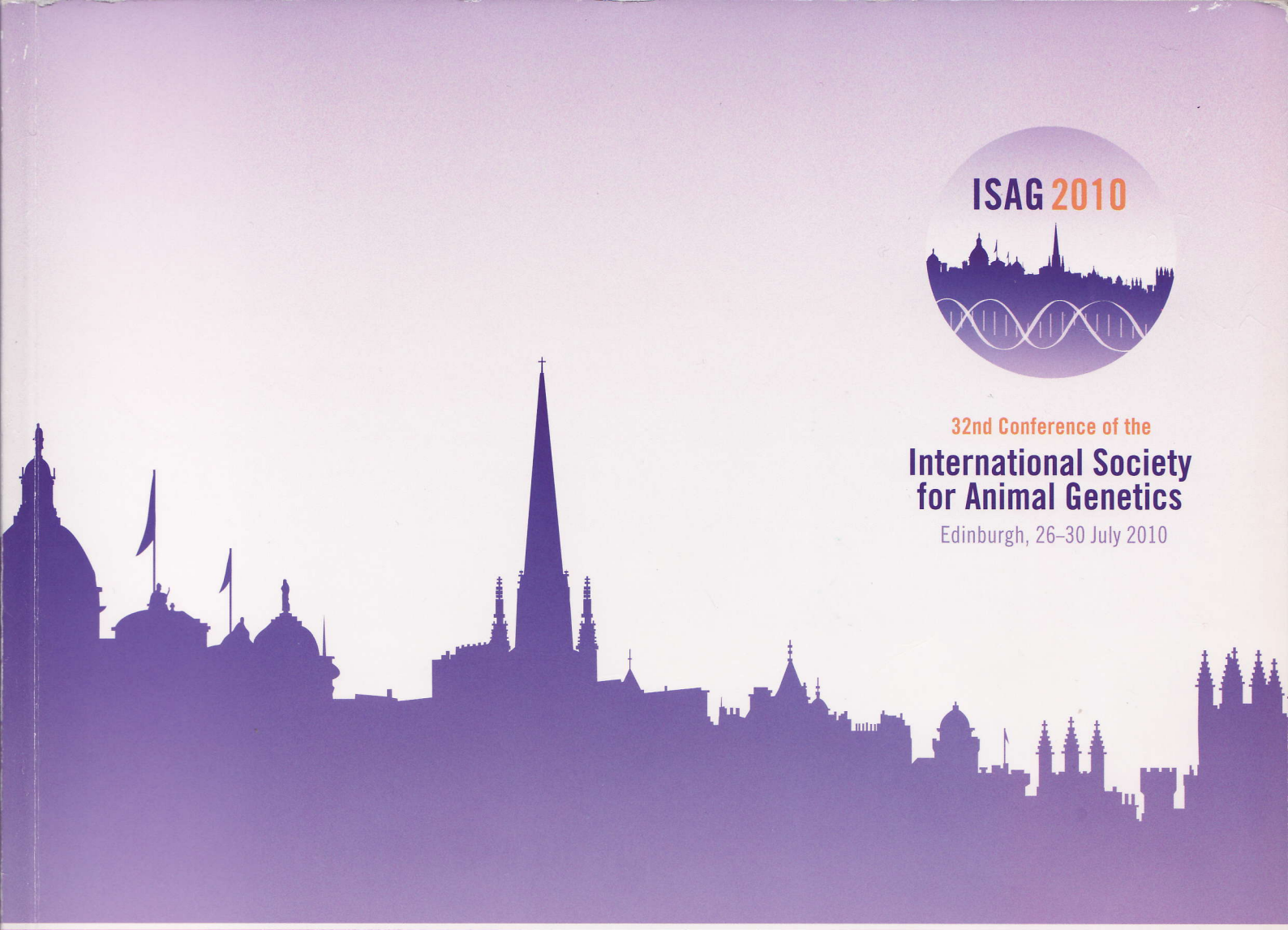

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P1001 Evolution and adaptive radiation of Humboldt penguin genus (*Spheniscidae*) using *Mhc* class II *DRB*-like gene region

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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.

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.

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.