genes involved in intramuscular adipogenesis, differential gene expression between the proliferation and differentiation phases in a clonal bovine intramuscular preadipocyte (BIP) cell line was profiled using serial analysis of gene expression (SAGE) (Mizoguchi et al, 2010; Anim Genet. 41). Of the 878 tags that showed differential expression (P<0.05), 377 were identified in the bovine RefSeq library. We selected 11 genes with dramatically changed expression (P<10^{-12}) (up-regulated genes: ADFP, ANPEP, BGN, CLU, FN1 and PTX3; down-regulated genes: VIM, SPARC, IGFBP6, SST and IGFBP4) after differentiation for further detailed studies. Differential gene expression levels were validated by quantitative real-time PCR in the BIPs, which were harvested 0, 3, 6, 9 and 12 days after adipogenic stimulation. Six of the genes (ADFP, ANPEP, BGN, CLU, PTX3, SST) showed up-regulation by day 3 post-differentiation induction (P<0.05). A further 4 genes (FN1, VIM, SPARC, IGFBP6) showed down-regulation from day 3 to the end of the culture (P<0.05). These results suggest that gene expression during adipogenesis in BIP cell lines was regulated at an early stage of differentiation. Our present study found gene expression profiles in 8 of the 11 genes confirmed by the previous data using SAGE, however we could not find gene expression profiles for the other 3 genes (FN1, SST, IGFBP4). These confirmed genes may be involved in the adipogenic processes of beef marbling. Next, we will study bovine intramuscular adipogenic mechanisms affecting marbling using molecular biological techniques and genetical approaches.

P4065 Seven nucleotide variations in sheep *DSG4* gene were in complete linkage disequilibrium

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Desmoglein 4 (DSG4) plays an important role in the regulation of growth and differentiation of hair follicles. A number of mutations in *DSG4* gene have been found to induce hypotrichosis in human, mouse and rat, implying it to be a potential candidate for wool traits in sheep. Using PCR-SSCP assay with three pairs of primers, a partial exon 16 and 3'UTR of sheep *DSG4* were screened in nine Chinese indigenous breeds and two imported breeds. PCR products of two fragments showed polymorphisms with genotypes designated as AA, AB, BB and BC, and DD, DE and EE, respectively. A and D alleles were dominant in all breeds, whereas B and E alleles were only found in Chinese breeds. C allele was very rare and only present in one Chinese sheep. Interestingly, polymorphisms in these two fragments were in complete linkage disequilibrium. Specifically, the combinations of genotypes between these two fragments were confined into AADD, ABDE, BBEE and BCDE. Sequences of the AADD and BBEE genotypes had six SNPs and one TTG insertion/deletion (indel). Among the SNPs, three were non-synonymous, while other two were synonymous, and the remaining one was located in the 3'UTR. The TTG indel was located in the coding region leading simultaneously to an amino acid substitution and an amino acid indel. Sequence of the BCDE genotype also possessed another missense mutation. The consequence of these significant variations present between the AADD and BBEE genotypes, needs further investigation.

P4066 Can we accurately predict the effective population size of a Thoroughbred horse population using SNP50 marker data?

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With many genomic methodologies relying on the presence of linkage disequilibrium (LD) between genotyped markers and related properties of the genome such as effective population size (N_e), it is important to investigate these properties within equine populations. In particular, we are interested in the potential application of genomic selection to horse populations. In order to make predictions of the potential accuracy of this approach, we require an estimate of N_e for our population. In this study, using Illumina SNP50 BeadChip genotype data for 817 UK Thoroughbreds, we examine the extent of LD between syntenic markers and use a formula for the expectation of r^2 (the squared correlation coefficient) to infer N_e for the UK Thoroughbred population. Effective population size was inferred both under the assumption of a constant population size and assuming linear growth. Under the assumption of a constant population size we estimated an N_e of around 100 for our population. Assuming linear growth, we observed a decrease in N_e since the distant past, reaching a minimum of ~90 at around twenty generations ago, followed by an increase until the present time. This pattern of changing N_e can be rationalised by current knowledge of the history of the Thoroughbred breed. Furthermore, N_e estimates derived from previous studies of inbreeding (based on UK Thoroughbred pedigree data) are in agreement with our predicted values of N_e. The relatively small N_e predicted implies genomic selection could effectively be implemented within this population using feasible sample sizes.

P4067 Transcriptional profiling of the adrenal gland for the identification of genes and pathways associated with stress response and aggressive behaviour

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Pre-slaughter stress is a key determinant of meat quality. An important factor of pre-slaughter stress is mixing of unfamiliar pigs because it may provoke aggressive behaviour. Fighting activates the hypothalamic-pituitary-adrenocortical axis, the sympatho-adrenomedullary system and increases physical activity which altogether alter metabolism of the skeletal muscle and ultimately meat quality. Stress responsiveness and aggressiveness are interrelated and both show large interindividual variation, which is partly attributable to genetic factors. To identify candidate genes for stress responsiveness and aggressiveness we performed transcriptome profiling of the adrenal gland. Animals of a commercial herd were mixed when loaded for transport to abattoir and aggressive behaviour (lesion count), physiological stress parameters (plasma levels of cortisol, creatine kinase, lactate, glucose) and meat quality (pH, color, water-holding capacity) were measured. Transcriptome of the adrenal gland of two groups of pigs, each comprising eight individuals, which differed significantly for aggressive behaviour and stress response, was compared using Affymetrix GeneChip® Porcine Genome Array. Statistical analysis revealed ~750 differentially regulated probe sets (representing ~650 annotated transcripts) at the nominal 5% significance level. Among the differentially regulated transcripts those from functional categories including lipid metabolism, small molecule biochemistry, endocrine system development and function and from canonical pathways including biosynthesis of steroids were over-represented according to Ingenuity Pathway Analysis.

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P4068 Resequencing of cattle QTL regions affecting udder health

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Many studies have identified quantitative trait loci (QTL) affecting important traits in cattle. Less success has been met in identifying the underlying genetic variation. Recent development in sequencing technologies has made it feasible to try to identify all variation in targeted regions of some hundreds of base pairs to several Mb. We have started resequencing QTL affecting udder health in cattle. Target enrichment was done using Agilent SureSelect solution hybrid selection, adapted to 454 sequencing as read-out. The targeted area comprised 8.2 Mb. After disposing repeat areas with RepeatMasker software, 55,500 120 bp RNA capture probes were created using eArray design with 2X tiling. Their specificity was tested using BLAT. Whole-genome fragment libraries from individuals with known QTL genotype were constructed using Roche 454 library preparation process. To increase yield we amplified the libraries by 15 cycles of PCR with Herculase II Phusion enzyme (Stratagene) using 454 adapter primers. In the hybrid selection step we added 2.5 µg of Bovine Hyblock DNA (Applied Genetics Laboratories) in order to block bovine repetitive sequence. Sequencing of the enriched chromosomal region was performed with 454 FLX sequencer using Titanium chemistry. We obtained on the average 78 % coverage of the regions with 4 -7 X depth. A bioinformatic pipeline was constructed for SNP detection and visualization. A set of validated SNPs will be genotyped in population samples to test for association with udder health.

These results are obtained through the EC-funded FP6 Project "**SABRE**"

P4069 Development of a novel PCR based analytical protocol for the characterization of the two variants of prolactin gene that affect milk yield in sheep breeds

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Prolactin is a lactogenic hormone which plays a significant role in milk production in mammals, and its depletion in sheep provokes severe reduction of milk secretion. Two different variants within intron 2 of the prolactin gene have been described (A and B) and this polymorphism has been recently proposed as a marker for future breeding schemes in dairy sheep. The present study fully characterized this polymorphism, resulting in a simpler and cost effective PCR-based assay for genetic identification in sheep populations. Up to now, the two variants A and B were identified by their difference in RFLP digestion patterns. This assay, however, is laborious since it requires the generation of a 2.5kb PCR fragment from genomic DNA prior to digestion, which is often difficult to obtain. By sequencing PCR products form AA and BB homozygous animals and performing alignments, we confirmed that the B variant results from a 23bp deletion (sequence: GGTGTTTCTCTTCATAAAGACTC) of the A variant of the prolactin gene. This finding assisted the design of new primers for the identification of prolactin polymorphism based on the size of the PCR product and relinquishes the need of RFLP digestions.

Using these developments, we genotyped an experimental flock of 380 Chios breed sheep and carried out association studies. In contrast to other sheep breeds, such as the East Friesian and the Serra da Estela, our preliminary data showed no significant effect of this gene on Chios first lactation milk yield. However, the effects of the prolactin gene merit more investigation.

P4070 Effect of *LEPR* genotype on hypothalamic expression of porcine candidate genes related with feed intake

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Leptin receptor is responsible of the hypothalamic control of feed intake and energy balance. It acts modulating the expression of several peptides at the arcuate nucleus. *LEPR* is a positional and biological candidate gene for growth and body composition. Association studies have been performed in experimental and commercial pig populations reporting significant effects on phenotype of c.2002C>T missense polymorphism (AF092422). The aim of this work was the evaluation of the effect of *LEPR* c.2002C>T genotype on hypothalamic leptin signalling genes´ expression on different genetic backgrounds. Hypothalamus samples were obtained from 67 pigs corresponding to two different experimental backcrosses of Iberian and either Landrace or Duroc origin, assuring similar representation of *LEPR* genotypes. Long-form of *LEPR*, Neuropeptide Y (*NPY*) and Cocaine and amphetamine regulated transcript (*CART*) cDNAs were quantified by RT-qPCR. The effect of genotype on gene expression was tested in a statistical analysis of normalized expression values, with a model including the effects of sex and breed/batch. Results indicate a very significant effect of c.2002C>T SNP on *LEPR* gene expression in both populations (p=0.003). Allele c.2002T, fixed in Iberian breed, shows lower *LEPR* expression that would lead to a lower leptin signalling, in agreement with the higher feed intake and fatness observed in this breed. Also, influence of *LEPR* genotype on *NPY* and *CART* genes expression was observed, although those effects were conditioned by genetic background. Joint results make this polymorphism a strong candidate to be the causal mutation of a highly significant QTL previously reported.

P4071 Effects of the polymorphism in the 5' regulatory region of *3β-hydroxysteroid dehydrogenase* gene on the androstenone level in backfat

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Androstenone is one of the steroid hormones and accumulated in fat causing primary boar taint. The porcine *3β-hydroxysteroid dehydrogenase* (*3β-HSD*) gene is considered a candidate gene for regulating androstenone level, because the 3β-HSD enzyme is due to a high rate of testicular androstenone biosynthesis and catalyzes the initial step of the hepatic metabolism of the androstenone with formation of the product β-androstenol. In the present study, seven new single nucleotide polymorphism (SNP) sites and one insertion/deletion (InDel) site in the 5' regulatory region of the 3β-HSD gene were identified by direct sequencing using DNA samples of four different breeds (Duroc, Yorkshire, Berkshire and Landrace). One SNP (nucleotides A or G) located at -1114 bp from the transcription start site was tested for the association analysis between genotypes and androstenone level. A total of 140 male pigs were genotyped by polymerase chain reaction-restriction fragment length polymorphism using *AluI* and measured for the accumulated androstenone level in backfat by gas chromatography-mass spectrometry. The SNP was significantly associated with the androstenone level in backfat ($P < 0.01$). The GG genotype had the lowest frequency (9.29 %) and the highest androstenone level among genotypes. Therefore, we suggest that negative selection for the genotype in the 5' regulatory region of the *3β-HSD* gene could down regulate androstenone level and consequently reduce boar taint. To clarify these suggests, the mechanistic studies are remained to reveal the effects of the SNP on the gene expression patterns.

P4072 Molecular Mechanisms Maintaining MHC Diversity

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As part of a long term study to fine-map MHC mediated resistance to the sheep nematode *Teladorsagia circumcincta,* we wish to determine the molecular mechanisms underlying both MHC diversity and immunity to nematodes. MHC diversity is maintained by balancing selection in both natural populations and livestock. Heterozygotes have superior disease resistance because they are able to recognise a wider variety of parasite molecules than homozygotes. There are two hypotheses to explain heterozygote advantage. Hypothesis A suggests that heterozygotes are more disease-resistant because allele A confers resistance to disease 1 while allele B confers resistance to disease 2. Hypothesis B suggests that heterozygotes are more resistant than homozygotes because they recognise more parasite molecules and mount more effective immune responses to each parasite. Theoretical considerations and experimental evidence favour hypothesis B. Hypothesis B implies that immune responses to most parasite molecules are protective. In our sheep - *T. circumcincta* system, animals with greater numbers of degranulated mast cells have fewer worms; this indicates a role for IgE in regulating worm numbers. Antigen specific IgE ELISA have been developed by us and others to three nematode allergens. IgE responses to each allergen are associated with a significant increase in resistance to nematode infection. These results argue against protection being mediated by a small proportion of nematode allergens. Therefore immunity to natural infection is likely to involve protective responses to a wide variety of nematode molecules.

P4073 Annotation of gene function using clustering of microarray results for gene expression

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Very large microarray datasets showing gene expression across multiple tissues and cell populations can give a window onto the transcriptional networks that underpin the differences in functional activity between biological systems. Clusters of co-expressed genes potentially indicate lineage markers and candidate regulators of cell function and predict functions for genes of currently unknown function. We have analysed a dataset comprising pure cell populations of mouse hemopoietic and non-hemopoietic cell types. Using a novel network visualisation and clustering approach, we identified expression signatures associated specifically with embryonic stem cells, mesenchymal cells and hematopoietic lineages. Analysis of selected gene clusters validated the prediction that gene function can be inferred by co-expression. A phagocyte expression cluster contained genes that may make up a 'pathway' for phagocyte differentiation. Promoters of these genes were enriched for binding sites for the Ets/PU.1 and MITF families. Another cluster was associated with the production of a specific extracellular matrix, with high levels of gene expression shared by cells of mesenchymal origin. This analysis has identified novel genes for characteristics of economic importance, including innate immunity and production traits such as muscle and fat and suggests that the clustering approach can be used to identify the function of other as yet unannotated genes.

P4074 Lipogenic and lipolytic gene expression profile of semitendinous muscle in Rasa aragonesa sheep

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Intramuscular fat is an important factor influencing the nutritional and technological quality of meat animal products. In this work four treatments varying on an intensification gradient according to the use of concentrates were studied: grazing alfalfa (GRE), grazing alfalfa with supplement for lambs (GRE-S), indoor lambs with grazing ewes (INT) and drylot (IND). The relative expression of *LPL, ACACA, FASN, FABP4, DGAT1, SCD, CPT1B, PRKAA2, LEP, PPARG* and *SREBP-1* gene was determined using RT- qPCR. Three specific housekeeping genes were used to normalize each set of results. The results of real time- qPCR showed that gene expression was significantly modulated by feeding system. Differences were found in *LPL*, *ACACA*, *SCD* and *CPT1B* gene expression. Lambs belonging to the ALF and IND-GRE group showed the lowest and highest levels of *LPL* expression respectively. The highest levels of *ACACA* gene were founded in IND-GRE group. Lambs belonging to the ALF and ALF+ S showed lower levels of *SCD* expression in comparison with IND-GRE and IND lambs. While *CPT1B* gene expression showed the lowest levels in IND- GRE group. This study shows that feeding system affect the expression of genes related with lipid metabolism.

P4075 *MTNR1A* is a potential candidate gene for seasonal reproductive activity and prolificacy in Rasa Aragonesa sheep breed

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Melatonin Receptor (MNTR1A) plays a key role in the control of the photoperiod-induced seasonality, which is mediated by the circadian levels of melatonin. Seasonality has proven to be under genetic control. The objectives of this study were to search for SNPs (single nucleotide polymorphisms) in *MTNR1A* gene and to associate them with seasonality trait in Rasa aragonesa sheep breed.

Total coding region of *MNTR1A* was sequenced and aligned among animals with extreme values for prolificacy and seasonal reproductive activity. Five SNPs were synonymous and other 5 were conservative mutations without effect on protein conformation. PCR products were digested with *Mnl*I and *Rsa*I, for two different animal designs. The daughter design was comprised across 3 different families with an average of 27 daughters per family, revealing a significant sire effect for seasonal reproductive activity ($P<0.0002$). Within-family analysis indicated significant genotype nested to sire effect in two families when digested with *Rsa*I enzyme. Finally, no significant effects were found for *Rsa*I and *Mnl*I genotypes in two groups of ewes with extreme values for prolificacy EBVs (n=235).

P4076 Genetic variability of Fatty Acid Synthase (FASN) gene in sheep

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In a previous study, a genome region significantly affecting the milk fat content of myristic and palmitic acids was detected on ovine chromosome 11 by using a sparse microsatellite map on an experimental Sardinian X Lacaune backcross population constituted of 10 half-sib families. Among the genes located in the proximity of the significant peak, Fatty Acid Synthase (FASN) was identified as a positional candidate gene since it codes for a multifunctional enzyme complex responsible for the *de novo* biosynthesis of fatty acids. Furthermore in bovine, previous studies found significant associations between SNPs in FASN and variation in the fatty acid composition of adipose and milk fat. Since only few ovine sequences were available, the bovine gene of 41 exons was used as reference. So far approximately 30% of the ovine gene has been sequenced on the 10 Sardinian x Lacaune F1 sires of 727 back-cross ewes. Six SNPs were identified in the introns: 5380 A>G, 5518 T>G, 5521 G>A, 5549 T>C, 9174 G>A, 10119 T>C and one in the 3'UTR: 19259 C>T. Two synonymous mutations were found in the exons (17890 C>A and 10019 C>T). Three mutations (10024 G>A, 18743 G>C and 18748 G>A) determine the substitution of serine with aspargine, glycine with alanine and alanine with threonine respectively. MAF ranged from 0.10 to 0.50 and the number of heterozygous sires ranged from 2 to 5. The found SNPs will be genotyped in the daughters in order to carry out an association analysis with fatty acid profile.

P4077 Hepatic Glucocorticoid Receptor NR3C1 Gene expression: the effect of sex in a slow growing chicken breed (*Gallus gallus domesticus*)

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The aim of the present study was to analyse the effect of sex on glucocorticoid receptor (GR) NR3C1 gene expression by RT-PCR. Birds' coping ability and behaviour are closely related to hypothalamus-pituitary-adrenal axis adaptation to differential stress, many stress response effects are mediated by GR which, modulating target gene transcription, controls gene expression, the reactivity level of the target cells is determined by receptor number and affinity. 48 one day old chicks (Valdarnese Bianca breed; 23 F and 25 M) have been reared in standard condition and humanely slaughter at organic production slaughter age (82 days). Hepatic GR mRNA content was investigated using one-tube, two temperature real time PCR for gene expression absolute quantification (standard curve method). Data were analysed using General Linear Model procedure of SAS statistic package, Student's t test was applied to last square means difference calculation. High GR mRNA content was found in samples' liver. No significant influence of the sex on GR expression have been recorded in the studied birds. Birds from standard intensive and organic production system are slaughtered before sexual maturity anyway, further analysis on sexual mature birds could be carried out to complete the study. Molecular biomarkers for absolute gene expression related to coping ability and behaviour represent powerful and effective early indicators for welfare evaluation both in intensive and organic poultry meat production.

P4078 RAD Sequencing: A method for linkage mapping and population genomics using next generation sequencing

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Next generation sequencing techologies are making a substantial impact on many areas of biology. However, genomic-scale population genetics studies, particularly from organisms with previously unsequenced genomes, remain prohibitively expensive. Restriction-site associated DNA sequencing, or RAD sequencing, is a high-throughput method that samples genomes at reduced complexity across target individuals. It promises to deliver high resolution population genetic data – thousands of sequenced markers across many individuals – for any organism at reasonable costs. I will demonstrate the potential of RAD Sequencing by presenting two ongoing pilot projects: the mapping of a disease resistance QTL in farmed Atlantic salmon, and the creation of high-density linkage maps of all 31 chromosomes of the diamondback moth Plutella xylostella, a major crop pest.

P4079 Integrating expression profiling and whole genome association in crossbred populations of commercial breeds for dissection of complex traits in pig

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Genetical Genomics is a useful approach for studying the effect of genetic variation on biological systems at the molecular level. Mapping transcription profiles which are correlated with traits allows discrimination of cis and trans effects which differentiate 'cause' and 'effect'. A promising mapping approach is association analysis in outbred stocks or different inbred strains. Here, we applied a whole-genome association analysis to hepatic gene expression traits in an pig commercial herd Pix(DLxDE). We focus on transcripts with expression levels that were correlated to fatness traits. A total of 150 crossbred pig were studied for whole genome transcript levels in liver by using the 24k Affymetrix expression microarrays and were subjected to genotyping using the 60k Illumina SNP chips. Lists of genes whose expression was significantly correlated with fatness traits were analysed for enrichment of functional annotation groups as defined in the Ingenuity Pathways Analysis Library. Lipid metabolism, molecule transport, carbohydrate metabolism as well as cell death signalling pathways were correlated with fatness traits. Regions affecting the transcription levels of these genes were mapped by whole genome association analysis and using an up-to-date annotation and localisation of Affymetrix probes sets and Illumina SNPs allowed discriminating cis and trans regulation. Genome-wide association of trait correlated transcripts is a powerful approach to the dissection of complex traits and their underlying molecular networks.

P4080 Association analyses of four genes within QTL for meat quality on q arm of SSC2

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Several quantitative trait loci (QTL) for different meat quality traits have been localized on the q arm of porcine chromosome 2 at position 55 – 78 cM. Association analyses were performed in a commercial Landrace x Chinese-European (LCE) crossbred population (N = 438) slaughtered at approximately 127 kg and an average age of 198 days with records for performance (growth, fat and meat accretion), meat quality (intramuscular fat – IMF, Minolta *L**, Minolta *a**, Minolta *b**) and backfat fatty acid composition traits. Polymorphisms within positional candidate genes cloned from homologous regions on HSA19 – *UBL5* (AM950288:g.543G>A), *RETN* (AM157180:g.1473A>G causing substitution Ala36Thr), *INSR* (AM950289: g.537T>C) and *CFD* (AM950287:g.257G>A) were located at positions 62.1, 64.0, 68.0 and 70.7 cM, respectively, on the current USDA USMARC map of SSC2 and had the following allele frequencies in the LCE: *UBL5 543G* – 0.57, *RETN 1473G* – 0.84, *INSR 537C* – 0.70 and *CFD* 257*G* – 0.73. The effects of alleles within the candidate genes on recorded traits were estimated using an animal model. Significant effects ($P < 0.05$) were found for IMF (*RETN*) and Minolta *L** (*RETN*, CDF). Significant allele substitution effects were 0.46 SD for IMF (*RETN*) and 3.1 SD for Minolta *L** (*RETN*, CDF). Suggestive effects ($P < 0.10$) on IMF (*UBL5*, *CDF*), Minolta *a** (*INSR*, *CDF*) and Minolta *b** were also observed. Our results support localization of QTL for meat quality traits in this region but a higher number of gene-tagged markers with known gene order are needed for detailed linkage disequilibrium mapping of these QTL.

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P4081 Polymorphism and association analysis of the porcine *SERPINE1* in the Meishan x Large White cross

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SERPINE1 (serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1) encodes for PAI-1. In humans PAI-1 levels have been found to be associated with obesity, serum triglyceride and total cholesterol and type 2 diabetes. We detected SNPs FN396538:g.566G>A in intron 3 and FN396538:g.154A>G in exon 3 causing amino acid substitution Ile159Val. Frequencies of alleles *566G* and *154A* in population under the study were 0.59 and 0.54, respectively. *SERPINE1* mapped approximately at position 8 cM on the current USDA USMARC linkage map of chromosome 3, within 95% confidence interval of QTL for fatness. The SNPs were genotyped on 565 animals of 12^{th}–15^{th} generation of the Meishan x Large White cross (PIC, Hendersonville, USA) with records for weight at end of test, life daily gain, testtime daily gain, loin depth, backfat depth. The polymorphism 566G>A was detected with *Mbi*I PCR-RFLP and SNP 154A>G with allele-specific PCR assay. In the whole population no associations between traits and the SNPs were revealed. However association analysis done within sexes showed that the SNPs 566G>A and 154A>G were associated with loin depth ($P = 0.01$) for females (N = 239) but no associations were observed for males (N = 323). The alleles *566G* and *154A* were associated with higher loin depth with allele substitution effects 0.65 and 0.56 SD, respectively. These results should be confirmed in other populations.

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P4082 Novel polymorphisms influencing muscular fatty acid profiles in cattle

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Fatty acid consumption is one of the consumers major concerns and has led to a decrease in beef meat consumption due to the relatively high concentration of saturated fatty acids (SFA) and low concentration of polyunsaturated fatty acids (PUFA). Understanding how the fatty acids participate in meat metabolism, influence flavour and juiciness, and which are the genes involved, enables improvement in meat quality. The aim of this study was to identify associations between Single Nucleotide Polymorphisms (SNPs) in candidate genes and fatty acids levels measured in 314 muscle samples of individuals belonging to 11 European bovine breeds and fed from weaning to adult weight on a similar diet. All individuals were genotyped for 71 SNPs located within 51 candidate genes involved in pathways implicated in meat production traits. Among the preliminary results, it is worth highlighting novel associations between six genes and fatty acid levels. These genes include peroxisome proliferator activated receptor gamma (PPARG), heat shock 27kDa protein 1 (HSPB1), RAR-related orphan receptor alpha (RORA), corticotrophin-releasing hormone (CRH), peroxysome proliferator-activated receptor-γ coactivator-1α (PPARGC1A) and lipoprotein lipase (LPL). We found PPARG associated with increase in PUFA (significantly for 22:5 n-3, 22:6 n-3, 20:5 n-3, and n-6 to n-3 ratio); HSPB1 with increase in n-6 to n-3 ratio; RORA linked to percentage of residual FA; CRH with increase in 20:4 n-6; PPARGC1A with amount of 18:0; and LPL with neutral 20:3 n-6 as relevant results. As meat quality is a complex trait, these results add information to improve meat from selected individuals.

