How to Search

D001

Polymorphous systems of blood of polecats, minks and sables

L.G. MARKOVICH[1], E.A. TINAEVA[1], A.M. MASHUROV[2], V.N. ORLOV[2], H.H.TKHAN[2] & N.I. KULIKOVA[1]

[1]V. Afanasiev Research Institute of Fur Bearing Animals and Rabbits, Rodniki, Moscow region, Russia; [2]Institute of Evolution Morphoiogy and Animal Ecology of the Russian Academy of Sciences, Moscow, Russia

Biochemical polymorphism of polecats of 3 color groups (n=366) was studied for the first time. Results were compared with minks (n=1212) and sables (n=806). IN polecats genetical polymorphism was discovered in systems of albumin, postalbumin, transferrin, posttransferrrin of serum and hemoglobin of red corpuscles. Methods of horizontal electrophoreses were used for identification of genotypes. We made a hybridological analysis and established codominanteous character of inheritance of all system alleles in polecats. Allele B predominated in albumin locus in golden and pastel polecats (0.4042 and 0.3980, respectively).

Albumin A predominated in mother-of-pearl polecats (0.4055). IN posttransferrin locus allele C (0.5052), allele C (0.5510) and allele A (0.4330) predominated in golden, pastel and mother-of-pearl polecats, respectively. High concentration of allele A was character for postalbumin locus and allele 4 (D) prevailed in hemoglobin locus of all types of polecats.

In minks there was the highest frequency of albumin B (0.6351), hemoglobin 3 (C) allele was absent. Sables were only notable for high frequency of posttransferrin B (0.6723). Average level of real homozygosity was within 39.1-43.2% in polecats. Hybridological analysis showed a high level of accordance of parent and progeny genotypes. Thus in polecats as well as in minks and sables there is a possibility to fulfill genetical control of animal's origin and directional selection of certain genotypes.

D002

Biochemical polymorphism of caged marmots (Marmota bobac)

E.A. TINAEVA[1], L.G. MARKOVICH[1], A.M. MASHUROV[2], N.S. ARTYUHOVA[1] & V.N. ORLOV[2]

[1]V. Afanasiev Research Institute of Fur Bearing Animals and Rabbits, Rodniki, Moscow region, Russia; [2]Institute of Evolution Morphoiogy and Animal Ecology of the Russian Academy of Sciences, Moscow, Russia

Adaptation of marmots began in 1989 from snaring 1500 animals in Rostov region and their further reproduction in state pedigree farm "Pushkinskiy", situated in Moscow region. For the first time biochemical polymorphism was studied on the second year marmot (n=153) by horizontal electrophoresis methods. Blood was taken from heart during slaughter. Two allele genes were detected in albumin locus, three allele genes were found out in postalbumin, transferrin and posttransferrin loci, and four - locus of hemoglobin of red corpuscles. 15 allele variants were determined altogether. In all systems level of homozygosity was within 50, 4-54,5%, that corresponded to 44, 9-45,1 theoretically expected percentage (x2>0,05). Body length of the marmots wasn't connected with definite alleles and genotypes of studied polymorphous systems. However connection between posttransferrin A allele and live weight of females was established. Stabley for 3 year posttransferrin A allele had been defining in marmot females which had higher weight in comparison with those which had alternative allele. Degree realization of possible variability in five loci was within 49,9-64,7 that testified to considerable genetical reserves of marmots of this population.

D003

Combined estimation of QTL effects using a mixed model in an inbred pig family*+

X.L. WU[1,2], C.B. JORGENSEN[2], K. CHRISTENSEN[2], J.N. JORGENSEN[2] & M. FREDHOLM[2]

[1]Lab of Molecular Biology, Hunan Institute of Animal and Veterinary Science, Changsha, P.R. China and [2]Department of Animal Science and Health, The Royal Veterinary & Agricultural University, Copenhagen, Denmark

An inbred pig family was started from a father-daughter backcrossing; inbreeding was continued, chiefly by performing sib mating, for five successive generations. This pig family provided an informative reference for QTL analysis and mapping, as well as for investigation of inbreeding and inbreeding depression. The use of this pig family in QTL analysis is limited however, by the small number of backcrosses, which consists of 39 pigs, of which only 30 have complete records of performance. Combined estimation of QTL effects is therefore suggested for QTL analysis in this family, with a view to increasing the number of animals and consequently increasing the power of detecting QTLs. The basic idea is to combine estimates of QTL effects from the backcrosses and the F_t generation using a mixed model by giving them different weights. The expectation of additive effect (a) in the combined estimation equals $a+w_1d$, which is biased by w_1d. The bias, however, is much smaller than that in backcross estimation if considering w_1 <1. The expectation of dominance effect in the combined estimation is unbiased. Single-locus analysis is utilized to illustrate the theory, using results of microsatellite genomic scanning and individual records of performance in the BC and F_3 generations of the CUP family. Analysis of variance has revealed the existence of a significant QTL on swine chromosome 17, which positively contributes to slaughter weight. Combined estimation shows that the additive effect of the QTL is 1.786 .733kg ($p<0.05$), and the dominance effect is 1.364 .194kg ($p>0.05$). Variance contribution of this QTL is estimated to be 9.60%. A comparison of different estimations has also been conducted. The estimate of additive effect in the backcrosses is the largest but not significant ($p>0.05$), and that of the F3 is the smallest ($p>0.05$). The combined estimates fall in between the BC estimate and F3 estimate, and are significantly greater than zero ($P<0.05$). The standard error for the combined estimation is the smallest, which is a sign of the improved power of detecting a QTL. Dominance effect of the QTL in the combined estimation is exactly the same as that in the F3 estimation.

* Thanks to Sino-Denmark Scientific Collaboration Committee (CDSCC), the Nature Science Foundation of China (NSFC), and Lab of DNA, KVL, Denmark for the financial support of this research

* This research was supported by the Nature Science Foundation of China (NSFC) and Danish Government Scholarship.

+ Correspondence author: nickwu@public.cs.hn.cn

D004

Association of crossbreeding parameters for growth and meat-production traits with average band-sharing of RAPD markers in commercial crosses of Duroc, Landrace and Large White Pigs*+

Q.S. SHI[1,2], X.L. WU[1,2], X.C. LIU[2], AND R.Z. LIU[2]

[1]Lab of Molecular Biology, Hunan Institute of Animal and Veterinary Science, Changsha, P.R. China, [2]College of Animal Science and Technology, Hunan Agricultural University, Changsha, P.R. China

By using individual records of growth, meat production, and genotypes of RAPD analysis in commercial crosses of Duroc, Landrace and Large White pigs from 1996 through 1998, crossbreeding parameters have been estimated for: average daily gain (ADG); dressing percentage (DRP); backfat thickness (BFT); percent of hind legs (HLP); and average band-sharing of RAPD markers. The results indicate that individual and maternal genetic effects are significantly greater than zero for all four traits of growth and meat-production ($p<0.05$). Effects of individual heterosis and maternal heterosis have been found to be significant for ADG ($p<0.05$), but not significant for DRP, BFT, and HLP ($p>0.05$). Positive maternal genetic effect and negative effects of individual heterosis and maternal heterosis have been revealed for ABS of RAPD markers. Correlation analysis shows that the total correlation between RAPD-marker ABS and traits of economical importance, when including all parameters at a time, is small (0.2735~0.4414) and not significant ($p>0.05$). The partial correlation, however, is greatly improved (-0.8240~0.8919) when considering one parameter at a time, or two in the case of heterosis. Partial correlations for maternal genetic effect and combined effects of individual and maternal heterosis between ADG and RAPD-marker ABS are significant ($p<0.05$), and the correlation for individual genetic effects between them is very significant ($p<0.01$). It is therefore concluded that the efficiency of predicting heterosis is low by simply applying total correlation. Alternatively, the efficiency will be much improved by investigating correlation of individual parameters between markers and traits of economic importance, instead of using all parameters. Increasing QTL coverage (percent of markers linked to QTL) is also expected to significantly contribute to increased accuracy of predicting heterosis. Correlation of additive effect between ABS of RAPD markers with ADG, DRP, and HLP is positive, but that of individual and maternal heterosis between them is negative. This result suggests that, while genomic similarity of crosses increases, individual genetic effect will increase (or decrease in certain cases), but the effects of individual heterosis and maternal heterosis will decrease.

* This research is sponsored by Nature Science Foundation of China (39600105) and Nature Science Foundation of Hunan Province(97JJY2005). Corresponding author: Dr. Wu Xiao Lin, email: nickwu@public.cs.hn.cn

D005

Microsatellite and blood protein diversity in the sheep breeds of Finland and North-Western Russia

M. TAPIO[1], I. MICEIKIENÉ[2] & J. KANTANEN[1]

[1]Agricultural Research Centre of Finland MTT, Jokioinen, Finland and [2]Lithuanian Veterinary Academy, Kaunas, Lithuania

Genetic diversity at 15 microsatellites and 7 blood proteins were examined in 9 sheep breeds or local populations, e.g. different Finnsheep colourtypes, two distinct Romanov populations and autochthonous populations from Russian Karelia. In addition, a Finnish Oxford Down population was used as a reference group. Within-population expected mean heterozygosities varied from 0.64 to 0.74 for microsatellites, and from 0.15 to 0.35 for proteins, respectively, with the Oxford Down being the least variable. No statistically significant differences in heterozygosity between the breeds were found, but this could be due to the limited number of loci analysed. It was estimated that a minimum of 20 to 35 polymorphic loci were needed to detect a true difference of 0.10 in heterozygosity between two populations by a paired t-test (α=0.05). However, an allelic deficiency was observed, e.g. in Oxford Down and the brown colourtype of Finnsheep, suggesting a recent decrease in their effective population sizes. Finite population size may also explain linkage disequilibrium between physically unlinked loci in some of the studied breeds. Statistically significant population differentiation was observed. θ values based on microsatellite data and protein data were 0.080±0.007 and 0.149±0.057, respectively. Respective Nei's *DA* distances varied between 0.051–0.351 and 0.018–0.156. Distances were used to construct a neighbour-joining tree illustrating the grouping of these breeds.

D006

Marker-assisted introgression experiment in mice

O. D. KOUDANDÉ[1], J.A.M. VAN ARENDONK[1] & F. IRAQI[2]

[1]Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands; and [2]International Livestock Research Institute, Nairobi, Kenya

African trypanosomiasis is an endemic disease affecting sixty million cattle in 37 African countries, with annual direct and indirect losses estimated at more than US$5 billion. Most West African *Bos taurus* breeds are genetically resistant to trypanosomiasis and chromosomal localization of cattle trypanotolerance quantitative trait loci (QTL) is underway. In an earlier experiment, using F_2 and F_6 crosses of susceptible (A/J) and resistant (C57BL/6) mouse strains, 3 trypanotolerance QTL were mapped to chromosomes 1, 5 and 17. A marker-assisted introgression experiment involving the two inbred lines has been set up to study the effectiveness of an introgression program. The experiment started in 1997 with reciprocal crossing of the two parental lines producing an F_1, followed by four generations of backcrossing to the susceptible strain, an intercrossing phase and a multiplication phase. During backcross and intercross, animals were selected based on markers flanking the identified QTL (5, 18 and 7 cM on chromosomes 1, 5 and 17, respectively). Ten groups of animals carrying different combinations of marker-genotypes were produced. These groups and the two parental lines were challenged with *Trypanosoma congolense* and trypanotolerance was measured as the survival time following infection over a period of 22 weeks. Difference in survival time between the two parental lines was 58 days.

D007

Towards a strategy for the conservation of the genetic diversity of European cattle

J.A. LENSTRA[1] , P. AJMONE-MARSAN[2], D.G. BRADLEY[3], B. DANELL[4], G. DOLF[5], S. DUNNER[6], G. ERHARDT[7], E. EYTHORSDOTTIR[8], HIENDLEDER[6], L.K HOLM[9], O. JANN[7], J. KANTANEN[10], G. MOMMENS[11], K. MOAZAMI-GOUDARZI[12], D. LALOË[12], I. MEDUGORAC[13], R. NEGRINI[2], I.J. NIJMAN[1], I. OLSAKER[14], C. RODELLAR[15], A. SANCHEZ[16], D. SMITH[17], A. VALENTINI[18], P. WIENER[17], J.L. WILLIAMS[17], M. ZANOTTI[19] & P. ZARAGOZA[15]

[1] Fac. Vet. Med., Utrecht, The Netherlands; [2] Univ. Catt., Piacenza, Italy; [3] Trinity College, Dublin, Ireland; [4] Dept. of Anim. Breeding Genet., Uppsala, Sweden; [5] Univ. Berne, Berne, Switzerland; [6] Fac. Vet., Madrid, Spain; [7] Justus-Liebig-Univ., Giessen, Germany; [8] Agric. Res. Inst., Reykjavik, Iceland; [9] Danish Inst. Agric. Sci. Tjele Denmark; [10] Agric. Res. Centre, Jokioinen, Finland; [11] PolyGenLab., Malle, Belgium; [12] Inst. Nat. de Recherches Agron., Jouy-en-Josas, France; [13] Ludwig Maximilian Univ., Munich, Germany; [14] Norwegian School Vet. Sci., Oslo, Norway; [15] Fac. Vet., Zaragoza, Spain; [16] Fac. Vet., Bellaterra, Barcelona, Spain; [17] Roslin Inst., Roslin, Scotland; [18] Univ. Tuscia, Viterbo, Italy; and [16] Univ. Milano, Milano, Italy.

There are growing concerns that the almost exclusive use of highly productive cattle breeds, which are the result of a systematic and intense selection, will lead to a reduction of genetic diversity and the disappearance of valuable phenotypes such as local adaptation and disease resistance. The project *'Towards a strategy for the conservation of the genetic diversity of European cattle'* from the European Community coordinates the efforts of several laboratories and has the following targets:

1. Microsatellite genotyping of 50 animals from 50 breeds with standardized set of 30 markers. This will combine and complement previous national and regional studies, which were done with different sets of markers.
2. AFLP genotyping of 20 animals from 50 breeds with 100 biallelic markers.
3. Analysis of the effect of selection by studying the diversity in loci coding for milk proteins, the myostatin locus and regions known to contain a QTL.
4. Formulation of recommendations for conservation It is expected that the RESGEN project will generate a unique and valuable set of data, which represents most of the genetic diversity of European cattle. This will lead to fundamental new insights in the relation between selective breeding and genetic diversity as revealed by different categories of molecular markers.

D008

Polymorphism of *Calpain* locus and relationship with meat tenderness in Piedmont cattle breed

L. DI STASIO, S. SARTORE, G. DESTEFANIS & A. BRUGIAPAGLIA

Department of Animal Science, University of Turin, Italy

Calpain-mediated degradation of myofibrillar proteins is known to be responsible for post-mortem meat tenderization. Polymorphism at calpain II regulatory subunit gene (*CAPN*) has been detected in several cattle breeds. Therefore the present investigation was carried out to study the variability of *CAPN* locus in the Piedmont breed and to determine whether the polymorphism is related to beef tenderness. Seventy-five unrelated subjects were analyzed for *CAPN* genotype by PCR-RFLP of genomic DNA from blood, and for meat tenderness by Warner-Bratzler shear force (WBs) on LTL at 1, 3, 7 and 11d post-mortem. GLM procedure was used for statistical analysis. The digestion of PCR products with *HhaI* revealed two alleles: $CAPN^{A}$ (900, 620 and 280 bp) and $CAPN^{B}$ (1520 and 280 bp), with frequencies of 0.24 and 0.76 respectively. No significant associations were observed between *CAPN* genotype and Wbs:

	WBs1	WBs3	WBs7	WBs11
AA	12.20	7.99	6.82	6.27
AB	10.70	6.57	5.79	5.49
BB	9.54	7.28	6.62	5.50

The results indicate that this polymorphic site at *CAPN* locus cannot be used to predict beef tenderness.

D009

Polymorphism in the Upstream Regulatory Regions of *BoLA-DRB* genes.

RIPOLI[1] M. V., S. DIAZ[2], F. N. DULOUT, P. PERAL-GARCIA & G. GIOVAMBATTISTA.
CIGEBA, Fac. Cs. Vet. UNLP. 60 y 118 CC 296, CP B1900AVW, La Plata, Buenos Aires, Argentina.

The *MHC-DRB* are highly polymorphic genes. These polymorphims were mainly located in the peptide-binding site (*PBS*). In human and mouse, variant sites were also reported in the upstream regulatory regions (*URR*). In bovine, the polymorphism of the second exon of the class II genes were extensively studied. However, to our knowledge, *URR* have not been studied yet. Consequently, the aim of this study was to analyze the *BoLA-DRB URR* polymorphism using single-strand conformation polymorphism (*PCR-SSCP*) technique. Genomic DNA from 37 Argentine Creole cattle of one paternal half-sib family was amplified for *URR* of the *DRB* genes by *PCR*. The oligonucleotide reported by Turco *et al.*, 1990 Immunog 32, 117-28 were used as forward primer, while the reverse primer were deduced from *BoLA-DRB3*, *HLA-DRB*, *SLA-DRB*, *H2-Mb2* and *H2-IAb* published sequences (from position -2 to 20). These oligonucleotides amplified a single product of the expected size (~ 220 bp) corresponding to previously reported data. Amplification products of the putative *URR* reveals four distinct patterns using *SSCP* method. In order to identify allelic variants, the PCR product from the sire was cloned. All bands detected in the sire could be explained by the observed patterns in the clones. These *SSCP* patterns appear to be inherited in a simple Mendelian fashion in the studied family. Additional studies, like DNA sequencing of the *PCR* product, are required to confirm the identity of the sequences and their implications with regulation of the *DRB* genes expression.

[1] Fellow of Universidad Nacional de La Plata (UNLP). [2] Fellow of Comisión Nacional de Investigaciones Científicas y Técnicas (CONICET)

D010

Preliminary *PCR-RFLP* analysis of *DQB* exon 2 polymorphism in Argentine Creole horses.

DIAZ[1], S. , E. E. VILLEGAS-CASTAGNASSO[2], G. GIOVAMBATTISTA, F. N. DULOUT & P. PERAL-GARCIA.

CIGEBA, Fac. Cs. Vet. UNLP. 60 y 118 CC 296, CP B1900AVW, La Plata, Buenos Aires, Argentina.

The *DQB* gene of the Equine Leukocyte Antigen (*ELA*) seems to be encoded by a single locus. Up to date, the complete coding sequence of *ELA-DQB* gene is known and thirteen *DQB* exon 2 sequences were reported in domestic horses (*Equus caballus*). Genotyping systems have been defined for *MHC* class II *DQ* molecules for a number of species, including cattle and humans. However, there is no widely used system for typing *DQB* gene in horses. The aim of this study was to analyze the *DQB* exon 2 polymorphism using the *PCR-RFLP* technique. Genomic DNA from 22 Argentine Creole horses of two paternal half-sib families was amplified using the oligonucleotide primers *GH28* and *GH29* (Gyllensten *et al.*,1990 Proc. Natl. Acad. Sci. USA 87,1835-9). The identity of the PCR products was confirmed by digestion with *Taq* I restriction enzyme. PCR products were digested with either *Eco* RI, *Hae* III, *Rsa* I, *Hinf* I and *Msp* I. Restriction fragments were separated by electrophoresis in 8% (19:1) acrylamide-bis acrylamide gels. In the sample studied, *Eco* RI digestion profiles were monomorphic, while the remaining enzymes showed from two to three distinct restriction patterns. The restriction sites present in the thirteen reported *DQB* sequences could only explain five out of ten observed restriction patterns. The remaining patterns would represent additional alleles or the result of coamplification of another class II gene. Further studies have to be done in order to improve the specificity of *DQB* primers to use *PCR-RFLP* as a typing method for *ELA-DQB*.

