A001

Analysis of genetic variation among five Chinese indigenous goat breeds by using bovine and ovine microsatellites

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Genetic variability has to be taken into account to guide genetic conservation programs. This study was conducted to determine the genetic variation between five Chinese indigenous goat breeds by using microsatellites from cattle and sheep. Blood samples from five Chinese indigenous goat breeds, Liaoning cashmere (33), Inner Mongolian cashmere (35), Tibetan cashmere (37), Wuan cashmere (49), and Matou goats for meat production (39) from Hubei province, were collected from random individuals. Fifteen pairs of microsatellite primers from cattle (BM1842, BM4621, BM6444, BM6506, BM757) and sheep (OarCP20, OarCP34, OarCP49, OarFCB11, OarFCB11, OarFCB128, OarFCB20, OarFCB266, OarFCB304, OarFCB48, MAF33, McM218) were tested to detect polymorphisms among those breeds. More than four alleles were found at six loci (BM4621, OarCP34, OarFCB11,OarFCB20, OarFCB304, OarFCB48) and generated a total of 57 alleles from the 5 breeds and 193 individuals analyzed. The other nine microsatellites were not used to assess genetic diversity because they were monomorphism or produced non-specific bands. Within-breed variation was analyzed. Heterozygosity, polymorphism information content and effective allele number had similar tendencies among breeds; Taihang had the highest values, followed by Neimonggol, Liaoning, Matou, and Tibetan breeds, in that order. Total gene diversity, average heterozygosity within each population, and coefficients of gene differentiation between breeds were calculated. The mean values of the three parameters are 0.831, 0.800, 0.038, respectively. The results show that variation among the breeds is low.

A002

Comparison between serological and molecular genetic tests for parentage control in pigs in Germany.

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For more than 40 years, blood typing for parentage control and blood type cards have been offered to pig breeders by the Goettingen Institute. The test sera were self produced and controlled in ISAG Comparison Tests. For blood group testing we used the following systems, depending on the available test sera: *A-S, D, E, F, G, H, K, L* and 3 electrophoretic systems: *EsD, GPI, PGD*. We calculated a cumulative exclusion probability (CEP) of 97.5% in 1980 over all breeds. After 20 years of intensive selection, we now decreased to a *CEP* of about 90%, which means that within the breeds gene frequencies have changed and thereby biodiversity was lost, particularly in the smaller German Large White and Hampshire breeds. (*CEP*: 94,52% in German Landrace, 83,31% German Large White, 91,93% Pietrain and 85,44% in Hampshire). After introduction of PCR techniques mainly the microsatellites raised in importance for identification of individuals and parentage control. In collaboration with the Institute in Vienna, we prepared and tested a multiplex PCR with 10 microsatellites . They were tested on the same pigs which were blood typed and CEP was 98,84% in German Landrace, 98,80% in German Large White, 99,57% in Pietrain, but only 94,51% in Hampshire. In all breeds, except the German Large White, the polymorphism information content (PIC) of the microsatellites SW240, SW857, S0155 and SW24 were the highest with more than 0.60. S0227 had the lowest *EP* in all breeds and was even fixed in Hampshire and should be replaced. The aim is to reach a CEP of more than 99.5% in all breeds.

A003

Potential for breed assignment of horses

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Degree of population demarcation was investigated in eight European horse breeds, of which four represent Norwegian breeds. Altogether, 306 individuals were genotyped for 26 microsatellite loci. Two approaches were applied to assess potential for correct breed assignment: simple allele sharing statistics, and genotype simulation based on breed-specific allele frequency distribution. A clear breed differentiation was detected in the phylogenetic analysis based on allele sharing, and 95% of the individuals clustered together with animals of the same breed. Even breeds with a short period of breed divergence formed distinct clades. Moreover, the majority of the simulated individuals were assigned to their source population, but with this method potential of correct assignment was negatively correlated with genetic variation within a breed. In conclusion, the study demonstrated obvious distinction among horse breeds, and simple allele sharing statistics provided the most reliable information for testing the breed of individual animals.