P4083 Differential gene expression profile between two porcine skeletal muscles: *longissimus dorsi* and *gluteus medius*

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In the last years, a number of QTL and expression studies on pig muscle phenotypes have been conducted, most of them focused on the *longissimus dorsi* muscle. Nevertheless, very few studies have considered the existence of functional differences between muscles. In the present study we have investigated differential expression patterns between two pig muscles with relevant commercial value: *longissimus dorsi* (LD) and *gluteus medius* (GM). The *GeneChip Porcine Genome* array (*Affymetrix*) was used to obtain the expression data of 40 muscle samples belonging to 20 commercial Duroc pigs. The class comparison of the expression data between both muscles was performed including the individual as a random effect. This analysis resulted in a total of 604 *Affymetrix* probes (574 single genes after annotation) showing significant differences in mRNA expression levels between GM and LD muscles (FDR<0.05). The most notable differences affected genes encoding factors that play a crucial role in morphogenesis (*HOXB6*, *HOXB7*, *HOXA9*, *HOXA10*, *PITX2* and *TBX*), being all of them overexpressed in the GM muscle. A gene ontology analysis was carried out to perform a functional classification of genes differentially expressed between both muscles. Under the biological process category, the gene ontology chart gathered a group of terms mainly related with muscle and vasculature development, fibre contraction process, protein folding, cell migration and response to stimulus. A metabolic and gene transduction network analysis is currently underway to identify the main pathways underlying these differences.

P4084 Identification of Equine Repetitive Element-1 (ERE-1) and four SNPs in horse myostatin *(MSTN)* gene

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Horses have a very high muscle mass to body weight ratio compared to other mammalian species. The myostatin *(MSTN)* gene has become a very important candidate gene for muscularity. Polymorphisms of the *MSTN* gene are associated with muscle hypertrophy phenotypes in several mammalian species. The aim of this study was to investigate the presence of single nucleotide polymorphsims (SNPs) in MSTN. The equine MSTN gene was sequenced for 96 unrelated horses representing twelve breeds (Thoroughbred, Arabian, Wielkopolski, Silesian, Percheron, Polish Heavy Draft, Haflinger, Norwegian Fjord, Hucul, Polish Konik, Welsh and Shetland Pony). Equine Repetitive Element-1 (ERE-1) was identified in the *MSTN* 5'-flanking region (g.66484673_66484974insERE-1 227bp) in 3 Thoroughbred and 1 Arabian horse. ERE-1 was not present at this site in the EquCab 2.0 sequence nor in the sequence derived for the other horses. In addition, four SNPs were detected at the following locations: promoter region g.66484304T>C, missense mutation in exon 2 g.66487142C>A, intron 2 g.66487453T>C and exon 3 g.66489990A>G (probably 3'UTR). Future studies will investigate the correlation of these polymorphisms on muscle mass and function.

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P4085 Gene expression patterns in four brain areas associate with quantitative measure of estrous behavior in dairy cows

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The decline noticed in several fertility traits of dairy cows over the past few decades is of major concern. Understanding of the genomic factors underlying fertility is limited. Here, we aimed to identify and study those genes whose expression levels associated with quantitative measures of a key fertility trait namely estrous behavior, among genes expressed in four bovine brain areas (hippocampus, amygdala, dorsal hypothalamus and ventral hypothalamus), either at the start of estrous cycle, or at mid cycle, or regardless of the phase of cycle. An average heat score was calculated for each of 28 primiparous cows whose estrous behavior was recorded for at least two consecutive cycles starting 30 days post-partum. Gene expression was measured in brain samples collected from these cows, 14 of which were sacrificed at the start of estrus and 14 around mid cycle. For each brain area, gene expression was modeled as a function of the orthogonally transformed average heat score values using a Bayesian hierarchical mixed model. Genes whose expression patterns showed significant linear or quadratic relationships with heat scores were identified. These included genes expected to be related to estrous behavior as they influence states like socio-sexual behavior, anxiety, stress and feeding motivation (*OXT, AVP, POMC, MCHR1*), but also genes whose association with estrous behavior is novel and warrants further investigation. Studying these genes and the biological processes they control improves our understanding of the genomic regulation of estrous behavior in dairy cows.

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P4086 Identification of uterine microRNAs in pigs

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MicroRNAs (miRNAs) are a class of single-stranded small (18-25 nt) non coding RNAs that have regulatory roles on gene expression at the post-trascriptional level controlling a wide range of biological processes. Several studies have shown the involvement of miRNAs during embryogenesis but little is known about the porcine miRNAs expressed in uterus during this period. An F_2 intercross was created from 3 Iberian (Ib) boars and 18 Meishan (Me) sows in order to study prolificacy traits in pigs. Fourteen F_2 IbxMe sows, classified into two groups regarding the number of embryos (NE) at 30-32 days of gestation as high (NE≥14; n=5) or low (NE≤11; n=9) prolificacy, were used to elaborate small RNA libraries from uterus. High-throughput sequencing with the 454 Genome Sequencer FLX Titanium (Roche®) was used to determine the level of miRNAs expression in sow uterus as well as to determine if differentially expressed miRNAs could be associated with prolificacy performance. Overall, 249 miRNAs were found when our deep-sequence dataset was aligned with miRBase v.15.0. The most abundant miRNAs in pig uterus at 30-32d of gestation were miR-125b, miR-200b, miR-200c, miR-23a and miR-23b. Several miRNAs showed differences in frequency between high and low prolificacy sows suggesting that miRNAs could be involved in embryo survivability during gestation in pigs. Quantitative PCR analyses are now being performed in order to validate in a more accurate way the results obtained with the deep sequencing approach.

P4087 Sequencing and Polymorphisms in the Porcine Glucocorticoid Receptor

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Both animal and human studies have shown that during early childhood and old age, the brain is extremely sensitive to stress. High levels of stress lead to release of glucocorticoids, which bind to the glucocorticoid receptor forming a complex that acts as a transcription factor. This regulates gene expression for development, metabolism, and the immune response. Data from the rat suggests that the GR gene is epigenetically altered in the offspring of stressed mothers. The importance of this observation to other mammalian species needs further investigation. The pig is the model organism chosen for this study as its size and physiology as well as the size and structure of its chromosomes are highly similar to that of humans. The glucocorticoid receptor gene sequence in pigs has recently been completed, however many of its structural variants (SNPs and CNVs) remain unknown. We have identified and sequenced the coding and promoter regions of the porcine GR gene via a comparison of the porcine genome to the published human sequences. The sequences and polymorphisms of the 9 porcine exons are currently being compared between different individuals. Preliminary results indicate that Duroc pigs have more polymorphisms. The sequence information produced in this study will hopefully allow those regions of the promoter already known, to undergo epigenetic modification in other species to be interrogated in pig.

P4088 Transcriptome alterations due to physiological normoxic (2% O_2) culture of embryonic stem cells

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A study carried out in ARK-Genomics looked to identify consensus transcriptional changes across three well characterised embryonic stem cell lines (ESCs) in response to being cultured in 2% oxygen (physiological normoxia) compared to 21% oxygen has been reanalysed. Previous studies of culturing ESCs at 2% have reported reductions in spontaneous differentiation, reduced spontaneous chromosomal aberrations, enhanced clonality and smaller, less complex cells. We took this data, previously analysed externally, and reanalysed using our standard laboratory software. ARK-Genomics, as part of its support for gene expression, has access to a wide variety of tools, the most commonly used within the laboratory being Partek Genomics Suite and Ingenuity Pathway Analysis (IPA). While mostly in agreement with the previous analysis, the use of the GO ANOVA tool in Partek followed by subsequent analysis in IPA generated previously unidentified changes in gene expression.

P4089 An INDEL polymorphism in DGAT1 3'UTR correlates with milk production parameters in Brazilian Guzerat dairy breed

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One of the strongest QTLs influencing milk production yet identified, DGAT1 gene provides a good example of the kind of work required to transfer a QTL from taurine to indicine breeds. In *Bos taurus* breeds, the SNP DGAT1 K232A, explains 2-50% of the variance in such parameters. DGAT1 232A allele has been correlated with lower saturated fat. We found low frequencies of DGAT1 232A in Brazilian Zebu breeds (0-2%). However, an allele being absent or rare, and therefore barely detectable in association studies, does not mean that the gene itself is not a QTL. Searching for breed specific polymorphisms, we sequenced DGAT1 3' UTR in 8 Guzerat individuals. We identified a new INDEL polymorphism. We screened the INDEL in a sample of 97 Guzerat cows evaluated for: total milk production (kg), lactation length (days), content of protein, fat, lactose and solids (kg), expected progeny difference (DEP), and breeding values (BV). Significant associations ($p = 0.05$) of INDEL polymorphism were found for BVs in 305 days, total fat and protein. Comparing to +/+, genotype +/- had a loss of 297 kg in production and genotype -/- a reduction of 113 kg (comparing to +/-). The heterozygote genotype is associated with a 9 kg reduction on BV for total fat, while genotype -/- causes an additional loss of 11.5 kg. A similar trend was observed for proteins, with losses of 12.4 kg (genotype +/-) and 16 kg (genotype -/-). These results confer additional support for DGAT1 as a QTL.

Support: FAPEMIG, CNPq, PRONEX/FAPEMIG.

P4090 Effect of aggressive growth implants and/or β-adrenergic agonist supplementation on CAPN1 and CAST expression and their association with shear-force youthful beef cattle

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The interaction of intracellular protease Calpain 1 (CAPN1), and its inhibitor Calpastatin (CAST), has been associated with meat tenderness. In this study, expression of CAPN1 and CAST were investigated in youthful beef *gluteus medius* and *semitendinosus* muscles in the presence/absence of aggressive growth implants (IMP) and/or β-adrenergic agonist supplementation (BAA), in Charolais x Red Angus verses Hereford x Aberdeen Angus crossbred steers (n=56). Expression of CAPN1 and CAST were measured via real-time PCR, and the MIXED procedure in SAS was used to determine factors affecting their expression. In *gluteus medius*, CAPN1 expression was different between the two breed crosses (P=0.047), and CAST approached significance due to IMP (P=0.069). In *semitendinosus*, CAPN1 expression was associated with the interaction of IMP*BAA*breed-type (P=0.031), while CAST expression approached a significant association with IMP*breed-type (P=0.069). Shear-force values obtained from *gluteus medius* and *semitendinosus* were investigated using MIXED with IMP, BAA, and breed-type in the model, plus their interactions, along with CAPN1 and CAST expression included as covariates. For *gluteus medius* muscle, shear-force values were significantly related to CAST and the interaction of IMP*BAA*breed-type (P<0.05). For *semitendinosus*, shear-force values were also related to CAST (P=0.005), while the interaction of IMP*BAA*breed-type neared significance (P=0.084). In both muscles higher CAST levels were associated with an increase in shear-force.

P4091 Association of annexin 1 protein with fat accumulation in bovine subcutaneous adipose tissues

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The fat components of red meat products have been of interest to researchers due to the health aspects of excess fat consumption by humans. We hypothesized that differences in protein expression have an impact on adipose tissue formation during beef cattle development and growth. Therefore, in this study we evaluated the differences in the discernable proteome of subcutaneous adipose tissues of 35 beef crossbred steers [Charolais x Red Angus (CHAR) (n=13) and Hereford x Angus (HEAN) (n=22)] with different back fat (BF) thicknesses. Using 2D gel electrophoresis analysis, approximately 541-580 protein spots were detected and compared in each crossbred group, and 33 and 36 protein spots showed expression differences between tissues with high and low BF thicknesses from HEAN and CHAR crossbred, respectively. The annexin 1 protein was found to be highly expressed in both crossbred steers that had a higher BF thickness ($p < 0.05$). On the contrary, no significant difference in expression of annexin 1 gene was observed in both crossbreeds, suggesting that posttranscriptional and/or posttranslational mechanisms may be involved in regulating this protein expression associated with fat formation. This result provides a basis for future studies to develop the protein marker for assessing animals with different BF thickness.

P4092 Possible association between flesh lipid and immune response genes in Atlantic salmon (Salmo salar)

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There is an urgent need to replace fish oil in aquaculture feeds with sustainable alternatives, such as vegetable oils (VO). Individual variability has been noted in the capacity of salmon to retain or biosynthesise long chain polyunsaturated fatty acids (LC-PUFA) when fed VO diets. It has thus been speculated whether combining genetic selection with changes in commercial diet formulations might be a viable strategy to meet worldwide growing demands for aquaculture products. This study was designed to analyse potential relationships between genotype and retention/biosynthesis efficiency of LC-PUFA in Atlantic salmon fed VO diets. From fifty families fed a 100% VO diet and phenotyped for flesh total lipid (TL) and LC-PUFA content, four families were chosen for liver microarray analysis. As LC-PUFA level is intimately associated with tissue TL, families containing higher and lower flesh LC-PUFA levels were compared at two TL levels. A two-way ANOVA was performed to identify genes that responded significantly to the factors "TL" and "LC-PUFA". This analysis revealed that only a few genes (about 8% of total genes) related to lipid metabolism were significantly affected by flesh TL level and none by LC-PUFA. A surprisingly high proportion of the genes responding to the TL (28%) or LC-PUFA (38%) factors were related to immune response. A slight but potentially meaningful correlation was found between both factors (somewhat higher for LC-PUFA absolute level) and resistance to infectious pancreatic necrosis (IPN), which should be further investigated in the future and might explain, at least partially, some differences in the expression of immune response genes between families.

P4093 Molecular identification of XY sex-reversed female and YY male channel catfish

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Production of channel catfish leads U.S. aquaculture, and monosex culture may provide higher production efficiencies. Determination of phenotypic sex is labor intensive and not practical for large scale culture. Catfish have an X-Y sex determination system with monomorphic sex chromosomes. Hormonal sex reversal can produce viable XY females which produce XY and YY male offspring when mated with normal XY males. The YY males are phenotypically identical to XY males and must be identified by progeny testing which requires 2.5 years or longer. Therefore a molecular method of sex determination would improve the efficiency of YY male production. Four microsatellite loci that were linked to the sex determining locus were used to genotype normal females and males and sex-reversed females from a pool of three full-sibling families from the 2005 year class (YC2005). Putative XY females were spawned with normal XY males to produce YC2007 offspring, and microsatellite analysis identified putative XX, XY, and YY genotypes. The YC2007 XY and YY males were mated naturally or manually in 2009 with normal XX females, and sex ratios of YC2009 offspring were determined by examination of reproductive tissues. The data showed that all offspring from four putative YY sires were male while 34-66% of offspring from six putative XY sires were male. These experiments demonstrated microsatellite allele genotypes could be used to predict sex phenotype in these families to improve the efficiency of production of all male populations.

P4094 Genome-wide association and candidate gene analyses for back fat thickness in Italian Large White pigs

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Fatness in pigs is an important trait that affects carcass value and consumers' acceptance of pork. On the other hand, an appropriate fat coverage of the legs is needed for dry-cured ham production. In addition, considering fat deposition traits, the pig could represent an interesting animal model for human obesity. Back fat thickness (BFT) is a measure that can be easily recorded and that is usually included as a target trait in selection programs in purebred pig populations and commercial pig lines. In this work we carried out a genome wide association (GWA) study using the Illumina PorcineSNP60K chip and a candidate gene analysis with additional 677 single nucleotide polymorphisms (SNPs) in order to identify genes and chromosome regions affecting BFT in the Italian Large White breed. The genotyped pigs were chosen among a population of about 12,000 Italian Large White animals individually performance tested at the Test Station of the National Pig Breeder Association (ANAS) using a selective genotyping approach based on the extreme and divergent estimated breeding value (EBV) for BFT (280 with most negative and 280 with most positive EBV). Results indicated that about markers in 60 genes are associated (P nominal value <0.10) with the target trait. Several chromosome regions affecting BFT have been identified in the GWA study and some of them overlap with the position of analysed candidate genes. These results support the need to combine both approaches to confirm and identify markers associated with BTF.

P4095 Hybridization-based targeted enrichment and second generation sequencing applied to domestic animals

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Sequencing of large genomic regions associated with phenotypic traits has been a major challenge in the past to identify causative mutations. New technologies such as selective enrichment of specific genomic regions coupled with massive parallel sequencing have the potential to reduce the time and resources needed to identify genetic variation underlying both complex and monogenic traits. CRB GADIE is a French National Biological Resources Centre that provides various services for the animal genetics community (http://crb-gadie.inra.fr/). We are conducting four different pilot studies in pig, chicken and bovine to evaluate different capture strategies. Our objective is to develop, test and validate protocols for selective enrichment, both in-solution and on arrays coupled with sequencing using second-generation sequencing platforms. Each pilot study addresses different scientific questions and will allow us to test the feasibility and limits of the method. CRB GADIE plans to offer this technology as a service to the animal genetics community.

We are currently testing selective enrichment and re-sequencing on a 3 Mb continuous region that harbour the causative mutation causing the Polled phenotype in cattle. We will present the protocol and the sequence analysis of the bovine data generated by capture on Nimblegen arrays followed by sequencing using the Illumina GAII instrument.

P4096 Update for genome-wide association analyses with osteochondrosis in Hanoverian horses

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Osteochondrosis (OC) is caused by a disturbance of the endochondral ossification of growing cartilage of the articular/epiphyseal complex resulting in retention and thickening of cartilage. These primary lesions can progress to cartilage flaps, subchondral fractures and osteochondral fragments (OCD). Articulations mainly affected in horses are the fetlock, hock and stifle joints. Genetic components play a major role in OC and detection of quantitative trait loci (QTL) has substantiated the involvement of genetics. The objectives of this study were to exploit the equine Illumina 50K beadchip for genome-wide association analyses with OC. Radiographies of joints and genotypic data were available for 300 Hanoverian horses. For genome-wide mapping we performed association analyses controlling for cryptic structure of the genotypic data, relationships among animals and other fixed effects. We compared logistic regression analyses using the GRAMMAR approach with mixed model analyses and linkage mapping. Associated loci explained more than 60% of the phenotypic variance and for all these loci candidate genes were identified. The most significant hits were on ECA4, 5, 6, 16 and 18. Evidence of association could be shown for further loci. These results should be useful for validation studies in other horse breeds and fine mapping of candidate regions.

P4097 Single nucleotide polymorphisms (SNPs) of Calpain1, Calpastatin and Cathepsin D genes and their association with beef quality traits in Piemontese cattle breed

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The interest of researchers and meat industry for beef quality (BQ) has grown dramatically, but routinely assessment of BQ is difficult and expensive to be carried out. Several gene polymorphisms have been reported to be involved in BQ. The aim of this study was to evaluate the effect of *Calpain1(CAPN1), Calpastatin (CAST)* and *Cathepsin D (CTS)* genes on share force, drip loss, cooking loss and meat color in 990 young Piemontese bulls. *CAPN1* SNP g.4558 G>A and CAPN1 SNP g.6545C>T, CAST SNP g.2870A>G, CAST SNP g.2959A>G, CAST SNP g.282C>G, and CTSD SNP g.77G>A were investigated by RFLP-PCR and ARMS-PCR techniques. An association study for the aforementioned SNPs was performed using Bayesian methodology via Gibbs sampling. The model included the effects of the fattening herd, the week of BQ analysis, the genotypes of the investigated loci and the additive genetic effects of animals.

A weak effect was observed only for CAST g.282C>G and *CAPN1* g.4558 G>A on drip loss and for CAST g.2959A>G on redness. Nevertheless, a 95% Bayesian confidence region of estimates included zero. These results suggest that genotypes at all investigated loci did not strongly affect BQ.

P4098 GWAS using a 60K SNP chip to explore genomic control of boar taint in pigs

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Androstenone and skatole accumulate in the fat of mature intact (i.e. non-castrated) male pigs. These compounds can be detected as boar taint - an offensive odour affecting the smell and taste of the cooked product. Genetic variation has been shown to exist for levels of these compounds and provides a more sustainable solution than castration for commercial pig production.

A pilot study using 2,700 SNPs identified SNPs around the *CYP2E1* gene on SSC14 explaining 5% of the phenotypic variation in skatole. The current study is a high density genome-wide association study utilising a 60K SNP chip to explore variation throughout the genome including biological pathways and interactions.

Data selected from 6,000 Danish Landrace pigs comprised of 500 individuals with high skatole (>0.3 µg/g), each matched with a low skatole litter mate. Skatole and androstenone were measured at slaughter. After quality control, genotypes from 44,967 SNPs on 938 individuals were included. Polygenic and fixed effects were accounted for using a linear model using ASReml2 software. GWAS on the residuals was implemented using GenABEL software.

Preliminary results confirm the effect of *CYP2E1*. This locus remains the strongest association for Skatole although interestingly does not affect levels of androstenone. Preliminary results for androstenone suggest a large effect on SSC5. Genetic variation in, and correlations with, traditional slaughter traits are also being investigated. Together these results present a unique opportunity to explore the biological mechanisms underpinning boar taint and provide breeding solutions for commercial pig production.

P4099 Genome-wide expression analysis of mammary epithelial cells (MEC) during early lactation in buffalo (*Bubalus bubalis*) and zebu cattle (*Bos indicus*)

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The molecular physiology underlying buffalo milk production is largely unknown. The purpose of this study was to understand the transcriptional differences between buffaloes and native cows during early stage of lactation. The study included milk purified mammary epithelial cells (MECs) from healthy Murrah buffaloes (2) and Sahiwal cows (2) at 15 days post calving. The Agilent 44K bovine array chip was employed for transcriptome analysis. High proportions of genes showed similar expression levels in MEC of both species. A total of 159 differentially expressed genes (DEG) were identified using cut off criteria: P<0.01 and change >2.0 fold. Out of these, 140 genes were up-regulated and 19 genes were down-regulated in buffalo MEC. The Gene ontology analysis associated DEG with many biological processes like actin filament-based process, sequestering of actin monomers, cytoskeleton organization, maintenance of protein location *etc.* Similarly several molecular functions *viz.* actin binding, cytoskeletal protein binding, cystein/serine-type endopeptidase inhibitor activity, enzyme inhibitor activity were found to be affected. Several genes were significantly up-regulated in buffalo MEC as compared to cow MEC; viz. beta-2 microglobulin (139), thymosin beta (77.7), osteopontin (46.4), LIM and SH3 protein (26.6), intercellular adhesion molecule-3 (20.3), heterogeneous nuclear ribonucleoprotein F (19.05), adenyl cyclase-associated protein 1 (16.1), and stearoyl CoA desaturase (15.3). Global expression analysis thus showed subtle differences in transcriptional pattern of certain genes at early lactation and holds promise in characterizing the transcriptome to better understand lactation biology of the two dairy species.

P4100 Single nucleotide polymorphism(SNP) in *MAF1* and *GPAA1* genes and association analysis with carcass and meat quality traits in Angus cattle

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The use of single nucleotide polymorphism(SNP) as detectable molecular markers is a promising alternative to the current methods of trait selection, when these markers are proven to be associated with traits of interest in animals. The bovine *MAF1* gene is located on chromosome 14 within a QTL region for fat yield and fat percentage. *GPAA1* gene serves as a general mechanism for linking proteins to the cell surface membrane. Together these genes underlie the QTL region on chromosome 14. Thus, we sought to evaluate the extent to which polymorphisms in these genes are associated with carcass and meat quality traits in Angus cattle. Single nucleotide polymorhisms (SNPs) were identified in *MAF1* and *GPAA1* genes by direct sequencing and genotyping for all SNPs was accomplished with the Sequenom MassARRAY system. Two synonymous SNPs in *MAF1* were identified (MAF1201 C>T; MAF1282 T>C) in exon-3 and another SNP (MAF1109 G>A) was identified in intron-3. In addition, a synomymous SNP (GPAA1229 C>T) was identified within exon-8 of *GPPA1*. Association analysis between each of the identified SNPs and carcass and meat quality traits was performed with 1139 head of Angus bulls, steers and heifers from two different locations. Among the three *MAF1* SNPs genotyped, only MAF1109 G>A showed some trend for marbling score (MS) (P=0.085) with no population x SNP interaction. Another SNP, MAF1282 T>C showed suggestive association for CalcYG (P=0.067) with a significant population x SNP interaction (P< 0.01). This was highly significant (P<0.01) within the California population with no significant association in ISU population. However, a Bonferroni correction showed no significant association of these SNPs with carcass or meat quality traits. The GPAA1229 C>T genotypes had no significant association with any of the traits in Angus beef cattle. Thus overall, we identified 3 SNPs in *MAF1* and a SNP in *GPAA1* of which none were significantly associated with carcass or meat quality traits in Angus cattle.

P4101 Identifying differentially expressed genes in lactating and non-lactating mammary gland of water buffalo (*Bubalus bubalis*) by PCR-based suppression subtractive hybridization technique

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To identify differentially expressed transcripts in lactating (LAC) and non-lactating (N-LAC) mammary gland tissues of water buffalo, the PCR-Select cDNA subtraction technique was employed. A total of 459 cDNA clones from reverse subtracted (N-LAC/LAC) and 93 cDNA clones from forward subtracted (LAC/N-LAC) libraries were selected randomly and sequenced. BLAST analysis with nr and EST database revealed a total of 162 cDNA unique sequences (139 in reverse and 23 in forward subtracted libraries) and 5 novel cDNA sequences (no homology with sequences in the public databases). A higher redundancy (1.7 to 22.4) was observed in the forward subtracted library, mainly due to the abundance of milk caseins (62%) transcrips. In contrast, the low redundancy (0.2 to 3.9) observed in the reverse subtracted library indicated a increased number unique mRNA transcripts that were expressed during the N-LAC stage. The differentially expressed transcripts were annotated based on Gene Ontology terms. Eighteen genes were selected, based on their frequency of occurrence in the two subtracted libraries, for validation via real time-PCR. Genes like beta-casein (β-casein), lactoferrin (LTF), calmodulin 2 (CALM2), elongation factor 5 (ELF5), solute carrier family 28 (SLC28A3) and osteonectin-SPARC were relatively abundant in the LAC stage while chemokine (C-X-C motif) ligand 10 (CXCl0), microsomal glutathione S-transferase 1 (MGST1), Janus kinase 1 (JAK1), Aldolase A (ALDOA), and eukaryotic translation elongation factor 1 alpha 1(EEF1A1) were up-regulated during the N-LAC stage. The present effort has helped to identify stage specific mammary gland transcripts and generated resources for identification and characterization of novel genes in water buffalo.