[1] Fellow of Comisión Nacional de Investigaciones Científicas y Técnicas (CONICET), [2] Fellow of Universidad Nacional de La Plata (UNLP).

D011

cDNA cloning of a swine major histocompatibility complex (SLA) class II DMA gene and genetic polymorphisms in the SLA-DMA, -DRB, and -DQB genes

A. ANDO[1], H. KAWATA[1], T. MURAKAMI[1], A. SHIGENARI[1], T. SHIINA[1], M. SADA[2], T. TSUJI[2], A. TORIU[3], Y. NAKANISHI[4], T. MITSUHASHI[5], K. SEKIKAWA[6] & H. INOKO[1]

[1]Dept. of Mol. Life Sci., Tokai Univ. School of Medicine, Isehara, Kanagawa, Japan; [2]Nat. Medical Cardiovascular Center, Suita, Osaka, Japan; [3]Nat. Livestock Industry Breeding Center, MAFF, Makabe-gun, Ibaraki, Japan; [4]Kagoshima University Lab. of Animal Reproduction, Kagoshima, Japan; [5]Nat. Inst. of Animal Husbandry, MAFF, Inashiki-gun, Ibaraki, Japan; and [6]Nat. Inst. of Animal Health, MAFF, Tsukuba, Ibaraki, Japan

In order to isolate cDNA clones from the swine histocompatibility complex (SLA) class II DMA gene, a Göttingen miniature swine spleen cDNA library was screened using the PCR products from the third exon in the human HLA-DMA gene. Seven cDNA clones were isolated, and the longest of these clones encodes a primary translated product of 260 amino acids. Amino acid comparative analysis and construction of a phylogenetic tree revealed that this clone was more closely related to the human and bovine DM α genes than to the genes for the conventional swine class II α chains, DRA and DQA. These results suggest that the SLA-DMA gene is expressed and may function, like HLA-DM, as an important modulator in class II restricted antigen processing in the SLA. Furthermore, we have analyzed genetic polymorphisms and allelic variations of the SLA-DMA, -DRB, and -DQB genes in five different breeds of swine. Based on sequences and PCR-RFLP patterns in the SLA-DMA gene, no allelic variation was recognized in the second exon, and five allelic variations were recognized in the third exon. These DMA alleles are defined by variation at four nucleotide positions. Two alleles among them result in an amino acid substitution. These results suggest that SLA-DMA has little polymorphism as observed in HLA-DMA and mouse H2-DMA. Sequence analysis of the second exon of the SLA-DRB genes after PCR amplification has allowed the identification of three and two novel alleles in the SLA-DRB1 and -DRB2 genes, respectively. In addition, two novel alleles were also identified from the sequence analysis of the second exon of the SLA-DQB gene.

D012

Development of a microsatellite parentage and identity verification test for dromedary racing camels

J. SASSE, M. MARIASEGARAM, M.K. JAHABAR ALI, S. PULLENAYEGUM, R. BABU, J. KINNE & U. WERNERY

Central Veterinary Research Laboratory, Dubai, United Arab Emirates

Camel racing in the U.A.E. is a traditional sport comparable to horse racing in the Western world. With the increase in value of transactions for these camels, a need arises for a test to confirm the parentage or identity of a camel. Since no microsatellite markers were available for *Camelus dromedarius*, two approaches were used: firstly, the evaluation of published South American camelid microsatellite primer pairs, and secondly, the *de novo* development of microsatellite markers. In the first approach a total of 37 recently published polymorphic microsatellites were assessed. According to our results, 24 of the 37 primer pairs amplified in *C. dromedarius*. The second approach involved the development of a small insert genomic DNA library. The library was screened with a DIG$(GT)_{15}$-labelled oligonucleotide. Positive clones were sequenced and primers designed to amplify the microsatellites resulting in 8 markers. Polymorphism of all 32 markers was evaluated in a group of 52 unrelated racing dromedaries. A total of 7 markers proved to be monomorphic, while 6 were dimorphic and 19 polymorphic with allele numbers ranging from 3-19 alleles. Mendelian segregation of alleles was confirmed in 9 paternal half-sib families with a total of 24 offspring-dam pairs. One marker was found to be X-linked, the rest were unassigned autosomal. A total of 16 highly polymorphic markers with probability of exclusion values ranging from 0.2-0.7 resulted in a total P_E of 0.99997. Current work focuses on the establishment of non-linkage and the multiplexing of the microsatellite markers. Suitable genetic markers were quickly developed with the two-tier system used.

D013

Relationships among the Spanish Pure Breed and other horses bred in Spain

J.L. VEGA-PLA, B. ALCAIDE & P.P. RODRIGUEZ-GALLARDO

Laboratorio de Grupos Sanguíneos, Servicio de Cría Caballar, Córdoba, Spain

The Andalusian, or Spanish Pure Breed, horse is the most emblematic Spanish horse from economical, zootechnical and cultural points of view. This study uses DNA microsatellites to complete previous studies of its biodiversity with classical markers. The main objective is typing a random sample of 850 animals including 150 Spanish Pure Breed and other breeds from the Iberian Peninsula. The methodology consisted of a whole blood DNA extraction, PCR amplifications, and allelic characterisation with silver stain technique. For routine typing we have been using the following multiplex groups with great success: M1 = *ASB2* + *AHT4*, M2 = *HMS2* + *HMS7*, M3 = *HMS3* + *HTG7* + *HTG6*, M4 = *HMS6* + *HTG4* + *VHL20*. Four of these groups (M2, M3, M4 and M1) are suitable for multiloading into a single line, although M4 and M1 require waiting 7 min between loads. We found a clear differentiation of Spanish Pure Breed from other horses bred in Spain, confirming historical data and data found in studies with blood groups and biochemical polymorphisms.

D014

Genetic analysis of the Chato Murciano pig and its relationships with the Iberian pig using microsatellites

A.M. MARTÍNEZ[1], B. PEINADO[2], C. BARBA, J.V. DELGADO, J.L. VEGA-PLA[3]

[1]Universidad de Córdoba, Córdoba, Spain; [2]Centro de Investigación y Desarrollo Agroalimentario, Murcia, Spain; and [3]Laboratorio de Grupos Sanguíneos, Córdoba, Spain

The Chato Murciano is a Spanish pig whose origin was in old Iberian pigs from the Mediterranean area. During the first quarter of the 1900s, it was crossbred with Berkshire, Tamworth and other European breeds to improve its production traits. In the last decade it was near extinction, but today there is great interest in conserving its genetic resources. The primary objective of this work is to elucidate and clarify the genetic relationships between this breed and the Iberian Pig using DNA microsatellites. This information could be interesting for the development of future conservation plans. Blood samples were taken from 80 animals. Microsatellites used in this research (25) are enclosed within those recommended by the ISAG-FAO advisory group for diversity studies in pig. Genotypes were compared with those obtained in previous studies of the Iberian Pig and the Duroc populations. Descriptive statistics, genetic diversity, and distances have been studied. Results have shown that the Chato Murciano pig has a low level of variability, probably because of the bottleneck effect. Also, this breed has shown allelic frequency differences and a great genetic distance from the Iberian pig at the microsatellite level. In spite of sharing the same origin, the influence of European pigs and the decreasing size of their populations could be the origin of divergence between these two breeds of pigs.

D015

Relative quantitation of *CSN3* allele mRNAs in heterozygous Holstein cows

J.R. WOOLLARD, B.W. KIRKPATRICK & M.R. DENTINE.

University of Wisconsin, Madison, Wisconsin, USA

Many alleles have been identified at the *CSN3* locus (A, B, C, E, F, G, H, I), of which three (A, B, & E) have been shown to be segregating in the U.S. Holstein population. A difference in the relative protein expression of the A and B alleles (B > A) has been shown previously. However, little is known about the relative expression of the mRNA of any of the three alleles in Holsteins. The objective of this study was to determine the relative amounts of the *CSN3* allele mRNAs expressed in heterozygous Holstein dairy cattle. Total RNA was extracted from cells obtained from milk samples taken post-milking near peak lactation of each cow. *CSN3* mRNA was amplified by RT-PCR using 5' fluorescent labeled primers. RT-PCR products were digested with *Hinf*I and size-separated on an ABI 310. *Hinf*I digests the A allele product resulting in bands of 92 and 186 bp while the B allele does not digest yielding the full length product of 278 bp. Bands were quantified by sizing the detected bands using Genescan™. Peak areas and sizes were extracted from data files using Genotyper™. Relative amount of allele A was determined by calculating the relative proportion of the 92 and 186 bp peak areas divided by the total peak areas of the 92, 186, and 278 bp peaks. A standard curve, using cloned cDNAs of each allele in known proportions, was used to predict the relative amount of A vs. B mRNA (R^2 = 0.956). 27 animals were analyzed in this way and yielded an average relative quantity of 43.3% (SEM = 1.98) for the A allele, which was significantly different from the expectation of 50% (T-test $p < 0.001$).

D016

Detection of polymorphism between full-sib calves by representational difference analysis

M. HIGUCHI[1], Y. NAGAMINE[1], N. MIYASHITA[2] & T. AWATA[2]

[1]Tohoku National Agricultural Experiment Station, Morioka, Japan and [2]National Institute of Animal Industry, Tsukuba, Japan

Representational difference analysis (RDA) is a subtractive hybridization method by which the difference between two complex genomes can be isolated. We applied this technique to detect the polymorphism between two Japanese Black full-sib male calves. Genomic DNA was digested with a restriction enzyme recognizing six base pairs and an oligonucleotide adaptor was ligated to the digest. PCR was used to amplify the populations of the fragments of approximately less than two kilobases in length (called mplicons . Both tester and driver amplicons were re-digested with the same enzyme and only tester amplicon was ligated to another adaptor. Then tester amplicon was hybridized with an excessive amount of the driver amplicon so that the fragments that existed only in the tester could be effectively amplified by PCR. The amplified fragments were hybridized with the same driver amplicon to remove pseudo-positive fragments and PCR was performed again. This hybridization-amplification step was repeated once more. After these operations, five to ten clear bands were visualized in agarose gel electrophoresis. Southern blot analysis revealed that some of these bands existed only in the tester, although others existed in both tester and driver. The result demonstrated the usefulness of RDA to detect the polymorphism among full-sib calves.

D017

Molecular weight determination of *L* system alloantigens by SDS-PAGE

K.A. AMEISS & W.E. BRILES

Northern Illinois University, DeKalb, Illinois, USA

The *L* system is one of twelve alloantigen systems in the chicken. White Leghorn populations generally are characterized by two alleles designated *L1* and *L2*. Genotypic effects have been demonstrated for both macrophage function and regression of Rous sarcoma-induced tumors. Total membrane protein was isolated from Leghorn erythrocytes of both homozygous and the heterozygous types. The isolates were run on eight and twelve percent SDS polyacrylamide gels under reducing and non-reducing conditions. After transfer, the membranes were probed with polyclonal alloantisera specific for either *L1* or *L2* antigens. Two pairs of bands were detected using horseradish peroxidase conjugated secondary antibodies and Amershams Enhanced Chemiluminescence kit. The *L1* and *L2* antisera detected identical bands for their respective homozygote genotypes and both detected the same bands for the heterozygote. The heavier pair had molecular weights of approximately 80 and 70 kD and the lighter pair 35 and 25 kD. Both reduced and non-reduced samples gave the same results. Therefore, the specific alloantisera made against *L* alloantigens collectively identifies four polypeptides of approximately 80, 70, 35 and 25 kD in White Leghorns.

D018

Polymorphism of 10 tetranucleotide repeat microsatellite markers in Korean dogs

Y.J. CHAE, K.Y. PYUN, M.H. LEE & H. LEE

College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University, Suwon, Republic of Korea

Microsatellite markers have been used for various purposes, from individual identification to population genetics. To obtain genetic data for a population of Korean dog breeds, DNA samples from Jindo, German Shepherd, and miscellaneous other dog breeds were analyzed for 10 different tetranucleotide repeat microsatellite loci (Francisco *at al.*, 1996 Mamm. Genome 7, 359). The genotyping was carried out using techniques of PCR, polyacrylamide gel electrophoresis, silver staining and DNA band size determination compared to standard marker DNAs. The results showed that Jindo dogs have a higher degree of polymorphism than German Shepherd for all markers analyzed. In the Jindo dog, 9 out of 10 loci have a high PIC value (>0.7), and 4 loci are over 0.9. On the contrary, the German Shepherd dog had only 4 loci with a PIC value exceeding 0.7. Even though the observed hetorozygosities were slightly lower than expected in all loci examined, the differences were not statistically significant. The results indicate that the Jindo dog has a larger gene pool than the German Shepherd. The number of loci with exclusion powers exceeding 0.6 was 8 in the Jindo dog and 5 in the German Shepherd. The present data may serve as a basis for developing a parentage testing and individual identification system for dogs in Korea.

D019

A *Cervus* genotyping kit based on automated fluorescent multiplex PCR for rapid characterization of genetic diversity in several deer populations

A. BONNET[1], S. THÉVENON[1], F. MAUDET[2] & J.C. MAILLARD[3]

[1]MNHN, Paris, France; [2]CIRAD-EMVT, Econap, University of Kasetsart, Bangkok, Thailand & [3]CIRAD-EMVT, Animal Health, Montpellier, France

Microsatellite loci are highly conserved among related species (Kühn *et al*, 1996) such as cattle and sheep (Moore *et al*, 1992). Transfer of a large number of polymorphic microsatellites between families of the *Artiodactyla* order has been demonstrated several times (Engel *et al*, 1996; Kuhn *et al*, 1996, Slate *et al*, 1998 and Roed, 1998). Therefore, a multi-allele system should be useful in studying the genetic diversities of populations. This study was developed using four different deer species (Rusa, Eld, Swamp and Vietnamese Sika deer). A set of 38 microsatellites derived from bovine and ovine origin were chosen based on two criteria: (i) known to amplify in other deer species (Red deer); and (ii) showing an interesting polymorphic level as described in previous studies (Slate *et al*, 1998 and Talbot *et al*, 1996). From these 38 screened markers, 30 gave an amplified product in the 4 deer species (78.9 %). Of those 30 markers, 14-20 (40%-60%) were polymorphic, depending on the species. Using 12 microsatellites polymorphic in the 4 species, we set up a unique multiplex PCR optimized for annealing temperature and reagent concentrations. The 12 primer sets were labelled with 3 different fluorochromes, depending on the allelic range for each species. The automatic analysis was performed using an ABI 377 sequencer and PE Genotyper software. This method for parentage testing or genotyping gave good and reproducible results for the 4 studied species as well as for several other tested deer species and subspecies. This tool could be considered a first generation “Cervus” genotyping kit useful for the rapid characterization of genetic diversity.

D020

Linkage mapping of rabbit microsatellite markers generated from chromosome-specific libraries

R. KORSTANJE[1], G.F. GILLISSEN[1], S.A. VERSTEEG[2], B.A. VAN OOST[2], L.F.M. VAN ZUTPHEN[1] & H.A. VAN LITH[1]

[1]Department of Laboratory Animal Science, Faculty of Veterinary Medicine, Utrecht University, The Netherlands; and [2]Department of Clinical Sciences of Companian Animals, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

The rabbit is frequently used as an animal model for human diseases like atherosclerosis and hypercholesterolemia and as a production animal for meat, wool and fur. In order to identify and localize the genetic factors which are involved in these diseases and traits, a dense genetic map of the rabbit is needed. In collaboration with Keygene N.V. (Wageningen) a genetic map containing about 280 AFLP markers in 23 linkage groups has been developed. Up until now we could only assign one AFLP linkage group to a specific chromosome (ocu 1). Extending the map with polymorphic microsatellite markers with known chromosomal location would increase the usability of such a map. The availability of flow-sorted chromosomes (Korstanje et al., 1999 Cytogenet. Cell Genet. 86(3-4):317-322) allows us to use a chromosome-specific approach. Flow-sorted chromosomes are randomly amplified (DOP-PCR) and enriched for CA-repeats. The DNA-fragments are ligated into vectors to facilitate sequencing. Primers spanning the CA-repeat are designed and the amplification products are tested for polymorphisms in a panel consisting of different rabbit breeds and strains. Polymorphic microsatellites are subsequently analyzed in an F_2-intercross and/or three backcrosses. With this method we have been able to incorporate microsatellite markers in the linkage maps of chromosomes 1, 3, 5, 6, 7, 12, 15, 18 and 19. Research is ongoing to complete the chromosomal assignment for all linkage groups.

D021

Identification of *BoLA-DRB3* in Japanese black cattle by PCR sequence-based typing

S. TAKESHIMA[1, 2], M. IKEGAMI[1], Y. NAKAI[2], M. MORITA[3]& Y. AIDA[1]

[1]RIKEN Tsukuba Institute, Tsukuba, Ibaraki; [2]Tohoku University, Sendai, Miyagi and [3]Livestock improvement association of Japan Inc., Maebashi, Gunma, Japan

The bovine leukocyte antigen (*BoLA*) class II genes code for highly polymorphic transmembrane glycoproteins that present antigenic peptides to helper T cells. The *BoLA-DRB3* is thought to be the most functionally significant, as it is actively transcribed and at least 97 alleles have been characterized for this locus. In this study, we developed the technique of polymerase chain reaction-sequence based typing (PCR-SBT) which can assign *DRB3* alleles in DNA sequence level and would allow fast screening of large numbers of animals. Since an alignment of the predicted amino acid sequences of the β1 domain encoded by previously characterized *DRB3* alleles appeared that 93 distinct alleles were divided into 8 allelic groups, we designed 8 kinds of sequence specific primers (SSP) for the specific amplification of exon 2 of all alleles in each group for sense primer. First, *DRB3* alleles were amplified from animals obtained from the 5th international BoLA workshop by the primer set of one of 8 SSP and a locus-specific primer for antisense and each PCR product was directly sequenced. The typing results of 53 samples obtained by SBT were coincident with results had been typed by PCR-restriction fragment length polymorphism (RFLP). Next, a sample of 200 Japanese black cattle had studies by this method. We identified 20 distinct published alleles and 4 new alleles. The three most frequently isolated alleles, *DRB3*1001*, **1601* and **1101* were accounted for 17.5, 12.8 and 11.0% of the alleles in population of this breed, respectively. Results of this study indicate that the *BoLA-DRB3* locus is highly polymorphic in Japanese black cattle.