A004

Microphylogenesis of antigens in blood of nine strains of Kazakstan bulls

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The progress in human and animal microevolution and phylogenesis study allows scientists to use serology blood testing in the systematization of molecular taxonomy, or the immunogenetic and biochemical classification of cattle strains. As a result of antigen funds, alleles and genotypes studying unique scientific data were obtained on genetic structure peculiarities and their evolution in bulls of basic cattle breeds (alatauskaja, auleatinskaja, kazakh whitehead, black and white, golshtinofrizskaya, brown latvian, simmental, red stepee, anglerskaja), which are used at the Kazakstan artificial insemination stations, taking into account fact that they influence significantly the allelofund lines and related groups of animals of corresponding strains of cattle. Indices of immunogenetical distance (Ds and Dn) between populations were then determined. Distance varied from 0.1107 to 0.2260, or by 11.53%. This means that the differences between breeds are almost the same as within the breeds. So the reserve of immunogenetical variability within the breeds is wide. In order to receive more intergrated and obvious picture of immunogenetical relations between investigated populations the dendroprogram and the pattern of these populations position in nonlinear coordinates plane were obtained. So studying cattle allelofund of blood genetical markers will help to preserve, use rationally and enrich the genetical resources of Kazakstan animals.

A005

Cytogenetical investigation and teratological screening of some Kazakhstan sheep breeds

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In 260 sheep of Karakul, Kazakh semifine wool and Edilbai breeds of different age and sex, the frequency of spontaneous chromosome aberrations was estimated. These sheep populations had different phenotypes, productivity level and were bred in different environment regions of Kazakhstan. To estimate qualitatively and quantitatively the mutation process dynamics induced by environmental mutagens and teratogens the frequency of lamb births with developmental anomalies was also studied. In Karakul sheep populations lamb anomalies were 1.5-2 times more frequent than in the Kazakh finewool sheep population (0.08±0.01% and 0.04±0.01%, respectively). In the course of teratological screening of the dam population, 474 ewes with different organ and tissue defects were found as well as 13 fetuses with developmental anomalies. Sixty-three types of inborn anomalies were detected, the descriptions of 18 types of inborn anomalies and 24 teratology variants were not observed in the available publications on sheep genetics. The specific feature of the research undertaken was the screening of the same sheep flocks for as long time as 2-3 generations changed one another. So the bone marrow cells chromosomes were studied not only for normal animals but also of 414 lambs with inborn anomalies, 50 spontaneous abortuses and fetuses of different age, 23 deadborn lambs, 14 infertile dams and 24 animals with different abnormalities. The extensive cytogenetical and teratological data allowed us to characterize the spontaneous and induced mutagenic process dynamics expressed at the chromosome, genome and morphologic levels in the breeds and animal populations bred in different environmental conditions. The unfavorable ecological situation on the vast territories of Kazakhstan includes the influence of many years long nuclear tests on the Semipalatinsk test territory and declined Aral sea level.

A006

Genetic markers at goats

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The knowledge of genetic markers, in particular of blood groups of goat until now is very much limited. Taking into account, that there is no systematization of blood groups of goat, because of generalities of antigenes sheep and goat while 4 systems of blood groups *A*-system (*Aa*- antigenes), *B* - system (*Bb*-antigenes), with *C*- system (*Ca* -antigenes), *R*-system (*R*- antigenes) are chosen. Alongside with these common antigenes the analysis of various breeds and crosses of goat on 41 alloreagents was conducted. Three breeds of goats (Altai Mountain, Orenburg, Saanen), and also two crosses 1/2 Saanen x Altai Mountain and 3/4 Saanen x Altai Mountain were investigated. The frequency of each antigene is calculated. On the basis of computer program of a method "Manhattan distances" their appropriate clasterisation was conducted. Dendrogramma which consists of 4-th dem is constructed: I - crosses 1/2 Saanen x Altai Mountain and 3/4 Saanen x Altai Mountain; II - actuates dem I and Altai Mountain. All of them form a separate cluster; III - to the given cluster adjoins Saanen goats; IV - actuates a Orenburg breed of goat. At investigated animals the frequency alleles *HBA, TFA* prevail. It is established, that the milk from Saanen goats with $_{s1}$-*Cn* by a genotype has best cheesemaking by properties, than with $_{s1}$-*Cn* and $_{s1}$-*Cn* by genotypes. Milk from an animal with a genotype as_1-*Cn* had mean speed of curtailing, the obtained bunch was of a dense, elastic consistence and appeared to be the most desirable for production of cheeses and cottage cheese. Milk from an animal with $_{s1}$-*Cn* genotype was fast contracted. The obtained bunch was fragile and fragile, that appeared to be not suitable for cheesemaking. On the contrary, milk from an animal with $_{s1}$-*Cn* genotype under rennet effect was contracted slowly. The bunch was received flabby on the consistence.