P4102 Using xenografts to unravel the genetic interplay between donor and host oyster contribution to pearl development

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Cultured pearl production consists of a complex biological process involving surgical implantation of a mantle allograft (from a donor oyster), along with a nucleus into the gonad of a 2nd recipient oyster (host). What is not known, however, is whether it is the genotype from the host and/or donor oyster contributing to pearl development. This knowledge would significantly aid in pearl oyster selective breeding. Mantle xenografts from two *Pictada* pearl oyster species, *P. maxima* and *P. margaritifera*, were used to further our understanding of the host and donor genetic contribution to pearl formation through 1) phenotypic and 2) molecular data. Here, host oysters were implanted with mantle tissue originating from either the same species (allograft) or the other species (xenograft). The phenotypic results showed that pearl quality (size, shape, colour, complexion and lustre) was affected by xenografts, in particularly by the donor oyster (significantly influencing size, colour, lustre and complexion).

In a further effort to elucidate the role the donor oyster has in pearl quality, this project aims to extend the results from this previous study by examining if the donor oyster DNA is transcriptionally active. Here I sequenced (Illumina, GA-IIx) RNA extracted from the pearl sac and obtained 14GB of sequences from the allografts and xenografts. Preliminary assemblies produced 62,000 contigs based on larger than 200 bp and maximum contig size of 6,134. Transcriptome profiling will allow us to identify for the first time, the origin of expressed genes involved in pearl development. Furthermore, the expression level of these genes will be discussed.

P4103 Response of the Black Tiger shrimp (*Penaeus monodon*) to induced silencing of a myostatin-like gene – implication for shrimp growth

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Myostatin (MSTN) is a key protein involved in development and maintenance of animal growth. Functional investigations from vertebrate models indicate that MSTN acts as a negative regulator on growth, whereby increased MSTN abundance inhibits both number and size of muscle fibres and consequently growth, making it an interesting target for the improvement of farmed animal productivity. A high degree of conservation is observed across vertebrate and invertebrate MSTN sequences suggesting that functions exerted by this protein have been maintained across the animal kingdom. Although intensively investigated in several mammals and fish, very few studies have targeted invertebrate MSTN genes where their primary function remains unknown. Herein, we report on the molecular characterization of a MSTN-like gene from the black tiger shrimp (*Penaeus monodon*). The *P. monodon* MSTN gene shares high sequence identity with other known crustaceans MSTNs such as the white shrimp *L. vannamei* (97.3%) and the tropical land crab *Gecarcinus lateralis* (82.6%) and contains all the putative functional sites observed in other known MSTNs. Relative tissue abundance of MSTN was measured using real time PCR. Levels of transcripts were highest in the heart. Similar levels of expression were observed in muscle, gills, stomach and eyestalk, while low abundance was detected in the hepatopancreas. In order to determine whether the function as a negative regulator of muscle growth has been maintained in crustaceans, we silenced the expression of MSTN for a period of 10 weeks by tail muscle injection of dsRNA designed to target the sequence corresponding to the biologically active C-terminal domain of the protein. Growth, survival and MSTN transcript abundance were measured during this 10 week experimental trial. Preliminary results suggest that crustacean MSTN has an opposite role in invertebrates like shrimp in that it may positively regulates growth. The implications of these results will be further discussed.

P4104 Validation of Ten Single nucleotide polymorphisms previously detected from six candidate genes in independent Hanwoo population (n=768)

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The objective of this study is to validate SNPs that were significantly associated with carcass traits in previous study. Previously ten SNPs having significant effect on carcass traits were detected from six candidate genes in progeny population (n=300). The six candidate genes were fatty acid binding protein 4 (FABP4), fatty acid binding protein 5 (FABP5), stearoyl-CoA desaturase (SCD), lipoprotein lipase (LPL), leptin (LEP) which are associated with fat metabolism and inositol(myo)-1-monophosphatase 1 (IMPA1), heat shock 27kDa protein 1 (HSPB1), inositol 1,4,5-triphosphate receptor type 1 (IP3R1), nephroblastoma overexpressed (NOV), exostosin-1 (EXT1) genes which are related to muscle growth. Ten SNPs detected from above six candidate genes were previously evaluated their association with carcass weight (CW), eye muscle area (EMA), back fat thickness (BF) and marbling score (MS) in Hanwoo progeny test population (n=300). In this study, we attempted to validate ten SNPs using generalized linear model in validation population (n=768) derived from all different sire and dam. Statistical analysis revealed that four SNPs detected from EXT1, FABP4 and IMPA1 genes showed marginal and suggestive effect on BF and EMA. However, one SNP detected from IP3R1 gene was significant with MS in validation population. This SNP could be useful for DNA marker accounting for marbling phenotype in Hanwoo.

P4105 Design of Multiplex primers for Next Generation Sequencer

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The latest massively parallel sequencing techniques on the Illumina GAIIx sequencer make it possible to generate many giga bases of nucleic acid sequence data in a single analysis. The sequencing of small nucleic acid templates such as bacterial or viral genomes results in many fold coverage, far in excess of what is required for analysis of the genome. Tagging of the sequence templates with a sample specific sequence tag allows multiple samples to be run in each of the 8 lanes of a flow cell, greatly increasing the sample capacity of a run and thus reducing the cost per sample. ARK-Genomics have designed and tested a set of 12 sequence adapters which fit directly into the Illumina sequencing protocols for DNA and mRNA sequencing. The 5 base tags are designed, in groups of 4 independent tags, to provide a balance of bases at each position in the tag, reducing distortion in base composition at the beginning of each sequence read. The codes can be expanded to increase the number of samples analysed whilst still allowing for the recovery of sequences with up to 2 base mismatches within the tag. The current sets of sequences used in the tags have also been incorporated into sequencing adapters for digital gene expression and miRNA sequencing.

P4106 Transcriptomics of *Macrobrachium rosenbergii* of the Malaysian Giant Freshwater Prawns

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Macrobrachium rosenbergii (de Mann, 1879), known as the Malaysian Giant Freshwater Prawn, is listed as one of the commercially important crustacean species worldwide and it is reflected by its phenomenal increase in its production number. Despite its economical potential, this growing industry is experiencing low production inefficiency, economic losses mainly due to viral diseases plaguing the farmed stocks, inbreeding depression (New, 2000) and selection of low performance brood stock. (Mather and Bruyn, 2003) Although it is crucial to genetically improve this organism through the rapid and effective marker assisted selection program, there is no previous selection work that has been scientifically documented in Malaysia and relatively little molecular understanding of this organism has been achieved compared to other shrimps such as *Litopenaeus vannamei* and *Penaeus monodon*. Therefore, the study initiated intensive molecular studies on *M. rosenbergii* by sequencing the transcriptome to provide sufficient biological knowledge, raw materials, and molecular tools to assist in constructing a systematic and effective genetic improvement and sustainability programs. Data analysis will lay the groundwork for the subsequent specific molecular and genetic studies to address the scientific hypothesis and biological problems of interest.

P4107 Carcass and Meat Traits and Expressions of Target Gene

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This study was conducted to evaluate the efficiency of selection for intramuscular fat (IMF) content in a population of purebred Beijing-You (BJY) Chickens. Female chickens from divergently-selected lines (selected-up (UL) and selected-down (DL)) for IMF content of the breast muscle were studied at 120 days of age. After five generations of selection, 38.39% difference of IMF in breast muscle was obtained (4.65 % in UL vs. 3.36% in DL). The IMF content in leg muscle was also higher in UL compared to DL ($P<0.05$). Results of this study revealed that there were significant differences ($P<0.05$) on growth and carcass traits as well as on meat quality between the two selected lines. The ultimate pH (pHu) was lower and lightness (L*) along with yellowness (b*) were higher in breast for UL birds compared to DL birds. Similar trends for pHu and L* value were observed in leg muscle. Furthermore, differences in mRNA expression of the heart fatty acid-binding protein (H-FABP) gene in breast and the lipoprotein lipase (LPL) gene in abdominal fat between UL and DL ($P<0.05$) suggest that these two genes are candidate genes affecting IMF.

POSTERS 5001 – 5100

Genetics and Disease

P5001 Haplotypes, genotypes and diplotypes of two non-coding indel sites influences the expression of *PRNP* in Japanese Black and Japanese Brown cattle

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23-bp insertion/deletion (indel) polymorphisms within the promoter region of prion protein gene (*PRNP*) have a tentative association to BSE susceptibility in German cattle. These polymorphisms cause changes in *PRNP* expression. We genotyped 218 genomic DNA samples from two Japanese cattle breeds for indel polymorphisms in the promoter and intron 1 regions and analysed the *PRNP* expression levels of medulla oblongata using real-time PCR. We found a significant correlation between indel diplotypes and *PRNP* expression (P 0.0413) in the promoter and a novel difference in *PRNP* expression ($P < 0.0001$) between the two breeds ($n = 40$). On genotype based analyses, our results clearly showed that while homozygous of the deletion allele (del/del) had the highest expression level of *PRNP* at the 23-bp indel site, the heterozygous (ins/del) has the highest expression level of *PRNP* at the 12-bp indel site in both cattle breeds. As for the alleles, we observed that the insertion (+) allele had a lower expression than the deletion (-) allele at both sites in both breeds. These results suggest that indel genotypes could modulate the *PRNP* expression levels in medulla oblongata and the 23-bp del/del genotype may be the main cause of higher expression of *PRNP* in cattle.

P5002 Expression and bioactivity of recombinant human lipoprotein lipase in milk of transgenic mice

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Lipoprotein lipase (LPL) is a rate-limiting enzyme, which catalyses the hydrolysis of the triacylglycerol components of circulating chylomicrons and very low density lipoproteins (VLDL) into glycerol and non-esterified fatty acids. LPL plays an important role in transportation and energy metabolism of plasma lipoprotein. Besides, a series of clinical symptoms of lipid metabolism disorders, atherosclerosis and obesity are concerned with changes of LPL activity and quantity, and disorders of LPL function. Now we have obtained 8 founder lines of transgenic mice expressing recombinant human lipoprotein lipase (rHLPL) specifically in mammary gland, and identified them at DNA level (PCR, Southern blot), organization level (RT-PCR, Q-PCR and Immunohistochemistry) and protein level (Western, Elisa and Activity assay). The highest level of rHLPL expressed in milk was up to 0.16 mg/ml. According to this model, we are now doing some interesting research about relationship between LPL function and milk lipid synthesis in mice mammary gland. While, this model can also be used to explore the possibilities of producing rHLPL in milk of large transgenic animals such as cows for therapeutic purposes.

P5003 A 700Kb region is linked to Progressive Retinal Atrophy in Tibetan Spaniels

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Generalized progressive retinal atrophy (PRA) is a hereditary disease characterised by retinal degeneration, and occurs in numerous dog breeds including the Tibetan spaniel (TS). Several different mutations have been identified that are associated with PRA in different breeds but a causal mutation has yet to be identified for the TS. A whole genome association study using DNA from 23 PRA cases and 10 controls identified a significant association on chromosome 10 spanning a 9Mb region from 61.85Mb to 70.70Mb, when correcting for multiple testing, with the most significant SNPs at 62.00Mb and 63.83Mb (p=0.0036). The initial 9Mb region contains 49 genes, none of which are immediate candidates for PRA. Retinitis Pigmentosa (RP) in humans is homologous to PRA in dogs and part of the TS PRA critical region is syntenic to the RP28 locus in humans. Fine mapping with microsatellite markers has reduced the critical region to 700Kb between 64.00Mb and 64.71Mb, containing 8 genes. The Lhasa Apso and Tibetan Terrier breeds can also be affected with PRA and are closely related to TSs. Work is underway to investigate and compare the haplotypes across the TS PRA region in PRA-affected dogs from all 3 breeds. Experiments to further investigate the 700Mb region by targeted resequencing are being considered. The lack of PRA- or RP-associated genes in this region indicates one of the genes will have a novel association with PRA in dogs and possibly also RP in humans.

P5004 Expression profiling of mRNA and microRNA in pigs infected with *Actinobacillus pleuropneumoniae*

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Actinobacillus pleuropneumoniae is a gram-negative bacterium that causes porcine pleuropneumonia, which is a widespread, highly contagious and often fatal respiratory disease in swine. In this experiment pigs were inoculated with *A. pleuropneumoniae* serotype 5b. Liver samples from non-inoculated pigs and experimental inoculated pigs were used to characterize the expression profiles of mRNA and microRNA genes using DNA microarrays and Solexa deep sequencing, respectively. The microarray analysis identified a large number of genes, which significantly differed in expression in infected versus non-infected animals. MicroRNAs are short single stranded RNA molecules that regulate gene expression by sequence specific binding to the 3´ untranslated region (3´UTR) of target mRNAs. The deep sequencing analysis determined the identity and abundance of nearly 400 microRNAs of which a portion was found to significantly differ in expression between the infected and non-infected animals. Target genes for differentially expressed microRNAs were predicted using microcosm Targets, which is based on the miRanda algorithm. Comparison of the two gene lists showed many common genes, which may suggest a causative relationship between changes in microRNA expression and target gene expression.

P5005 MiR-21 may affect porcine oocyte meiosis retrieval through its target MEK4

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MiRNA activity was found suppressed in mouse oocytes, so we wanted to know whether it was also suppressed during porcine oocyte maturation. We found that miRNA expression profiling was dynamic changed in porcine oocytes cultured *in vitro* (IVM) for different times. All the differently expressed miRNAs were upregulated when comparing mature oocytes with immature ones. During these miRNAs miR-21 expression was down regulated after GVBD, while up regulated when maturation retrieval was blocked using IBMX. This suggested that miR-21 might participate in porcine oocyte meiosis retrieval. MEK4 is the member of MAPK pathway which plays a key role in controlling cell cycle. Inversely correlated with miR-21, we found that MEK4 was up regulated and occurred some unknown modification as soon as meiosis retrieval. Then using luciferase assay we verified MEK4 was the direct target gene of miR-21. These results suggested that miR-21 may switch porcine oocyte meiosis retrieval through MEK4 which was different from miRNA activity suppressed in mouse oocytes.

P5006 Molecular cloning and characterization of porcine *Pkd1*: Implication for the construction of pig ADPKD disease model

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Mutations in the *Pkd1* account for most cases of ADPKD (Autosomal Dominant Polycystic Kidney Disease) in human. As pigs are extensively used as human disease models, we cloned the porcine *Pkd1* cDNA with a length of 14,209bp which encodes a peptide of 4,305 aa--Polycystin-1. The deduced Polycystin-1 is highly homologous to human Polycystin-1 with overall identity and similarity values of 81% and 87% respectively. The genomic sequence of porcine *Pkd1* is located in the chromosome 3 derived BAC CH242-207G4, which is identified by using human *Pkd1* cDNA to blast against the NCBI pig HTGS database. The porcine *Pkd1* has 46 exons spanning about 50kb and is adjacent to *TSC2* that is also found in the BAC in a tail-to-tail manner. A polypyrimidine tract was found in the intron 22 but absent in the intron 21. Using real time RT-PCR we found that *Pkd1* has the highest expression level in the pancreas but is lowest in the small intestine both in the adult and nascent pigs. There is a tendency, if not significant, that the expression levels of *Pkd1* in the adult organs are lower than that of neonate's except the pancreas. At the protein level, faint 450 kDa bands were detected by western blot with loading mass of 300 μg of total proteins and confirmed that *Pkd1* has a low expression level. Besides, we identified an alternative acceptor site in the intron 9 resulting in an additional 11-aa-fragment between C-type lectin and LDL-A like domains.

P5007 Cloning and expression analysis for *Hoxc8* gene in chicken

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It had been widely accepted that the *Hox* genes performed important function during development stages of vertebrate animal including chicken, but the genome sequence and expression profile were not deeply discovered based on the poor data in the region released from the chicken genome map especially the *Hox* C cluster. Our study focused on the *Hoxc8* gene in the C cluster of 39 *Hox* genes which may be located in the E22 linkage group in chicken genetic map. After detecting 3 *Hox* genes by the RT-PCR method in two different chicken breeds, we found that the *Hoxc8* showed a tissue special expressed differentiation in 4 embryo developmental stages (E8, E10, E12, E16). Expression profile of skin was done by real-time PCR from developmental stage E13 to E21 in two breeds. For the poor sequence information in the candidate gene region, RACE experiment was done to elongate the mRNA full length from 1476 nt to 2248 nt. The genome sequence in the candidate gene region was obtained by BAC shotgun sequencing, and 7 large scaffolds were established. We discovered that the candidate gene comprises 3 exons and 2 introns. Compared with human, mouse and zebrafish, the *Hoxc8* gene in chicken showed one more exon in gene structure. We expect to Ifill gaps in the genome region of *Hoxc8* and also discover the basic biology function in chicken by candidate gene analysis after the study is finished.

P5008 Genome-wide Association Study for Susceptibility to Coliform Mastitis in Sows

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Coliform Mastitis as part of the Postpartum Dysgalactia Syndrome in sows is an important disease after parturition. Regarding sows' and piglets' health and welfare this disease has a relevant economical impact. A possible genetic background of this disease has been discussed, but has never been investigated in detail. The aim of the current study was therefore to detect loci affecting the susceptibility to Coliform Mastitis in a genome-wide association approach. The study was designed as a family-based association study with matched sampling of affected sows and healthy half- or fullsib control sows on six farms.

For this reason, 490 sows were genotyped for 62,163 Single Nucleotide Polymorphisms (SNP) using the Illumina Porcine SNP60 BeadChip. After quality control applying an individual call rate threshold of 0.95, a maximum missingness per SNP of 0.1 and a MAF of 0.05, 483 sows (260 affected versus 223 healthy control sows) and 50,791 SNPs remained for further analysis.

In a first approach, we used a simple score test for association (procedure qt-score in R-package GenABEL). Accounting for the fixed effects of breed and farm, raw p-values were corrected using genomic control method. Applying a cutoff for the corrected p-values of 0.0001, we identified a significantly associated region on porcine chromosome 1. Detailed results and further analyses considering also pedigree-structure will be presented at the conference.

P5009 Identification of Single Nucleotide Polymorphisms (SNPs) and Linkage Disequilibrium (LD) Analysis in PRNP gene of Brazilian Locally Adapted and Commercial Sheep Breeds

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The mammalian PRNP gene has a strong impact on the susceptibility/resistance to Transmissible Spongiform Encephalopathies (TSEs) and several polymorphisms at the coding region of this gene have been correlated to different risk levels of scrapie development in sheep. The aim of this study was to prospect SNPs in three different regions of PRNP gene in locally adapted and commercial sheep breeds from Brazil, and to evaluate LD levels of these polymorphisms. PCR fragments from a total of 96 animals from 13 different breeds were resequenced to prospect SNPs in the Promotor, 3'-UTR and coding regions, spanning approximately 1850pb of the gene. Sequence analysis identified eight unreported polymorphisms: one in the Promoter, two in the 3'-UTR and five in the coding region. In addition to these new SNPs, 27 previously reported SNPs were observed. LD analysis was performed with 35 SNPs using Haploview software revealing two regions in strong LD (D' values between 0.94 and 1): the promoter region and the 3'-UTR. The latter shows a high degree of correlation between alleles, as indicated by high r^2 values varying from 0.69 – 1. These results are similar to other reports and reinforce the great genetic diversity contained in this gene. Studies underway by the International Sheep Genomics Consortium indicate Brazilian breeds are highly similar to Mediterranean and African breeds. However, our results indicate that a significant amount of genetic variability is specific to South American breeds and suggest this information could be used for refining genetic models for this disease.

P5010 Foal Immunodeficiency Syndrome: Understanding the genetics and developing a carrier test

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Over the past decade, Fell Pony foals have been subject to a severe immune deficiency and progressive anaemia, known as Foal Immunodeficiency Syndrome (FIS), formerly known as Fell Pony Syndrome. Foals appear normal at birth but within a few weeks begin to lose condition and suffer from multiple clinical signs associated with the syndrome. Despite extensive treatment foals die or are euthanised on the basis of lethargy, severe anaemia and persistent infections before sixteen weeks of age. To date there have been no validated cases where foals have survived this disease. Pedigree analysis strongly suggests that a genetic lesion, which is autosomal recessive in nature, is responsible for this disease. A homozygosity mapping approach using 286 microsatellite markers identified a marker on ECA26 as showing complete concordance with the disease phenotype and a nearby second marker showing a less pronounced pattern. This association was replicated in a genome-wide association study using the EquineSNP50 Beadchip (Illumina Inc., San Diego), confirming that ECA26 was the location of the mutation. Fine mapping with affected foals was undertaken using additional SNPs generated during the sequencing of the horse genome (http://www.broadinstitute.org/ftp/distribution/horse_snp_release/v2/). A homozygous, identical-by-descent block spanning ~1.2Mb was identified as the critical region. This was re-sequenced in five animals using the Roche® GS FLX Titanium platform, in an attempt to identify the causal mutation. Identification of an associated variant, an exonic SNP which causes an amino acid substitution, has led to the development of a diagnostic test for FIS.

P5011 A genome wide-association study for racing performances in Thoroughbreds

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Thoroughbreds are a very famous breed of racehorses in the world and have been repeatedly bred to produce excellent racehorses. In this study, the heritability (h^2) of racing performances in Japanese Thoroughbreds was estimated on the basis of quantitative or categorical trait models using lifetime earnings on JRA. The minimum heritability estimates were approximately 0.07–0.18 in quantitative trait model analyses, suggesting that the racing performance is heritable. The differences on heritability estimates revealed in categorical trait model analyses by using different re-categorizations of the traits were remarkable, which indicates the presence of major genetic factors affecting the racing performance. A genome-wide association study (GWAS) was performed between superior and inferior Thoroughbreds concerning their racing performances with lifetime earnings. One genomic region, containing the myostatin gene and spanning approximately 0.6 Mb on chromosome 18, was identified as the candidate genetic region. This region has 4 SNPs, namely, g.65809482T>C ($P = 1.05E-18$), g.65868604G>T ($P = 6.47E-17$), g.66493737C>T ($P = 9.06E-16$), and g.66539967A>G ($P = 3.35E-14$). In these SNPs, the dominant models for the risk (better performance) alleles were strongly associated with the better racing performance in Japanese Thoroughbreds. Especially, in female Thoroughbreds (OR = 4.69) the racing performances were more strongly associated with the loci than in male (OR = 2.92). These findings suggest the presence of single genetic factor including the myostatin gene with a major effect on the racing performance in Japanese Thoroughbred racehorses. We also report a retrospective cohort study for the 4 SNPs using over 3,000 horses.

P5012 Bovine proteins containing poly-glutamine repeats are often polymorphic and enriched for components of large transcriptional regulatory complexes

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In humans there are more than 40 rare neurodegenerative diseases linked with repeat instability mutations. A distinct subset of these diseases is caused by expansions of polymorphic trinucleotide repeats; typically CAG repeats encoding poly-Q tracts in proteins. Normal variation in some of these highly conserved genes has been implicated in morphological differences between species and phenotypic variations within species. The current investigation identified 184 bovine poly-Q encoding genes (Q≥5) including 27 genes whose orthologs in human and mouse did not contain poly-Q tracts. The bovine poly-Q proteins typically had ubiquitous expression patterns and unusually large sizes. Analysis of gene ontology terms (DAVID) revealed that these proteins were strongly enriched for functions associated with transcriptional regulation and many formed large physical interaction networks in the nucleus where they presumably act cooperatively in transcriptional regulatory complexes (InnateDB analysis). In addition, CAG repeats in some bovine genes impacted mRNA splicing thereby generating unusual tissue-specific transcriptional diversity. The poly-Q encoding genes were prioritised using multiple criteria for their likelihood of being polymorphic and then the highest ranking group was experimentally tested for polymorphic variation within a cattle diversity panel. Extensive variation was identified. There were marked differences in allele frequencies in *Bos taurus taurus* and *Bos taurus indicus* cattle. The transcriptional diversity generating poly-Q proteins, combined with their physical interactions in large nuclear complexes controlling the transcription of many other genes, suggest that polymorphic variation in multiple components of these complexes have potential to greatly amplify their combined individual effects on phenotype.

P5013 X-linked hypohidrotic ectodermal dysplasia in cattle caused by an L1 derived cryptic exon in the *EDA* transcript

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X-linked hypohidrotic ectodermal dysplasia (XHED) is a well described syndrome in humans, mice, cattle and dogs. In all species the syndrome is characterized by defects in morphogenesis of ectodermal derived tissues such as the integument, teeth, nails, sweat glands and hair, resulting in e.g. hypohidrosis and hypotrichosis.

More than one hundred different mutations in the *EDA* gene have been described as the cause of XHED in humans alone, and in cattle, two different mutations have been reported; one deletion including exon 3 and one intronic point mutation disrupting correct splicing. We present here a new genetic type of bovine XHED, where the synthesis of *EDA* is compromised by insertion of a 161 bp long cryptic exon between exon 1 and exon 2 in the *EDA* transcript. The remainder of the transcript is spliced correctly, but due to a frame-shift inflicted by the 161 bp exon a stop codon is introduced in the beginning of exon 2 resulting in a truncated protein. The cryptic exon is an exact match of a fragment of the L1-Bt element.