D022

Analysis and frequency of *OLA-DRB1* alleles in Suffolk, Cheviot, Corriedale sheep

S. KONNAI[1, 2], Y. NAGAOKA[1], M. ONUMA[2] & Y. AIDA[1]

[1]RIKEN Tsukuba Institute, Tsukuba, Ibaraki and [2]Hokkaido University, Sapporo, Hokkaido, Japan

To investigate the genetic diversity of the sheep MHC (OLA) class II *DRB1* locus, we amplified exon 2 of *OLA-DRB1* alleles by polymerase chain reaction (PCR), and determined the nucleotide sequences from both strands after cloning. Studying 78 sheep of 3 breeds such as Suffolk, Cheviot and Corriedale, 25 *OLA-DRB1* alleles were identified with frequencies ranging from 0.7% to 17.5%. Nineteen alleles were similar to those reported previously; and 6 new alleles. At the protein level, the identity between these alleles is rather high, ranging from 80.5% to 100%. Moreover, by a two-step PCR followed by digestion with restriction endonucleases *Rsa*I, *Sac*I, *Sac*II and *Hae*III, the 19 different restriction fragment patterns were identified that exactly matched the patterns predicted from DNA sequences. Thus, only 19 of the 27 sequenced *DRB1* alleles may be distinguished by PCR-RFLP. The 18, 14 and 9 allele types were identified in Suffolk, Cheviot and Corriedale sheep: their allelic frequencies differed within 3 breed sheep. Most frequently isolated alleles *OLA-DRB1n*2*, *OLA-DRB1n*23* and *OLA-DRB1n*22* accounted for 28.8%, 25.0% and 25.0%, respectively of the alleles in the population of Suffolk, Cheviot and Corriedale. Results of this study demonstrated that the *OLA-DRB1* locus is highly polymorphic in Suffolk, Cheviot and Corriedale sheep.

D023

A BAC library of elite genomic BOVINE DNA constructed in a modified BAC vector

W. BAWDEN, J. MARTYN, T. WEBSTER, N. NGUYEN & K.A. ZUELKE
Victorian Institute of Animal Science, Melbourne, Victoria, Australia

Our aim is to clone and analyse multigenic trait loci in cattle. Therefore, we constructed a bovine genomic DNA library in a BAC vector that would enable ready manipulation and ligation of inserts. We replaced the polylinker of the vector pBeloBACII with one containing multiple 8-base rare-cutting restriction sites to generate the modified vector, pRazorBAC. Chromosomal DNA from the leukocytes of a purebred Holstein bull was partially digested with *Hind III* and then resolved via pulsed-field agarose gel electrophoresis (PFGE). Optimal PFGE separation was achieved by first applying short pulse times in the "reverse" direction to run smaller fragments off the "short" end of the gel (i.e. behind the wells) and then reversing the current to return the DNA through the origin and into the gel. Size-selected DNA was then electroeluted from the gel and ligated to *Hind III* digested pRazorBAC. The most critical parameters that affected DNA insert size were DNA preparation, size selection and elecroporation conditions. DNA of superior integrity was obtained through electroelution into dialysis tubing rather than agarase digestion from the agarose gels. This BAC library consists of approximately 100,000 clones (average insert size 120 kb; approximately 3-fold genome coverage) and represents a valuable resource for our ongoing structural and function dairy genomics research.

D024

Assessment of biodiversity in a wide range of chicken breeds by genotyping DNA pools for microsatellite loci

S. WEIGEND[1], J. HILLEL[2], M.A.M. GROENEN[3], M. TIXIER-BOICHARD[4], A. KOROL[5], V. KIRZNER[5], P. FREIDLIN[2], R.P.M.A. CROOIJMANS[3], A. VIGNAL[6], K. WIMMERS[7], S. PONSUKSILI[7], P.A. THOMSON[8], T. BURKE[8], A. MAKI-TANILA[9], K. ELO[9], A. BARRE-DIRIE[1,] L.A. ZHIVOTOVSKY[10] & M.W. FELDMAN[11]

[1]Insitute for Animal Science and Animal Behaviour Mariensee, Federal Agricultural Research Centre Braunschweig, Germany; [2]Department of Genetics, Faculty of Agriculture, The Hebrew University, Rehovot, Israel; [3]Institute of Animal Sciences, Wageningen Agricultural University, Wageningen, The Netherlands; [4]Institut National de la Recherche Agronomique, Centre de Recherches de Jouy-en-Josas, France; [5]Institute of Evolution, University of Haifa, Israel; [6]Institut National de la Recherche Agronomique, Centre INRA de Toulouse, France; [7]Institute of Animal Breeding Science, Rheinische Friedrich-Wilhelms-Universitat Bonn, Germany; [8]Department of Animal & Plant Sciences, University of Sheffield, Sheffield, United Kingdom; [9] Agricultural Research Centre, Institute of Animal Production, Jokioinen, Finland; [10] Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia; and [11]Department of Biological Sciences, Stanford University, California, USA

During domestication, a vast range of genetic diversity has been accumulated in the chicken. Each breed or strain might comprise a unique set of genes. A widely accepted assumption is that potentially important genetic resources are represented in those populations that are most divergent within the species. In a project on chicken biodiversity (AVIANDIV) funded by the European Commission, eight laboratories collaborated to assess the genetic variation between and within a wide range of chicken breeds. In the first phase of the project, genetic diversity of 43 chicken breeds has been evaluated by genotyping a set of 22 microsatellites. The genotyping was carried out on breed-specific DNA pools made from 50 individual samples per breed. The estimated heterozygosity (Gene Diversity) varied between 26% and 66%, with an average of 47%. The average number of alleles per locus across breeds was 10.1. Using allele frequencies and sizes, four kinds of genetic distance were estimated. Based on these estimates, the mean genetic distance from other breeds (MGD) was calculated. This measure may indicate the degree to which a particular population shares its genomic polymorphism with the other analyzed populations. It was concluded that Red Jungle Fowl, traditional unselected breeds and broilers are highly polymorphic, and all had relatively small MGD values, in contrast, to both highly selected breeds (layers and experimental lines) and some local fancy breeds. The latter populations were characterized by low polymorphism, relatively high MGD values and are outliers from the spectrum of the detected biodiversity.

D025

Multi-primer target PCR for rapid identification of bovine *DRB3* alleles

S.A. LEDWIDGE[1], Z.H. JIANG[1], B.A. MALLARD[1], J.P. GIBSON[2] & G.B. JANSEN[1]

[1] University of Guelph, Guelph, ON, Canada and [2] ILRI, Nairobi, Kenya.

BoLA DRB3 is the most polymorphic MHC class II gene in cattle. Currently, more than 70 bovine *DRB3* alleles have been characterized. The traditional method of typing based on PCR-RFLP is expensive, laborious and unable to distinguish all the alleles. Consequently, in order to efficiently screen large numbers of animals for the presence or absence of alleles associated with increased risk (DRB3.3*23 (DRB3*1501 & 1502)) and decreased risk (DRB3.2*16 (DRB3*2701-2703, 2705-2706)) of mastitis in Canadian Holsteins, we have developed a multi-primer PCR-based method. Two outer primers are used to amplify exon 2. Simultaneously, two inner, allele-specific primers amplify the individual alleles. Initially, 36 animals previously typed by PCR-RFLP were tested using the multi-primer target approach. The alleles tested include 20 of the original 30 *DRB3.2* alleles and all those at a frequency of greater than 5% in the Canadian Holstein population. All animals carrying alleles DR3.2*16 or DRB3.2*23 were correctly identified and there were no false positives. We are currently confirming the validity of this technique by typing 50 daughter/dam pairs first by the multi-primer approach, then by PCR-RFLP. Results can be obtained in four hours for the cost of a simple PCR reaction. As a result, this method can be used for rapid screening of a population for a specific allele and has the potential to be modified for numerous *DRB3* alleles.

D026

Variation at microsatellite loci in the Lantang, Dahuabai and Landanrace breeds of swine

J. Q. LI, Y. S. CHEN & C. WANG

Department of Animal Science, South China Agricultural University, Guangzhou 510642, P R China

Fifteen highly polymorphic microsatellites were utilized to study the relationship between the Lantang, Dahuabai and Landrace breeds of swine. Lantang and Dahuabai are Chinese native breeds that are highly reproductive and very good meat tasty, but low daily gain and very fat. Although Lantang and Dahuabai are produced in the same province of China, they have had limited contact until recent years because Lantang was only produced in a little county with high inbreeding. Because of this situation, it is questioned whether the two breeds represent the same gene pool in Guangdong province. In order to more clearly define the relationship between Lantang and Dahuabai, Landrace was selected to serve as a potentially unrelated reference breed. Heterozygosity levels, F_{st} and genetic distance were calculated from the allele frequencies in each breed. Heterozygosity levels indicated that Lantang was highly inbreeded. F_{st} values indicated that Lantang and Dahuabai are moderately different. Genetic distances indicated breeds were related in following order (from most to least): Lantang, dahuabai, and Landrace.

D027

Use of microsatellites to analyse genetic variability in four populations of *Columba palumbus*

P. ABRANTES[1] & D. DIAS[1,2]

[1]*Departamento de Zoologia e Antropologia, Faculdade de Ciências da Universidade de Lisboa, Portugal,* [2]*Centro de Biologia Ambiental/Departamento de Zoologia e Antropologia, Faculdade de Ciências da Universidade de Lisboa, Portugal*

Columba palumbus (woodpigeon) is a very important game bird in European countries such as Portugal, Spain and France, so, it is essencial to understand the amount of genetic variability remaining within the existing populations and also the extent of gene flow between them. Eight microsatellite *loci* were studied in different populations of *C. palumbus* from Azores Islands (n = 23), Portugal Continental (n=33), Spain (n = 30) and France (n = 29). Genomic DNA was extracted from feathers samples using phenol/chloroform and commercial kits and analysed in 6% polyacrylamide gels using a fluorescence 4200S Li-Cor automated sequencer. The primers used were CliμDo1, CliμD16, CliμD17, CliμD32,CliμD19,CliμT13 and CliμT17 from *C. livia* (domestic pigeon breeds) and PTC3 from *Ficedula hypoleuca* (pyed flycatcher). Results showed that PTC3 and CliμD32 were monomorphic . The other ones were polymorphic with allele variation ranging from 5 to 12. Preliminary analyses of CliμT13, a tetranucleotide, showed dinucleotide variations in 6 individuals from Azores, Portugal Continental and France populations. The PCR products will be sequencing to confirm if we can assume this mutation as a population and/or specie specific marker.

D028

Genetic distance among bovine populations estimated with AFLP markers

P. AJMONE-MARSAN[1], R. NEGRINI[1], E. MILANESI[1], C. GORNI[1], M. OTSEN[2], A. VALENTINI[3], M.T.R. KUIPER[4] & J.A. LENSTRA[2].

[1] Università Cattolica del S. Cuore, Piacenza, Italy[2]; Fac. Veterinary Medicine, Utrecht, The Netherlands; [3] Università della Tuscia, Viterbo, Italy; [4] Plant Genetics Systems, Gent, Belgium

Relative genetic distances of cattle individuals within and among European breeds were estimated on the basis of biallelic AFLP markers. The accuracy of these estimates was evaluated by bootstrap analysis. In a panel of 47 Holstein-Friesian cattle, genetic distances correlated with the additive relationship derived from herdbook data. In a panel of 116 animals from three different Italian breeds the mean pair-wise genetic distance within breeds was 89 % of the value across the breeds. A Principal Co-ordinates analysis of AFLP markers clearly clustered the animals according to the breeds. In a panel of 45 animals of 14 European breeds, we found a significant clustering of black-pied dairy animals and British breeds, but not of French or beef cattle. Our findings indicate that (a) AFLP can provide quantitative estimates of genetic distances of individuals; (b) breed formation had only a small effect on the overall diversity; and (c) genetic distances of breeds depend partly on geographical origin. These findings may be relevant for conservation efforts and breeding strategies.

D029

Detection of origin between two bloodlines of Romosinuano cattle using microsatellite screening.

R.A. BRENNEMAN[1], C.C. CHASE, JR.[1], T.A. OLSON[2], G.A. ROHRER[3] & S.W. COLEMAN[1]

[1] USDA, ARS, SubTropical Agricultural Research Station (STARS), 22271 Chinsegut Hill Road, Brooksville, FL 34601; [2] University of Florida, Gainesville, FL, 32611 and [3] USDA, ARS, U.S. Meat Animal Research Center (MARC), Clay Center, NE, 68933.

Two primary bloodlines of Romosinuano cattle exist (i.e., a Costa Rican [COR] and a Colombian [COL] bloodline) and have been imported for evaluation at STARS near Brooksville, Florida. The two bloodlines are phenotypically indistinguishable except for occasional scurs or rare white spotting observed in the COR bloodline. The objective of this study was to determine whether or not the genetic variation between the two bloodlines was significant and how accurately an individual's bloodline of origin could be determined based on genetic markers. The COR bloodline originated through upgrading from Hereford dams at the University of North Carolina from 1948-50's and was imported in 1990-92 as frozen embryos from the Centro Agronomico Tropical de Investigation y Ensananza (CATIE), Turrialba, Costa Rica. The COL bloodline was imported in 1996 from Venezuela as frozen embryos with no evidence of outcrossing from the pedigrees tracing to Colombian origins. Forty-seven individuals from each bloodline were identified and screened across 46 microsatellite loci selected by proximity to published carcass merit QTL, usefulness in previously published genetic distance studies, or chromosomal location maximizing genomic coverage. Unique alleles (n=66, COR and n=47, COL) were detected in 41 of the microsatellite systems comprising up to an allele frequency of 0.511 for a single system. Polymorphism information content values (both maximum and average) and average heterozygosities for the 46 systems were (COR) 0.891, 0.752, 0.571 and (COL) 0.918, 0.705, 0.579, respectively, facilitating correct bloodline assignments.

D030

The use of microsatellites for measuring genetic diversity of European local beef cattle breeds for conservation purposes

J. CAÑON[1], P. ALEXANDRINO[2,3], A. BEJA-PEREIRA[2], I. BESSA[2], C. CARLEOS[4], Y. CARRETERO[1], S. DUNNER[1], N. FERRAND[2], D. GARCIA[1], J. JORDANA[5], D. LOLOË[6], A. SANCHEZ[5], K. MOAZAMI-GOUDARZI[6]

[1]Facultad de Veterinaria. Universidad Complutense de Madris, España, [2]Centro de Estudios de Ciência Animal-ICETA/Universidade do Porto, Portugal; [3]Faculdade de Ciencias da Universidade do Porto, Portugal, [4]Facultad de Ciencias, Universidad de Oviedo, España; [5]Facultad de Veterinaria, Universidad Auronoma de Barcelona, España, [6]Inst. National de Recherches Agronominques, Jouy-en-Josas, France.

This study was undertaken to determine the genetic structure, the genetic relationships, and the genetic diversity of a set of 18 local cattle breeds from Spain, Portugal and France using 16 microsatellites. Heterozygosites, estimates of Fst, genetic distances, dendrograms, multivariate, diversity analyses and assignment tests were performed. Heterozygosities ranged from 0.54 in the Pirenaica breed to 0.72 in the Alentejana breed. Seven per cent of the total genetic variability could be attributed to differences among breeds (mean F_{st} -0.07; $P<0.01$). The six computed genetic distances have been compared and no correlation was found to be significantly different from 0 between distance based on population effective size and those which use the sizes of the alleles. Support for internal nodes in phenograms estimated by bootstrapping was, in general, low, except for the Alistana/Mirandesa and the Salers/Aubrac groups, which appeared with an occurrence of 94% and 96% respectively. Multivariate analysis distinguished 4 breed groups. The diversity of the breeds was measured by the Weitzman's recursion approach which suggests that the most important breeds to be preserved are those included into two clusters: the one formed by Mirandesa and Alistana breeds, and the other one composed of the Sayaguesa and Tudanca breeds. The hypothetical extinction of one of those clusters presents a 17% of loss of diversity. In addition, the variation between breeds was sufficiently high as to assign individuals to their breed of origin with a probability of 99% for simulated samples.

D031

Homozygosity mapping approach for the Chondrodysplasia gene in Dexter Cattle

J.A.L. CAVANAGH[1], I. TAMMEN[1], P.A.W. HARPER[2], H.W. RAADSMA[1] & F.W. NICHOLAS[3].

[1]Reprogen, The University of Sydney, Camden, NSW, Australia; [2]NSW Department of Agriculture, Goulburn, NSW, Australia and [3]Department of Animal Science, The University of Sydney, Sydney, NSW, Australia.

Dexter cattle are a dwarf breed of cattle originating in Ireland which have been bred in Australia for several decades. There have been reports of mutant, aborted foetuses in this breed of cattle, described as chondrodysplastic foetuses. The affected foetuses display disproportionate dwarfism, a short vertebral column, marked micromelia, a relatively large head with a retruded muzzle, cleft palate and protruding tongue and a large abdominal hernia. Dexter chondrodysplasia is considered to be inherited in an incompletely dominant manner. As part of an approach to controlling the disease in Australia, the Australian Dexter Association has chosen to support research to develop a DNA test to prevent carrier/carrier matings. A homozygosity mapping approach is being used to localise the disease gene. Firstly, 12 candidate genes were identified by searching for diseases with similar phenotype in other species. Using comparative mapping, nine regions on the cattle genome were selected. A total of 90 microsatellite markers were used to cover these regions. A selection of animals was genotyped and the results analysed by searching for regions of homozygosity in the affected samples. Of the 90 selected markers, one demonstrated a homozygous pattern amongst the affected samples, but not among the parents and unrelated animals. A gene in this region is currently being screened for mutations. If a mutation is found, a DNA based heterozygote test can be developed.

D032

Genetic polymorphism of Goat k-casein

E. BUDELLI[1], P. BOLLA[1], A. CAROLI[3], S. JÄGER[2] & G. ERHARDT[2].

[1]University of Milano, Milano, Italy; [2]University of Giessen, Giessen, Germany and [3]University of Bari, Bari, Italy.

Investigation on milk protein polymorphism in goat mainly concerns *CSN1S1* fraction, which is characterized by a high qualitative and quantitative genetic variability. Isoelectic focusing (IEF) in ultrathin polyacrylamide gels with carrier ampholytes was used to demonstrate *CSN3* polymorphism in milk samples of Italian (Orobica n=36; Saanen n=60) and German goat breeds (Weisse Deutsche Edelziege n=85; Bunte Deutsche Edelziege n=25; Thüringer Waldziege n=57). A genetic polymorphism resulting in three phenotypes (A, AB, B) could be demonstrated at *CSN3* locus in addition to the already described polymorphism in *CSN1S1, CSN1S2* and *CSN2*. The further *CSN3* casein band exhibited a more cathodic migration than $CSN3^{A}$. $CSN3^{B}$ can only be resolved using an ultranarrow pH range pH 4-5.5. Otherwise there is an overlapping by *CSN1S2* C and other protein fractions. After chymosin action, the genetic polymorphism was also observed in the para-k-casein fraction. Thus, the further allele might correspond to the B variant described by Di Luccia *et al* (1990). The genetic basis of $CSN3^{B}$ was confirmed by genetic studies. The frequency of $CSN3^{B}$ ranged from 0.36 (Orobica) to 0.26 (Bunte Deutsche Edelziege). The populations were in Hardy-Weinberg equilibrium at *CSN3*. $CSN3^{B}$ occurred in low frequency (<0.03) in Thüringer Waldziege and in the related breeds Weisse Deutsche Edelziege and Saanen. The results indicate that goat *CSN3* locus is more polymorphic than described until now and studies are in progress to evaluate the influence of the different *CSN3* genotypes on cheesemaking properties as already demonstrated in bovine milk.