LINE elements with an effect on a phenotype in cattle have been described only once before. L1 derived cryptic exons have not been described in cattle nor has it been described in relation to the *EDA* gene or XHED in any species. In general, cryptic exons derived from transposable elements are potentially an overlooked phenomenon since a number of reports on cryptic exons fail to recognize the transposable element origin of the sequence.

P5014 Microphthalmia in Texel sheep is associated with a missense mutation in the paired-like homeodomain 3 (*PITX3*) gene

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Microphthalmia in sheep is an autosomal recessive inherited congenital anomaly associated to the Texel breed. It is characterized by extremely small or absent eyes, and affected lambs are absolutely blind. For the first time, we use a genome-wide, high-density ovine SNP array for positional cloning of a Mendelian trait in sheep. Genotyping 23 cases and 23 controls on the 50k ovine SNP chip allowed us to localize the causative mutation for microphthalmia to a 2.4 Mb interval on the virtual genome sequence of sheep chromosome 22 by association and homozygosity mapping. The *PITX3* gene is located within this interval and encodes a homeodomain-containing transcription factor involved in vertebrate lens formation. An abnormal development of the lens vesicle was shown to be the primary event in ovine microphthalmia. Therefore, we considered *PITX3* a positional and functional candidate gene. An ovine BAC clone was sequenced, and after full-length cDNA cloning the *PITX3* gene was annotated. Here we show that the ovine microphthalmia phenotype is perfectly associated with a missense mutation in the evolutionary conserved homeodomain of PITX3. We thus have identified a candidate causative mutation for microphthalmia in Texel sheep.

P5015 Genetic and evolutionary analysis of Toll like receptor 7 of Indian *Bos grunniens* (Yak) and *Bos frontalis* (Mithun)

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Toll-like receptor 7 (TLR7) is an important immune receptor recognizing ssRNA virus ligands and helps in the elimination and enhancement of adaptive immunity. No information is available on TLRs of *Bos grunniens* (Yak) and *Bos frontalis* (Mithun), which are reared in high altitude areas and sustains the livelihoods of marginal or poor farmers of these areas. The aim of present study was to genetically characterize and analyse the evolutionary lineages of Yak and Mithun TLR7. Successful full length amplification of TLR7 genes was achieved using specific self designed primers with expected amplicon size. PCR products were cloned in pGEMT-Easy vector followed by transformation in *E. coli* Top10 strain and sequenced. Sequence analyses of Yak and Mithun TLR7 genes showed more than 99.2% sequence homology with other *Bos indicus* and *B. taurus* breeds. The evolutionary lineage findings clustered Yak and Mithun more close to bovine species. Point mutations were seen at 25 nucleotide positions in Yak while only at 7 positions in Mithun, with corresponding amino acid changes at 8 positions in Yak and no change in Mithun. SMART analysis of Yak and Mithun protein domain architecture revealed variations in leucine rich repeats (LRR) region, which plays an important role in the pathogen recognition. Homology modelling of TLR7 revealed horseshoe shaped structure with 5 alpha helices. One additional alpha helix present in Yak was not seen in other species. Present study showed existence of more genetic variability in TLR7 gene of Yak than Mithun, including the LRR region of TLR7.

P5016 Transcription patterns and polymorphism of the non classical MHC class I genes in pig

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The major histocompatibility complex (MHC) class I genes comprise classical (Ia) and non classical (Ib) genes. The highly polymorphic class Ia molecules are widely expressed, present peptides to cytotoxic T cells and modulate the activity of natural killer cells. In contrast, class Ib genes are predominantly expressed in immunotolerant organ sites, notably at the foeto maternal interface during pregnancy in humans, show limited polymorphism and can be alternatively spliced. In pig, little information is available on the class Ib genes SLA-6, -7 and -8. Our aim was to study the transcription patterns and polymorphism of these genes. RT-PCRs with gene specific primers were carried out. Full length transcripts were characterized resulting in the annotation of 8 exons for SLA-7 and -8 and 7 exons for SLA-6. For SLA-8, no additional transcript was found. For SLA-6, four additional transcripts were detected and for SLA-7, an alternative spliced variant was found in the 3'UTR of the gene after the termination codon suggesting possible post-transcriptional regulation. For polymorphism studies, long PCRs suitable for amplification of the three genes from the five prime downstream to the three prime non-coding sequence were designed and MeLiM pigs harbouring spontaneous melanomas were characterized. Sequencing results confirmed the low level of polymorphism of the three genes. In order to start functional studies, ongoing experiments aiming at detecting expression of the proteins at the cell surface will be carried out in the near future. Our overall results will provide very new data on these enigmatic SLA-Ib molecules.

P5017 Genotyping of Gray coat color using hair samples in Thoroughbreds

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Identification of coat color is necessary for ensuring registration of particular horse breeds such as Thoroughbreds. Because the Gray represents a gradual loss of hair pigmentation, it is occasionally difficult to identify it when registering new born foals; genotyping of the Gray at an early stage must provide useful information. The LA-PCR genotyping for *STX17* gene is available; however, the sensitivity of this method has been dependent on the condition of DNA samples. We investigated an applicable procedure for the genotyping using hair samples. Allelic frequency on the *STX17* in a Thoroughbred population was assessed and further the present method was utilized to the Gray genotyping in new born foals. It was concluded that the use of purified DNA from hair samples collected within at least three months before the analysis consistently led to successful LA-PCR genotyping. The allelic frequencies in the population were estimated to be 0.026 (*G*) and 0.974 (*g*), and observed genotypes were completely concordant with the registered phenotypes. It was also confirmed that all of 14 stallions and 576 mares registered as the Gray possessed the *G* allele. Based on the genotypes, 664 foals born from the Gray stallions and/or mares could be classified into 307 Gray (3 *G/G* and 304 *G/g*) and 357 non-Gray horses, providing complementary information to the registration. In this analysis, a few non-Gray horses showed white hairs interspersed among pigmented hairs could be found. Regarding these cases, the Gray genotypes and segregation data may suggest the influence of another causative gene.

P5018 Identification of the arachnomelia mutation in Brown Swiss cattle by sequence capture and massively parallel sequencing

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Arachnomelia is a monogenic recessive inherited disease in Brown Swiss cattle. Affected calves are non-viable and show characteristic skeletal features, such as elongated limbs and facial deformities. A ~7 Mb interval of bovine chromosome 5 was previously shown to carry the mutation causing the arachnomelia phenotype in Brown Swiss cattle. Two individuals (one arachnomelia affected calf assumed to be homozygous across the entire sequence interval including the causative variant and a healthy partially inbred cow carrying one copy of the critical chromosome segment in its ancestral state and one copy of the same segment with the arachnomelia mutation) were selected for an array-based sequence capture and massively parallel sequencing approach to re-sequence the entire critical interval. This revealed a single base insertion leading to a premature stop codon in the coding sequence of the sulfite oxidase gene (*SUOX*) which is perfectly associated with the arachnomelia phenotype. The genetic makeup of several partially inbred cattle provides extremely strong support for the causality of this mutation. Our findings suggest a previously unnoticed role for sulfite oxidase in bone development and can immediately be applied to remove this deleterious mutation from the cattle breeding population.

P5019 Mapping a gene responsible for natural resistance to Rift Valley Fever virus in inbred rats

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The Rift Valley Fever virus (RVFV), a phlebovirus which presents an epidemic and epizootic threat in sub-Saharan Africa, Egypt, and the Arabian Peninsula, has recently gained attention as a potential weapon of bioterrorism due to its ability to infect both livestock and humans. Inbred rat strains show similar characteristic responses to the disease as humans and livestock, making them a suitable model species. Previous studies have shown differences among various inbred rat strains in susceptibility to RVFV hepatic disease, including a higher susceptibility of Wistar-Furth (WF) rats compared to a more resistant Lewis (LEW) strain. Further study revealed that this resistance trait follows the pattern of a dominant gene inherited in Mendelian fashion. A congenic WF.LEW strain resistant to infection with RVFV was derived from the susceptible WF and resistant LEW strains, and a subsequent genome scan revealed two prospective regions for the location of the gene, one on chromosome 3 and the other on chromosome 9. Through backcrossing, genotyping, and subsequent challenges of three N1 litters with RVFV, the ~2MB region on the distal end of chromosome 3 was determined to contain the gene conferring resistance. The use of markers to detect recombination in further backcross generations resulted in the identification of two recombinants in this original congenic region of chromosome 3. Through RVFV challenges, these have narrowed the prospective region to ~500KB. Future sequencing and expression studies will facilitate the identification of a candidate gene and mechanism, which will potentially become the basis for developing new preventive measures against the virus.

P5020 Transcript expression analysis in tracheobronchial lymph nodes of Pseudorabies virus infected pigs

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This study addresses the critical relationship between Pseudorabies virus (PRV) and its host at a transcriptional level during the course of an infection. RNA isolated from draining tracheobronchial lymph nodes (TBLN) specimens from 5-week old pigs clinically infected with a feral isolate of PRV (FS268) and uninfected were interrogated using Illumina Digital Gene Expression (DGE) Tag Profiling to better understand the physiopathology of infection and the immune response at a cellular level. Over 100 million digital gene expression tag sequences were observed, representing 4,064,189 unique 20-base sequences collected from TBLN at time points 1, 3, 6 and 14 days post-infection. Multidimensional statistical tests were applied to determine which changes in tag abundance were significant and tags were annotated with transcriptomic information. The experimental results have been integrated with previous studies to develop a robust model of swine respiratory virus infection.

P5021 Analysis of innate and adaptive immune response in pigs after vaccination: a study based on genetics and functional genomics

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Resistance to disease and immunocompetence in livestock production are complex issues. Our aim is to study the genetic determinism of immune response in pig without focusing on a particular pathogen. A population of 443 60-day old castrated Large White males recorded for production traits was scored for 54 immunity parameters three weeks after vaccination against *Mycoplasma hyopneumoniae*. Blood cell subpopulations were counted by hemograms and flow cytometry was performed to detect cell subsets including IgM+ (B lymphocytes), γδTCR+ (γδ T lymphocytes), CD4+ and/or CD8+ (αβ T lymphocytes), CD16+/CD2+ (NK cells) and CD16+/CD172a+/MHCII+ (monocytes) cells. Innate immunity parameters included phagocytosis tests, *in vitro* production of IL1B, IL6, IL8, TNFA and IL12 from stimulated blood cells, IFNA release after blood stimulation by a viral antigen, measurement of C-reactive protein and haploglobin in the serum. For adaptive immunity, parameters included antigen-specific and non-specific cell proliferation, *in vitro* production of IL2, IL4, IL10 and IFNG from stimulated blood cells and quantification of total antibodies (IgA, IgM and IgG) and specific IgG levels in the serum. Genetic analyses revealed that 44 parameters show moderate to high heritabilities with an average of 0.4 +/- 0.2. The transcriptome of leukocytes from animals with contrasted immune response parameters was studied using a porcine generic array enriched in immunity-related genes. Our results show that this molecular phenotype is informative for part of the immune traits. Our overall results confirm that many immunity parameters are under genetic control and that including a molecular phenotype is relevant to refine immunity phenotypes.

P5022 Fine mapping the genetic component of the immune response post immunisation

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Breeding for disease resistant livestock and more efficacious vaccines could improve the economic and animal welfare burdens that infectious disease poses to both, developed and developing countries. We aim to identify key genes and pathways that control variation in immune response, knowledge that may aid both breeders and vaccinologists. A herd of Charolais and Holstein cattle (n=982) were initially genotyped with 165 microsatellite markers genome wide, and the F2 backcross generations were phenotyped for their immune responses (IgG1, IgG2, T-cell and IFN-γ) to a 40-mer Foot-and-Mouth Disease Virus (FMDV) peptide (n=195) and also (total IgG responses) to a Bovine Respiratory Syncytial Virus (BRSV) vaccine (n=467) across several time points. From a Linkage Association study, broad regions of the bovine genome were identified as significantly linked to the immune responses post immunisation. Further genotyping of the F2 animals in these regions using non-synonymous single nucleotide polymorphisms (SNPs) (n=274), placed in proximity to genes of relevance to immunity increased the levels of linkage disequilibrium. This enabled a Genome Wide Association Study to be conducted. The results indicated that several regions of the bovine genome play a significant role throughout the immune response to both the FMDV peptide and the BRSV vaccine. These regions include: the Major Histocompatibility Complex (MHC) on chromosome 23 and the Toll-like Receptor Cluster 1/6/10 on chromosome 6. Thus we conclude that key pivotal pathways maybe shared in eliciting and maintaining an immune response to differing types of antigens.

P5023 Fine mapping of quantitative trait loci for tick resistance on BTA5

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In tropical regions, the cattle tick *Rhipicephalus (Boophilus) microplus* is a major threat to cattle production systems. The identification of molecular markers linked to tick resistance would provide a better strategy for selecting resistant animals. Previous studies by our group detected QTL on BTA 5 for tick resistance in a Gyr x Holstein F_2 population. This QTL was detected with moderate resolution because markers were relatively far apart from each other. Five more markers were added to BTA 5 in the region spanning the QTL in order to reduce the confidence interval. Blood samples were collected from 480 animals (including parental, F_1 and F_2) and genomic DNA was extracted. PCR products were detected by capillary electrophoresis in the MegaBACE 1000 DNA sequencer. Statistical analyses were performed with GridQTL software. New association analyses were performed after saturation of the new markers and the same QTL was detected in the previously mapped location. The confidence interval on BTA 5 was reduced from 20 to 10 cM. The phenotypic variation explained by this QTL was 3.67% and the negative additive effect indicates that Gyr animals harbor alleles that increased resistance to tick. The smaller confidence interval increases the chance to identify candidate genes that explains tick resistance.

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P5024 Microarray analysis of differentially expressed genes in the medulla oblongata of Spanish sheep naturally infected with scrapie

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The pathogenesis of natural scrapie and other prion diseases remains unclear. The determination of transcriptome variations in infected versus control animals might clarify some molecular mechanisms of the pathology. In addition, it may allow the development of new tools for diagnostics and therapy. The aim of this work was to identify disease associated alterations in the gene expression profiles in Medulla oblongata (MO) during the symptomatic phase of natural scrapie. The gene expression patterns in MO from 7 sheep naturally infected with scrapie were compared with 6 controls using the CVI custom 4x44K microarray platform containing 13k 60-mers oligos representing previously sequenced clones from a custom normalized cDNA library of sheep Peyer's Patch, tonsil and brain. The array was supplemented with all publicly available transcripts from NCBI/EBI databases. Over 350 significant probe sets displayed expression changes greater than 2-fold. From these probes we identified 148 genes, many of them encode proteins that are involved in immune response, ion transport, cell adhesion, and transcription. We also confirmed many earlier published regulated genes found in animal models with induced scrapie. Finally, we investigated the relationship between gene expression profiles and the appearance of the main scrapie related lesions: Prion deposition, gliosis and spongiosis. In this context, the potential impacts of these gene expression changes in MO on scrapie development are discussed.

P5025 Evaluation of candidate genes for late onset hereditary juvenile cataract in the Boston Terrier

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Although a mutation for the early onset form of hereditary juvenile cataract in Boston Terriers has been identified in the *HSF4* gene, a more prevalent form of juvenile cataract occurs in this breed that is usually detected at 4-6 years of age. This cataract appears in the anterior cortex of the lens and may start as a unilateral punctuate cataract but progresses to bilateral cataract with a spoke-like formation as the dog ages. In this study exon scans of five candidate genes were performed. Eighteen Boston Terriers were included in the exon scan: ten dogs affected with late-onset juvenile cataracts, two dogs affected with cataracts at 2 years of age that tested negative for the *HSF4* mutation, three dogs affected with the early onset juvenile cataracts (*HSF4 mut*), one dog unconfirmed clear for late onset juvenile cataract past 8 years of age and two dogs that were certified to be free of cataracts past the age of 8 years. The genes sequenced were *CRYGD, CRYGE, CRYGF, MIP* and *SORD1*. Several polymorphisms were identified including a base substitution and insertion 54bp upstream from exon 1 of *CRYGD*. In addition, 39 microsatellites associated with 20 additional cataract candidate genes were screened against the DNA of 26 Boston Terriers.

P5026 Achondroplasia in UK Cheviots fine-mapped to 360 Kb region of chromosome 3

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Achondroplasia is an autosomal recessive disease causing limb malformation and non-viable offspring in the Cheviot breed. To map the causal mutation, eight affected individuals, an additional eight obligate carriers and 27 unaffected Cheviots were genotyped at 49,034 SNP using the *ovine* SNP50 BeadChip. A genome wide case – control analysis identified strong association ($p < 1.5 \times 10^{-15}$) to three SNP on sheep chromosome 3 (OAR3) in an 8.0 Mb interval from 152 – 160 Mb. Homozygosity mapping using genotypes from the eight affected animals revealed a single region, spanning 82 SNP and 4.0 Mb from positions 153 – 157 Mb, was common to each animal. To narrow the critical interval further, haplotype analysis was conducted for each of the asymptomatic carrier animals. Importantly, one obligate carrier retained homozygosity for the disease haplotype through all but a small region, thus narrowing the critical interval to 360 Kb containing 11 SNP. Analysis of this region of OAR3 revealed no compelling positional candidate genes, however *COL2A1* which causes a similar phenotype in humans is positioned approximately 4 Mb upstream. The study demonstrates the power of the *ovine* SNP50 BeadChip to efficiently finemap single gene disorders and represents an important step towards identification of the causal mutation.

P5027 The sequence variation of Heat Shock Protein70 gene and its effect on heat stress resistance in chicken

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In the present study, gene sequencing was used to detect the genetic viariation of chicken *Hsp70* gene, 34 SNPs and 2 indel mutations were found in 102 individuals from 10 breeds. Bioinformatics analysis indicated that 7 variation sites might make transcription factor binding sites disappear or change among these 36 variation sites in the *Hsp70* gene complete sequence. These mutations might influence the expression of the chicken *Hsp70* gene. One hundred and fifty-two Lingshan chickens and 159 White Recessive Rock chickens were used in the association analyses. The distribution frequency of genotypes of 3 SNPs (A-69G, C+909A and T+1476C) was highly diversity ($P < 0.01$) in Lingshan chicken and White Recessive Rock. A-69G and T+1476C were significantly associated ($P<0.05$) with CD4+/CD8+, and suggestive association was found between A-69G and H/L and between T+1476C and T3. Moreover, A-69G, C+276G and C+909A sites were present in the same haplotype block and this block was highly significantly associated ($P < 0.01$) with CD4+/CD8+, which indicated that A-69G possibly associate with CD4+/CD8+.

P5028 Studies of ncRNAs of potential relevance for resistance towards *Actinobacillus pleuropneumoniae* (APP)

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Respiratory diseases are one of the major problems in the pig industry with severe consequences in respect to both animal welfare and economy. The gram negative bacterium *Actinobacillus pleuropneumoniae* (APP) is responsible for pleuropneumonia, a highly contagious and often fatal porcine respiratory disease. The incidence of chronic pleuritis in Denmark has been increasing steadily since the beginning of the 1980s. Thus, it is of great interest to obtain detailed information about the molecular mechanisms involved in host-pathogen interaction. An extensive study based on a genome scan in an extended pig pedigree characterized for the APP infection has been conducted. The scanning resulted in the identification of three QTL regions associated with resistance towards APP infection.

The objective of the present project is to characterize ncRNAs that are differentially expressed in RNA extracted from infected versus non-infected lung tissue, with special focus on the ncRNAs lying in the QTL regions.

Solexa deep sequencing and microarray analysis of RNA isolated from infected and non-infected lung tissue have revealed significant differences in ncRNA expression between the two tissues. Many microRNAs seem to be up regulated in the non-infected vs. infected samples, some of them encoded within the QTL regions. In contrast, many snoRNAs are up regulated in the infected lung tissue. These results provide information about ncRNAs of potential functional importance in regard to resistance towards *APP* lung infection. SNP analysis and additional functional studies needs to be performed in order to confirm the functional relevance of the ncRNA candidate genes.

P5029 A nonsense mutation in the optic atrophy 3 gene (*OPA3*) causes dilated cardiomyopathy in Red Holstein cattle

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Cardiomyopathies are severe and degenerative disorders of the myocardium. They are highly correlated with heart failure and increased mortality. Dilated cardiomyopathy is the most common form of human cardiomyopathy with an incidence of about 5.5/100,000 cases *per annum*.

Bovine dilated cardiomyopathy (BDCMP) was observed worldwide during the last three decades in cattle of Holstein-Friesian origin. The disease affects Swiss Fleckvieh and Red Holstein animals. BDCMP-affected individuals have episodes of global heart enlargement with ventricular dilatation, reduced wall thickness and systolic dysfunction. BDCMP is a late onset disease and follows an autosomal recessive mode of inheritance. Initially, the disease-causing locus was mapped to a 6.7-Mb interval on bovine chromosome 18 (BTA18) based on a pedigree bred for this disease. We narrowed down this region to 1.0 Mb by applying a combined homozygosity mapping and association-based strategy. Subsequently, we fine mapped the interval of interest to 240 kb. By re-sequencing this chromosomal region we were able to identify the causative mutation for BDCMP. A nonsense mutation was found in the bovine optic atrophy gene (*OPA3*). Previously, mutations in this gene were reported to cause 3-methylglutaconic aciduria type III with optic atrophy 3 in humans. We provide convincing genetic and functional evidence demonstrating for the first time, that a naturally occurring mutation in the *OPA3* gene causes dilated cardiomyopthy. Our results revealed an unexpected and novel role of the *OPA3* gene and may also be relevant to elucidate the genetic background of human dilated cardiomyopathy.

P5030 Molecular characterization of equine mesenchymal stem cells used in cell therapy

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In the equine orthopedic field, mesenchymal stem cells (MSCs) are currently used in clinical applications for the treatment of injuries because of their ability to self-renew and differentiate into various tissue lineages under specified conditions. The goal of the cell therapy based on MSCs is to repair and restor functionality of the damage tissue. There are two main sources of MSCs, bone marrow (BM-MSC) and adipose tissue (AT-MSC). The objective of this work is the molecular characterization and comparison of BM-MSC and AT-MSC used in cell therapy. Proliferation and viability assays were carried out showing differences between BM-MSC and AT-MSC. The expression of undifferentiated and differentiated markers was assessed by real time RT PCR. Cells from nine horses were used to determinate the surface marker profile of BM-MSC and AT-MSC studying the expression of: CD29, CD31, CD34, CD44, CD45, CD49d, CD73, CD90, CD105, CD106, CD146 and CD166. CD29, CD44, CD90 and CD105 markers were confirmed by flow citometry. Both cells expressed common surface markers being CD34 a differential marker. The osteogenic and adipogenic potential of MSCs from both sources were quantified in five horses measuring specific genes associated with each cell lineage. For osteogenic differentiation the expression of *RUNX2, SSP1, ALP, COL1A1* and *BGLAP* was quantified. In general, apart from *COL1A1*, all markers were up-regulated as differentiation went by in both MSC types. For adipogenic differentiation *PPAR*γ and *LPL* expression was also measured. These results were confirmed using staining techniques.

P5031 Searching for genetic factors affecting ovine claw horn resistance against footrot

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Footrot is one of the most important diseases in sheep production flocks. Conventional therapeutic and prophylactic measurements are in many cases not successful. As footrot is an infectious disease of the claw, genetic differences in immune mechanisms as well as in claw characteristics have to be considered. Heritabilities for morphological traits of the ovine claw are in the low to intermediate range while for histological parameters high values were estimated. Therefore, claw horn phenotypes and gene variants related to claw quality parameters are excellent candidates to elucidate genetic resistance to footrot. In this study, claw horn and blood samples were collected in footrot-affected flocks from 450 Merinolandschaf ewes of known age and body weight while they were examined for clinical signs of footrot. The water absorption capacity and histological parameters as number of horn tubules per mm^2, area of individual horn tubules and total area of horn tubules are determined in the claw horn samples. Keratin 5 (*KRT5*), transglutaminase 1 (*TGM1*) *and* keratinocyte growth factor (*KGF*) were chosen as candidate genes for claw horn quality, and their complete coding regions and exon-flanking parts were sequenced in order to identify genetic variation in and between different sheep breeds as well as in the sampled populations. In all three genes, several nucleotide substitutions were found which will be genotyped in the sampled sheep for association studies regarding footrot status and claw horn traits.

P5032 Refined mapping of the *Escherichia coli* F4ab/F4ac receptor locus (*F4bcR*) on pig chromosome 13

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Enterotoxigenic *Escherichia coli* (ETEC) with fimbriae of the F4 family are one of the major causes of diarrhea and death among neonatal and young piglets. Bacteria use the F4 fimbriae to adhere to specific receptors expressed on the surface of the enterocytes. F4 fimbriae exist in three different antigenic variants, F4ab, F4ac and F4ad, of which F4ac is the most common. Resistance to ETEC F4ab/F4ac adhesion in pigs has been shown to be inherited as an autosomal recessive trait. Previous genome scans with microsatellites have shown that the locus ETEC F4ab/ac locus (*F4bcR*) is located in the q41-region on pig chromosome 13. One of the possible candidate gene located in this region, the Mucin4 (*MUC4*) gene, showed a nucleotide polymorphism co-segregating with the *F4bcR* alleles. Recently, we discovered a boar from a Swiss experimental with a recombination between *F4bcR* and *MUC4*. We have tested several SNPs downstream of *MUC4* and our results suggest that the locus for *F4bcR* is distal to the MUC4-gene.

P5033 Structural and functional characterization of ovine interferon gamma gene: Its role in nematode resistance in Rasa Aragonesa sheep breed

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The aim of this study was to explore the role of interferon gamma (*INFG*) gene in nematode resistance of Rasa Aragonesa sheep breed. Ewes were selected based on faecal nematode eggs count during two years. Finally, a total of 71 Rasa Aragonesa ewes with extreme values for nematode eggs count were included for this study. For expression studies, thirty-one animals with the most extreme values were drenched ten days before experimental infection with *Haemoncus contortus*, and slaughtered three weeks post-infection. Necropsy data and samples from spleen and mesenteric lymph node were taken. The total coding region sequence of ovine interferon gamma has been sequence from ten resistant and susceptible animals. Ten SNPs were found: six in promoter region, one in intron 1, two in exon 3 and one in intron 3. Sequencing of intron 1 revealed a microsatellite, which has been associated with nematode resistance. The substitutions found in exon 3 were found to be synonymous. Association studies between faecal nematode eggs count and polymorphisms were carried out. Finally, resistant (n=16) and susceptible (n=15) ewes were used to evaluate the relative gene expression of *INFG*. The relative expression of *INFG* was determined using standard curve method in spleen and mesenteric lymph node of Rasa Aragonesa ewes. Eight housekeeping genes (*RPL19, GAPDH, YWHAZ, B2M, UBC, SDHA, G6PDH* and *ACTB*) were evaluated to normalise *INFG* results in nematode infection of resistant and susceptible animals. *YWHAZ* and *G6PDH* genes were chosen as the most stable genes.

P5034 Genetics of dwarfism in Miniature Horses

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The goal of miniature horse breeding is to select for a horse with small stature and exhibiting the same body proportions as those of a full size horse. Unfortunately, dwarfism occurs within miniature horse populations, resulting in reduced stature but also diverse conformational defects and embryonic losses. Since dwarf offspring can be produced by normal parents, breeders assume a recessive mode of inheritance for the trait. Genome Wide Association assays were performed using the Illumina Equine SNP50 chip for 46 miniature horses, 19 of which were classified as dwarves. The data were analyzed using PLINK. An association was identified with SNPs on ECA1 following a Monte Carlo correction for number of SNPs tested (EMP= 0.019). The associated SNPs were near the gene *Aggrecan* (*ACAN*); mutations of ACAN are known to cause dwarfism in people, cattle and mice. Surprisingly, haplotype analysis did not identify a common haplotype among the dwarf horses. Instead, a minimum of 5 haplotypes were identified among the dwarf miniature horses. Sequencing the ACAN gene led to discovery of 3 alleles associated with dwarfism, designated alleles 2, 3 and 4. The three alleles are characterized as one missense mutation and two deletions. All horses homozygous for allele 2 and heterozygous for 2/3 and 2/4 were dwarves. We have not seen homozygotes for 3 and 4 nor 3/4 heterozygotes and assume them to be lost as embryos. At this writing we anticipate discovery of a 5th allele for ACAN associated with dwarfism.

P5035 Identification of anti-avian influenza virus immune-related genes

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The differential display reverse-transcription PCR was used to identify altered genes in spleen cells from chickens with/without injection of avian influenza virus (AIV) vaccine. A total of 40 Abor Acre broiler chickens were selected randomly in this study, 20 of which were injected with inactivated influenza virus H5 vaccine, and the other 20 were used as controls. A total of 13 ESTs (expressed sequence tags) were found using DDRT-PCR and reverse northern dot blot, 9 of which in the injected group, and the other 4 in the control. All 13 ESTs were compared with nucleotide sequences deposited in the nr database and the dbEST database of Genbank using BLAST. DD3, DD4, DD6, DD9, DD11 and DD12 ESTs had highly similar nucleotide sequences with ESTs existing in nucleotide databases but with unknown functions. DD10 and DD13 were found similar to chicken mRNA for ribosomal protein L7a. DD1, DD2, DD5 and DD8 ESTs had no significant similarity with existing genes or ESTs, and were regarded as new ESTs. The four new ESTs were submitted to GenBank (accession numbers: EB714185, EB714186, EB714187, and EB714188). This lays a foundation for further study of anti-AIV genes and for producing transgenic chickens that can resist AIV. In conclusion, our findings indicate that Chicken ribosomal protein L7a gene and unknown functional genes of other ESTs may provide candidate genes for studying antiviral genes.

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P5036 SLA haplotypes and microsatellite marker analysis in Korean native pigs

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The porcine MHC (Major Histocompatibility Complex), encoding the SLA (Swine Leukocyte Antigen) genes, is one of the most significant regions associated with immune-rejection response to successful allo- or xeno- transplantation. In this study, three SLA class I (*SLA-1, SLA-3, SLA-2*) loci and three SLA class II (*DRB1, DQB1, DQA*) loci were investigated in the previously unidentified Korean native pig (KNP) population that was closely inbred in the research station in Korea. Total thirteen KNPs from four generations were genotyped for the SLA alleles and haplotypes were investigated using PCR-SSP (Sequence-Specific Primer) method. As the results, homozygous SLA class I genes, Lr-56.0/56.0 (*SLA-1**11XX, *SLA-3**03XX, *SLA-2**15XX) and, homozygous SLA class II genes, Lr-0.30/0.30 (*DRB1**11XX (1101/11ac21), *DQB1**05XX, *DQA**02XX), were identified. This indicated that these KNPs had Lr-56.30/56.30 homozygous haplotype. Based on results of fifty microsatellite (MS) markers, 17 MS markers were fixed in all generations and the fixed alleles are increasing to 26 loci for the fourth generation. Two markers, S0069 and SW173, were heterozygous for all the animals tested. Observed and expected heterozygosities were calculated and the average inbreeding coefficients for each generation were also calculated. In the fourth generation, the average inbreeding coefficients was 0.732 and this may increase with further inbreeding process. Analysis of the SLA haplotypes and MS alleles can give important information for the breeding this pigs for xenotransplantation studies.

P5037 The role of epigenetic and its applications on embryonic and pluripotent stem cell technology

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Epigenetic mechanisms deliver an effective stability of gene expression during the earliest stages of mammalian development. Genome-wide epigenetic reprogramming occurs at stages when developmental potency of different cells changes. This epigenetic reprogramming is likely to be needed for totipotency, correct initiation of embryonic gene expression, and early lineage development in the embryo. Nowadays, embryonic and pluripotent stem cells in a different species of mammalians are an attractive resources for new therapeutic approaches for several different diseases by tissue regeneration. On the other hand, stem cells derived from the inner cell mass have been used to examine the epigenetic pathways that regulate pluripotency, differentiation, and lineage commitment. Also these embryonic stem cells can differentiate into all cell types of the body. Epigenetic mechanisms such as post-translational histone modifications, chromatin remodeling, alterations in nuclear architecture, DNA methylation and small non-coding RNA-mediated regulatory events are considered as the essential factors to control the heritable cellular memory of gene expression during development and functional changes. The purpose of the present review is to provide a brief overview of the current approaching in relation to epigenetic mechanisms which extent the development of strategies to induce tolerance and improve stem cells especially for therapeutic aspects.

P5038 Genetical characterization of the first Polish scrapie cases

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Scrapie, one of the fatal transmissible spongiform encephalopathies (TSEs) occuring in sheep, has two known forms with different etiology: classical and atypical. Polymorphism of three codons (136, 154, 171) of the *PrP* gene has been associated in many studies worldwide with susceptibility to classical scrapie. Until now only one *PrP* gene variant coding a phenylalanine in codon 141 has been found to be associated with the atypical scrapie cases. Another recently identified interesting candidate gene for scrapie susceptibility in sheep is an *SPRN* gene coding for Shadoo protein (Sho). An indel mutation found in Sho was associated with typical scrapie occurrence.

Here we determined polymorphism of *PrP* and *SPRN* gene in five atypical scrapie cases and in one classical scrapie case and compared this results with control group of healthy animals of five representative Polish sheep breeds.

The *PrP* genotypes of scrapie affected sheep were: $A_{136}L_{141}H_{154}Q_{171}$/ALRQ, ALRR/ALRQ, ALRR/AFRQ, ALRQ/AFRQ, ALRR/ALHQ (atypical cases) and ALRQ/VLRQ (classical case). Other identified SNP in *PrP* coding region were A428G (in protein: H143R; observed only in control group), G670A (E224K) and two silent mutations: A691C (231R) and C711G (237L). In the coding region of SPRN we found six mutations: C218T (V73A), four silent SNPs: C87G (29G), A195G (65A), A303G (101A) and C342T (114Y) and one 6-nucleotide indel. Identified in hydrophobic region of Sho indel of two alanins was observed in both healthy (n=151) and scrapie affected animals (n=6). No association with disease occurrence could be detected due to the low number of scrapie sheep.

P5039 LUPA, a European initiative taking advantage of dog genomics to decipher complex human diseases

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Dogs suffer from the same range of diseases as humans do but the genetic complexity underlying disease is reduced due to their population structure. Consequently the number of samples and SNP markers needed to perform whole genome association studies are significantly reduced. The LUPA project is taking advantage of this extraordinary genetic model. Veterinary clinics are collecting DNA samples from large cohorts of dogs suffering from well defined diseases of relevance to human health (cancers, inflammatory diseases, epilepsy…). Once the cohorts are built, DNAs are sent to a centralized genotyping facility. Together with Illumina, LUPA developed a new high-density canine SNP (170K) array with an even coverage across the dog genome and good representation in a large variety of breeds. The SNP genotypes are stored in a central database and made available to the participants who analyze the data. Ten thousand samples will be genotyped in total; half of them have been processed already. As CNVs may play a role in several disorders LUPA has screened for CNVs in several dog breeds using CGH and identified > 2500 CNVs with an average number of 70 per dog. Following GWA and fine-mapping, candidate genes are chased at the molecular level. Complex diseases begin to be unraveled pointing out molecular pathways not always suspected in similar human conditions. Monogenic disorders are also investigated and these studies have led to the discovery of new genes implied in corresponding human diseases. So the approach is successful in giving new insights into the pathogenesis of diseases.

P5040 The porcine systemic response to pleuropneumonia studied by transcriptional profiling of liver and tracheobronchial lung lymph nodes using multiplexed mRNA-Seq

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Actinobacillus pleuropneumoniae (Ap) is a gram-negative bacterium that causes porcine pleuropneumonia, which is a widespread, highly contagious and often fatal respiratory disease in swine. A total of 44 pigs were experimentally inoculated with Ap serotype 2 or 6 and samples of liver and tracheobronchial lung lymph nodes were collected 6, 12, 24 and 48 hours after experimental inoculation, as well as from six non-inoculated control pigs. Transcriptional profiles of the liver samples have been generated by preparation of 12-plexed mRNA-Seq libraries followed by sequencing on an Illumina GAIIx (51+7 cycles) obtaining more than 200 million tag sequences. The 12-plexed mRNA-Seq libraries of the lung lymph node samples have presently (April 2010) been prepared and are to be sequenced. The PCR amplicons of the liver libraries were quantified using both a fluorometer and a qPCR assay, including the use of a sequence-titrated, in-house control library. The libraries were diluted to 6 pM based on the qPCR assay, except for a single library set which was duplicated and diluted based on the fluorometer measurements as well. Analysis of the obtained sequences revealed that the qPCR based quantifications reduced the cluster density variability as compared to fluorometer based quantifications. Furthermore, it was found that the fluorometer based measurements tended to deviate for dilute as well as for more concentrated libraries. Following the sequencing of the lung lymph node samples analyses are to be conducted to study the time and serotype dependent transcriptional response to Ap infection.

P5041 Characterization of immunorelevant candidate genes in spleen of a rainbow trout selection strain

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Rainbow trout (*Onchorynchus mykiss*) is a species of salmonid, which account for 27% of the European aquaculture production. Besides a set of abiotic stressors, pathogens such as *Aeromonas salmonicida* cause dramatic losses in salmonid aquaculture annually. The long-time selected rainbow trout strain BORN (Germany), which has been bred from Danish STEELHEAD trout in brackish water, tolerates several stressors to a bigger extent. A preliminary infection experiment with an *A. salmonicida* bacteria injection dose of $1x10^4$ CFU revealed that cumulative mortality among STEELHEAD trout was 2.8-fold higher than among BORN trout causing an 87% cumulative mortality of STEELHEAD trout by day 24, whereas cumulative mortality of 31% among BORN trout did not increase after day 14. We aim to evaluate BORN trout as model for stress regulation and immune defence and to identify differentially regulated genes that are involved in very early immune mechanisms responsible for elevated resistance. Comparative microarray analysis of splenic tissue in healthy BORN and STEELHEAD rainbow trout were carried out. Two promising candidate genes that showed a significant expression difference are encoding for Interleukin-1 receptor-associated kinase 3 (IRAK3; 2.6-fold expression difference) and the Complement Factor D (CFD; 5.8-fold expression difference). IRAK3 as a negative regulator of TLR-signaling has a unique role in preventing excessive innate immune response. Complement Factor D plays an essential role in the initiation and propagation of the alternative pathway of complement activation.

P5042 A unique genetic defect on chromosome 3 is responsible for fatness in the Berlin Fat Mouse

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This study aimed at the mapping and estimation of genetic and sex effects contributing to the fat phenotype of the Berlin Fat Mouse Inbred line 860 (BFMI860). These mice accumulate 24% total fat mass at ten weeks of age under a standard diet and serve as a model for body fat distribution in livestock. 471 mice of a (BFMI860xC57BL/6NCrl) F_2 intercross population were analysed for body composition at ten weeks when mice have finished their rapid growth phase. The most striking result was the identification of a novel locus on Chr 3 at 40 Mb explaining 39% of the variance of total fat mass in the F_2 population. The BFMI860-allele effect was recessive. Animals homozygous at this QTL had on average 3 g more total fat mass than the other two genotype classes. The effect was evident in all white adipose tissues, brown adipose tissue and also in liver. The position of the Chr 3 effect is syntenic to a region on Chr 13 in pigs. Additional loci for total fat mass and different white adipose tissue weights with minor effects were detected on mouse Chr 5 and 6. Many loci including the Chr 3 QTL affected males and females to a different extent. This suggested that metabolic and regulatory pathways differed between the sexes. The major QTL on Chr 3 for fatness and its interaction with sex is unique and makes the BFMI860 mice an interesting resource for the discovery of novel genetic factors predisposing extensive body fat.

P5043 *IL4R* gene and recurrent airway obstruction in Swiss Warmblood horses

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Recurrent airway obstruction (RAO) in horses is the result of an interaction of genetic and environmental factors and shares many characteristics with human asthma. Many studies have suggested that interleukin-4 receptor gene (*IL4R*) is associated with this disease. In our previous work, we performed a QTL analysis for RAO in two Swiss Warmblood families. In this study we detected a QTL on ECA13 in one of the two families, which indicates that genetic differences between the two families exist. The *IL4R* gene is located within the ECA13 QTL. In this study we analyzed the expression of the *IL4R* mRNA in cells from broncheoalveolar lavage fluid (BALF). *IL4R* expression was increased in RAO-affected offspring during exacerbation in the family where the ECA13 QTL was detected but not the other family. This indicates that the QTL influences *IL4R* expression. Horses from the QTL family were then genotyped using the illumina equine SNP50 chip, which led to the identification of an RAO-associated haplotype GTGCTC around the *IL4R* gene associated with RAO in family 1. This haplotype includes 6 SNPs between BIEC2-215736 and BIEC2-216142, spanning over a 452287 bp region on ECA13. Additionally we genotyped 366 unrelated Warmblood horses on the SNP chip. In this cohort of unrelated horses the RAO-associated haplotype from the family had a frequency of 12.5% among 186 cases and of 9.0% among 180 controls. This difference was not statistically significant.

P5044 Signal regulatory protein-beta family expression correlates with the bovine macrophage immune response to infection with *Theileria annulata*

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Theileria annulata is the protozoan parasite responsible for tropical theileriosis which is endemic from Southern Europe to Asia. Indigenous Sahiwal (*Bos indicus*) cattle have greater resistance to *T. annulata* infection than the more productive Holstein (*Bos taurus*) breed which suffers a prolonged inflammatory response. The parasite has a complex life cycle with the schizont stage infecting and inducing proliferation in host macrophages (mϕ) by unknown mechanisms. Sahiwal and Holstein-derived mϕ have distinct transcriptome profiles, in particular signal regulatory protein (SIRP) beta which is present at 24.5 fold higher levels in Holstein mϕ. Since SIRPs are known to regulate inflammatory responses, we hypothesized that this difference in expression may underlie the observed genetic resistance to *T. annulata* infection. SIRPs, which are expressed primarily by myeloid cells, include SIRPα which inhibits mϕ function and SIRPβ which activates mϕ by association with DAP12. There is little information about bovine SIRPs or their role in *T. annulata* infection and the aim of this study is to further our understanding of this protein family. *In silico* analysis has shown that *SIRPA, SIRPB1* and *SIRPD* genes have a similar chromosomal arrangement to human *SIRP* genes, and putative genes for *SIRPG, SIRPB2* and *SIRPB3* have been identified. A bovine homolog of human pseudo-gene *SIRPA2* has not been found. Expression of *SIRPB1, SIRPB2* and *SIRPB3* differs between Holstein and Sahiwal *T. annulata* infected mϕ, and this difference may alter the balance of activating and inhibitory signaling and consequently determine how the infection progresses.

P5045 Splenic gene expression after infection of broiler chickens with *Escherichia coli*

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Infection of poultry by avian pathogenic *E.coli* (APEC) is an animal-health, animal-production, and food-safety concern. Non-antibiotic-based methods to enhance resistance of poultry to APEC, such as genetic enhancement of innate resistance capabilities, are desirable but depend upon a better understanding of the underlying mechanisms. The aim of this study was to identify genes with differential expression in the chicken spleen, related to host response to APEC. Four-week-old male broiler chicks, in four replicates, were orally infected with APEC. Forty spleens were analyzed using the 44K Agilent chicken whole genome microarray. Samples were grouped based upon contrasts of: infected vs. non-infected, vaccinated vs. non-vaccinated, necropsy 1 vs. 5 days post infection, and, for the non-vaccinated, challenged birds, severe vs. mild lesion scores. A novel reference design was used in which each experimental treatment was paired on the array with its non-challenged, non-vaccinated, day 1 necropsy birds as a reference. Dye swapping was performed between experimental replicates, with two references each labelled with Cy5 or Cy3. LOWESS normalization was applied to each array. Elements with a signal to noise ratio < 3 for all arrays were excluded from analysis. We fitted a linear mixed model to the data in R. Contrasting the samples from non-vaccinated, challenged birds with mild vs. severe lesions, there were more significantly differentially expressed genes on day 5 than day 1. This suggests that gene action as late as 5 days after infection may be critical in defending against an APEC infection.

P5046 Genome Wide Association Study of equine insect bite hypersensitivity

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Allergic eczema, also known as insect bite hypersensitivity (IBH), is the most common skin disease in horses and affects numerous breeds worldwide. The disease manifests as an itching dermatitis and without protective measures, continuous scratching may cause open wounds, leading to the risk of infections. Secondary symptoms include lichenification, crusts, dandruff and alopecia. The allergy primarily has the characteristics of an IgE mediated type I hypersensitivity reaction but a delayed-type hypersensitivity reaction also seems to be present. Strong evidence points towards proteins in the saliva of various species of the biting midges *Culicoides* as being the main allergen. Insect bite hypersensitivity is generally chronic and clearly seasonal, determined by the activity of insects. We have estimated the heritability of IBH for Icelandic horses born in Sweden at 0.30 (s.d. < 0.2), leading us to search for genes that regulate IBH susceptibility.

We utilized the EquineSNP50 BeadChip for genotyping and the data was used to conduct a genome wide association study (GWA). Several of our affected horses were related, so we chose to use matched healthy half sibs as controls to overcome the problem of stratification. All individuals were selected to be as unrelated as possible on the maternal side and controls were picked from high-risk areas. In total, the horse material consists of 104 affected and 104 healthy horses, sired by 42 different stallions. Affected horses were classified into three different groups depending on the severity of symptoms. The result from the GWA is currently being analyzed.

P5047 A genetic analysis of interval censored single intradermal comparative tuberculin test data from the bovine tuberculosis control programme in Northern Ireland

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In Northern Ireland all cattle are tested routinely with an annual single intradermal comparative tuberculin test (SICTT). Following herd breakdown, this test is repeated at 60-day intervals, until all cattle are negative. This results in the onset of *Mycobacterium bovis* infection being recorded in an interval-censored manner. The aim of this study was to test the ability of an interval censored Bayesian mixed model to infer infection status and time until infection and therefore to predict the genetic parameters of onset of *M. bovis* infection following herd breakdown using simulated SICTT data. Phenotypic records of time to onset of infection following herd breakdown were simulated across a four-generation pedigree. Fourth generation records (n=10,000) were retained, and analysed using a linear mixed model. Phenotypic records were then converted to an interval censored data structure based on the 60 day testing interval imposed post herd breakdown, and analysed using the Bayesian generalised mixed model. Right censoring at 10%, 50% and 80% were tested. Posterior modes of heritability of time to onset of infection following herd breakdown were 0.15, 0.14, 0.13 and 0.08 from the non-censored, 10%, 50% and 80% censored data set respectively; with all 95% highest posterior density intervals overlapping with the 95% confidence intervals of the heritability estimate (0.15, SE=0.02) from the linear model. This model will now be used to estimate the heritability of time to onset of *M. bovis* infection following herd breakdown in the Northern Ireland Holstein Friesian dairy cows from field SICTT data.

P5048 Are we capturing all the relevant genetic variance when analysing infectious disease data?

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Following an epidemiological SIR model, infectious disease prevalence in a population may be affected by host genetic variation in susceptibility, infectivity and/or recovery rate. Infectivity, i.e. the propensity of transmitting infection upon contact with another individual, is expressed by infectious individuals but visible in susceptible individuals to different extent depending on their susceptibility. Genetic variation in infectivity or recovery rate can therefore be considered as associative genetic effects, as the genotype of infected individuals affects the disease status of susceptible group members. Current genetic analysis of disease data generally interprets variation in prevalence as variation in susceptibility alone. Hence, associative genetic effects are currently ignored.

This study examines what impact genetic (co)variation in epidemiological traits affecting disease transmission has on the estimation of genetic variation from field data, and how much of it is truly due to susceptibility. Data was generated by running a stochastic simulation of an SIR model recording the disease status of individuals at regular intervals. Genetic variation was introduced in infectivity, susceptibility and recovery rate by sampling the parameters for an individual from a normal distribution with known variances. Epidemiological parameters were then assumed to be correlated to different degrees. Preliminary results suggest symmetry between infectivity and susceptibility in determining the course of the epidemic in the absence of a correlation between parameters, and that correlation between epidemiological parameters affect the estimates of genetic parameters for predicted disease status. The study constitutes an important first step to including associative genetic effects in genetic analyses of disease data.

P5049 Quantitative trait loci associated with osteochondrosis in Standardbred trotters

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Osteochondrosis (OC), a disturbance in the process of endochondral ossification, is a common and clinically important disease that affects developing joints in animals and humans. The purpose of this study was to identify quantitative trait loci (QTL) associated with osteochondrosis dissecans (OCD) at the intermediate ridge of the distal tibia in Norwegian Standardbred trotters (NST) using the *Illumina* Equine SNP50 BeadChip whole genome single nucleotide polymorphism (SNP) assay. Radiographic data and blood samples were obtained from 464 NST yearlings. Based on the radiographic examination 162 horses were selected for genotyping; 80 cases with an OCD at the intermediate ridge of the distal tibia, and 82 controls without any developmental lesions in the examined joints. When conducting a case-control genome wide association study (GWAS), regions on chromosomes (ECA) 5, 10, 27 and 28 showed moderate evidence of association ($p < 5 \times 10\text{-}5$) with OCD in the tibiotarsal joint. Two SNPs on ECA10 represent the most significant hits ($p = 9.31 \times 10\text{-}7$). Putative QTLs on ECA 5, 10, 27 and 28 represent interesting areas for future research, validation studies and fine mapping of candidate regions. Results presented here represent the first GWAS of OC in horses using the recently released *Illumina* Equine SNP50 BeadChip.

P5050 A simple and efficient method for DNA extraction from low amounts of animal bones

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DNA extraction from bones is a routine procedure in service labs doing parentage testing as well as in forensics when there is no other biological material available. In bones, the DNA is present in low amounts, it can also be degraded and the presence of inhibitors has been frequently reported. Therefore, many methods have been described to extract DNA based on processing quite a lot of bone powder to overcome these limitations. We tried different techniques focused on scaling-down the whole procedure. The chosen method uses Dextran-blue as a carrier for removing the PCR inhibitors through a selective ethanol precipitation. It was initially assayed on cattle bone remains. Due to the good quality and high rate of recovered DNA, the starting material can be less than 0.25 ml of powder bone and sample handling can be sized to 1,5 mL tubes. The whole procedure can be completed in a few hours and the DNA is suited for STR amplification. The protocol worked very well for all cattle bones tested, included one boiled for several hours. On equine bones, however, DNA recovery is much lower and highly dependent of the bone. Thus, when Dextran-blue performance is low, we tried another approach starting from thin pieces of bone that were exhaustively decalcificated until material was soft enough to be treated as a cartilage tissue. DNA was then extracted using the DNeasy Blood and Tissue Kit (Qiagen). This technique can also be handled on a reduced scale and completed in a short time.