D033

Genetic relationships among some Criollo populations bred in Caribbean area

G. CERIOTTI, R. RIZZI, G.M. KONDO, P. BOLLA, A. CAROLI[1] & F.M. CERUTTI

University of Milano, Milano, Italy and [1]University of Bari, Bari, Italy

The polymorphisms of 19 genetic systems, 9 blood groups, 5 blood proteins and 5 milk proteins were investigated in a Carora population (Criollo x Brown Swiss) bred in Venezuela to characterize the genetic structure. Since 9 loci (ALB, TF, CA, HB, CSN1S1, CSN2, CSN3, LALBA, LGB) were in common with other researches carried out in three Criollo populations of Caribbean area, the data were pooled and submitted to statistical analysis in an attempt to define their genetic relationships. In addition, three breeds (Holstein Friesian, Brown Swiss and Zebu) were included in the study for their possible relations with the investigated breeds. The gene differentiation coefficient Gst was computed by DISPAN program and the genetic distances were calculated by PHYLIP program. Moreover, Principal Component Analysis was performed. The results show a clear and expected separation among Zebu, Holstein and Brown while the other breeds are rather closed to the last two ones. The more interesting loci for gene differentiation were ALB, CSN1S1, LALBA. These results confirm that the traditional blood and milk genetic polymorphisms provide an efficient marker system to discriminate *Bos taurus* from *Bos indicus* and to quantify the blood percentage among cross-bred cattle populations.

D034

Cloning and characterisation of CYP51 transcripts in pig

M. COTMAN[1], P. LAZAR[1], D. ROZMAN[2]

[1]Laboratory for Blood Groups of Animals, Institute for Health Care of Pigs, Veterinary Faculty; [2]Medical Centre for Molecular Biology, Institute of Biochemistry, Medical Faculty, University of Ljubljana, Slovenia

Lanosterol 14 α-demethylase (CYP51) is a microsomal cytochrome P450 enzyme involved in postsqualene cholesterol biosynthetic pathway. CYP51 removes 14 α- methyl group from lanosterol, forming FF-MAS (**f**olicular **f**luid **m**eiosis **a**ctivating **s**terol). The gene shows remarkable conservation between species and is the only member of the cytochrome P450 superfamily whose sequence is conserved across phyla. The quantitity of CYP51 mRNA varies between tissues. The highest expression is in mammalian (mouse, rat, human) testis. Besides somatic transcripts, shorter CYP51 transcripts are present in testis of these species. These shorter transcripts are germ cell specific, with the highest level of expression in round and elongated spermatids. The goal of our study is to determine the expression of CYP51 in pig and to evaluate if a shorter, testis specific CYP51 transcript is found also in testis of this mammal. RACE (**r**apid **a**mplification **c**DNA **e**nds) PCR techniques was used for characterisation of pig CYP51 transcripts. Combination of classical PCR and RACE-PCR on the pig testis cDNA library enabled isolation of three polyadenilated CYP51 cDNA clones with lenghts 1620 bp, 1623 bp and 1725 bp. A CYP51 cDNA from pig liver which has been reported in GenBank recently, has a longer 3' untranslated end compared to the testis clones. Northern analysis showed two CYP51 mRNAs (3.1 kb and 2.2 kb) highly expressed in pig testis. The 3.1 kb trancript is probably identical to the liver one. The partial pig testis polyadenylated clones which end at 1620 bp, 1623 bp and 1725 bp probably all belong to a broadened 2.2 kb long transcript. Immunohistochemical studies of pig testis show highest level of CYP51 protein expression in Leydig cells and in round and elongated spermatids. Colocalisation of the electron transferring enzyme, NADPH citochrom P450 reductase, indicates that the CYP51 protein is functional. Preliminary results of immunogold EM in mouse testis show CYP51 localised in the membrane of smooth endoplasmic reticulum in Leydig cells and round spermatids and on the acrosomal membrane in round and elongated spermatids. High levels of CYP51 mRNA and protein expression in germ cells sugessts that CYP51 may play a fundamental role in reproduction *via* formation of meiosis activating sterols MAS.

D035

Lipogenesis genes and fatness in the chicken

S. DAVAL[1], S. LAGARRIGUE[1], F. PITEL[2], C. LE NIGEN[1], A. VIGNAL[2], B. LECLERCQ[3] & M. DOUAIRE[1]
[1] INRA-ENSA, Rennes, France, [2] INRA, Toulouse, and [3]INRA, Nouzilly, France.

Molecular genetic mechanisms involved in the meat chicken fatness were investigated, based on the lipid metabolism pathway. Experiments were conducted in experimental lean and fat chicken lines obtained by divergent selection on abdominal fat weight. Several genes coding for enzymes of fatty acid synthesis (ATP-citrate lyase [ACL], acetyl-CoA carboxylase, fatty acid synthase, malic enzyme [ME], stearoyl-CoA desaturase), an apolipoprotein (apolipoprotein A1 [apoA1]) and a transcription factor (C/EBPα) were studied. A first set of experiments was done to compare the hepatic transcription rates (run on assays) and mRNA levels of the quoted genes between lean and fat chickens. These criteria were significantly higher in the fat line for the ACL, ME and apoA1 genes. This result suggested that these genes contribute to the fattening variability. Besides, a cosegregation analysis of ACL and apoA1 alleles and adipose tissue weight was achieved in full- and half-sib F2 families derived from a cross between the fat and lean lines. In the studied families, no relationship between the gene sire alleles and the trait values appeared, meaning that these genes could not be considered as responsible for the phenotypic variability. These results emphasized the need of complementary analyses (gene expression and genetic association) in the search for genes of quantitative trait.

D036

Characterization of Epitheliogenesis Imperfecta in *Equus caballus*

L.DLIETO AND E. G. COTHRAN

University of Kentucky Lexington KY USA

Epitheliogenesis Imperfecta (EI) is a severe, mutilating mechanobullous disease in horses. Affected foals have been observed in the American Saddlebred and Belgian Draft breeds. Inheritance patterns indicate that it is an autosomal recessive disease. Transmission electron microscopy (TEM) of skin from affected horses revealed a separation between the epidermis and dermis at the level of the Lamina Lucida. TEM also revealed reduced numbers of small abnormal hemidesmosomes. Based on these findings the three subunits of the Laminin 5 protein were identified as possible candidate genes. Linkage disequilibrium analysis was performed using markers chosen based on information from the horse/human comparitive map and the equine gene map. The analysis suggests an association between EI and Equine chromosome 5, a likely location of the Laminin α3 and Laminin γ2 subunits.

D037

Isolation and characterization of equine ß-casein cDNA

T. LENASI, M. DEBELJAK, I. ROGELJ & P. DOVC

University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Groblje 3, SI-1230 Domzale, Slovenia

Lactoprotein genes belong to the best studied loci in most farm animal species. However, until recently the only equine lactoprotein cDNA sequence available in the GenBank was cDNA sequence encoding β lactoglobulin. We focused our research on milk protein genes in horse because of the possibility to use mare milk as replacement for cow's milk for children, sensitive to cattle milk proteins. From the lactating mare mammary gland mRNA was extracted and used for cDNA synthesis. The cDNA was cloned into the pBluescript vector and propageted in competent *Epicurian Coli Cells*. Screening of positive colonies was performed using DIG-labeled PCR product representing the 445 bp fragment of the equine *β-casein* (*β-CN*) cDNA, obtained with heterologous primers based on bovine *β-CN* sequence. Sequencing of the cDNA was performed on ABI Prism 310 Genetic Analyzer. The obtained cDNA sequence is full length at the 3'-end, but 41bp shorter than the bovine homologue at the 5'-end. However, the exact determination of the transcription start site has not been performed. The entire coding region for equine *β-CN* has 75.1%, 74.4 %, 73.8%, 72.3%, 69.9% sequence identity with camel, porcine, human, bovine and rabbit *β-CN* mRNA, respectively. The sequence identity within the signal peptide coding region is considerably higher than in the remaining parts of the cDNA sequence.

D038

Sequence variation in the mitochondrial DNA control region of wild African cheetahs (*Acinonyx jubatus*).

R. FREEMAN[1], D.E. MACHUGH[5], S. M[C]KEOWN[2], C. WALZER[3], D.J. M[C]CONNELL[1], [4] & D.G. BRADLEY[1]

[1]Smurfit Institute of Genetics, Trinity College, Dublin 2, Ireland; [2]H.E. Sheikh Butti Bin Juma al Maktoum´s Wildlife Centre, Dubai, United Arab Emirates; [3]Salzburger Tiergarten Hellbrunn, A- 5081 Anif, Austria; [4]Fota Wildlife Park, Carraigtwohill, County Cork, Ireland and [5]Department of Animal Science and Production, University College Dublin, Belfield, Dublin 4

The control region is the most rapidly evolving segment of the mitchondrial genome and its sequence should provide a sensitive assay of residual genetic variation in cheetahs, which are the most widely quoted example of a genetically depauperate species. 525 bp from this area were sequenced and analysed for 20 *Acinonyx jubatus* and one *Felis catus*. These sequences were analysed and compared with published sequences from another domestic cat, 20 ocelots (*Leopardus pardalus*) and 11 margays (*Leopardus weidii*). The intra-specific population divergence in cheetahs was found to be less than in the other cats. However variation was present and two main haplogroups which correspond to a north-south geographical division are discernible. The 80bp RS2 repetitive sequence motif previously described in other felids was found in four copies in cheetah. The two central repeat units in cheetah show homogenisation which may have arisen by convergent evolution.

D039

Arthrogryposis in Piedmontese cattle breed: a genetic analysis.

A. GALLI, M. ZANOTTI*, G. BONGIONI, L. DEL BO, M. BONA, R. ALEANDRI, M. LONGERI*.

*Istituto Sperimentale 'L. Spallanzani', Rivolta d'Adda, Cremona and *Istituto di Zootecnica, Faculty of Veterinary Medicine, University of Milan, Italy.*

Arthrogryposis is a congenital syndrome affecting a considerable number of new-borns in Piedmontese cattle. It is characterised clinically by limb juncture hyperflexion and muscle atrophy at birth. The high frequency within families, particularly in males, and similarity with other animal and human heritable syndromes (particularly Spinal Muscular Atrophy), justify a study on this disorder by genetic approach. The present work is part of a wider study on arthrogryposis in Piedmontese breed. The aim of this work was the study of this syndrome by disease frequency analysis at population level and by candidate gene approach. Starting from 1980 syndrome incidence in the breed has been analysed on a studbook database. The *Survival Motor Neuron* 1 (SMN1) gene has been chosen as candidate gene. By comparative alignment on DNA sequence databanks, several primer pairs have been drawn to amplify a great part of the gene. RT/PCR from spinal cord of 2 affected calves and one control, plasmid cloning and ABI Prism 377 P.Elmer sequencing have been performed. From population data the disease resulted inherited as a recessive trait, showed an incidence of 1.8% on the total new-born population and an incomplete penetrance in female. Complete sequences (from exon 1 to 7) and sequences with exon 2 or 5 deletions have been found by SMN1 cDNA clones sequencing, indicating an alternative splicing mechanism, not directly correlated to the presence or the absence of the syndrome in our samples. Our results could suggest a pathological effect conditioned by a modulation of the mRNA maturation or the presence of gene duplication.

D040

Mapping of QTL involved in growth, backfat thickness and intramuscular fat content in pigs. Regional RH mapping in the QTL region identified on chromosome 7.

C. GENET[1], J.P. BIDANEL[2], C. RENARD[2], N. IANNUCCELLI[1], J.C. CARITEZ[3], J. GRUAND[4], F. BOURGEOIS[5], Y. AMIGUES[5], C. ROGEL-GAILLARD[2], J. RIQUET[1], J. MOUROT[6], A. BARBOSA[2], C. CHEVALET[1], L. OLLIVIER[2], J. GELLIN[1] & D. MILAN[1]

[1] INRA Toulouse, 31326 Castanet-Tolosan ; [2] INRA Jouy, 78352 Jouy en Josas ; [3] INRA Le Magneraud 17700 Surgères; [4] INRA Rouillé, 86480 Rouillé; [5] Labogena, 78352 Jouy en Josas & [6] INRA Saint Gilles, 35590, L'hermitage. FRANCE.

A QTL detection experiment has been performed at INRA since 1991 in F2 crosses between Meishan (MS) and Large White (LW) pig breeds. Six F1 males and 23 F1 females issued from 6 LW boars and 6 MS sows produced 530 F2 males and 573 F2 females. F2 animals were measured for a large number of traits related to growth, backfat thickness, carcass composition and meat quality. QTLs were evidenced on chromosomes 1, 4, 7, 8 and 11 for growth traits, on chromosomes 1, 6, 7, 8 and X for backfat thickness, on chromosomes 2, 4, 7, 17 and X for carcass composition traits and on chromosome 7 for intramuscular fat content. To refine position of QTL, production of backcross animals and haplotype analysis on various populations are underway. On chromosome 7, the Meishan allele has a positive effect on growth, backfat thickness and intramuscular fat content. The most likely position of the QTL is close to SLA region. Construction of a BAC contig and sequencing of the SLA Class I region is performed (see Renard et al, this congress). To improve knowledge of the region surrounding SLA, we simultaneously developed a high-density RH map of swine chr7p-q12 using IMpRH radiation hybrid (RH) panel. We added on the RH map 23 new loci (8 microsatellites, 14 genes and one EST). Our results show that gene order is conserved between chromosomal region HSA 6p and SSC 7p-q12. All these studies will help to identify gene(s) responsible(s) for QTL effects observed on chromosome 7.

D041

The relationship of sequence homology between species to within species rate of polymorphism: evidence from 108 pig genes

Z. JIANG[1], J. P. GIBSON[1, 2] & A. M. GIBBINS[1]

[1] Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada and [2] ILRI, PO Box 30709 Nairobi, Kenya

The genetic dissection of complex traits is one of the most challenging problems in livestock genome mapping. A dense set of nucleotide polymorphic markers, together with automated genotyping approaches brings the prospect of widespread use of linkage disequilibrium to map quantitative trait loci (QTL) in livestock. We report here the results of a pilot study on characterization of nucleotide polymorphisms in 108 porcine gene-fragments (one fragment per gene). We identified 134 nucleotide polymorphims in 37.5 kb of porcine genome, including 120 SNPs, 9 sequence repeat variations and 5 deletion/insertions. We observed differences between exon and intron regions in the rates and types of polymorphisms and in proportion of transition and transversion that occurred at CpG dinucleotide sites. In coding sequences, the rate of synonymous and non-synonymous SNP decreased significantly with increase of homology among mammalian species ($P < 0.0001$). In introns, those that exhibited variation in interspersed repeats, such as SINE, LINE, MIR, MER and STR, between species also exhibited twice the frequency of within breed nucleotide polymorphism than introns without interspersed repeats. This study suggests strategies that would maximise the efficiency of developing genetic markers within genes in order to facility mapping of mammalian genome.

D042

Molecular and functional characterization of coat color mutations at the *I/KIT* locus in the pig

E. GIUFFRA, A. TÖRNSTEN, J. KIJAS and L. ANDERSSON

Swedish University of Agricultural Sciences, Sweden

Together with *Extension/MC1R*, Dominant White (*I/KIT*) is the major coat color locus in pigs and has been extensively characterized at the molecular level. *KIT* encodes a tyrosine kinase (mast/stem cell growth factor receptor) with an important role in several tissues during embryonic development and in adult animals. In mice, most of the viable *KIT* cause a variety of pigmentation defects due to altered expression which are often associated to long-range genomic rearrangements. Four *KIT* alleles have been documented in the pig so far: Dominant white (*I*), Patch (I^P), Belted (I^{Be}) and the recessive wild type allele (*i*). *I* and I^P are associated with a long-range duplication that contains the entire coding region of *KIT*, while the molecular basis of I^{Be} has still to be unraveled. The further characterization of these mutations is of great interest, in that provides the opportunity to gain insight in the regulatory mechanisms of tissue-specific expression. We characterized the *KIT* expression profile in various adult tissues of *I/I*, I^{Be}/I^{Be} and i/i animals by Northern Blot analysis and quantitative RT-PCR (TaqMan technology). We show that *KIT* expression is dramatically reduced in the lung of I^{Be}/I^{Be} animals, stressing the similarity between I^{Be} and some mice W mutants, while in *I/I* animals both copies of *KIT* are expressed in most tissues. We also carried out physical characterization of the same alleles by Pulse Field Gel Electrophoresis (PFGE), and constructed a BAC contig spanning *KIT* and its flanking region. Current work is aimed to refine the precise location of the duplication breakpoint proximal to *KIT*.

D043

Genetic diversity analysis and parentage control in Korean native cattle using Microsatellite

S.K. HAN, H.D. BYUN & E.Y. CHUNG[1]

Laboratory of Molecular Genetics, Department of Dairy Science, Faculty of Animal and Life Science, Kon-Kuk University, Seoul, Korea and [1]College of Life Science and Natural Resources, Sangji University

Korean native cattle have been characterized by using 8 microsatellite markers (ETH3, ETH10, ETH225, TGLA122, TGLA126, TGLA227, BM1824 and BM2113) and parentage control have been investigated. Allele frequencies were calculated and used for the characterization of Korean native cattle. The diversity value, number of alleles per locus, heterozygosities and PIC values, is calculated for each marker on the basis of frequency data. And exclusion probabilities is calculated and determined on exclusion efficiencies for each marker. The number of alleles varied between 6 and 13 with an average number of alleles per locus of 8.75. ETH3, ETH10, ETH225, TGLA122, TGLA126, BM1824 and BM2113 display 7, 9, 7, 13, 7, 12, 6 and 9 alleles, respectively. Among them TGLA122 display most (13) and BM1824 display smaller numbers (6) of allele. The highest heterozygosity is observed at TGLA227 (0.880). In contrast, TGLA126 is the least heterozygosity (0.644). The mean expected heterozygosity is 0.789. Polymorphic information content is observed from 0.608(TGLA126) to 0.861(TGLA227) and mean polymorphic information content is 0.757. This result exhibit that Korean native cattle is higher polymorphic than other European improved cattle breeds. The exclusion probability is observed from 0.427(TGLA126) to 0.748(TGLA227). The highest exclusion probabilities are observed with TGLA227(0.748) and ETH10(0.740), whereas TGLA126 is found to be the least exclusion probability (0.427). The total exclusionary power of all markers is 0.999. This result exhibit to be suitable for parentage control in Korean native cattle using 8 microsatellite polymorphisms investigated in this study.

D044

Simultaneous PCR of equine *SRY* and *AMEL*, and its application in infertile mares.