P5051 A mutation in the mitofusin 2 gene (*MFN2*) is perfectly associated with inherited neuropathy in Tyrolean Grey cattle

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Tyrolean Grey cattle represent a local breed with a population size of ~5000 registered cows. In 2003, a previously unknown neurological disorder was recognized in Tyrolean Grey cattle. The clinical signs of the disorder are similar to those of Weaver in Brown Swiss cattle but occur much earlier in life. The pedigrees of the affected calves suggest monogenic autosomal recessive inheritance and nearly all affected animals trace back to a single female. We studied the neuropathology of this disease and histologic examination of peripheral nerves showed an axonal degeneration. Genotyping 14 cases and 30 controls using the illumina BovineSNP50 BeadChip allowed us to localize the causative mutation to a 3 Mb interval on cattle chromosome 16 by association and homozygosity mapping. Haplotype analysis of 14 additional carriers that were not parents of the genotyped cases narrowed the interval further down to 2 Mb. The *MFN2* gene is located within this interval and encodes a mitochondrial membrane protein that participates in mitochondrial fusion and contributes to the maintenance and operation of the mitochondrial network. A recessive inherited human axonal neuropathy (Charcot-Marie-Tooth disease-2A2) is caused by *MFN2* mutations. Therefore, we considered *MFN2* a positional and functional candidate gene and performed mutation analysis in affected and control Tyrolean Grey cattle. We did not find any non-synonymous variants in the coding sequence. However, we identified a perfectly associated silent SNP in the coding region of exon 19 of bovine the *MFN2* gene. Marker assisted selection can now be used to eliminate this neuropathy from Tyrolean Grey cattle.

P5052 T_H1 and T_H2 cytokines mRNA levels in Brazilian Somalis crossbreed sheep resistant and suceptible to *Trichostrongylus colubriformis* infection

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Cytokines are proteins that play a central role in immune mechanisms involved in defense against gastrointestinal nematodes infections. The present study used the real-time PCR methodology to quantify Brazilian Somalis crossbreed sheep cytokines (IL-4, IL-13, TNF-α and IFN-γ) in two groups: one resistant and other susceptible to *Trichostrongylus colubriformis* infection. From a Somalis sheep herd, 75 young animals were kept together on pasture without anthelmintic treatment for 4 months. The eight most resistant and the eight most susceptible animals were chosen based on the mean of fecal egg counts and slaughtered for recover the parasites and small intestine tissue samples collection. RT-PCR was performed using the LightCycler PCR and SYBR Green I dye. SDHA (succinate dehydrogenase complex subunit A) was used for normalization and the relative quantification of genes was calculated by REST software. Resistant animals presented lower EPG counts than susceptible animals (1312,5 and 5081,6, respectively; $P<0.0001$) and 3 fold less specimens of *Trichostrongylus colubriformis* ($P<0.05$). Only IL-13 was up-regulated in resistant animals ($P<0.02$) and the other three genes analyzed, IL-4, TNF-α and IFN-γ were down-regulated in this group, although not significantly ($P>0.05$). IL-13 is a cytokine that stimulates the T_H2 response, leading the host to quickly and efficiently respond to the infection and contributes to the parasite expulsion. Although IL-4 acts together with IL-13 in this process, IL-13 is independent to eliminate the parasite for itself. It can be inferred that in the resistant animals a bias of T_H2 type response was activated.

P5053 Whole genome re-sequencing of Japanese Black cattle for SNP discovery in critical regions for *Marbling-2, CW-2* and *FMA*

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Marbling-2 and *CW-2* are QTL for marbling and carcass weight, and *FMA* is a genetic disease for Forelimb-girdle Muscular Anomaly. *Marbling-2* and *CW-2* have been mapped with progenies of Bull A, while *FMA* has been mapped with a progenies of Bull B. The critical regions for *Marbling-2, CW-2* and *FMA* were 4.6-Mb (BTA7), 0.9-Mb (BTA6) and 2.4-Mb (BTA26), respectively. To identify responsible/causative genes or SNPs from these relatively large regions, we performed whole genome re-sequencing of Bull A and B that are maternal half-sib.

A single-end library and three size-different paired-end libraries were prepared according to the manufacture's protocol, followed by sequencing with 36-bases reads using Genome Analyzer *II* (Illumina).

More than 3 x 10^9 reads were generated from Bull A and B, respectively, of which 11.5-M reads (412.3-Mb; x 52.03) and 11.7-M reads (415.3-Mb; x 58.96) were mapped to the target regions. Nearly 90% of the target regions were covered by ≥10 depth in both Bull A and B, and 21,136 (Bull A) and 19,598 SNPs (Bull B) were detected in comparison with Btau4.0 reference sequence. Based on criteria that Bull A is heterozygous for *Marbling-2* and *CW-2*, and Bull B is heterozygous for *FMA*, responsible or causative SNP will be searched among the SNPs.

P5054 Annotation of the immunity-related genes in the pig genome

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Resistance to disease and control of immune responses are complex processes that may be studied at several levels including individuals, populations, species, and phylogenetic groups. Indeed, there are mechanisms and responses shared between species and others that are species-specific, partly due to co-evolution of hosts and pathogens. Large scale sequencing of complex genomes provides unique opportunities to annotate large sets of genes and their structural variations for further comparative analyses. An automatically annotated draft assembly of the pig genome was released with Ensembl 56 in October 2009. The Immune Response Annotation Group (IRAG), comprising scientists working on resistance to disease and immunity in swine, was established to identify shared and species-specific immune responses and refine the annotation of immunity-related genes. A list of close to 1700 genes was drawn using information from gene ontology annotation (GO: 0002376 for immune system process), a core set of genes involved in host pathogen interplay (Jenner and Young, 2005), and gene sets under positive selection in humans (Barreiro and Quintana-Murci, 2010) and cattle. Manual annotation of these genes has begun using the WTSI Otterlace software. Alignments of genomic contigs with publicly available mRNAs, ESTs and proteins are analyzed to delineate exon and intron boundaries, thus providing a refined functional annotation of genes. Preliminary results have identified gene duplications and many new alternative splice variants for known genes. Our future challenge will be to identify the underlying biology specific to the pig species that can be identified from this major annotation effort.

P5055 Gene expression and pathways analysis of white blood cells from cattle orally challenged with Bovine Amyloidotic Spongiform Encephalopathy (BASE) one year post-infection

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Bovine Amyloidotic Spongiform Encephalopathy (BASE) is a recently discovered atypical form of BSE, transmissible to primates, and has been proposed as an equivalent of sporadic Creutzfeldt-Jacob Disease in man. Although transmissible, it is unknown whether BASE is acquired through infection or arises spontaneously. In the present study, the gene expression patterns of white blood cells (WBCs) from five cattle one year after oral BASE challenge were compared with negative controls using a custom microarray containing 43,768 unique gene probes.

A total of 140 genes were found to be differentially expressed between BASE and control animals with a log fold change of 1.5 or greater. Of these, 70% were up-regulated in the infected animals. Microarray data were then confirmed by qRT-PCR. The majority of the differentially expressed genes are related to immune functions, and several belong to the same pathways as revealed by KEGG analysis. In particular, BASE animals appear to have significantly modified expression of genes linked to the differentiation of subsets of immune cells, T and B cell development and activation, Natural Killer activity and inflammatory response. Samples from the same animals were examined by qRT-PCR at different time-points for the most interesting pathways to define when changes in gene expression are first observed and their kinetics.

The potential impacts of these gene expression changes will be discussed, together with their potential use as biomarkers and their contribution to better define PrP related gene functions in immune cells.

P5056 Prediction of the Kappa Casein genotype based on adjacent SNPs in Brown Swiss cattle

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Kappa Casein genotype is associated with the SNPs in the region where the Kappa Casein gene is mapped. In the present study, 695 Brown Swiss cattle with known genotypes of Kappa Casein and genotypes of 54k SNPs were analysed. The animals represent a random sample of Brown Swiss population in Switzerland. 78 animals had Kappa casein genotype AA, 296 had AB and 321 BB. 20 SNPs between 60.4 and 61.7 Megabase on chromosome 6 were selected due to calling rate and allele fractions. Between SNP UA-IFASA-6142 and the Kappa Casein 406 (58%) genotypes were concordantly. The SNP with the lowest accordance had 220 (32%) concordat genotypes. The analyses of allele combinations resulted in rare combinations predicting the Kappa casein genotypes based on grouping. The prediction of Kappa casein genotypes on association due to linkage disequilibrium was not efficient. Kappa casein as a monogenetic trait will further be predicted based on segregation or will be genotyped.

P5057 Comparative kinetics of *E. coli* vs. *S. aureus* specific activation of key-immune pathways in the Mammary Epithelial Cell: *S. aureus* elicits a delayed response dominated by IL-6, but not IL-1A or TNF-alpha

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Identification of principles causing the different aetiology of clinical and subclinical mastitis is important to developing preventive measures against this disease. We used primary bovine mammary epithelial cells (pbMEC) as a model and challenged them for one, three, six and 24 hours with inactivated *E. coli* or *S. aureus* mastitis pathogens. Affymetrix microarrays were used to analyse extent and kinetics of the pathogen-specific inflammatory response. The alteration in gene expression in pbMEC challenged with *E. coli* is much stronger and faster than in *S. aureus* stimulated cells. Most of the genes whose expression is strongly up-regulated in *E. coli* challenged pbMEC are belated and weaker regulated after *S. aureus* stimulation. The genes which are exclusively and most strongly up-regulated by *E. coli* may be clustered into a regulatory network with Tumor necrosis factor alpha (TNF-alpha) and Interleukin 1 (IL-1) in a central position. Expression of these master cytokines is not significantly regulated by *S. aureus*. In contrast, IL-6 expression was early up-regulated by the *E. coli* as well as the *S. aureus* challenge. Many of the genes which are late induced by both pathogens are known to be regulated by IL6-signalling. We suggest that the *E. coli* specific strong induction of TNF-alpha and IL-1 expression is causative for the severe inflammatory symptoms of animals suffering from *E. coli* mastitis. *S. aureus* fails to induce these master cytokines in MEC, eventually resulting in a moderate inflammation and a subclinical outcome of the udder infection.

P5058 Genome-wide Association Study for immune Traits in Swine

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Increased disease resistance through improved general immune capacity would be beneficial for the welfare and productivity of farm animals. The physiological similarity between swine and humans provides a promising way to enhance researches on the immune system of swine during the past few years, since swine can be used as an animal model for biomedical research. To identify genomic regions responsible for immune traits in the pig, a genome-wide association study was conducted. 11 traits, including proportions of $CD4^+$, $CD8^+$, $CD4^+CD8^+$, $CD4^+CD8^-$, $CD4^-CD8^+$, $CD4^-CD8^-$ and $CD4^+/CD8^+$, levels of IgG, INF-γ and IL-10, and INF-γ/IL-10, were measured in a composite pig population, which consisted of 568 piglets from 4 Landrace, 16 Large White, and 3 Songliao Black (a native breed of China) boar families. At the 21th day of age, all piglets were vaccinated with modified live CSF vaccine. Blood samples were collected when the piglets were 20 and 35 days of age, respectively. All the piglets were genotyped for 62,000 single nucleotide polymorphisms (SNP) using the Illumina porcineSNP60k BeadChip. 46,079 SNPs were selected after quality control for association tests between SNPs and each immune trait considered based on a single-locus LD regression model. A total of 11 SNPs were found to be associated with 5 immune traits at a chromosome-wise significance level (corrected for the number of SNPs on each chromosome). Most of the significant SNPs fall into the regions which harbour a number of known immunity-related genes. Furthermore, several significant SNPs are located within the QTL regions reported in previous studies. Results herein lay a preliminary foundation for further identifying the causal mutations affecting swine immune capacity in follow-up studies.

P5059 Investigation of the mode of inheritance of cardiac accessory pathways in Labrador retrievers

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Accessory pathways are abnormal electrical conduction routes in the heart and cause a form of arrhythmia, a supraventricular tachycardia called orthodromic atrioventricular reciprocating tachycardia (OAVRT). This is typically seen in young adult dogs who present with signs of weakness or congestive heart failure. Treatment involves lifelong therapy with anti-arrhythmic drugs (with variable outcome and the possible drug refractoriness) or radiofrequency ablation which is expensive and performed at only 3 centres (including R(D)SVS) worldwide. The mode of inheritance of OAVRT in humans and dogs has not been fully elucidated. Analysis of 22 cases seen at the R(D)SVS has shown a predisposition to OAVRT in non-yellow, male Labrador retrievers. Detailed analysis of the pedigrees of 12 dogs (11 male, one female; 5 black, 7 chocolate) showed a high degree of inbreeding in five (F at least 0.1) but very low level of inbreeding in five others (F under 0.01). A distant common ancestor was found repeatedly in the pedigrees of eleven dogs, in both paternal and maternal lines for ten of these. Two dogs also shared a different ancestral line. The remaining dog was closely related to one of these. There were two affected full siblings and a half-uncle/nephew pair as well as several inbred cousin relationships. We are currently studying the relatives of affected dogs to ascertain whether some show subclinical manifestations of the condition, prior to determining the mode of inheritance and risks to relatives.

P5060 Analysis of gene expression in cumulus oocyte complexes from Holstein Friesian repeat breeder cows

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In the dairy industry worldwide, reproductive disorders are a major cause of economic losses, whereas in recent decades a declined fertility associated with increased milk production have been widely reported. Fertility failures have multifactorial origin: environment, nutrition, management practices and pathologies. However, some fertility problems can not be directly attributed obvious causes. A repeat breeder was defined as a cow which does not become pregnant after three inseminations despite no clinically detectable reproductive disorders can be diagnosed. In this study an approach based on gene expression was used in order to increase knowledge on this issue.

Transcriptomes of cumulus oocyte complexes (COCs) collected from three Holstein Friesian repeat breeder and three normally fertile control cows were compared. During the experiment all the animals were maintained under the same conditions of environment, feeding and management. For each animal, up to 40 COCs were collected through repeated sessions of Ovum pick up in absence of hormonal stimulation. COCs were immediately plunged into liquid nitrogen and stored at -80°C until analysis. For each subject, RNA of pulled COCs was extracted and hybridized on GeneChip® Bovine Genome Array (Affimetrix). Analysis of gene expression profiles of repeat breeder and control groups, revealed 178 genes with a log fold change higher than 1.5 and 30 genes higher than 2. A total of 20 genes potentially involved in reproductive processes were considered for the validation by qRT-PCR. The data obtained in this work provide information for potential genes associated to repeat breeding in dairy cows.

P5061 Prion protein genotypes in goats from Slovenian scrapie outbreak

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Polymorphism of the prion protein gene (*PRNP*) is an important and well understood factor influencing the susceptibility and incubation period in scrapie in sheep. It has led to the development of breeding programmes based on the selection of sheep genetically resistant to Classical scrapie. In goats, the association of specific *PRNP* genotypes with resistance to transmissible spongiform encephalopathies (TSE) remains unclear. Goats from the classical scrapie outbreak in Slovenia were examined to determine *PRNP* genotypes in diseased animals. Animals were evaluated for the presence of detectable protease-resistant prion protein (PrP^{Sc}) in the obex region of the brain, histopathological changes associated with scrapie and *PRNP* genotype. In four goats (from 99 goats in the flock) the accumulations of PrP^{Sc} in the obex were detected by the rapid test and confirmed by immunohistochemistry. All scrapie-affected goats detected by rapid test carried the WW_{102}, AA_{136}, SS_{138}, II_{142} NN_{146}, RR_{154}, QQ_{171} and RR_{211} *PRNP* genotype. In three goats with confirmed scrapie silent mutations were observed at codon 138 (agc → agt).

P5062 Transcription patterns of beta-defensins mRNA in bovine mammary gland during bacterial infection

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Antimicrobial peptides are key molecules in local host defense. They have a broad spectrum of activity against Gram-positive and Gram-negative bacteria as well as fungi and enveloped viruses. The aim of this study was to determine the mRNA expression of chosen bovine β-defensins genes in mammary gland of dairy cows with infection caused by coagulase negative or positive staphylococci. The study was performed in secretory tissues and gland cisternal epithelium. The quantitive real-time PCR approach with taqMan probes and a set of stable, specific reference genes was used. Studies are based on the assumption that there are differences in mRNA abundance of beta-defensin genes in mammary gland tissues in infected cows. The mRNA level of investigated genes was higher in gland cisternal epithelium than in secretory tissues. Moreover, the abundance of LAP (lingual antimicrobial peptide) mRNA in epithelial tissues of gland cistern was higher than level of mRNA of BNBD10 (bovine neutrophil beta-defensin 10).

P5063 A Genome-Wide Association Study to isolate key Single Nucleotide Polymorphisms (SNPs) and Copy Number Variations (CNVs) involved in the molecular biology of fatness using microarray technology

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The pig is an excellent model organism for the study of fatness in humans due to similarities in size and physiology. Obesity is a disease where excess amounts of fat tissue exist in the body. This can cause a variety of illnesses including coronary heart disease, strokes and cancer. In agriculture, the control of relative fat and lean phenotypes is important and can be culture-dependent. This study therefore aims to identify Single Nucleotide Polymorphisms (SNPs) and Copy Number Variants (CNVs) that are associated with fat or lean phenotypes. In a preliminary study, 96 known individuals (48 fat and 48 lean) from Sire Line Large White pigs were interrogated using the Illumina porcine SNP chip. The data were analysed by logistic regression analysis assuming additive and dominance models, taking into consideration the Estimated Breeding Values (EBVs) of the pigs. Depending on the type of statistical analysis, our study revealed between 5 and 22 significant SNPs associated with fatness or leanness. The CNV analysis is ongoing. The study will be extended to further individuals from different breeds e.g. Duroc and Titan.

P5064 Characterisation of the porcine lung transcriptome using high-throughput pyrosequencing

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Transcriptome characterisation using next generation sequencing allows the global description of the genes expressed in a tissue or organ, the discovery of novel genes or alternative splicing events. In addition we can identify sequence variation (SNPs) and get information about transcript abundance. Our objective was to investigate animals previously not affected by lung disease and those that had been affected. To this end lung tissue samples were collected, separately pooled and tagged before sequencing using the Roche/454 FLX platform. We sequenced about one million reads that were clustered and mapped to the current pig genome reference sequence. Identified genes or clusters were annotated for functional classes and mined for singe nucleotide polymorphisms. In addition, we compared gene expression between sample groups in order to investigate possible changes in the lung transcriptome that may be related to previous infection.

P5065 Arthrogryposis multiplex congenita (AMC) a recessively inherited disease in Sus scrofa

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Arthrogryposis multiplex congenita (AMC) is a common malformation of joints in mammals. The typical symptoms can be caused by extrinsic or genetic factors. A genetically caused variant of porcine AMC was identified in the Swiss Large White population. The AMC diseased piglets show symptoms of persistent flexion of the limbs, overbite, deformation of the spinal cord and perinatal death. The disease is autosomal recessively inherited and the mutation was mapped on porcine chromosome 5 to a 3.2 Mb region between marker *SW152* and gene *CNTN1*. After analysing a family with the 60K SNP Chip from Illumina Inc. we found 60 SNPs in the AMC region. However, due to inbreeding we face a loss of heterozygosity in the region of interest and the interval could not be narrowed. Therefore, the experimental herd was expanded and we are currently genotyping a three generation pedigree in order to further restrict the AMC region.

P5066 Genes for resistance to melanoma: Can we reveal them in Old Kladruber horses?

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Melanoma is a highly prevalent disease in grey horses. The duplication in the *syntaxin 17*, causing coat colour (greying with age) is also associated with the formation of skin melanoma. A small proportion of horses do not present melanomas even in higher age. The aim of this study was to identify effects of further candidate genes involved in mechanisms of disease in a small, isolated and partially inbred (F_X=0.10) model population of Old Kladruber grey horses. Fifty breeding mares were examined by visual inspection and classified as negative (n=26, age 7-23 years) or positive (n=24, age 7-18 years) for the presence of melanoma. Association analysis with 50 microsatellite loci and with 31 polymorphic markers in expressed genes was performed. Standard two-sided chi-square and/or exact Fisher tests were used to assess associations between polymorphisms detected and variations in the presence of melanoma. Bonferroni corrections for multiple comparisons (81) were made. One microsatellite (*HTG004* on ECA9, P_{corr}=0.032) and one candidate gene (*TLR4* on ECA25, P_{corr}=0.0081) were significantly associated with the "resistance" to melanoma in this population. Additional genes (*CD14 molecule* on ECA14 and *matrix metallopeptidase 16* (*membrane-inserted*) on ECA9) were significantly associated in composed genotype analysis (*CD14/TLR4/MMP16*, P_{corr}=0.00081). Associations with immunity-related genes, although biologically plausible, need to be further investigated. If confirmed, they can contribute to our understanding of the complex nature of this important and common disease of grey horses and can also be used for a conservation programme for this endangered breed.

P5067 A comparative study of cELISA and real-time PCR Assays for detection of *Babesia equi* (Theileria) in Italian Horses

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Equine babesiosis is a horse tick-borne disease that affects horses, caused by two hemoprotozoan parasites, *Babesia equi* and *Babesia cabali*. Epidemiological studies on Italian horses populations have already shown that the *Babesia equi* is more common than the *Babesia* caballi. In this study, equine blood samples were collected in some Italian stables in which several horses had showed symptoms of *Babesia* disease during the last 12 months. An enzyme- linked immunosorbant assay (cELISA) was performed in order to verify the presence of antibody against *Babesia equi* in horse serum. Blood samples were then extracted and a real-time PCR was performed to find parasite DNA. Results of Real Time PCR assay were then compared with the cELISA test results. To date, a total of 60 samples have been analyzed with both methods: 24 samples (40%) were positive and 2 samples were possible positives to *Babesia equi* with the cELISA assay and 27 (45%) samples were positive with Real-Time PCR. Discrepancies between the Real-Time PCR and the immuno-enzymatic methods are due to the different phases of *Babesia equi* infection. More samples will be analysed and further studies will be necessary for the establishment of a fast and reliable method of detection for *Babesia equi* in the Italian horse population.

P5068 BLV-CoCoMo-qPCR: Quantitation of bovine leukemia virus proviral load using CoCoMo algorism

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Bovine leukemia virus (BLV) is associated with enzootic bovine leukosis. Infection by BLV can remain clinically silent, or it can emerge as a persistent lymphocytosis and, more rarely, as B-cell lymphomas in various lymph nodes after a long latent period. BLV infects cattle worldwide, imposing a severe economic impact on the dairy cattle industry. Here, a new quantitative real-time PCR method using Coordination of Common Motifs (CoCoMo) primers was developed to measure the proviral load of all BLV variants. We selected the long terminal repeat of BLV, which duplicated in one proviral genome sequence for BLV amplification. From 356 of sequences collected from GenBank database, 99 kind of individual BLV-LTR sequences were determined and designed 42 degenerate primers using the CoCoMo algorism which developed for design the PCR primers for multiple strains of viruses. We selected CoCoMo 6 and 81 primer sets out of 72 primers sets which reveal the best specificity investigated by PCR and melting-curve analysis. BLV copy number was normalized to the amount of cellular DNA by quantitative determination of the bovine leukocyte antigen (BoLA) *DRA* gene. The specificity, sensitivity, quantification ability of BLV-CoCoMo-qPCR were estimated by an amplification of other retroviruses, a serial dilution-Nested PCR method, syncytium formation method and immunodiffusion test. BLV-CoCoMo-qPCR could detect BLV of worldwide cattle which were failed to detect by previously developed Nested-PCR. Interestingly, we succeeded to confirm that copy number of BLV were clear increased depend on the disease progression induced by BLV.

P5069 Homozygosity mapping of a putative recessive gene associated with leg weakness in a commercial pig population

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Leg weakness is of welfare and economic importance in pigs. A severe leg deformity syndrome, significantly affecting piglet survival, has recently appeared in a commercial line of pigs. Quantitative genetic analyses revealed a strong genetic component, suggestive of a single recessive gene controlling the trait. This study aimed to map the putative QTL using homozygosity mapping. Under this hypothesis, assuming full penetrance, all individuals in the affected group will be homozygous for the putative QTL and this homozygosity will extend to the region surrounding it, with none in the control group. Ten full-sib pairs (one affected and one control sib) were genotyped for the Illumina 60k pig SNP chip. After stringent quality control, 39,201 segregating SNP covering 2.1 Gbp of the pig genome across all 18 autosomes were used. A region was identified on chromosome 15 where all affected animals were homozygous for the same haplotype extending 62 consecutive SNPs (8.55Mbp length). The longest homozygosity region in the controls was 23 SNP, on a separate chromosome. One control animal shared the homozygote haplotype of the cases, suggesting that any putative QTL located in this region is either not of full penetrance or resulted from a recent mutation event so that not all copies of this haplotype carry the causative mutation. Further genotyping is being carried out to confirm the region as the location of a putative QTL. The results from this study will provide the required information to control and eliminate leg deformity affecting this pig line.

P5070 Integrating comparative expression profiling data with association of SNPs to Salmonella shedding for improved food safety and porcine disease resistance

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Salmonella in swine is a major food safety problem, as a majority of US swine herds are *Salmonella*-positive. *Salmonella* can be shed from colonized swine and contaminate neighboring pigs and a) slaughter plants and pork products; b) edible crops when swine manure is used as a fertilizer; and c) water supplies if manure used as crop fertilizer runs off into streams and waterways. A potentially powerful method of addressing pre-harvest food safety at the farm level is through genetic improvement of disease resistance in animals. In this research we describe a successful strategy for discovering genetic variation at candidate genes associated with disease resistance in pigs, by integrating our recent global gene expression analysis of the porcine response to *Salmonella* with literature information on important candidate genes. We identified single nucleotide polymorphism (SNPs) in these functional candidate genes, and then genotyped three independent pig populations with data on *Salmonella* fecal shedding or internal burden (total n=377) at these loci. Out of 31 SNPs genotyped, we found 21 SNP segregating in at least two populations with minor allele frequency of 15% or greater. Statistical analysis revealed thirteen SNPs associated with *Salmonella* fecal shedding or tissue colonization with estimated proportion of false positives ≤ 0.21 and estimated *p*-values from 0.002 – 0.09. The genes with associated SNPs included GNG3, NCF2, TAP1, VCL, AMT, CCR1, CD163, CCT7, EMP1 and ACP2. These associations provide new information on the mechanisms of porcine resistance to *Salmonella* and may be useful in improving genetic resistance to this bacterium.