T. HASEGAWA[1], T. KATILA[2], A. MÄKINEN[2], F. SATO[1] & N. ISHIDA[1]

Equine Research Institute, Utsunomiya, Japan[1]; and University of Helsinki, Helsinki, Finland[2]

Chromosomal abnormalities are common in infertile mares with small gonads lacking follicular development or oestrus cycles. To help the diagnosis prior to karyotyping, a quick method for sex determination of horses was developed. Simultaneous amplification of equine *sex-determining region of Y chromosome gene* (*SRY*) and *amelogenin gene* (*AMEL*) accomplishes to determine the presence of both sex chromosomes and *SRY*. By the agarose gel electrophoresis, PCR products from normal stallion samples represent 1 *SRY* band and 3 *AMEL* bands (*AMELX*, *AMELY* & *AMELX* / *AMELY* heteroduplex), besides normal mares show single *AMELX* band. In 3 XY-mares tested, no *SRY* bands were detected while 3 *AMEL* bands were detected as well as normal males. This method enables a quick diagnosis for the infertile mares prior to karyotyping.

Genbank Accession: AB004572 (*SRY*)
: AB032193 (*AMELX*)
: AB032194 (*AMELY*)

D045

Allelic variation at the porcine α-inhibin(*INHA*), β_A-inhibin/activin (*INHBA*) and β_B-inhibin/activin (*INHBB*) loci

S. HIENDLEDER[1], R. GRANDKE[2] & V. DZAPO[1]

[1]Department of Animal Breeding and Genetics, Justus-Liebig-University, Giessen, Germany and [2]A.I. Station Giessen e.G., Giessen, Germany

Inhibins and activins, members of the *TGF-β* superfamily, are involved in the male and female reproductive axis and in growth and differentiation. *INHA* maps to SSC15 where evidence of a QTL for ovulation rate has been reported. We have investigated the porcine *INHA*, *INHBA* and *INHBB* genes of up to 33 boars of the Pietrain (14), Deutsche Landrasse Sauenlinie (11), Deutsche Landrasse Universal (3) Deutsches Edelschwein (1), Large White (1), Schwäbisch Hällisches Schwein (1) and Meishan (2) breeds for allelic variation by Southern blot RFLP analysis with 10 restriction enzymes using full length porcine and rat cDNA probes. *INHA* was highly polymorphic, where *Bam*HI, *Bgl*II, *Hind*III, *Pvu*II, *Sac*I and *Taq*I detected a large number of polymorphisms that describe at least 7 different alleles. *Msp*I was also polymorphic with the *INHA* probe, but could not be consistently typed. *Hind*III, *Msp*I, *Sac*I and *Xba*I detected RFLPs at the *INHBA* locus which identify up to 6 alleles. *INHBB* was less variable with at least 3 alleles detected by *Bgl*II and *Taq*I. Published porcine cDNA sequences were used to design primers for PCR amplification of 730 bp (*INHA*), 843 bp (*INHBA*) and 667 bp (*INHBB*) fragments that contain the mature subunits and a portion of the pro region of these genes. PCR products derived from Wild Boar, Meishan, Pietrain and Edelschwein were cloned and are currently being sequenced to evaluate the extent of coding region sequence polymorphism.

D046

Diversity in the ovine MHC *DQA* region

J.G.H. HICKFORD, S-M. SLOW, H.J. RIDGWAY, H. ZHOU & A.K. COUP

Lincoln University, New Zealand.

Approximately 2000 sheep from five different breeds have been genotyped at their MHC *DQA1* and *DQA2* loci using Southern hybridisation. This revealed 8 alleles at the *DQA1* locus and 16 alleles at the *DQA2* locus. A rapid PCR-SSCP typing system was developed for each of the loci and this, along with DNA sequencing, revealed a further 6 alleles at *DQA2*, some of which appear to be breed-specific. It is expected that further polymorphism will be found as more breeds and sheep are screened. Sequence analysis revealed that some alleles had potentially ineffective splice sites at the end of the hypervariable second exon. This suggests these alleles may be inactive, or possibly pseudogenes. Sequence alignment showed that some *DQA2* alleles that had greater sequence homology to bovine *DQA3* sequences, than to other ovine *DQA2* sequences.

D047

Association of single nucleotide polymorphisms in the growth hormone receptor gene with blood serum IGF-I concentration and growth traits in Angus beef cattle

W. GE, M. E. DAVIS, H. C. HINES & K. M. IRVIN

Animal Genetics Laboratory, Department of Animal Sciences, The Ohio State University, Columbus, OH, USA

To evaluate the association of four single nucleotide polymorphisms (SNPs) in the bovine growth hormone receptor (GHR) gene with blood serum IGF-I concentration and growth traits, 470 Angus calves divergently selected for blood serum IGF-I concentration at the Eastern Ohio Resource Development Center were examined for SNP genotypes as described earlier (Ge *et al*., 2000 J. Anim. Sci., in print). Blood serum IGF-I concentrations on d 28, 42, and 56 after the beginning of postweaning test, mean IGF-I concentration, postweaning gain, and weights at birth, weaning, and beginning and end of the 140-d postweaning test were analyzed using animal models and the MTDFREML computer program. The full animal model included year and season of birth, sex, selection line, age of dam, age of calf and four SNPs as fixed effects, and direct genetic, maternal genetic, and permanent environmental effects as random effects. Maternal genetic and/or permanent environmental effects were deleted from the full animal model if the effect accounted for less than 20% of the total phenotypic variance. One SNP located in the promoter region of the bovine GHR gene was significantly associated with all IGF-I concentrations. Significant association with IGF-I concentration on d 28 and 42 of the postweaning test was also found for another SNP located in the 10^{th} exon. A third SNP showed significant association with mean IGF-I concentration and tended to be associated with other IGF-I concentration measurements (P = .10). More tests are needed to fully evaluate the association of these SNPs with blood serum IGF-I concentrations and growth traits.

D048

Null-alleles in the standard set of loci for cattle parentage control

A. HØJ PETERSEN & C. BENDIXEN

Danish Institute of Agricultural Sciences, Research Centre Foulum, Tjele, Denmark.

In cattle parentage control, DNA typing of microsatellite markers is becoming an increasingly useful tool and many cattle laboratories are now processing towards a complete shift from the conventional blood typing to DNA typing. Different panels of microsatellites have been evaluated in the last 3 international cattle comparison tests, and a set of guidelines concerning marker set, exclusion parameters, reference sample and nomenclature has been proposed and will be further discussed at the present ISAG Conference. Another important topic for discussion is how and where to report instant problems, like discovery of null-alleles, to ensure that all laboratories get the information in order to keep parentage control a dynamically evolving procedure with a high degree of confidence. We report here the identification of three null-alleles that were discovered during routine parentage control using the Cattle StockMark Kit II from PE Biosystems. Two null-alleles were discovered in *TGLA122* in two different breeds, and one null-allele was discovered in *INRA023*. New primers were designed outside of each of the original primer sites to amplify the silent alleles, and the amplified fragments were cloned into pCR2.1 (Original TA Cloning Kit from Invitrogen). Sequencing of the clones revealed point mutations in the original primer sites. Lowering of the annealing temperature from 61 to 56 degrees Celsius during PCR with the Cattle StockMark Kit II resulted in amplification of all three “null”-alleles.

D049

Calcium chloride induction of an enhanced green fluorescent protein gene driven by the metallothionein promoter

H.A. HOSTETLER, P. COLLODI & W.M. MUIR

Purdue University, West Lafayette, Indiana, USA

Many of the initial experiments with transgenic animals used a combination of a metallothionein promoter and a growth hormone gene. This promoter was chosen for its inducibility by the presence of heavy metals (i.e. zinc, cadmium, lead, and copper). However, there were concerns that the promoter was active, even in the absence of such heavy metals. The sockeye salmon metallothionein promoter was used to drive the enhanced green fluorescence protein (*eGFP*) gene in transfected Chinese Hamster Ovarian (CHO) cells. Aberrant expression of the transgene shortly after calcium-phosphate transfection, and in the absence of heavy metals, was noted. After establishment of stable transfected cell lines, we found that concentrations as low as 12.5 mM of calcium chloride were sufficient to induce *eGFP* expression under control of this promoter. Thus, it is possible that leaky expression of metallothionein driven transgenes is the result of calcium-based induction.

D050

Microsatellite DNA testing for parentage verification of thoroughbreds

H. KAKOI, S. NAGATA & M. KUROSAWA

Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan

For individual identification and parentage verification of horses, especially thoroughbreds in Japan, we developed a microsatellite DNA typing with the routine 12 markers, AHT4, AHT5, ASB2, ASB17, HMS3, HMS6, HMS7, HTG4, HTG10, TKY19, TKY28 and VHL20, and the backup panel of four markers, ASB15, LEX33, TKY3 and UCDEQ425. This method consisted of multiplexing PCR procedures, and it showed reasonable amplification of all PCR products. Sizing precision with standard deviations of PCR fragment size at all loci was confirmed to be less than ± 0.4bp. Using the routine panel, 2,124 thoroughbreds in Japan were analyzed. Observed allelic frequencies provided a discrimination power of 6.74×10^{-12}, and it was considered that there were no null alleles at 12 loci in this population based on agreement between observed and expected heterozygosities with no significant deviation from H-W equilibrium. The exclusion probability (PE) on the routine panel was 0.9997 and a combined PE of the routine and backup panels was exceeding 0.9999. These results demonstrate that the present DNA typing is so useful for individual identification and parentage verification of thoroughbreds.

D051

Establishment and characterization of 600 canine (*Canis familiaris*) cDNA clones

B. KAZMIERCZAK[1], H. MURUA ESCOBAR[1], L. BORMANN[1], I. NOLTE[2]; & J. BULLERDIEK[1]

[1] Center for Human Genetics, University of Bremen, Bremen, Germany, [2]Clinic for Small Animals, Hannover, Germany

The dog shows a variety of disorders well suited as a model for understanding the molecular mechanisms underlying corresponding human diseases. Nevertheless, canine molecular genetics is still far behind that of human and mouse. A limiting step in using the dog as an animal model is the still small number of cloned genes. We established a cDNA library from canine testis tissue and picked random clones for analysis. By sequencing and characterizing 500 cDNA clones we showed that based on the gene level the homology between man and dog is much higher than estimated so far. The characterized cDNA clones showed a homology of 85 % - 100% to known human genes or ESTs, as e.g. the fragile X mental retardation syndrome related protein 1 (*FXR1*), interacting with *FMR1* (fragile-X-mental retardation), the Huntingtin interacting protein or the ubiquitin specific protease 4. In addition, we compared the sequence homology in the open reading frame and the 3'UTR separately. As the dog is well accepted now as an animal model for analyzing human diseases, it is very important to characterize and provide sequence data for canine genes in the near future. Combining these data and mapping genes will be a powerful tool for analyzing many human diseases as well.

D052

Canine Mitochondrial DNA, a Novel Criminal Case Study

M. S. KETCHUM, D. THOMPSON, & P. W. WOJTKIEWICZ

Shelterwood Laboratories, Carthage, TX, USA, North Louisiana Criminalists Laboratory, Shreveport, LA, USA

Evidence from an attempted capital murder was submitted for analysis using canine STRs. The evidence was in the form of canine hair that was retrieved from the crime scene. An attempt at STR analysis failed due to the fact that the hair was shed hair with no follicles. The hairs were then cleaned, ground and prepared for extraction. Mitochondrial DNA was extracted and amplified using two primers designed to amplify hypervariable region one in the dog. The product obtained produced template DNA to be sequenced using two more primers. One primer was 5' and the other 3'. The resulting products were quantified on an agarose gel prior to sequencing. Sequencing was performed with an ABI 377 Sequencer using the Big Dye Terminator sequencing kit by Perkin Elmer. Sequences were consistent with published sequences for canine HV1. Upon analysis of the sequences, there was heteroplasmy observed at a site known to be polymorphic in the evidentiary samples. The same heteroplasmy was seen in the known samples submitted with the case. Therefore, the suspect was linked to the crime scene on the strength of this evidence. This case establishes that the use of mitochondrial DNA from animals can be a valuable tool to the forensic community.

D053

Identification and characterization of a second interferon-regulated bovine Mx gene

T. KOJIMA[1], K. OSHIMA[1], H. WATANABE[2] & M. KOMATSU[1]

[1]Department of Animal Production, Chugoku National Agricultural Experiment Station, Oda, Shimane, Japan and [2]Bio-oriented Technology Research Advancement Institution, Tokyo, Japan

Mx proteins belong to the GTPase family, and are induced by type I interferons (IFNs), including IFN-tau, and which express antiviral activity. We have isolated a new bovine Mx gene (Mx2) which is distinct from bovine Mx1 from an endometrial (caruncular) cDNA library in pregnant Japanese Black Cattle (Day30 of gestation). We analyzed the structure of the isolated gene and performed genomic PCR using genomic DNAs from eight bovine breeds to investigate whether or not all breeds possess both Mx genes. Further, semi-quantitative RT-PCR analysis was performed to evaluate levels of mRNAs coding for bovine Mx1 and Mx2 in the endometrium (caruncle and intercaruncle) during the non-pregnant to pregnant period. Mx2 has a single functional open reading frame as same as Mx1, although the 5' untranslated region and the 5' coding region of Mx2 cDNA were rather different from the regions of Mx1. As a result of genomic PCR, it was indicated that eight bovine breeds all had both Mx genes, so the new bovine Mx was strongly suggested to be bovine Mx2. Further, both Mx genes were expressed from the non-pregnant to pregnant period, especially Day 17 and 20 of gestation when trophoblast secretes IFN-tau. In conclusion, cattle have at least two functional Mx proteins which might provide antiviral activity and/or perform a fundamental function in the endometrium, especially in early pregnancy.

D054

Sequencing and mapping of PERVs (Porcine Endogenous Retroviruses) in Westran pigs

J. H. LEE[1], G. C. WEBB[3], J. BURGESS[2], Y. CHEN[1], P. O'CONNELL[2] & C. MORAN[1]

[1]Department of Animal Science, University of Sydney, NSW 2006, Australia. [2]National Pancreas Transplant Unit, Westmead Hospital, NSW 2146, Australia. [3]Department of Animal Science, Waite Campus, Glen Osmond, The University of Adelaide, SA, Australia and Dept Obstetrics and Gynaecology, The Queen Elizabeth Hospital, Woodville, SA, Australia.

Pigs are regarded as a potentially good source of organs and tissues for transplantation into humans. However, porcine endogenous retroviruses have emerged as a possible problem as they can infect cultured human cells. Two main types of pig retrovirus, determined by envelope protein, PERV-A and PERV-B are widely distributed in different pig breeds and a third less common type, PERV-C has been recognised. Endogenous retroviruses have been analyzed from the Westran (Westmead transplantation) inbred line of pig, specially bred for biomedical research. Thirty one 1.8 kb *env* PCR product clones were sequenced after preliminary screening with the restriction enzymes *Kpn*I and *Mbo*I. Five recombinant clones between A and B were identified. 55% of clones (17/31) sequenced have stop codons within the envelope protein-encoding region, which would prevent the retrovirus from making full length envelope protein recognizable by cell surface receptor for the virus. The endogenous viruses have been physically mapped by FISH (Fluorescence *In Situ* Hybridisation) using PERV-A and PERV-B envelope clones as probes. Preliminary FISH data suggest that there are 15 plus PERVs (6 PERV-A and 9 PERV-B) and the chromosomal location of these are quite different from European Large White pigs. The sequences and mapping results of inbred Westran pig suggest that there are relatively few PERV integration sites compared with commercial pigs and further a large proportion of clones are defective due to premature stop codons in the envelope gene.

D055

Predicting Heterosis Using Biochemical and RAPD Markers in Animal Species

X.C. LIU[1], X. L. WU[1,2], R.Z. LIU[1], Q.S. Shi[1], B.N. XIAO[2], Q.J. XIAO[2], and J. JIANG[2]

[1] Hunan Agricultural University, Changsha 410128, China, and [2] Hunan Institute of Animal & Veterinary Science, Changsha 410131, China

The animal industry has a history of using breed crosses and/or strain crosses in commercial production, mainly to take advantage of heterosis. Association of heterosis of economic traits with biochemical and RAPD markers were therefore investigated, which involved 23 alleles at 8 polymorphic biochemical loci in poultry populations and 5 RAPD markers amplified by 2 random primers in swine stocks. Genetic distance between parental populations based on markers of polymorphic blood groups and isozymes were favorably associated with percent heterosis in poultry. However, neither RAPD-based parental difference nor average band sharing was predictive of the level of heterosis in swine. The result was not an evidence that RAPD markers were not promising for predicting heterosis, but rather that choosing proper markers and populations were more important than choosing types of markers. Sequent random sampling experiments on choosing biochemical markers applied in predicting heterosis supported the hypothesis that association of heterosis with markers might varied greatly with markers employed. Therefore, improper use of markers in predicting heterosis was likely to result in deviation.

D056

Mitochondrial DNA diversity and possible dual origins for BOS INDICUS

D.A. MAGEE[1], P. KUMAR[2], C.S. TROY[1], D. FULLER[3], J.F. BAILEY[1], R.T. LOFTUS[1] & D.G. BRADLEY[1]
[1]Smurfit Institute of Genetics, Trinity College, Dublin 2, Ireland; [2]Animal Genetics Department, Indian Veterinary Research Institute, Bareilly, India and [3]Institute of Archaeology, University College London, United Kingdom.

The origins and demographic history of domesticated cattle are controversial. The two major domestic cattle forms, taurine (*Bos taurus*) and zebu (*Bos indicus*), are differentiated primarily by the absence or presence of a hump, and are regarded as separate subspecies. Prior analysis of mitochondrial DNA (mtDNA) control region sequences has suggested that zebu cattle were domesticated independently from taurine cattle, presumably from a different aurochsen subspecies from the Indian subcontinent. In an attempt to address the origins of indicine cattle we have examined a 240 bp segment of the mtDNA control region from seven Indian breeds. Phylogenetic analyses revealed that haplotypes generally fall into two distinct groups corresponding to a geographical north-south division. These observations suggest more than one wild ancestral strain of indicine cattle.

D057

Genetic relationships between nine French Horse breeds.

J.C. MERIAUX[1], X. ROGNON[2], & D. LALOE[3].

[1]LABOGENA, Jouy en Josas, France ; [2]Département des Sciences Animales, INA P-G, Paris, France and [2] Station de Génétique Quantitative, INRA, Jouy en Josas, France.

The aim of this study is to investigate the genetic variability of a semiferal french breed namely *Pottok* (PO; n = 461) and its genetic relationships with two breeds from the same area *Landais* (LA; 78) and *Merens* (ME; 21), a draught breed *Breton* (BR; 88) and five French race and riding breeds, namely *Trotteur Français* (TF; 1836), *Selle Français* (SF; 1452), Thoroughbred (TB; 4065), Arabian (AR; 1080), Anglo-Arab (AA; 214). The analyses were performed using a set of 11 microsatellites (AHT4, AHT5, ASB2, HMS1, HMS3, HMS6, HMS7, HTG4, HTG6, HTG10 and VHL20) including the international parentage panel. Phenetic trees were generated by UPGMA with Nei's standard genetic distances and Neighbor-Joining with Cavalli-Sforza's chord distances. Multidimensional analysis was processed by correspondence analysis, which allows horse breeds and alleles to be ploted simultaneously. All phenograms showed a similar topology, with two major clusters. One group involved the three Pyrenean-"*Aquitaine*" breeds, *i.e.* PO, ME and LA, and the draught breed BR. A second cluster grouped TF, SF, AA and TB. Within this group, TF, originated from "old" French populations, was well differentiated from the others. SF and AA were still close to the Thoroughbred, resulting from its great genetic contribution. Finally, Arabian was in an intermediate position. Multidimensional analysis showed a similar pattern. These results are discussed on the light of historical and genealogical data, considering especially the possible ancient contribution of Arabian to Pyrenean-"*Aquitaine*" breeds.

D058

Analysis of hereditary diseases genes in Lithuanian and Finnish cattle populations

I. MICEIKIENE[1], J. KANTANEN[2] & J. KUCINSKIENE[1]

[1]Lithuanian Veterinary Academy, Kaunas, Lithuania and [2]Agricultural Research Centre of Finland MTT, Jokioinen, Finland

BLAD (Bovine Leukocyte Adhesion Deficiency), Citrullinaemia and DUMPS (Uridine Monophosphatase Synthesis Deficiency) genes were examined in Lithuanian and Finnish Holstein-Friesian cattle populations. Testing of mutations was performed by PCR-RFLP method with genomic DNA isolated from blood, semen and hair roots. Rapid method for extraction DNA from hair roots was optimised. No DUMPS or Citrullinaemia genes were found in Lithuanian and Finnish cattle populations. BLAD gene with the frequency of 3,7% within bulls, 6,7% within cows and 27% within daughters of the BLAD gene carriers bulls was found in Lithuanian HF cattle population. Pedigree analysis of bulls BLAD gene carriers showed that the bulls were paternal or maternal linked to the bull Osborndale Ivanhoe - ancestor of the mutation.No statistically significant differences in morfphological and biochemical blood testing between heterozygous for BLAD gene and having no mutation cows were found. No homozygote for BLAD was identified in tested population. No BLAD gene was found in Finnish Holstein-Friesian cattle population.

D059

The *RN* gene involved in meat quality in pigs encodes a muscle specific isoform of a regulatory subunit of AMP-activated protein kinase

D. MILAN[1], J.T. JEON[2], C. LOOFT[3], V. AMARGER[2], A. ROBIC[1], M. THELANDER[2], C. ROGEL-GAILLARD[4], S. PAUL[3], N. IANNUCCELLI[1], L. RASK[5], H. RONNE[2], K. LUNDSTRÖM[2], N. REINSCH[3], J. GELLIN[1], E. KALM[3], P. LE ROY[4], P. CHARDON[4] & L. ANDERSSON[2]

[1] INRA, 31326 Castanet-Tolosan, France ; [2] Swedish University of Agricultural Sciences, 75124 Uppsala, Sweden ; [3] Christian-Albrechts-University, 24098 Kiel, Germany, [4] INRA, 78352 Jouy en Josas, France and [5] Uppsala University, 752 23 Uppsala, Sweden

The *RN* gene has an unfavorable effect on meat quality in pigs. The dominant *RN*$^-$ allele affects not only yield of cured cooked ham but also tenderness and mellowness of meat. In order to identify the *RN* gene, we developed a high resolution linkage map using more than 1000 meiosis, a local Radiation Hybrid map in Pig and in Human, a BAC contig of 2.5 Mb, and a linkage disequilibrium map using Hampshire boars. We finally sequenced a BAC containing markers in total linkage disequilibrium with the *RN*$^-$ allele. Analysis of BAC sequence allowed us to identify a gene showing 63 % identity with a regulatory subunit of AMP-activated protein kinase (AMPK). The whole coding sequence of this new gene has been determined using RT-PCR and 5' RACE. Northern blot analysis revealed that this new isoform is specifically expressed in muscle. A non-synonymous mutation in this gene has been found in complete concordance with the presence of *RN*$^-$ allele. The mutation affects a protein domain well conserved from human to yeast. The mutation can be easily detected by PCR-RFLP and thus eradicated from populations in which it segregates. The discovery of this new muscle specific isoform of an AMPK subunit, and the identification of the *RN*$^-$ mutation, may also provide insights into muscle physiology in general as well as in the pathogenesis of disorders in energy metabolism, such as diabetes mellitus.

D060

DNA-based methods for *Mhc B* and *Rfp-Y* haplotyping in chickens

M.M. MILLER[1], R.M. GOTO[1], G.M. IGLESIAS[1,2] & W.E. BRILES[3]

[1]Beckman Research Institute of the City of Hope National Medical Center, Duarte, CA, USA; [2]Faculty of Veterinary Sciences, University of Buenos Aires, Buenos Aires, Argentina and [3]Northern Illinois University, DeKalb, IL, USA

Genetic variability in chickens at the major histocompatibility complex is extensive. Accurate *Mhc* typing in chickens is made particularly complicated by the arrangement of structurally related *Mhc* genes into two clusters (*B* and *Rfp-Y*) that transmit haplotypes independently of one another. Genetic recombination occurring apparently within both *B* and *Rfp-Y* further increases the complexity of the *Mhc* variability that may be present within breeding populations. Methods are needed that will allow polymorphism at *B* and *Rfp-Y* loci to be detected separately and yet not require the determination of allelic sequences or the development of cluster-specific alloantisera. Such methods would be especially advantageous for efficiently working out *Mhc* variability in previously uncharacterized genetic stock. To develop such methods available, we have designed a series of DNA probes for Southern hybridization and a series of primer sets for SSCP assays that are specific for loci within the *B* and *Rfp-Y* gene clusters. We have tested these and found that they reveal heritable differences at *B* and *Rfp-Y* among individuals in fully pedigreed families. We further demonstrate the utility of these methods in revealing *Mhc* variability in a commonly available line of SPF chickens and anticipate their use in the characterization of *Mhc* variability in additional genetic stocks.

D061

SNP screening at the myostatin gene level in European cattle breeds

M.E. MIRANDA[1], S. DUNNER[1], Y. AMIGUES[2], M-Y. BOSCHER[2], F. BOURGEOIS-BOSSAERT[2], J. CAÑÓN[1], O. CORTÉS[1], M. GEORGES[3], L. GROBET[3], R. HANSET[3], P. MAUGRION[4]& F. MÉNISSIER[4].
[1]Fac. Veterinaria UCM. Madrid. Spain. [2] LABOGENA, Jouy-en-Josas. France. [3] Fac. Vetérinaire. Univ. Liège. Belgium. [4]. INRA-SGQA Jouy-en-Josas. France.

The Muscular Hypertrophy syndrome (MH), which appears in many cattle breeds, is produced by mutations in the GDF8 gene encoding the myostatin protein involved in muscle growth regulation. The higher lean value this phenotype shows in some cattle breeds allows overcoming the often-coupled dystocia problems. As a result, certain breeds show a high occurrence of the phenotype while others do not. Although several mutations have been described, there are still some MH individuals belonging to different breeds which show a wild type myostatin sequence.

EuroMH (BIO4-CT98-0421) is a UE project grouping eleven breeds from France, Spain and Belgium. Its aim is to track all possible mutations accompanying different phenotypes, and address one of the main concerns of breeders, which is to know the status of their breed in order to allow a better management of the trait. For this purpose and as a first part of the project, the use of the SSCP (Single Strand Conformation Polymorphism) technique has allowed to detect all possible mutations, and to find new mutations in some breeds. These new mutations affect the first two exons corresponding to the latency-associated peptide and are silent (D108D, T129T) or missense (S105C, D182N). The OLA (Oligonucleotide Ligation Assay) technique has later been used to set up a one-tube assay to genotype all mutations in every individual of any breed, facilitating the mutation screen for management purposes.

D062

Mitochondrial D-loop nucleotide sequence variation in central and south american creole cattle breeds

M.M. MIRETTI[12], H.A. PEREIRA Jr[1], J.A. FERRO[1], M.A. LARA[2], M.A. POLI[3] & M.J. NAVES[4]

1 Depto. de Tecnologia, FCAV, UNESP, Jaboticabal, SP, Brasil; 2 Depto. de Genética, FMRP, USP Ribeirão Preto ,SP Brasil; 3 Inst. de Genética CNIA-INTA, Castelar, Argentina, 4 INRA, Unité de Recherches Zootechniques, Petit Bourg, Guadeloupe (FWI)

Creole cattle are the descendant from those bovines brought by spaniards during the first 100 years of colonisation. The cattle mated and reproduced freely suffering bottlenecks and expansion periods. Cross-breedings with introduced breeds could also have occurred in the history in some country. The aim of this works is to analyse the relationships among Creole Cattle breeds as determined by mtDNA D-loop sequence. It has been obtained the nucleotide sequence of aDNA fragment (850bp) comprising the ^{Thr}tRNA gene and most of D-loop region of 28 animals representing 6 Creole Cattle breeds from Argentina (Argentinean Creole, AC), Guadalupe (Guadalupe Creole, CG) and Brazil (Mocho Nacional, MN, Caracu, CC, Curraleiro, CU, Pantaneiro, P). These sequences were aligned using CLUSTALX, then phylogenetic trees were constructed by neighbor-joining procedure. Considering all breeds, 8 haplotypes were found. Two haplotypes (# 3, # 4 and #5, #7) were exclusive of AC and GC respectively. None of the 4 haplotypes found in these breeds was dominant. In contrast 2 haplotypes (#1 and #6 were predominant in the brazilian breeds). As expected, an increased transition/transversion bias was noted. All the sequences obtained were included within the taurine group of the dendrogram, indicating the absence of any Asian zebu introgression. Even though, this do not discard the possibility of contamination with other zebu strains. mtDNA d-loop sequence of other native breeds is under investigation.

D063

Cloning and characterization of microsatellites from enrichment library of equine genome

T. TOZAKI[1,2], S. MASHIMA[1], K. HIROTA[1], N. Choi-MIURA[2] M. TOMITA[2] & N. MIURA[1]

[1]Laboratory of Racing Chemistry, Utsunomiya, Japan, and [2]Department of Physiological Chemistry, School of Pharmaceutical Sciences, Showa University, Tokyo, Japan

Microsatellite analysis is useful for the construction of a linkage map and the parentage testing of equine. We constructed the equine genomic library enriched for DNA fragments containing (CA)n and (CAG)n repeats. The method includes the hybridization capture of repeat regions using biotin-conjugated oligonucleotides, nucleotide substrate-biased polymerase reaction with the oligonucleotides and PCR amplification. About 95% of the sequenced clones contained for the (CA)n repeats. In addition, about 90% of these clones had over 12-repeats, thus are more likely to be polymorphic. Microsatellites containing (CAG)n repeats were obtained at the ratio of one per 3~4 clones. Those results indicate that the enrichment value is about ~104 -fold, resulting in a significant reduction in the time and cost of microsatellite cloning. In this study, 55 clones containing (CA)n repeats and 66 clones containing (CAG)n repeats were identified. The number of the CA and CAG repeats ranged from 10 to 28 and from 4 to 33, with an average repeat length of 18.2 and 8.5 units, respectively. The flanking sequences of (CA)n and (CAG)n repeats of some microsatellites were identical with a few interruptions, suggesting that the identical sequences may be novel equine repetitive elements. Thus the primers to amplify the microsatellites must be carefully designed when multiplex PCR is performed.

D064

DNA microsatellite analysis in Austrian pigs for parentage control

D. NECHTELBERGER[1], C. KALTWASSER[2], I. STUR[1], J.-N. MEYER[2], G.BREM[1], M. MUELLER[1] & S. MUELLER[1]

[1] Institute for Animal Breeding and Genetics, Vienna University of Veterinary Medicine, Austria and [2] Institute for Animal Breeding and Genetics, University of Goettingen, Germany.

We have compiled a DNA microsatellite profiling system for individual identification and parentage control in pigs. The system was designed to be suitable for use in both German and Austrian herd-book breeding. We have combined 10 porcine DNA microsatellite markers for fluorescent labelled multiplex amplification and developed a standby battery of 5 markers. The microsatellites in our batteries are selected from the recommended panel of markers for diversity studies. The PCR fragments are analysed on an ABI PRISM™310 Genetic Analyzer using Genescan 2.1 and Genotyper 2.1 software. The French PiGMaP reference DNAs were used to calibrate the fragment sizes. The multiplex system of 10 markers contains the microsatellites *S0090, S0101, S0155, S0355 S0386, S0005, SW24, SW240, SW857* and *SW951*. In Austria we have investigated the two most important Austrian breeding populations, Large White and Piétrain. In our material the highest polymorphism information contents (*PIC* > 0.70) were present at the following loci and breeds: *SW24, SW240* and *S0005* in Piétrain, *S0005, SW857* and *S0355* in Large White. The combined exclusion probability of the 10plex battery exceeded the desired value of 99.5% in both investigated breeds: *CEP* 99.76% in Austrian Large White, *CEP* 99.74% in Austrian Piétrain.

D065

Assessment of the genetic diversity of sub-Saharan African sheep populations using microsatellite markers

A.W. MUIGAI[1], P.C. WATTS[2], J. HIRBO[1], S. KEMP[2], J.E.O REGE[3] & O. HANOTTE[1]

[1]International Livestock Research Institute (ILRI), Nairobi, Kenya; [2]School of Biological Sciences, University of Liverpool, UK and [3]ILRI, Addis Ababa, Ethiopia.

Indigenous African sheep have evolved in diverse environments and represent unique combination of genes that underline productive and adaptive traits. These populations are today often at risk of extinction through crossbreeding, replacement with exotic breeds, unstable political climate and environmental disasters. Our purpose is to understand the genetic diversity among the indigenous sub-Saharan African sheep populations in order to develop a rational approach to their conservation, utilization and improvement. Fifteen indigenous African and six reference breeds (North Ronaldsay, Swaledale, Dorset (UK), Lanzhou (China), Karakul (Central Asia) and Awasi (Syria)) were characterized with 23 autosomal microsatellite loci using fluorescent genotyping (ABI 377 DNA sequencer). Observed heterozygosities range from 0.623 ± 0.038, Blackhead Persian (South Africa), to 0.776 ± 0.0243, Ossimi (Egypt). Neighbor-joining tree (Da), principal component and multidimensional scaling analysis reveal two main clusters of sheep. The first cluster includes the East and South African fat-tailed sheep; the second cluster includes the West African thin-tailed sheep, the North African fat-tailed sheep (Ossimi), the fat- and thin-tailed reference breeds from outside Africa. The results suggest that there are at least two major genetically distinct groups of sheep in Africa, possibly corresponding to two different phases of sheep introduction within the African continent.

D066

Parentage testing with microsatellites in cattle: pitfall twins

H. FRITZ-HIRNI, J. MUNTWYLER & M.L. GLOWATZKI-MULLIS
Institute for Animal Breeding, University of Berne, Switzerland

Over 90% of multiple pregnancies result in anastomoses. In microsatellite typing one could assume that there would be enough DNA to display all alleles of co-twins. In routine case work we also test for freemartinism. Criteria that a female calf is considered sterile: more than two alleles, microsatellite profile typical for chimaerism, discrepancies blood-hairbu-lb analysis, male specific amplification product. Among 85 tests 2 cases (one described) had similar results: In the blood DNA of the twin female calf (male co-twin slaughtered) male-specific Bov157 was detectable but no chimaerism in any 11 microsatellites (standard set). Blood-hairbu-lb comparison showed that in 3 microsatellites only the co-twin's genotype was displayed in the blood; not the slightest peaks for the genuine alleles were perceptible. Since hairbu-lb testing is not always possible in parentage verification the phenomenon of hidden genotypes can be a cause of false exclusions in microsatellite typing too.

D067

Characterization of the caprine T cell receptor alpha chain

[1]A. FLURI, [2] G. BERTONI AND [1]G. OBEXER-RUFF

[1]Institute of Animal Breeding, Berne, Switzerland. [2] Institute of Veterinar Virology, Berne, Switzerland.

The examination of T cell receptor (TCR) repertoires plays an important role in the study of immune mediated diseases involving expansion of particular T cells. Expanding T cells can be traced by following the expression of the different TCR variable (V) chains in these cells using specific tools. In order to complete our study on the caprine TCR repertoire, which we have started by characterizing the different V regions of the TCR β chain, we have generated a cDNA library of TCR α chains. Total RNA, isolated from caprine peripheral blood mononuclear (PBMCs) was retro-transcribed and the cDNA used for AnPCR as described elsewhere. The amplified fragments were cloned and sequenced. From a total of 67 clones, 56 were functionally rearranged and classified according to their human counterpart. Sixteen caprine-specific Vα and 28 Jα regions could be identified. Based on this sequence information, it will be possible to develop Vα and Jα specific oligonucleotide probes and PCR primers. These tools, together with the previously developed Vβ specific reagents, will permit an identification of expanding T cell clones in several immunopathological processes such as caprine arthritis encephalitis (CAE) virus-induced arthritis, a unique natural model to study human rheumatoid arthritis, or Cowdriosis, induced by the hemoparasite *cowdria ruminantium*, which affects goats and other ruminants. Both are infectious diseases of goats where the clinical course appears to be controlled by immunogenetic factors that may also influence the T cell response to the infecting agents. Vα and Jα TCR specific markers will help to analyze and compare the T cell response in disease susceptible versus disease resistant animals.

D068

Diversity of the prion-protein gene *PRNP,* in Norwegian sheep

I. OLSAKER, K. G. GAUSTAD, A. ESPENES & B. HØYHEIM

The Norwegian School of Veterinary Science, MGA-Genetics, Oslo, Norway

The prion-protein in its normal form (PrP^{C}) exists as a cell surface protein in tissues of mammals. Prion diseases like scrapie, BSE and CJD, are associated with conformational change of PrP^{C} followed by intracellular accumulation of the abnormal form of the protein (PrP^{Sc}) in the central nervous system. This leads to vacuolization and malfunction of the brain and finally to the death of the diseased individual. The conformational change is catalyzed by exposure to the abnormal protein. Several allelic variants of the prion-protein gene (*PRNP*) have been observed in sheep and some variants are associated with increased susceptibility to scrapie. On this background we have analyzed a total of 250 animals from 11 Norwegian sheep breed groups. The samples were typed for variants in the coding region of *PRNP* by sequencing a PCR product covering bp 340 to bp 740. The reported polymorphisms at codons 136, 154 and 171 are covered by this approach. The animals were also typed for a microsatellite in the 5' region of the gene. For codons 136, 154 and 171 five haplotypes were found as reported in the literature. In addition we found polymorphisms at codons 137, 141 and 151. The microsatellite displayed five alleles. We observed large *PRNP* variation within and between the Norwegian sheep breeds, but all groups including the original Old Norwegian sheep showed a certain frequency of the *PRNP* haplotype associated with the majority of scrapie cases in Norway.