P5071 Nramp1 expression in water buffalo (*Bubalus bubalis*) blood cells

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NRAMP1 (natural-resistance-associated macrophage protein) determines the resistance or susceptibility of the host to intracellular pathogens influencing the initial phase of bacterial cellular infections and regulating macrophage activation. Different $(GT)_n$ microsatellite repeats (where n is 13,14,15 or 16), localized in the 3' UTR of the gene, have been associated with either susceptibility or resistance to *Brucella abortus* infections in buffalo. However, contradictory results were reported, referring either to $(GT)_{16}$ carriers or to $(GT)_{13}$ carriers as resistant animals to brucellosis. In a previous work we did not find significant differences in the frequency of $GT_{(n)}$ variant genotype between serologically positive/negative animals. The aim of this study was to evaluate NRAMP1 gene expression in a serologically negative herd of 42 buffaloes. qPCR experiments were performed for a total of 27 animal differing at the $(GT)_n$ microsatellite repeats. NRAMP1 expression was evaluated in RNA from total blood and, subsequently, in RNA from peripheral blood mononuclear cells (PBMC) and in RNA from blood depleted of PBMC cells (PMN/G). The comparison was performed between $(GT)_{12}/(GT)_{12}$ and $(GT)_{12}/(GT)_{16}$ carriers, because no homozygous $(GT)_{16}/(GT)_{16}$ was found in this herd. Our results showed that NRAMP1 expression in PBMC is lower than in PMN/G ($P<0.006$). On the other hand, we noted no statistically significance fold expression difference between the $(GT)_{12}/(GT)_{12}$ and $(GT)_{12}/(GT)_{16}$ groups ($P<0.1614$).

P5072 Effect of systemic progesterone on retinol binding protein gene expression and secretion in the bovine uterus

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Altered systemic progesterone (P4) concentrations, affects the timing and duration of endometrial gene and protein expression, which impacts conceptus development. The objective was to examine how P4 concentrations during the early luteal phase affected retinol binding protein-4 (RBP4) gene and protein abundance. Following oestrous synchronisation, heifers were assigned to Low, Normal or High P4 groups (N=5). Endometrial tissue and uterine flushings were recovered from the uterine horn ipsilateral to the corpus luteum on Day 7 and 13. RBP4 gene and protein expression was quantified using qRT-PCR and western blotting respectively.

Heifers in the low P4 group had lower plasma P4 concentrations throughout the cycle and heifers in the high group had higher P4 between Days 4-7 compared with controls ($P<0.05$). RBP4 gene and protein expression was higher on Day 13 compared with Day 7 in heifers having high and normal P4. However, there was no difference in RBP4 protein abundance between Day 7 and 13 in heifers with low P4. P4 had no effect on RBP4 expression on Day 7. On Day 13 of the oestrous cycle RBP4 gene expression was over 2-fold higher ($P<0.001$) in high and control P4 groups compared with the low P4 counterparts. In addition, RBP secretion was over 2-fold higher ($P<0.05$) in the high P4 group compared with the low P4 group on day 13.

In conclusion, systemic progesterone modulates uterine RBP gene and protein expression in a time and concentration dependant manner and thereby histotroph composition a critical factor for early embryo survival.

P5073 Profiling of porcine microRNAs and their involvement in *in vitro* infection with Aujeszky Disease Virus (ADV)

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MicroRNAs are small noncoding RNAs (18-25nt) which have emerged as important regulators of infectious disease status, being mostly involved in the host-virus interactions. Documenting the profile of miRNA expression in normal tissues provides a baseline for comparison with pathological states and can detect tissue-specific/enriched miRNAs as biomarkers. ADV is an α-herpesvirus which can affect pigs with tropism for respiratory and nervous system tissues. In order to increase the repertoire of porcine miRNA and to analyze the relation between host miRNA expression profile and ADV infection, we elaborated 2 sets of libraries: a) swine kidney samples from 7 different breeds (health status), b) PK-15 cells non infected and infected with an attenuated vaccine (Begonia) and a virulent strain (NIA3) of ADV (in vitro infection). We have determined the swine kidney microRNAome and detected 140 out of the 175 known swine miRNAs as well as other 154 orthologous. The most expressed miRNAs in swine kidney were miR-200b, miR-125b, miR-23a and miR-126. Differences in miRNA expression among breeds were detected and some miRNAs appeared to be breed-specific. We also determined some miRNAs in PK-15 cells, like miR-10a, miR-23b and miR-183, which increased their expression level after infection with both ADV strains. Moreover, some miRNAs, like miR-126-5p, miR-135b and miR-339-5p, were only expressed after Begonia or NIA3 infection, which could be involved in virulence process. A third library including olfactory bulb and trigeminal ganglia samples from pigs non infected, and infected with Begonia or NIA3 strains has been performed and is currently under analysis.

P5074 A Genome-Wide Association Study for Melanoma predisposition in the MeLiM biomedical swine model by using the porcineSNP60 BeadChip

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Melanoblastoma-bearing Libechov Minipigs (MeLiM) represent an excellent biomedical model for human hereditary cutaneous melanoma, as they develop spontaneous melanomas at birth with a strong clinical and histological similarity to human counterparts. A genome-wide QTL scan performed on a MeLiM x Duroc backcross (375 animals) with 153 microsatellites showed a complex genetic architecture of melanoma predisposition in MeLiM pigs, with at least five major QTLs localized on different porcine autosomes. In order to gain more insight, we have genotyped this population with the PorcineSNP60 BeadChip. After removing SNPs having <5% of minimal allele frequency or which genotypes were missing in >30% of individuals, 47,124 of 64,232 SNPs were retained for the subsequent analyses. Mendelian inconsistencies were removed with the aid of the PedCheck software and represented roughly 0.005 % of the obtained genotypes. Single marker association analyses for ten traits related to melanoma predisposition were performed using an animal model fitting the SNP additive and dominant effects, sex, skin color and the polygenic effect estimated from the backcross pedigree. In summary, over 25 genomic regions in 14 chromosomes showed association ($P < 5x10^{-5}$) with at least one trait, with the main effects localized on chromosomes 1, 2, 3, 5, 7, 8, 13, 14, 15 and 16. The results were replicated in R by using regression models adapted to the studied phenotypes. A substantial number of effects, including a highly associated region on chromosome 5, were not identified on the previous QTL analyses and represent novel targets for the ongoing follow-up studies.

P5075 High-resolution chicken MHC genotyping using a SNP panel

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The B region of the chicken Major Histocompatibility Complex (MHC) is a major locus for the control of infectious diseases, comprising multiple genes with very high levels of polymorphism. It has been defined by serology (Briles & Briles 1982), and more recently with an atypical marker, LEI0258 (Fulton et al. 2006) or by sequencing target regions or genes (Hosomichi et al. 2008). In order to cover the entire B region, increase the resolution of genotyping and understand the evolutive history of the different alleles, we developed a panel comprised of 96 SNPs. These SNPs were selected from a list of more than 4,500 SNPs identified by comparison of sequences available in databases, and by a resequencing approach of 24 PCR in 48 different chicken populations, including local breeds. The average distance between two consecutive SNPs from the panel is 2,256 bp (min: 79 bp, max: 11,763 bp). This panel was used to genotype 480 samples, mainly international reference lines, using an Illumina GoldenGate assay. The samples had previously been fully characterized by serology and molecular genotyping. Genotypes were obtained for all the samples and almost all the SNPs thus technically validating the panel. This panel will allow classification of the MHC B alleles corresponding to the different serology, and establish their evolutive relationships by determination of recombination breakpoints. Moreover the SNP genotypes have revealed far more variation in this region than was previously indicated by serology and LEI0258 marker.

P5076 Not all simply inherited diseases in dogs are easy to find using GWAS

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The Australian kelpie is a working dog developed from crosses with smooth collies in the 1870s. An ataxia caused by cerebellar abiotrophy (CA), loss of Purkinje cells, is widespread in the breed. The disease appears to have a simple recessive inheritance. We have attempted a genome wide association study using Affymetrix Canine V2 SNP Chip with 12 affecteds and 20 controls. No significant association was found using Plink. There is a single large region of homozygosity common to all affecteds on CFA3 of about 5 Mb with 44 genes. This region is also homozygous in a number of controls as well as some of the unaffected parents of cases. There are no obvious candidates in the region. Microsatellites in the region also show homozygosity in affecteds but those with longer repeat regions and higher mutation rates show some differences between the haplotype from parents passed on to cases to those not passed on. Our hypothesis is that the CA mutation has occurred on a common haplotype. We used Nimblegen Capture arrays and 454 sequencing of 2 affecteds and a control to sequence the region and identified about 2000 differences between the affecteds and control. Only 8 of these change an amino acid in a protein. None of these is consistent with being the cause of the disease when tested in a larger sample. The sequencing would not identify duplications or insertions of interspersed repeats. We are examining the remaining SNPs and a looking for insertions and duplications in the region.

P5077 c-kit and melanoma predisposition in pigs: Sequence variants, association analysis and gene expression

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In human and rodents, c-kit has been shown to be involved in melanocyte development and melanoma progression. Although this gene has also been extensively studied in pigs due to its role on coat colour, its possible relation with porcine melanoma predisposition remains unexplored. The objective of this work was to characterize c-kit genomic variants and mRNA expression in a porcine model of cutaneous melanoma, the MeLiM pigs, which develop spontaneous tumours at birth that are clinically and histologically similar to the human melanomas. After discarding the presence of the c-kit duplication and the splice mutation in intron 17 in the MeLiM animals tested, we screened the whole coding sequence and parts of the introns for polymorphisms between MeLiM and Duroc pigs. We found 1 in/del and 41 SNPs among which 2 are non-synonymous variants. We genotyped the most informative non-synonymous SNP in a backcross pedigree MeLiM X Duroc, and performed an association analysis with melanoma-related traits. A significant association was found with several traits defining the aggressiveness of the tumours, after a correction for sex and coat colour. Furthermore, preliminary data showed a differential expression of the gene between tumours and healthy skin. All these results are consistent with a potential role of c-kit in pig melanoma development.

P5078 Characterization of swine leukocyte antigen (SLA) polymorphism reveals a breed-specific constriction of SLA gene diversity in Pietrain pigs

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The porcine major histocompatibility complex (MHC) harbours the SLA (swine leukocyte antigen) class I and II gene clusters. The SLA genes are highly polymorphic; they encode a series of cell-surface glycoproteins which function mainly in presenting antigenic peptides to T cells, therefore representing one of the most important determinants in swine immune response to infectious disease and vaccination. In Austria, the majority of commercial pigs are F2 descendants of F1 Large White/Landrace hybrids paired with Pietrain boars. The repertoire of SLA alleles and haplotypes present in Pietrain pigs thus has an important influence on that of their descendants. In this study, we characterized the SLA class I (SLA-1, SLA-2, SLA-3) and class II (DRB1, DQB1, DQA) genes of 27 purebred Pietrain pigs using a combination of the high-resolution sequence-based typing (SBT) method and a low-resolution PCR-based method using allele-group sequence-specific primers (PCR-SSP).

A total of 15 class I and 11 class II haplotypes were identified in the studied cohort. The most common SLA haplotype Lr-43.14 (SLA-1*11XX-SLA-3*04XX-SLA-2*04XX-DRB1*0901-DQB1*0801-DQA*03XX) was identified in ten animals with a frequency of 18.5%. Three class I and two class II haplotypes appeared to be novel that have never been reported in other pig populations, suggesting a breed-specific constriction of SLA gene diversity in Pietrain pigs. In conclusion, this study may facilitate a better understanding of the influence of SLA genes on various immunological and pathophysiological conditions. It may also facilitate the design of more effective vaccines aiming to improve the overall swine health in Austrian commercial pigs.

P5079 The use of a high-density SNP array for identifying genetic regions controlling parasite resistance in sheep

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Gastrointestinal parasites have a profound detrimental effect on sheep production. To reduce these economic losses, it would be advantageous to identify sheep that are genetically resistant to parasites. As previously reported, a sheep flock segregating for genes controlling parasite resistance has been created at Louisiana State University. The flock includes 378 F2 offspring of five F1 sires produced from Gulf Coast Native (resistant) and Suffolk (susceptible) crosses. Using fecal egg counts (FEC) for *Haemonchus contortus* and packed cell volume (PCV) measurements taken after natural challenges, 25 "resistant" animals and 9 "susceptible" offspring were identified and then genotyped with the Illumina Ovine SNP50 BeadChip. Using PLINK software, a SNP around 34 Mb on chromosome 7 (OAR7) was significantly associated with parasite resistance ($p < 3.44E-06$). This SNP correctly predicted resistant/susceptible status in 23 of the 24 informative offspring. Other associations were found on OAR5 and OAR14, although at lower significance levels. These regions will be examined for potential candidate genes associated with parasite resistance.

P5080 Association of Toll-like receptor 4 single nucleotide polymorphisms with incidence of infectious bovine keratoconjunctivitis (IBK) in cattle

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Toll-like receptor 4 (TLR4) is a receptor protein identifying pathogen molecules mainly associated with Gram-negative bacteria. Our objective was to investigate the association of nucleotide polymorphisms in TLR4 with infectious bovine keratoconjunctivitis (IBK), or pinkeye, incidence in American Black Angus cattle. We used animals with previously calculated breeding values for IBK susceptibility to identify two SNPs in TLR4; Int1 (G/A) in intron1 (-26 Ex2 position) and Ex3 (C/T) in exon3 (1678 position). We collected IBK incidence information on 370 calves raised in Iowa at two time points – June or August (disease season) and October (at weaning) and genotyped them using PCR-RFLP protocols for the two TLR4 SNPs identified. In statistical models including year, pasture management group and SNP, the Int1 SNP had a significant effect on IBK infection rates both in-season ($P < 0.05$) and at weaning ($P < 0.01$), whereas the Ex3 SNP was not significant ($P > 0.79$) at either time point. LSMeans estimates for genotype effects from this model indicate Int1-A/A animals exhibit more IBK infection than Int1-G/G animals (62.1% vs. 36.1% infection rate). Furthermore, the Int1 SNP alone could account for 2.1% of phenotypic variation in IBK infection during the disease season and 3.0% of phenotypic variation in IBK infection at the time of weaning. These data indicate there is a relationship between Int1 genotype and the rate of IBK infection in Black Angus cattle.

P5081 Screening for associations of horse immune gene polymorphisms to EAV infection in horses from Argentina

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Equine Viral Arteritis (EVA) is a systemic infection in equids with variable outcome, ranging from subclinical infections to severe disease, and also has the capacity to induce abortion in pregnant mares. Persistent infections in stallions play a major epidemiological role in the dissemination and perpetuation of EAV. The disease is caused by a virus of the *Arteviridae* family. In 2001, as the result of an outbreak in Argentina, the virus was isolated from semen samples for the first time in South America. The aim of this study was to investigate polymorphisms in two immune response related genes (*ELA-DRA, TNF*-α) and three microsatellite loci (UM011, TKY08, LEX52) located within or near the ELA in order to identify associations with susceptibility to EVA infection. Silla Argentino (N=168) horse samples were serologically tested to EAV by the virus neutralization method during 2002, 2004 and 2006. Genotyping was performed by Pyrosequencing three SNPs on *TNF*-α promoter and *ELA-DRA* second exon, and STR genotyping was performed in automated DNA sequencer. Allelic and genotypic frequencies, heterozygozity, number of alleles and Linkage disequilibrium (LD) were estimated. All markers showed polymorphism. LD was confirmed between *ELA-DRA* and *TNF*-α ($p<0.01$). Association analysis in108 EAV-infected and 60 EAV-non-infected horses did not show significant differences in the allele or genotypic frequencies between infected and non-infected horses. No association could be established between the serological condition and the studied polymorphisms. However, some genotypic and haplotypic combinations showed differences in the incidence between infected and non-infected groups.

P5082 Whole-genome association analyses for identifying genomic regions related to scrapie in sheep

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Scrapie is an infectious disease of sheep that affects the central nervous system and is classified as a transmissible spongiform encephalopathy (TSE). Sheep infected with scrapie have served as an animal biomedical model for human prion diseases. The aim of this study was to use the ovine Illumina SNP50 BeadChip to identify genomic regions that contain genes controlling the incidence of scrapie. Samples genotyped with the array were collected in 1980 from sheep that were deliberately inoculated with scrapie-positive tissues. Quality control measurements applied to the SNP data included minor allele frequency (MAF) ≥ 0.01, genotyping rate ≥ 95%, and missing genotype rate ≤ 5%. The cleaned SNP data set comprised 66 individuals and 50,592 SNPs. SNPs significantly associated with scrapie infection were detected using PLINK Whole Genome Analysis Toolset. To date, 14 SNPs on seven ovine chromosomes were positively associated. Chromosomal regions containing the significant SNPs will be examined for possible candidates using a gene pathway simulation. The results of this study will aid in the eradication of scrapie in sheep.

P5083 Functional genomics studies of bovine monocyte-derived macrophages (MDM) stimulated *in vitro* with *Mycobacterium bovis, M. bovis*-BCG and *M. avium* subsp. *paratuberculosis* (MAP)

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To identify key host response genes to mycobacterial diseases in cattle, a functional genomics approach was undertaken to investigate bovine macrophage gene expression patterns in response to stimulation with *Mycobacterium bovis*, attenuated *M. bovis* bacillus Calmette-Guérin (BCG) and *M. avium* subsp. *paratuberculosis* (MAP) – the causative agent of Johne's disease. Purified monocyte-derived macrophages (MDM) from seven unrelated Holstein-Friesian females were used for separate *in vitro* challenge experiments using the three bacterial species (MOI – 2:1). Total cellular RNA was extracted from challenged MDM samples at intervals of 2 h, 6 h and 25 h post-infection and prepared for pan-genomic gene expression analyses using the high-density Affymetrix® GeneChip® Bovine Genome Array with features representing more than 23,000 gene transcripts. These gene expression data can be used for systems biology reconstruction of macrophage cellular pathways underlying host interactions with *M. bovis, M. bovis*-BCG and MAP. Important gene expression changes can be identified and validated using real time quantitative reverse transcription PCR (qRT-PCR). Analyses of these data will: (1) provide a panel of candidate resistance/susceptibility genes that can be used for subsequent population resistance/susceptibility studies in field-infected and control animals; (2) offer a greater understanding of the host responses against these mycobacteria; (3) lead to improvements in disease diagnostics; and (4) enhance vaccine development. Here we present preliminary analyses and results from these experiments.

P5084 Identification of a new QTL for resistance to *Salmonella* carrier-state

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The ability of chicken to carry *Salmonella* without displaying disease symptoms leads to an invisible propagation of *salmonella* in poultry stocks and contributes to human infections through the consumption of contaminated meat or eggs. Using chicken lines more resistant to carrier-state would thus improve both animal health and food safety. Previous studies identified several QTL for resistance to carrier-state in a dedicated F2 cross. To measure resistance, animals were orally infected at one week of age with 5 x 10^4 bacteria of the same phage type 4 strain 1009 of *S. Enteritidis*. The numbers of colonies forming units (c.f.u.) in caeca were counted five weeks post inoculation. However, this first genome scan was incomplete due to a lack of informative markers. Indeed, only 19 chromosomes out of the 38 autosomal pairs plus Z and W chromosomes existing in chicken, were covered with more than one marker. To complete the genome coverage, we produced a new set of 480 informative SNP markers, selected amongst 9216 SNP tested for polymorphism in the F1 individuals of our QTL detection design. Genotyping was done with the Illumina GoldenGate assay. To improve detection power, the families studied were extended to 378 F2 individuals. These new data led to the identification of a new QTL for resistance to carrier-state on chromosome 14, which was not included in previous analyses. To our knowledge this is the first report of a QTL or candidate gene related to *Salmonella* resistance detected on chicken chromosome 14.

P5085 High probability of exclusion and sex identification in goat genealogical control through 22plex PCR

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The development of a system allowing verification of animals' genealogy as well as their unequivocally identification throughout their life is very important as it is one of the weaknesses in the Selection and Breeding Programs for goats mainly due to the reproduction systems, based on the use of large number of females with several males more than artificial insemination. The Central Veterinary Laboratory of Algete (LCV) has developed a high-exclusion capacity, *multiplex* PCR system which amplifies 21 microsatellite markers (BM1818, CSRD247, ETH152, HSC, ILSTS008, ILSTS019, ILSTS030, ILSTS087, INRA005, INRA006, INRA023, INRA063, INRA172, MAF65, MAF209, McM527, OarFCB20, SRCRSP5, SRCRSP8, SRCRSP23 and TGLA53), and a sex marker (AME) in a PCR *multiplex* reaction. All of them have been selected from the proposed list by the International Society for Animal Genetics (ISAG) for the 2009-10 Goat International Comparison Test and used for studies in this species. Primers were adapted to achieve a final configuration allowing all markers to be analyzed together. Robotic procedures were implemented to minimize the risk of genotyping errors and parentage verification mistakes.

The proposed system has been designed to process a very large number of samples in a short space of time ensuring a perfect reliability and traceability of the obtained results. Probability of exclusion reached is 99,9999612%. We are developing a secondary panel of 19 additional microsatellite markers to be used in cases where there is a need for greater capacity of exclusion.

P5086 Immunological suppression in cattle challenged with tick *Rhipicephalus* (*Boophilus*) microplus

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Bos indicus breeds are more resistant to cattle tick *Rhipicephalus* (*Boophilus*) *microplus* than *Bos taurus*. Several factors might be involved in the breeds' variation regarding resistance to tick, including skin characteristics and immune activation. The aim of this work was study the expression of immune response genes of challenged cattle. From a Gyr x Holstein F2 population, six highly resistant and six highly susceptible animals were selected. The animals were infested with 20 k tick larvae and peripheral blood was collected 0, 24 and 48 hours after tick infestation. RT-PCR analyses were performed to evaluate the expression of FOXP3 and IL-10 genes. No difference was detected in the expression of these two genes in the susceptible animals during the 48h period. In the resistant animals, after 48 hours of infestation, FOXP3 and IL-10 were up-regulated by 30.3 ($p<0.001$) and 31.6 ($p<0.0001$) fold change, respectively. After 24 hours of infestation, FOXP3 and IL-10 were up-regulated by 67.4 ($p<0.0001$) and 26.5 ($p<0.0001$) fold change, respectively. In the comparison between resistant and susceptible groups of animals, FOXP3 and IL-10 genes showed up-regulation 24 hours after infestation in the resistant group by 5.4 ($p=0.013$) and 10.9 ($p=0.030$) fold change, respectively. After 48 hours of infestation, only FOXP3 showed up-regulated expression in the resistant group by 8.5 ($p=0.014$) fold change. These results suggest that immune response against tick in the resistant animals is already under suppression in the early 24 hours after infestation.

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P5087 Cloning and characterization of the rabbit neonatal Fc receptor

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IgG is the most important and abundant immunoglobulin in mammals. IgG protects against viral, bacterial and parasitic infections. The neonatal Fc receptor (FcRn) is an Fc receptor which is similar in structure to MHC class I proteins. Multiple functions have been shown for neonatal Fc receptor, as it mediates maternal transfer of IgG to offsprings, responsible for the maintenance of serum IgG and albumin levels and plays an important role in immune complex phagocytosis and antigen presentation. There is an increased interest in studying the effect of the therapeutic monoclonal antibodies that potentially cross the placenta and influence fetal ontogeny. Rabbits are potential candidates as "alternative" to the use of nonhuman primates as the maternofetal transfer in the last part of gestation is at a level similar in humans. Like primates, rabbit maternal IgG transport occurs during the fetal life and mediated by the FcRn through the yolk sac. Although, there were several studies in analyzing this IgG transport in rabbit yolk sac, the rabbit FcRn has not been cloned and its presence has not been analyzed in this tissue. In order to have a better understanding in this valuable model, first we cloned the rabbit FcRn using 5' and 3' RACE PCR and found that the coding region of the rabbit FcRn alpha-chain shows high similarity to the other mammalian FcRn heavy chains, so far analyzed, as it is composed of three extracellular domains, a transmembrane region and a cytoplasmic tail. We also characterized the rabbit FcRn alpha chain by detecting its expression in monocytes, macrophages, fetal placenta, yolk sac and amnion, though this last one presented less FcRn expression than the others, by RT-PCR. We detected this receptor in the endothelial cells of the placental capillaries as well as in the apical region of endoderm cells by immunohistochemistry. We also found that the rabbit FcRn, purified from the yolk sac, binds IgG in a pH dependent manner, like its other mammalian orthologues. These results confirm that the rabbit Fc receptor in the rabbit yolk sac is a bona fide FcRn receptor.

P5088 Genome-wide SNP association analysis for loci conferring Marek's disease

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Marek's disease (MD) is a T cell lymphoma of domestic chickens caused by Marek's disease virus (MDV), a highly oncogenic and contagious cell-associated alpha-herpesvirus. Since the 1970s, MD has been controlled by vaccination. However, due to continuous viral evolution with increasing virulence, and dominant susceptibility to MD in large numbers of commercial chicken flocks, MD remains as a highly potential threat to poultry industry worldwide. The host susceptibility and resistance to MD are controlled by genomic variation. QTLs conferring MD have been reportedly identified on GGA 1, 2, 4, 7, 8, 12, and 17. This study explored a panel of 57,636 SNPs to identify loci conferring MD in a F_2 population derived from two highly inbred experimental lines of chickens, highly resistant or susceptible to MD. A total of 2,610 SNPs were determined being informative among the F_2 chickens challenged with a very virulent plus (vv+) strain of MDV. We detected significant association between MD and 172 SNPs distributed on chromosomes 1, 3, 15, and Z by Marker-Trait Association analysis with a generalized linear mixed model using SAS Proc GLIMMIX procedure for binary trait analysis while taking into account of the pedigree structures (p values ranged from 0.049 to 0.000089). Whilst the results are in good agreement with reported QTLs on chromosome 1, the SNPs identified on chromosomes 3 (22 SNPs), 15 (20 SNPs), and Z (65 SNPs) present new possibility of loci or QTLs conferring MD and deserve further confirmation in other populations.