D069

Molecular basis of esterase D polymorphism in pig

T. OMI[1], S. TSUCHIDA[2], A. ONISHI[3], S. IWAMOTO[1], & E. KAJII[1]

[1]Dept. of Legal Medicine & Human Genetics, Jichi Medical School, Japan, [2]Veterinary Surgery, Nippon Veterinary & Animal Science University, Japan and [3]National Institute of Animal Industry, Animal Breeding &Genetics, Japan

Human esterase D (EC 3.1.1.1) is a member of the carboxylic ester hydrolase family ubiquitously distributed in the cells of the most tissue. The genetic polymorphism of the esterase D (EsD) isozyme is well known one of most useful genetic markers of population studies in human or animals. Two human EsD cDNAs, EsD1 and EsD2, are differed by one base nucleotide exchange G to A resulting in the amino acid substitution Gly191Glu (Tsuchida et. al., 1994 Hum. Genet. 93, 255). We demonstrated the isolation of the pig EsDA cDNA from a Ohmini pig, and showed the pig EsDA cDNA had an open reading frame composed of 849 bp nucleotides, which encoded a predicted protein of 282 amino acids. The pig EsDA cDNA exhibited 86% identity and 87% similarity with the human EsD1 cDNA. Furthermore, we found that the two alleles, EsDA and EsDB, were characterized by a missense mutation in the pig EsD polypeptide. These results will provide useful information for future research into the molecular evolution of the ESD gene in mammals.

D070

Genetic structure of several populations of Pottoka-Basque Poney

I. PASCUAL[1], M.V. ARRUGA[2], J.I. INTXAUSTI[1], L.V. MONTEAGUDO[2] & M.T. TEJEDOR[2]

[1] Servicio de Ganadería de la Diputación Foral de Vizcaya. Bilbao (Spain) and [2] Laboratorio de Citogenética y Genética Molecular. Facultad de Veterinaria. Zaragoza (Spain).

The Pottoka-Basque Poney is a local horse breed, traditionally used in countryside and mine work and presently bred at large. Its main interest is its potential for meat production in mountain areas and for sportive and cultural uses. To characterize this breed four serum protein loci (*AL, A1B, TF* and *GC*), eight enzyme loci (*PGD, GPI, PGM, MDH, SOD, LDHA, LDHB* and *ME1*) and three microsatellite loci (*VHL20, HMS7* and *AHT5*) were studied. Four populations from different geographic locations (72 individuals) were analysed. Allele frequencies, observed and expected heterozygosity values, percentage of polymorphism and mean number of alleles per locus were estimated for each population. Mean values of F_{is}, F_{it} and F_{st} are significant (F_{is}=0.06772 ± 0.08594, $p<0.05$; F_{it} =0,16105 ± 0.11791, $p<0.01$; F_s=0.10011 ± 0.05757). Only Population 1 shows a significant global F_{is} value (F_{is}=0.17647 ± 0.15475, $p<0.01$). Global F_{st} values for pairs of populations are significant, except for Populations 1 and 2. Gene flow between these two populations is possible, in the light of their geographic proximity. Significant levels of inbreeding in Population 1 can be caused by a small efective size or a particular reproductive scheme.

D071

Differential display RT-PCR revealed expression difference caused by culture condition in bovine embryos

Y.Q. YUAN, A. VAN SOOM, A. DE. KRUIF, A. VAN ZEVEREN, L. PEELMAN

Department of Animal Nutrition, Genetics, Breeding and Ethology, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, B-9820 Merelbeke, Belgium

Department of Reproduction, Obstetrics and Herb Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820, Belgium.

The synthetic oviduct fluid (SOF) system is widely used in the bovine embryonic culture in vitro, similar to the situation in bovine uterus 5% oxygen stress in applied in SOF system. But some other culture systems, for instance COC and TCM199 systems, still employ 20% oxygen tension in the culture atmosphere. Excess oxygen could form the reactive oxygen species (ROS), which has been reported relating to the induction of programmed cell death. Therefore, we applied 2%, 5% and 20% oxygen stress to our SOF system respectively, and used the sensitive differential display RT-PCR to study the gene expression in embryos which derived from the SOF system with different oxygen stress. To investigate the differential gene expression, one 17-base- pair anchor primer and four 10-base-pair random primers were applied in the RT-PCR. The most prominent different bands were excised, cloned and sequenced to identify the corresponding genes.

D072

A Parentage Test For Rhesus Macaques Using Human Primers for Microsatellite Markers

M.C.T. PENEDO, K.I. CORDOVA, T. WARD, D.G. SMITH, M. BAUTISTA, J. ROBERTS, M. MARTHAS & A.T. BOWLING

University of California, Davis, CA, USA

Rhesus macaques (*Macaca mulatta*) are the primary source of non-human primates for biomedical research. Experimental animals are derived from captive breeding colonies maintained by primate centers. Pedigree assignments have been mostly based on behavioral observations and the use of methods such as DNA typing for parentage determination has been limited. We identified 13 microsatellites in Rhesus that are amplified by heterologous human primers using a single PCR protocol and two sets of multiplexes. One set contains primers for D3S1768, D7S513, D7S794, D11S925, D13S765, D16S403 and D17S804. The other set contains primers for D6S276, D6S291, D6S1691, D8S1106, D10S1412 and D18S72. The HSA 6 markers are linked to the major histocompatibility complex (MHC) in humans. In a sample of 107 animals, the number of alleles ranged from 6 to 21 and heterozygosities ranged from 0.309 to 0.905. The expected probabilities of exclusion (PE) are 0.999 for one-parent (PE_2) and 0.998 for no-parent (PE_1) comparisons. The 13 markers provide a highly effective parentage test for macaques. The HSA 6 markers are useful as a screening tool to evaluate association between MHC markers and disease traits.

D073

A new protocol for genotyping microsatellites in Goats

F. PILLA[2], M. BLASI[1], A. BRUZZONE[2], & A. LANZA[1]

[2]Università degli studi Molise Campobasso, Italy, and [1]Laboratorio Gruppi Sanguingni, Cremona-Potenza

Because of the small economic value of single animals in this species the parentage test must be very low money and time consuming. For this reason a protocol was developed with a number of microsatellite loci large enough to give a good percentage of success in parents identification with the smaller economic and time effort possible. Five economically important italian goat breeds (Camosciata, Derivata di Siria, Ionica, Maltese Saanen), have been genotyped for 15 microsatellites loci with a protocol of multiplex PCR. It consists of two multiplex amplifications one of 9 (INRA005, MAF65, SR-CRSP24, ILST S19, SR-CRSP5, INRA063, SR-CRSP8, PZ963 and HSC) and one of 6 microsatellites (SR-CRSP23, FCB20, ILST S87, McM527, INRA023 and CSRD247) loci and their allele size scoring by automated fluorescent DNA sequencing. Parentage exclusion probability has been calculated for each locus and for all 15 loci together and is equal to 0.999. The first set of 9 microsatellites was enough in most cases to give a satisfactory paternity identification. Not very much is known about the genetic diversity of goats in Italy so allelic frequencies have been also used to evaluate the genetic variability (He ranged between 0,4 and 0.9, number of alleles from 3 to 16) and to calculate genetic distances. The population structure for the 5 breeds studied so far, gives a picture of inbreeding in each single breed with an over all heterozygote deficiency accompanied by an heterogeneity in the total goat population.

D074

Study of two candidate genes for production traits in pig: the lysosomal proteinase Cathepsin B (*CTSB*) and the proteinase inhibitor Cystatin B (*CSTB*).

V. RUSSO, L. FONTANESI, R. DAVOLI, L. NANNI COSTA, M. CAGNAZZO and J. MILC.
DIPROVAL Sezione di Allevamenti Zootecnici, University of Bologna, Reggio Emilia, Italy.

Excessive softness is a defect of dry cured hams which seems related to a higher *postmortem* activity of lysosomal cysteine proteinases, such as cathepsin B, in pig muscles. Using a candidate gene approach we studied two genes: Cathepsin B (*CTSB*) and Cystatin B (*CSTB*) which codes for an inhibitor of the cathepsins. A fragment of the porcine *CTSB* gene encompassing part of exon 6, the intron and part of exon 7 was amplified by PCR and sequenced. SSCP analysis revealed 4 alleles that have been characterized by sequencing. *CTSB* was assigned to porcine chromosome 14 by linkage and physical mapping. A fragment of the *CSTB* gene, encompassing part of exon 2, the intron and part of exon 3, was amplified and sequenced. The two alleles identified by PCR-RFLP have been sequenced and a non-synonimous mutation in exon 3 was identified. *CSTB* was localized to porcine chromosome 13 by linkage and physical mapping. Allele frequencies at the *CTSB* and *CSTB* loci have been studied in a sample of 390 pigs (Large White, 118; Landrace 53; Duroc, 57; Belgian Landrace, 29; Hampshire, 22; Piétrain, 32; Meishan, 14; Calabrese 9; Cinta Senese 14; Casertana, 14; Nera Siciliana, 28). Association analysis of the two loci with several meat quality and production traits (cathepsin B activity, ultimate pH, back-fat thickness, lean content, ham weight and average daily gain) was performed in a sample of 220 pigs (Large White, 128; Landrace, 38; Duroc 54) coming from central Sib-Test stations. The results indicate a significant association of *CSTB* ($P \leq 0.01$) with average daily gain.

D075

Use of Single Nucleotide Polymorphisms for low cost parentage testing in cattle & sheep

G. S. SELLICK[1], H. KOJEVNIKOFF[1], H. W. RAADSMA[2], & C. D. K. BOTTEMA[1]

[1]University of Adelaide, Adelaide, Australia and [2]University of Sydney, Camden, Australia

Increasingly efficient methods for the discovery and typing of single nucleotide polymorphisms (SNPs) has sparked a new revolution in DNA-based genetic studies. Single nucleotide substitutions can now be detected by methodologies which can be miniaturized and automated, and consequently, can be exploited for low cost parentage and identity testing in livestock. Published cattle and sheep sequences were used to amplify DNA segments from sets of unrelated animals and SNPs were identified by detecting differences in dideoxyfingerprinting (ddF) patterns. The presence of a SNP was confirmed by bi-directionally sequencing PCR products. Approximately 200 SNPs have been discovered and the allele frequency of the SNPs will be determined by sequencing pooled DNA samples. Addition SNPs will be generated by sequence information obtained by the end-sequencing of FISH-mapped bacterial artificial chromosome clones.

D076

Differential expression of mRNA in ileal Peyer's patches from sheep inoculated with scrapie agent and in matched controls.

G. SKRETTING, A. ESPENES, R. HEGGEBØ, K. G. GAUSTAD, B. HØYHEIM & I. OLSAKER.
The Norwegian School of Veterinary Science, MGA, Oslo, Norway.

Prion diseases are a group of neurodegenerative diseases that also can be infectious. The infectious agent is thought to be the prion protein (PrP) which has been conformationally tranformed (PrP^{SC}). The *PRNP* gene is highly expressed in brain, however, expression is also detected in several other tissues. It has been shown that *PRNP* expression is essential for development of the disease. Whether other proteins are involved in this process is not well understood. In brain tissues of mice and hamster with an experimental prion disease, up-regulation of several genes has been reported. In an effort to unravel factors involved in the initial steps of the disease development, we are studying differences in mRNA expression in ileal Peyer's patches from lambs orally inoculated with scrapie agent and control lambs matched with respect to age and *PRNP* genotype. Increased levels of PrP have been observed 1 week after inoculation in Peyer's patches and also preliminary results indicates increased levels of PrP mRNA in the same tissues. We are in the process of identifying differences in expression using representational difference analysis (RDA) of cDNA on Peyer's patches from both control and scrapie infected animals. This technique, using cDNA instead of genomic DNA, is very sensitive. Subtracting cDNA from normal tissue with cDNA from infected tissue and vice versa will identify both down-regulated and up-regulated genes. Results from this study will be presented.

D077

Polymorphism in the tyrosinase (TYR) gene in the PIG and its influence on coat color and quantitative traits

K. SIEBEL[1], M. REISSMANN[1], H.J. WAGNER[2], K. WIMMERS[3] & T. HARDGE[1]

[1]Institute of Animal Sciences, Humboldt-University Berlin, Germany; [2]Institute of Veterinary Biochemistry, Free University Berlin, Germany; [3]Institute of Animal Breeding Science, Friedrich-Wilhelms-University, Bonn, Germany

Tyrosinase (*TYR*) is known to be a decisive factor in the metabolic pathway leading to melanine, the pigment giving color to the mammalian coat. Mutations in the *TYR*-gene have been shown to be responsible for phenotypic changes as impressive as the albino phenotype which is known to influence not only coat color but also quantitative traits in mice. Though widely distributed among mammalian species, no such phenotype has ever been observed in the pig. Based on the partial cDNA-sequence of the mouse (D00440) and man (M27160) we amplified a 820 bp fragment of exon 1 of the porcine *TYR*-gene on Chr. 9 by heterologous PCR. The sequence is homologous to the mouse sequence by 84,4 % and to the human sequence by 87,9 %. We detected a single-base-polymorphism (C / T) that created a restriction site for MluN I. Based on a PCR-RFLP (forward: AATGCTCCTGGCTGTTTTGTA; reverse: CTGCCAGGAGGAGAAGAAGGATGCT) we genotyped 212 F_2 animals of a Duroc and Miniature Pig intercross resource population and additionally purebred animals (Saddleback, Large White, Piétrain, Hampshire, Duroc, Miniature Pig). The mutation tested was polymorphic in all breeds except the Miniature Pig. No correlation could be detected between the polymorphism and the coat color phenotype in the resource population. An association analysis between the tyrosinase genotype and quantitative traits (growth, carcass and meat quality) revealed a suggestive influence of the tyrosinase genotype on growth traits in the time period 35th to 100th day of age.

D078

Phylogenetic analysis of some Caucasian cattle breeds

A.M. MASHUROV, K.H. THANH , S.N.KASHTANOV[1] , A.F.NAZAROVA , R.O.TSAREV, V.N.ORLOV, L.V.STRELCHENKO & N.S.STRELCHENKO[2]

Institute of Ecology and Evolution RAS, Moscow, Russia, [1]Institute of General Genetics, Moscow, Russia, [2]ABS Global Inc. De Forest, WI, 53532-0459, USA

The results in the field of blood groups of 9-11 EA-systems – 22045 animals of 6 breeds, four of which are reared in the Caucasus, have been summarised. Immunogenetic distances (d) between the breeds have been calculated and a phylogenetic dendrogramme has been constructed. The smallest distance (d=0,1090) is observed between the Caucasian Brown and Swiss Brown breeds, the largest distances (d=0,249) and (d=0,281) between the Red Megrel and Red Steppe and Small Caucasian, correspondingly. On the dendrogramme all the 6 breeds are distributed into three clusters. One of them includes the Caucasian Brown and Swiss Brown breeds, the second includes the Khevsurskaya and Red Megrel breeds, and the third – the Small Caucasian and Red Steppe Breeds. Such a distribution of the breeds on the dendrogramme is in good agreement with the real interrelation of the breeds with known history and genealogy of their formation.

D079

Neuronal ceroid-lipofuscinosis in Australian Merino sheep

I. TAMMEN[1], R.W. COOK[2], F.W. NICHOLAS[3] & H.W. RAADSMA[1]

[1]Reprogen, The University of Sydney, Camden, NSW, Australia; [2]NSW Department of Agriculture, Wollongbar, NSW, Australia and [3]Department of Animal Science, The University of Sydney, Sydney, NSW, Australia.

The neuronal ceroid-lipofuscinoses (NCLs) are a group of autosomal recessive neurodegenerative diseases characterized by the accumulation of autofluorescent lipopigment in a variety of tissues. Clinical features of NCL are dementia, loss of vision, motor disturbances, and premature death. At least eight different forms of NCL occur in humans and the disease has been identified in various animal species, including sheep. Recently NCL was reported for the first time in Merino sheep in Australia. The aim of this project is to characterise the genetic defect in Merino sheep and to compare it to a clinically and genetically well defined form of NCL in South Hampshire sheep. Due to the limited number of animals and the lack of pedigree structure a homozygosity mapping approach was used successfully to localise the disease gene in Merino sheep to the same region on chromosome 7 in which the NCL gene was recently mapped in South Hampshire sheep. This region shows conserved synteny to a region on human chromosome 15 in which the human NCL variant CLN6 was mapped. NCL in Merino and South Hampshire sheep are therefore regarded as animal models for the human variant CLN6 and further investigations are undertaken to identify the disease gene.

D080

Effects of leptin polymorphisms on bovine carcass traits

K. TESSANNE, M. E. DAVIS, H. C. HINES, W. GE & J. A. RIGGENBACH

Animal Genetics Laboratory, Department of Animal Sciences, The Ohio State University, Columbus, OH, USA

Effects of leptin polymorphisms on bovine carcass traits were examined in 179 Angus bulls divergently selected for blood serum IGF-I concentration at the Eastern Ohio Resource Development Center. The bulls were born in spring and fall of 1995 and 1996, and in spring of 1997 and 1998. Microsatellites BM 1500 and WDMS (Wilkins & Davey, 1997 Anim. Genet. 28, 376) were genotyped by denaturing PAGE following PCR amplification. SNPs LSNP (Lien *et al*., 1997 Anim. Genet. 28, 245) and FSNP (Fitzsimmons & Schmutz, 1998 PAGVII, P493) were genotyped by agarose gel electrophoresis following digestion by *Bsa*AI and *Aci*I restriction enzymes, respectively. Carcass traits included hot carcass weight, ribeye area, KPH, backfat thickness, marbling score, quality grade, and yield grade. Ultrasound measurements of ribeye area and backfat thickness were made at days 56 and 140 of the postweaning period. Data were analyzed using SAS general linear models procedures. Fixed effects included in the model were year and season of birth, IGF-I selection line, age of dam, leptin genotypes, and a covariate for age of calf. Interactions among the BM 1500, WDMS, LSNP, and FSNP genotypes were tested and were generally found to be unimportant (P>.10). Significant effects were found for FSNP upon ribeye area (P=.03), FSNP upon hot carcass weight (P=.03), and WDMS upon ultrasound ribeye area at day 56 (P=.01). Noteworthy relationships (P<.15) were observed between BM1500 and ultrasound backfat thickness at day 140, WDMS and ultrasound back fat thickness at days 56 and 140, and WDMS and hot carcass weight.

D081

Polymorphism of the *MC1R* gene and feather color in the Chicken : between breeds diversity and within family analysis.

M. TIXIER-BOICHARD, J.L. COVILLE & G. COQUERELLE

INRA- Lab. Génétique Factorielle, 78352 Jouy-en-Josas Cedex, France

The *Melanocortin 1 Receptor* is a candidate gene for the *Extension* locus in the chicken, which controls the distribution of black pigment over the body (Takeuchi et al., 1996, B.B.A., 1306, 122-126). In the present study, the relationship between a missense mutation of *MC1R* (Glu92Lys due to substitution G →A274) and allelism at the *E* locus was investigated with two approaches : (i) a family of 132 chicks segregating for the (G →A274) mutation was produced by mating an heterozygous sire with black phenotype to 8 non-carrier dams (ii) 25 chicken breeds were sampled within the European project AvianDiv, showing various phenotypes corresponding to 5 different alleles known at the *E* locus. The (G →A274) mutation was detected with a PCR-RFLP test. The results from the within-family study showed a tight linkage between *MC1R* and *E* loci because all chicks (n=65) showing the extended black phenotype at hatch (*E*E* allele) were heterozygous carriers of the mutation, but 4 chicks classified as brown (*E*EB*) at hatch were also carriers and showed red plumage at 8 weeks. The between-breeds analysis showed that 4 out of 5 black breeds were homozygous carriers and the 5th one was heterozygous, but the mutation was also found in 3 out of 4 breeds carrying the *E*ER* allele and in 2 brown breeds. Most of the wild-type and the wheaten breeds did not carry the mutation, but 2 over 10 appeared to be segregating. It was concluded that the *MC1R* (G →A274) mutation was necessary but not sufficient to determine the extended black phenotype in the chicken.

D082

Comparison of rapid methods for the detection of mutations in the myostatin locus in cattle breeds of Central Italy.

A. VALENTINI[1], C. MARCHITELLI[1], E. MILANESI[2], P. AJMONE-MARSAN[2], F. FILIPPINI[3] & A. NARDONE[1]

Università della Tuscia, Viterbo, Italy[1];Università Cattolica del S. Cuore, Piacenza, Italy[2] & ANABIC, Italy[3]

In several European beef breeds double muscling can be attributed to mutations at the myostatin locus. While in some populations the alleles are fixed, in others they are still in segregation and there is an objective interest for a genotyping-assisted selection in order to build up homozygous double muscled breeds. We have recently described a new mutation causing double muscling in some individuals of Marchigiana breed. A genotyping of all performance tested bulls of this and of other related breeds of Central Italy (Chianina and Romagnola) is in progress using different techniques, as sequencing, RFLP-PCR, allele specific PCR and BESS (Base Excision Sequence Scanning). Also cows surveyed on farms and showing a relevant muscle development are genotyped. On the 60 Marchigiana individuals analyzed so far we found only the wild type and the described mutation, therefore it seems safe to proceed with rapid and cost effective methods like allele specific PCR, which proved to be quicker and more repeatable than RFLP-PCR. Interestingly, none of the 120 individuals of Chianina and Romagnola analyzed showed such a mutation, although it is reported that in 18th century Marchigiana was crossed with these breeds. For Chianina and Romagnola, the BESS technology allows a quicker screening relatively to sequencing, while revealing at least half of the present mutations and partially identifying also the heterozygotes. The BESS protocol standardized for the specific locus and the first results are reported.

D083

Primer extension preamplification: a tool in embryo diagnostics.

L.H.P. VAN DE GOOR, W.A. VAN HAERINGEN, H. VAN HAERINGEN

Dr. Van Haeringen Laboratorium b.v. P.O. Box 408, 6700 AK, Wageningen, The Netherlands

With the advent of techniques like IVF, IVP and multiple ovulation and embryo transfer, molecular DNA tools to determine genotypes of bovine preimplantation embryos have become of great importance for animal breeding strategies. Biopsied embryos remain capable of normal development after deep-freezing and transfer to recipients. However, only a limited number of cells can be removed from the embryos without markedly reducing their viability. Since the amount of DNA that can be isolated from this few cells is limited, it has been tried to adjust and improve the primer extension preamplification (PEP) for whole genome amplification of embryonic bovine DNA. The PEP method is a PCR-based in vitro method for amplification of a large fraction of the DNA sequences present in a single haploid cell by repeated primer extension using a mixture of 15-base random oligonucleotides.

The PEP amplification efficiency fluctuated much between experiments and between samples amplified during the same experiment. Stringent and sterile setting up conditions are very imported to avoid contamination. Other problems of the PEP method are preferential amplification of one allele at heterozygous loci and non-reproducible results between different primer batches. The just discussed problems rise serious doubts about the potential of a successful and reproducible PEP procedure for the production of good quality bovine embryonic DNA that can be used as template for subsequent PCRs. Other methods are currently under development to achieve the same goal.

D084

Functional characterization of the Atlantic salmon MHC class II promoters.

O. VESTRHEIM, M. SYED & M. LUNDIN.

The Norwegian School of Veterinary Science, Dept. of morphology, Genetics and Aquatic biology, Box 8146 Dep., N-0033 OSLO, NORWAY

The Major Histocompatibility Complex (MHC) molecules are necessary components of the primary immune response. The MHC class II are dimeric cell surface glycoproteins expressed primarily on antigen presenting cells and B-cells. They present short polypeptides to the T-cell receptors which thereby induces the immune response. We have isolated the MHC class IIβ and MHC class IIα promoters from Atlantic salmon (Salmo salar). In order to investigate the activity of the promoters they were cloned into LacZ and Green Fluorescent Protein (GFP) reportergene plasmids and used for transient transfection in an Atlantic salmon head kidney cell line called SHK-1. Preliminary positive results were achieved. In addition, the function of the MHC class II promoter/LacZ reporter constructs is investigated by injection into somatic tissue of the Atlantic salmon. Immune response was measured after two weeks of injection for both the constructs.

D085

Generation of transgenic pigs by sperm-mediated gene transfer using a linker protein (mAb C)

K. CHANG[2], M. WU[3], M. JIANG[4], H. LO[2], C. CHEN[1], Y. LIU[5], F. LING[1], AND K. WANG[1].

1. BioAgri Corp. City of Industry, California, USA; 2. Dept. of chemistry, Soochow University, Taipei, Taiwan; 3. Dept. of physiology, Taiwan Livestock Research Center, Tainan, Taiwan; 4. Dept. of Anesthesiology, UCLA, California, USA, and 5. Center for Craniofacial Molecular Biology, USC, California, USA

Sperm-mediated gene transfer has been recognized as a potentially powerful method to make transgenic animals for many years. It might overcome the difficulty of microinjection reported to have only limit success in higher or larger animals. A monoclonal antibody C (mAb C) has been identified after screening many hybridomas immunized with mouse sperm cells. mAb C is reactive to a surface antigen on mouse sperm cell and is also cross-reactive with sperm cells from many different species such as pig, cow, sheep, goat and chicken. mAb C has been characterized as a basic protein, and is shown to bind DNA through ionic interaction. Therefore exogenous DNA can be specifically bound to the sperm surface via mAb C, and successfully integrated into the chromosome of offsprings in mouse and pig. Furthermore expressed foreign proteins can be detected in the serum of transgenic pigs. Diluted pig sperm cells were mixed with mAb C to form a sperm-mAb complex and pSEAP-2 reporter gene (Clontech) was added to react with the sperm-mAb complex. Then half-million treated sperm cells were injected into each side of the oviduct from ovulating pigs by surgical oviduct fertilization. Forty-three pig offsprings have been analyzed, 30% of piglets' tails were shown to contain exogenous DNA integrated into host genome by Southern blot analysis and PCR. Furthermore 65% of these offsprings were found to express human placenta alkaline phosphatase secreted into the serum. All these data suggest that transgenic pigs can be generated with greatly improved efficiency by sperm-mediated gene transfer using the linker protein, mAb C.

D086

Glycogen storage disease type IV: an inherited deficiency in glycogen branching enzyme in the Quarter Horse

T.L. WARD, S.J. VALBERG, D.B. NAHEY, H. HIRARAGI & J.R. MICKELSON
University of Minnesota, St. Paul, MN, USA

Glycogen storage disease type IV (GSD IV) is an hereditary disease characterized by abnormal polysaccharide inclusions and a decreased glycogen branching enzyme (GBE) activity. Clinically, GSD IV can range from liver failure to fatal cardiomyopathy to mild skeletal muscle myopathies. We now have documented the occurrence of fatal form of GSD IV in seven Quarter Horse foals (1), cardiac arrest (3) and stillborn (1). Increased serum activities of liver and muscle enzymes and leukopenia were present (5). 3 foals died suddenly by four weeks of age and 3 foals were euthanized by 7 weeks of age. Muscle, heart and liver samples from the foals were devoid of normal PAS staining for glycogen and contained abnormal PAS positive inclusions. Accumulation of unbranched polysaccharide was suggested by an alteration in iodine absorption spectra in isolated polysaccharide from tissues of affected foals. Assays for GBE function in multiple tissues showed virtually no activity, whereas activities of control enzymes compared to a normal control. Western immunoblot assays detected no GBE protein in the liver of an affected foal and diminished levels in one suspected carrier as compared to a control horse. Pedigree analysis further supports and automosal recessive trait. Clinical, histological, biochemical, and genetic finding in these horses is analogous to GSD IV as exhibited in humans and cats and may represent an important cause of neonatal mortality in Quarter Horses.

D087

Study of genetic diversity of sub-Saharan African goat populations using microsatellite loci

P.C. WATTS[1], S.W. CHENYAMBUGA[2, 3], J. HIRBO[2], P.S. GWAKISA[3], G.C. KIFARO[3], S.J. KEMP[1], J.E.O. REGE[4] & O. HANOTTE[2].

[1]School of Biological Sciences, University of Liverpool, Liverpool, UK; [2]International Livestock Research Institute, Nairobi, Kenya; [3]Sokoine University of Agriculture, Morogoro, Tanzania and [4]International Livestock Research Institute, Addis Ababa, Ethiopia.

There are at least 90 breeds of African goats. They represent a unique genetic resource due to their adaptation to the local environmental conditions of the tropics. Phenotypic and genetic characterisation of this unique livestock genetic resource is urgently needed for their efficient conservation and management. In this study we report the genetic diversity and relationship of 18 indigenous African goat breeds using 18 microsatellite loci. Within-breed genetic diversity was estimated by the number of alleles per locus and the average observed heterozygosity. Genetic differences between breeds were estimated by Nei's standard genetic (Ds) distance. Breed relationships were assessed by Neighbour-Joining trees, principal component analysis and multidimensional scaling. Average number of alleles per locus ranged from 4.61 to 6.44. Average observed heterozygosity was high (0.661 ± 0.036 (Afar) to 0.552 ± 0.038 (Newala). Ds ranged between 0.033 ± 0.013 (Ugogo and Masai) to 0.477 ± 0.145 (Tswana and Tanzanian Coast). The 18 African breeds cluster in four different groups, the Southern African breeds (Tswana, Ndebele, Venda, Landim), the Ethiopian-Kenyan breeds (Boran, Galla, North East Highland, Afar), the Nigerian breeds (Red Sokoto, West African Dwarf) and the Tanzanian breeds (Ujiji, Sukuma, Masai, Ugogo, Mbeya, Newala, Tanzanian Coast). In general, the relationships among breeds reflect their geographic origins more than the morphological classification based on sizes and the shape of the ears.

D088

Identification and confirmation of differentially expressed genes in developing pig fetuses

S. R. WESOLOWSKI, N. E. RANEY & C.W. ERNST.
Michigan State University, East Lansing, Michigan, USA.

Growth and development of pig fetuses is controlled by the coordinated expression patterns of multiple genes. The objective of this experiment was to identify differentially expressed genes in developing pig fetuses using the differential display reverse transcription PCR (DDRT-PCR) technique. Total RNA was extracted from 3 whole 21 d fetuses and from the anterior, medial, and posterior sections of three 35 d and three 45 d fetuses. For DDRT-PCR assays, RNA samples were pooled from anterior, medial, and posterior sections for each 35 and 45 d fetus. Sixteen differentially displayed fragments were excised, PCR re-amplified, and cloned. DNA sequence analysis resulted in identification of eleven unique gene products. Pig cDNAs were identified with homologies to *collagen type XIV (COL14A1), complement component C6 (C6), vitamin D binding protein (DBP), titin (TNT), craniofacial developmental protein (CFDP1), DNA binding protein B (DBPB), KIAA0456 protein*, and *epsilon globin*. Three novel pig ESTs were also identified. Differential expression of *COL14A1, C6*, and *DBP* was confirmed by northern blot analysis. For those three genes, relative abundance of mRNA increased in fetuses from 21 to 45 d of gestation. The spatio-temporal differential expression of *DBP* was discovered by northern blot analysis with the anterior, medial, and posterior samples. Relative mRNA abundance for *DBP* was high in posterior samples and undetectable in anterior samples. These results provide new information concerning gene expression in developing pig fetuses and indicate that DDRT-PCR is a powerful technique for identifying differentially expressed genes.

D089

Polymorphisms of the porcine *C3* - a candidate gene for generalized defence power

K. WIMMERS[1], S. PONSUKSILI[1], S. MEKCHAY1, T. HARDGE[2] & K. SCHELLANDER[1]

[1]Institute of Animal Breeding Science, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany and [2]Institute of Animal Science, Humboldt University of Berlin, Invalidenstr. 42, 10115 Berlin, Germany.

The complement system is an important defence mechanism of innate immunity. It mediates phagocytosis, controls inflammation and interacts with antibodies in immune response. The complement factor C3 is the central component of the complement system. The classical and alternative pathway of complement activation lead to the formation of a convertase that cleaves C3 to C3a and C3b, the central event of the complement activation. C3b in turn activates the terminal lytic complement sequence, which is identical in both pathways. Because of its central function within the complement cascade C3 is a potential candidate for complement activity. High complement activity can be expected to contribute to increased generalised defence power. We determined the cDNA-sequence of the porcine *C3-gene* (GeneBank accession number AF154933). The porcine cDNA (5131 bp) shows 82% identity to the human homologue. The sequence codes for 1661 amino acids that show 86% identity to the human C3 protein. Primer pairs where derived in order to amplify overlapping fragments of about 500 bp suitable to be screened for polymorphism by DGGE and comparative sequencing. PCR-fragments were produced from liver cDNAs of eight animals of the breeds Duroc, Hampshire, Pietrain, German Landrace, Duroc x Berlin Miniature Pig (F1 and F2) and were screened for polymorphism. Three polymorphic sites were found. PCR-RFLPs were derived suitable to be applied on genomic DNA. The polymorphic sites were found to segregate in our porcine F2-resource families (Berlin Miniature Pig x Duroc) in Mendelian manner.

D090

Genetic polymorphism of 12 microsatellites in the Polish native horse Konik Polski

B.GRALAK, C.NIEMCZEWSKI, Z.JAWORSKI & M.ZURKOWSKI

Institute of Genetics and Animal Breeding, Jastrzebiec, 05-551 Mroków, Poland

Konik Polski is the native horse breed kept in Poland as genes reservation. This study is concerned with the conservation of its biodiversity and autochthonous genetic resources. Genetic polymorphism of 12 microsatellite loci (*AHT4, AHT5, ASB2, HTG4, HTG6, HTG7, HTG10, HMS2, HMS3, HMS6, HMS7, VHL20*) was studied in 215 individuals. Genotyping was performed on an ABI 377 DNA Sequencer. The results showed high polymorphism of 10 tested markers (6-9 alleles). Locus *HTG6* was monomorphic and in locus *HTG7* 3 alleles were identified. The highest heterozygosity was observed in loci *HMS6* (0.87), *ASB2* (0.82) and *HTG5* (0.79). The values for heterozygosity of 8 microsatellite markers ranged from 0.65 to 0.75.

D091

Genetic polymorphism at *Mhc B* and *Rfp-Y* in the Camperos broiler chickens

G.M. IGLESIAS[1], R.M. GOTO[2], M.C. MIQUEL[1], O.J. LOPEZ[1] & M.M. MILLER[2]

[1]Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Argentina and [2]Beckman Research Institute of the City of Hope National Medical Center, Duarte, CA, USA

Camperos broiler chickens are produced at INTA, Argentina (see L. Soria et al., this volume). They are the F1 generation of two cross-breeds that have been maintained essentially without selection for 7 generations. With the aim of determining the degree of *Mh*c variability in these broilers, 51 individuals (15 males from the male parental breed, 18 females from the female parental breed and 18 Camperos) were analyzed with restriction fragment pattern typing methods utilizing probes specific for *B-G*, *B-F* and *Y-F* genes. The two *B-G* probes used in this analysis were: *G412*, a 752 bp probe generated by PCR from exon 1 (signal peptide), intron 1, and exon 2(IgV-like domain) of a Camperos *B-G* gene; and *bg28*, a 525 bp cDNA probe corresponding to a portion of exon 2 (IgV-like domain), exon 3 (transmembrane domain) and exons 4-11 (heptad domains) of a Leghorn *B-G* gene. We were able to identify a total of at least 45 *B-G* genotypes among the 51 samples. Some genotypes were distinguishable only with *G412* and others only with *bg28* suggesting that restriction fragment polymorphisms in these chickens are likely present in exons corresponding to both extracellular and intracellular domains. A *B-F*-specific probe revealed far fewer restriction fragment patterns at *B-F* loci in these chickens even though there is other evidence for *B-F* variability. The *Y-F* specific probe, *163/164f*, revealed the presence of at least 44 different *Y-F* genotypes assorting independently from the *B-G* genotypes. Thus, there is very high genetic variability at the *Mhc B* and *Rfp-Y* gene clusters in this broiler breed, considerably greater than that present in the Leghorn control population used in this study.

D092

Genetic biochemical polymorphism of Yaroslav cattle and its intercommunication with other representatives of Bovine

A.F. GORBACHEV, A.M. MASHUROV[1] , V.F. MAXIMENKO[2], A.V.BUDNIKOVA[3], A.F. NAZAROVA[1] , S.M. ALKHUTOV[1], & L.V.STRELCHENKO [1]

Ural research Institute of Agricultural, Ekaterinburg, Russia; [1] Institute of ecology and evolution RAS, Moscow, Russia; [2] Yaroslav Research Institute of Animals Breeding and Forage Production, Yaroslav region, Russia; [3] All-Russian Institute of Animals Breeding, Moscow region, Russia

There were investigated 62 antigenes of 11 EA-systems by 2845 animals of Yaroslav breed. We did not discover the Z' and M antigenes (in EAA and EAM systems accordingly). It was calculated the indices of similarity between Yaroslav cattle and with 86 other representatives of Bovinae (n=110000). The least similarity (r=0,5108-0,6821) this breed showed with buffaloes, yaks, aurochs and zebu and with 3 beef breeds: Chianina, Limousine, Svetlaja Akvitanskaja (r=0,7152-0,7343) and the largest similarity with the 4 craniological groups (r=0,8566-0,8730): B.t. brachyceros (Alatoo, Aberdin-Angus, Pinzgau), B.t. primigenius (Black and White ukrainian, Angeln) and B.t. brachycephalus (Red humped). This is demonstration that the Yaroslav cattle was created on the basis of Middle Russian cattle use of the method of nationality selection. Were calculated frequencies of alleles some blood proteins: TFA=0,481, TFD=0,467,TFE=0,052; HBA=0,899, HBB=0,101; CPA=0,192, CPB=0,808; CAS=0,848, CAF=0,152; fixed at 3 alleles of esterasa : ESS, ESP and ESO, and Alkaline Phosphatase: PPS, PPO and PPF. The frequencies alleles of milk proteins was following: CASA1B=0,807, CASA1C=0,193; CASBA=0,759, CASBB=0,238, CASBC=0,003; CASKA=0,383, CASKB=0,617; LGBA=0,350, LGBB=0,650. The Yroslav breed very discriminated from Europe breeds more frequencies of CASA1C allel.