P5089 Study association between polymorphisms of intron 13 and 14 of ABCG2 gene and milk production traits

A QTL affecting milk production traits was previously mapped on chromosome 6 in dairy cattle. The most significant associations were found between the A/C polymorphism located in exon 14 of ABCG2 (ATP binding cassette subfamily G member 2) and milk production traits. The SNP (Single nucleotide Polymorphism) of A to C in exon 14 of bovine ABCG2, causing the substitution of tyrosine to serine at protein position 581 (Y581S) and increases milk yield and decreases fat and protein concentration. The aim of this research was to study polymorphism of partial sequences of intron 13 and intron 14 of ABCG2 gene in Iranian population of Holstein bulls and their association with milk production traits.

Genomic DNAs of 105 bulls were extracted from semen samples using high Pure PCR template preparation kit. Primers were designed with Oligo software and utilized in PCR, whereas the PCR fragments were then sequenced. Some SNPs detected for the first time in intron 13 and intron 14 as compared with NCBI sequence databases. Statistical analysis indicated association between some new mutations in intron 13 and 14 and Breeding Value of quantitative traits. mutations in bases numbers 4133 (T→C) and 4137 (T→G) of intron 13 had significant effect on Fat Percent ($P<0.05$) and mutations in bases numbers 2 (T→C) and 55 (G→C) of intron 14 resulted in significant effect on Fat and Fat Percent ($P<0.05$).

P5090 Structural analysis of the porcine antiviral Mx1 gene promoter region with potential functional importance

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The porcine Mx1 gene promoter was identified from PK15 cells and found to contain important regulatory sequences similar to other IFN and virus-inducible promoters. The polymorphic sites within the promoter sequence showed to increase the activities after IFN-∞ 2b treatment. Some genotypes turned out to be characteristic for Asian native pig breeds. In this study we have cloned and characterized the porcine Mx1 gene promoter from 36 individuals representing 6 European and a Thai native breed. DNA fragments containing the full-length Mx1 gene promoter region were screened *in silico* for polymorphisms. Twenty-seven nucleotide substitutions and 6 indels were detected. The most extended indels led to fragments consisting of 806, 821, and 848 bp. The fragments of 821 and 848 bp were specific for animals of the Thai native breed. The wild type fragment of 806 bp was taken to search for putative binding sites *in silico*. Bioinformatics helped to detect several binding sites, i.e. three Sp1 (A, B, and C), a NF-kB, and 2 AP-2 (B and C) sites. It became evident that the position of the putative transcription start site was identical in each of the three fragments. Three SNPs lie in Sp1 binding sites A and B and one SNP is specific for NF-kB. To address the potentially functional role of these polymorphic sites, their responsiveness to interferon (IFN∞/β) will be quantified using dual-luciferase reporter assays. The identification of different regulatory responses of variants could later be used to improve the innate resistance of pigs by genetic selection.

P5091 Predicting a 1.5Mb genomic interval for canine lupus erythematosus using GWAS

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Cutaneous exfoliative lupus erythematosus (LE) is an autosomal recessive disease unique to German short-haired pointer dogs, comparable to the chronic, inflammatory autoimmune LE in man, for which more than 20 genes have been implicated with disease risk to date. The large number of potential candidate loci combined with often weak association prevented in depth screening of the dog population thus far. We have applied a GWAS study utilizing indisputably diagnosed canine patient material to identify the genomic region responsible for the phenotype. A set of 13 affected and 21 non-affected individuals initially predicted a defined 1.5 Mb region on CFA18 when analyzed with a combination of association and autozygosity mapping. The results were confirmed by follow-up genotyping and resulted in a marker predicting disease. To gain insights into the minimal number of samples necessary for such studies, we randomly confined each subset, affected and normal, and reapplied the statistical analyses. While 8 individuals per group still suffice to suggest the correct genomic area with the combined approach, individual methods fail to locate the disease locus even with 10 and more samples. These results strongly emphasize the importance of sufficient sample size with adequate diagnosis, accurate prediction of the mode of inheritance, and combination of available statistics to minimize efforts related to disease mapping.

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P5092 Allelic variants of ovine prion protein gene (PRNP) in Brazilian local sheep breed Santa Ines

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There are few reports about PRPN polymorphism related to resistance/susceptibility to Scrapie in Brazilian local sheep Santa Ines, although its population is estimated in more than four million heads. A total of 467 blood samples of healthy sheep (both sexes and different ages) were genotyped for codons 136, 154 and 171 using a dual fluorescent multiprobe assay based on real time PCR. Individuals were subdivided in four groups: Dorper – DO (91), Suffolk – SU (53), Santa Ines – SI (169) and ½ Dorper ½ Santa Ines – DS (154). Wild-type ARQ/ARQ, commonly related with high risk to Scrapie infection w as the most frequent in all breeds, with prevalence of 50% in SI, 40% in DS, 38% in DO and 64% in SU. The resistant genotype ARR/ARR was detected in all breeds, with higher frequency in DO (11%) followed by 8% in SI, 5% in DS and 2% in SU. VRQ/VRQ genotype, considered the most susceptible, was absent in Suffolk and present in low er percentage in DO (1%), DS (1%) and SI (2%). Another important genotype related with high risk of developing this disease (A RQ/VRQ) w as found in DO (22%), DS (11%) and SI (6%). Considering the high frequency of the most susceptible genotypes in Santa Ines, and its relevance for the local sheep breed, the use of a fast and a cost-effective method for routine PRPN genotyping in sheep is a vital instrument to select resistant animals if considered the emergence of this disease in Brazil.

P5093 Association of canine Slc11a1 gene polymorphism and susceptibility to visceral leishmaniasis

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Visceral leishmaniasis is a serious worldwide public health problem. There is evidence that the Slc11a1 (Nramp1) gene may be associated with dog susceptibility to this zoonosis in dogs. The present study analyzed intron 1 microsatellite DNA regions as well as three SNP and the G-stretch in the promoter region of this gene by PCR and direct sequencing. To date, we have evaluated 168 samples from owned dogs, mostly mongrel, more than two years old and living in an endemic area for visceral leishmaniasis (Araçatuba, SP, Brazil). Positivity for the presence *of Leishmania* kDNA in blood samples was observed in 56.5% of the dogs. After direct sequencing, analysis of the promoter region showed that 55% of the samples had 8 Gs allele, 39% 9 Gs, and 6% 7 Gs, not statistically associated to *Leishmania* positivity. Allele frequency for intron 1 microsatellite 145 was of 40%, for 149 of 34% and for 141 of 26%; Chi-square test showed association of allele presence and PCR results for kDNA (p=0.003). All dogs were shown to be monomorphic for the SNP in the promoter region (TAG). Nineteen haplotypes were observed by the combination of the three marker classes (SNP, G-stretch and microsatellite). Although not statistically associated to negativity by Fisher´s test (p=0.2351) a higher percentage of negative dogs showed the haplotype TAG-8-141/141. More samples are under analysis in order to prove the hypothesis that allele 141 may be associated to resistance to visceral leishmaniasis.

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P5094 *Piscirickettsia salmonis* inhibits γIFN and γIP expression in *Salmo salar* susceptible families

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Piscirickettsia salmonis, the SRS causative, is an intracellular pathogen that seems to have evolved strategies to avoid eradication by macrophages, affecting several cultured marine fish species worldwide. Previous results have shown that during SRS outbreaks, *Piscirickettsia salmonis* can be detected *in vivo* inside head kidney and spleen macrophages, but it can also survive and replicate inside challenged fish macrophages maintained *in vitro*. This behaviour has been described for other intracellular pathogens, like mammalian rickettsiae and *Mycobacterium* species. Even though the mechanism that leads to the survival condition has not been completely elucidate, the inhibition of phagosome–lysosome fusion seems to be one of the main strategies. γIFN and the γIFN induced protein, γIP are needed to phagosome–lysosome fusion, and previous results demonstrated that in mammals during *Mycobacterium* infection both are inhibited. The aim of this study was to evaluate the expression level of γIFN and γIP in five *Salmo salar* families challenged with *Piscirickettsia salmonis* differing on their susceptibility to this intracellular pathogen. There were significant differences between both susceptible and resistant families for γIFN and γIP expression measured on head kidney. Resistant families showed an upregulation by 4 fold change on the log scale compared to the susceptible families. This suggests that one of the main organs related to immune response is inhibited in more susceptible families, suggesting a *Piscirickettsia salmonis* action mechanism.

P5095 Polymorphisms in *DMRT1* coding and promoter regions are not causative for swine SRY-negative XX sex reversal

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SRY-negative XX sex reversal is an inherited or sporadically occurring disorder, where testis development appears in the absence of *SRY*. Although the molecular background of this intersexuality syndrome in pigs is unknown, it was proposed that familial cases might be inherited as a single autosomal recessive trait. Because of the autosomal status of *DMRT1* (SSC1q21), its sexually dimorphic expression in swine gonads and its strong significance in vertebrate testis development, the molecular analysis of this gene was performed in previously reported 3 intersexes (38,XX, *SRY*-negative), the progeny of a single boar of a Polish commercial farm. The first two exons (coding functional DM domain) and the promoter region with 5'UTR were sequenced and compared with male and female control pigs and with the reference sequences available in Ensembl (ENSSSCG00000005237) Entrez (NM_214111, AF426435) and Sscrofa9 databases. Three different polymorphisms were found in the coding region, one indel type polymorphism (DNA 142_144indelAGC; protein S47_G48indelS) and two silent SNPs (DNA G432A and G492A). The promoter region seems to be highly polymorphic (nucleotide positions refer to AF426435): 14 SNPs (G359C, C390T, T501C, G649T, T743C, A951G, T953C, A1381T, A1383T, A1384T, A1390T, A1392T, T1887C, G2432A) and 4 indel type polymorphisms (738_739indelC, 1026_1027indelC, 1375_1377indelAT, 1666_1667indelT). However, sequences from healthy male and female controls were concordant with those for the intersex pigs. Moreover, it was noticed that Entrez sequence AF426435 differed significantly from controls and from the current draft genome sequence (Sscrofa9) database. In order to evaluate this phenomenon more animals need to be investigated.

P5096 Assessing the impact of feed efficiency and fertility in beef cattle

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Before commercializing animals on the basis of feed efficiency it is crucial to rule out negative effects with other important traits such as fertility. Some studies in swine feed efficiency have indicated a relationship between high efficiency and low fertility, but results are conflicting. In order to investigate if this relationship existed in a population of beef cattle selected for increased feed efficiency, we measured the number of progenies from 16 sires from both low (efficient) and high (inefficient) residual feed intake (RFI). Calves were born in spring 2008 from 9 low RFI sires and 7 high RFI sires. The average progeny number was 13.72 ± 13.29 progenies per sire in the high RFI (inefficient) sire class versus 16.00 ± 11.98 progenies per sire in the low RFI (efficient) sire class. Preliminary results show that there is no significant difference between the number of progenies sired by either efficient or inefficient sires ($P < 0.73$). Breeding soundness evaluation was also performed and there were no significant difference in sperm motility, morphology, concentration and disposition between low and high RFI sires.

P5097 Genetic control of ovine heritable arthrogryposis multiplex congenita (OHAMC)

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An outbreak of Ovine Heritable Arthrogryposis Multiplex Congenita (OHAMC) was recently described in a Salz breed situated in the region of Aragón (Spain). In the light of its similarity with the previously described Bovine Heritable Arthrogryposis Multiplex Congenita (BHAMC) in the Angus breed, an autosomal recessive inheritance pattern was proposed for this disease. As the flock had been submitted to selection for several generations to improve some productive traits, the owner preferred not to introduce rams from external origin. In the present report, we describe the design and use of a low cost approach based up on the use of a multiplex set of microsatellite markers (INRA0023, CSRD0247, MCM527, OarFCB0020, INRA005 and MAF0065) on both the existing and the replacement rams, in order to identify those of them siring affected lambs. Even if not all of the affected animals were referred to our laboratories, the number of cases has reduced from 12% to less than 1%. What is more, a 17% prolificacy increase has been detected, probably due to the descent in the intrauterine foetal deaths, which could also be related to OHAMC. The cost/benefits ratio of this technical approach can be considered to be excellent.

P5098 MHC II Risk Haplotype Associated with Canine Chronic Superficial Keratitis in German Shepherd Dogs

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Canine chronic superficial keratitis (CSK) is a progressive autoimmune ocular disease often leading to blindness if untreated. Characteristic for CSK is progressive, bilateral vascularisation, fibrous tissue formation and pigmentation of the anterior corneal stroma. Although CSK is found in many breeds it is most prevalent in German Shepherd Dogs (GSDs). Since the major Histocompatibility Complex (MHC) class II is associated with several autoimmune diseases in dogs we investigated the possible role of DLA-DRB1, -DQA1 and -DQB1-genes in GSDs affected with CSK. Our study population included 25 healthy controls and 30 CSK-diagnosed dogs. Most of the affected dogs were females suggesting a female predisposition. We identified eleven unevenly distributed haplotypes in the study cohort. One of the haplotypes, DLA-DRB1*01501/DQA1*00601/DQB1*00301 was significantly associated with the CSK dogs (OR=2.7, CI=1.17-6.44, p= 0.02). We found also that overall homozygosity of the MHC II locus increases risk for CSK (OR=4.37, CI=1.27-18.46, p=0.02) and homozygosity of the risk haplotype by 7-fold (OR=7.6, CI=1.21-202.58, p=0.03). This study identifies a MHC II risk haplotype for CSK in GSDs and further supports the autoimmune origin of the disease.

P5099 Quantification of scrapie risk in ovine milk: a new Real-Time PCR approach to improve the dairy product safety

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Scrapie is a fatal neurodegenerative brain disease affecting sheep and goat and is one of the known transmissible spongiform encephalopathies (TSEs). Genetic resistance to classical scrapie is associated with polymorphisms at three sites on the Prion Protein gene (PrP), at codon 136, 154 and 171. This implies the possibility to select animals with scrapie resistant genotypes for breeding. Scrapie has not been considered a zoonosis; anyway the risk to human health is posed by the possible presence of BSE in sheep. There is a risk of the transmission of scrapie from ewe to lamb via milk or colostrums, therefore the use of milk and milk products from a flock with classical scrapie may carry a TSE exposure risk for humans and animals (EFSA Journal, 2008).

This work describes a Real-Time PCR assay for codon 171 PrP allelic quantification by sampling the bulk milk. The quantitative PCR reactions were performed by ABI Prism 7900 Sequence Detection System and TaqMan® 3'minor groove binding (MGB) probes. The analytical performance of the Real-Time PCR typing protocol for PrP codon 171 was evaluated in terms of precision, accuracy, LODs and LOQs.

The proposed assay allows to quantify the scrapie risk in ovine bulk milk and represents an accurate and high-throughput method for the routine quality control of milk and dairy products. The dairy industry represents a strategic area within the agricultural and food economy; the possibility to increase consumers' security in relation to the assumption of scrapie-free dairy products will represent a competitive advantage on the market.

P5100 Genetic analysis of a glomerulonephropathy segregating in a pedigree of French Mastiff.

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A juvenile glomerulonephropathy (JGN) has been reported among a pedigree of French Mastiff. Affected dogs develop symptoms usually during the first year of life with polyuria, polydipsia and signs of azotemia. The urinary protein/creatinine ratio is increased in all dogs. At the histopathology level, cystic glomerular atrophy with hypercellularity, capillary wall thickening and expansion of the mesangial matrix are observed. Under electron microscopy, the basal glomerular membrane is focally wrinkled with sparse small membranous electron-dense inclusions. Sixteen affected dogs are derived from eight different litters. All affected dogs are consanguineous as common ancestors were found in maternal and paternal lineages. Diseased dogs are born from clinically healthy parents, and there is no gender predisposition. The proportion of affected dogs within a litter ranged from 10 to 43 percent suggesting an autosomal recessive mode of transmission. A genome wide association (GWA) study using the 50K canine SNPs Affymetrix array on 30 healthy and 10 affected dogs did not reveal any region significantly associated with the disease. However all 10 affected dogs were found to share a haplotype block of 5Mb with 5 of them being homozygous, suggesting potential allelic heterogeneity at the putative disease locus. A new genome wide scan with data coming from the 170K Illumina array did not point out another linked region nor could reduce the length of the shared haplotype. Candidate genes lying within the interval are currently under investigations together with expression studies in kidney tissues from affected dogs versus healthy ones.

POSTERS 6001 – 6007

Genetics and the Next Green Revolution

P6001 Expression of recombinant human anti-HBV antibody in milk of transgenic mice

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Hepatitis B is one of the critical public health problems globally caused by hepatitis B virus (HBV), which could lead to cirrhosis, liver failure even death. Passive immunization of HBV plays an important role in post-exposure prophylaxis with clinical applications often requiring large amounts of antibody. As an alternative to the *in vitro* production of recombinant proteins, expression of monoclonal antibodies (mAbs) in the milk of transgenic animals is currently used being associated with low production costs and high activity. In this study, eight founder lines of transgenic mice were generated by co-microinjection of the two cassettes encoding the heavy- and light-chains of a neutralizing anti-HBV antibody, respectively. The expressed heavy- and light-chains of the mAb were correctly assembled and modified in the mammary gland as detected by western blotting. Expression levels of the antibody were detected by ELISA and the highest level was up to 9.1 mg/ml. No direct relationship between the transgene copy number and the amount of mAb was observed, implying that the integration site of the transgene had a greater effect on the expression level. The binding specificity of anti-HBV mAb to HBsAg was assayed by IRMA, showing that the samples had high activity with the highest level up to 2800 mIU/ml. The neutralization test of anti-HBV mAb is yet to be conducted. This work suggests that a large-scale and efficient production of the anti-HBV mAb in the milk of transgenic farm animals would be feasible in the future.

P6002 Association of markers on bovine chromosome 5 with birth and weaning weight in Hereford cattle raized under extensive conditions

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Genetic markers have been used to assess the association of economically important traits with cattle under intensive feeding conditions; however, there is still the need to ascertain the usefulness of these markers under extensive production systems. Bovine chromosome 5 has been widely studied because several QTL have been detected. Microsatellites neighboring the Myogenic factor 5 (Myf5) gene, BP1; and Insulin-like Growth Factor 1 (IGF1) gene, ETH10, IGF1 and RM029, were selected to establish their association with BLUPs (Best Linear Unbiased Predictors) for direct Birth Weight (dBW), direct Weaning Weight (dWW) and maternal Weaning Weight (mWW). Two unrelated herds were used for this objective, one commercial and the other experimental. Significant associations ($P \leq 0.05$) between dWW and all BTA5 loci (BP1, ETH10, IGF1, and RM029) were detected. Additional associations were observed between mWW and BP1. dBW was significant associated ($P \leq 0.05$) with ETH10 genotypes and with the interaction IGF1*Herd. In particular, the region near BP1 marker could be contributing to the rare positive correlation between dWW and mWW previously found in the INTA Balcarce Station experimental herd. We confirmed marker association with growth traits in two BTA5 regions close to previously reported QTL obtained in intensive feeding conditions; these regions are affecting dBW, dWW and mWW also in a pasture based system.

P6003 Comparison between bivariate and multivariate joint analysis on the selection loss for growth traits in beef cattle

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Selection of 431,224 animals based on growth traits (birth weight (BW), weaning weight (WW), post-weaning gain (PWG) and muscle score (MUSC)) was investigated in a Nellore beef cattle population comparing bivariate and multivariate joint analysis. The model of multivariate joint analysis was assumed to be the correct one for selecting the best animals and maximize the response to selection, since it is impossible to select for just two traits separated without affecting other important traits. The objective of this study was to estimate the possible selection loss when the selection decision was based on the bivariate model. Different selection intensities were applied (top 10, 1%, 10% and 30%) and the selection loss was estimated as the percentage of decreasing on EPD results comparing the bivariate method to the multivariate. The comparison resulted in selection loss for all traits studied for the four different selection intensities and the results showed that the selection loss partly depended on the selection intensity, whereas a tendency of a decrease in the percentage of loss occurs as the selection intensity increases. The percentages of loss for the different selection intensities ranged between 9.09 and 2.27 for BW, 8.43 and 2.43 for WW, 2.16 to 1.36 for PWG and 6.25 to 2.44 for MUSC. The importance of using more complex algorithms that approximate the estimated value calculation to the real value should not be ignored, considering that the computational advances allow in short amount of time that multivariate solutions can be provide with more accurate results.

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P6004 Calcium Carbonate crystal size is highly heritable and positively correlated with egg shell quality in laying hens

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The shell of the hen's egg consists of columnar calcite crystals and a small amount of organic matrix that influences the growth and size of the crystals. Crystal size determines a ceramic's properties including strength. This study was undertaken to determine the role of genetics in determining crystal size and how this was correlated with measurements of eggshell quality.

Crystal size was measured on shell samples using a collimated X-ray beam using the total average intensity of the diffracted X-rays spots. Eggs from 880 Rhode Island Red hens from 32 sire families were used in this study, two eggs from each hen were analysed. Heritabilities were estimated using a model including the fixed effect of hatch date and the random effects of sires and dams within sires. Parameters were estimated by REML. Genetic correlations were estimated from a bivariate mixed model.

Heritability estimates for crystal size were high (0.61±0.18). Crystal size was genetically correlated with thickness of the palisade layer (0.51±0.20) and breaking strength (0.45±0.25). However this positive correlation is contrary to the view that smaller crystals give superior mechanical properties. This suggests the ability to grow larger crystals is important in the elaboration of a stronger shell in nature and increases our understanding of the fundamental material properties of the eggshell. The measurement shows considerable promise for use in genetic selection to improve egg safety and quality.

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P6005 Comparison of genomic evaluation methods for estimating breeding values and mapping QTLs using heterogeneous mouse data

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The objective of this study was to examine the performance of genomic evaluation methods to estimate total breeding value and map QTLs. Five Bayesian methods were used to estimate SNP effects: BayesA, BayesB, empirical best linear unbiased prediction, Bayesian least absolute shrinkage and selection operator (LASSO) and a mixture model approach (MIXTURE). Traits studied were weight at 6 weeks (WT, n=1,924), body length (BL, n=1,839), serum alkaline phosphatase activity (ALP, n=1,536), and serum high density lipoprotein (HDL, n=1,454), for which we used data that were collected at the Wellcome Trust Centre for Human Genetics, Oxford University. For the latter two traits, it has been reported that functional causative genes were located on the identified chromosome regions. A total of 10,134 SNPs were used in the analyses. Cross-validation was conducted to assess predictive ability of the methods. For WT and BL, locations of SNPs with relatively large effect detected by BayesB were similar to those reported in a previous study. For these traits, LASSO yielded slightly higher predictive ability than the other methods, but the differences among the methods were small. For ALP and HDL, SNP effects estimated with MIXTURE were comparable to those with BayesB, and the positions of major QTLs suggested by the estimates were almost the same as those of functional causative genes reported. The predictive ability in MIXTURE for these traits was equivalent to that of BayesB. The MIXTURE, that requires relatively minimal a *priori* information, would be one of the better methods to use.

P6006 Microsattelite and SNP analysis for parentage verification using bovine nasal samples with Performagene™•LIVESTOCK

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Parentage analysis of livestock animals is becoming increasingly utilized in the agriculture industry to improve breed characteristics and herd efficiency. To date, a significant portion of parentage analysis is done using microsattelites, however, parentage can also be determined using SNP genotyping. With the increasing usage of genomics for identifying production traits and parentage, the demand for efficient and reliable solutions for sample collection and processing is increasing. Performagene•LIVESTOCK is an all-in-one DNA sample collection, stabilization and extraction kit that enables more efficient parentage analysis by streamlining sample collection and processing. Using the Performagene•LIVESTOCK DNA collection kit developed by DNA GENOTEK, Inc., we demonstrate the reliable collection and processing of high yield, high quality genomic DNA from bovine nasal samples. The samples were collected from the nostrils of purebred Holstein cattle during routine parentage analysis and purified using the Performagene•LIVESTOCK protocol. Microsattelite and SNP analysis of the genomic DNA obtained shows the suitability and reliability of the sample method for use in parentage analysis.

6007 Adult cattle clones as a tools to identify specific features of the epigenome for improved genetic selection and sustainable animal breeding.

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Epigenetic mechanisms are now considered as potential heritable sources of phenotypic variation in addition to DNA sequence polymorphism. To address the relationship between epigenetic and phenotypic variations in cattle we use sets of adult healthy animals obtained from the same genome either by embryo splitting (monozygotic twins, n = 24) or by nuclear transfer (NT clones, n = 38). We first check that these animals were genetically highly similar if not identical using RDA, several microsatellite, and SNP array (54K) and CNV analyses. By quantifying individual levels of global DNA methylation, we provided evidence that animals issued from the same genome exhibited a large variability in the extent of their methylated cytosine content (de Montera et al., Cellular Reprogramming, 2010). This provides a first indication that the same initial cattle genome can tolerate a wide range of epigenetic variability. By using specific gene and pyrosequencing analysis we have started to identify specific features of the epigenome that could provide a molecular signature of genome flexibility and robustness for animals obtained by conventional breeding. These signatures could become new co-variables in a molecular genetic testing program for improved selection and sustainable animal breeding.

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