A007

Genomic constitution of the wild gynogenetic triploid crucian carp (*Carassius auratus langsdorfi*)

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The so-called ginbuna (*Carassius auratus langsdorfi*, Japanese silver crucian carp) is widely distributed in Japan and occurs in the triploid form as well as the diploid form. Remarkably, the triploid ginbuna reproduces gynogenetically in nature, giving rise to clonal offspring. However, little is known about the genetic background or origin of the triploid due to a lack of diagnostic morphological features. Our goal is to elucidate it through DNA analyses. We have so far been focusing on characteristic repetitive DNA sequences and mitochondrial DNA of the ginbuna, and have proposed that the triploid ginbuna might have arisen from hybrids to which the diploid ginbuna, the ancestor of the goldfish, and unknown (sub)species collectively contributed. To foster a better understanding of the triploid genomic constitution, we isolated novel genetic markers using the genomic subtraction method Representational Difference Analysis (RDA). Three series of RDA (restriction enzymes, *Bgl*II, *Hin*dIII or *Bam*HI; subtraction of triploid ginbuna amplicon from diploid ginbuna or goldfish amplicon) yielded three valuable markers; two probes detected restriction fragments existing only in most of the triploid ginbuna and all goldfish examined, whereas a probe detected a fragment in a particular clonal line of the triploid ginbuna and several individuals of the diploid ginbuna. This study provides additional evidence for the genomic contribution by the ancestor of the goldfish and the diploid ginbuna to the triploid hybrid. Further isolation of genetic markers is ongoing.

A008

SWISS-PROT/TrEMBL/InterPro/CluSTr and animal genetics

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SWISS-PROT is a manually curated protein sequence database that strives to provide a high level of annotation, a minimal level of redundancy, and a high level of integration with other databases. SWISS-PROT and its computer-annotated supplement TrEMBL provide the user with a tool for various aspects of animal genetics. The highest represented domestic animals are cow (2556 entries), chicken (2409 entries) and pig (1969 entries). InterPro, an integrated resource for protein families, domains and functional sites, and CluSTr, a database of sequence clusters, are new tools for proteome analysis with pointers to SWISS-PROT and TrEMBL.

A009

Mouse Genome Database: A resource for comparative genomics

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The entire sequence of the human and mouse genomes will be available within the next few years. Genomics initiatives on agriculturally important animal species are underway to provide cross-reference to the mouse and human maps. As data from these projects are integrated, functional genomics will use animal models to identify candidate genes for analogous functions, determine gene interactions in different contexts and finally assess their contribution to human phenotypes. Ultimately, comparative genomics will be used to address some of the most fundamental issues in evolutionary and basic biology. The Mouse Genome Database (MGD) is the community database for the laboratory mouse dedicated towards providing an integrated representation of mouse genomic and biological information. MGD currently provides a scientifically curated homology dataset primarily extracted from literature for a select group of mammalian species. The coming flood of genomic sequence data from human and mouse will shift the emphasis of data curation from literature-driven to curator-driven annotation. MGD has developed a set of criteria that support homology assertions to aid in this annotation. Oxford grid displays and whole-genome map views can also be used to generate chromosome-wide and genome-wide graphical representations of homology. A feature of gene annotation in MGD is the use of controlled vocabularies for the description of the molecular function, biological process and cellular component of gene products as part of the Gene Ontology (GO) project. These terms can be used as attributes of gene products across species aiding in the development of comprehensive comparative maps and facilitating queries across multiple databases. This will aid in the transfer by inference of biological information to non-model organisms.

A010

The use of a genetic algorithm in mapping of multiple interacting QTL

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Here we describe a general method for improving computational efficiency in simultaneous mapping of multiple interacting quantitative trait loci (QTL). The method uses a genetic algorithm to search for QTL in the genome instead of an exhaustive enumerative (step by step) search. It can be used together with any method of QTL mapping based on a genomic search, since it only provides a more efficient way to search the genome for QTL. The computational demand decreases by a factor of about 130 when using genetic algorithm-based mapping, instead of an exhaustive enumerative search for two QTL in a genome size of 2,000 cM using a resolution of 1 cM, for example. The advantage of using a genetic algorithm increases further for larger genomes, higher resolutions and searches for more QTL. We show that a genetic algorithm-based search has efficiency higher than, or equal to, a search method conditioned on previously identified QTL for all epistatic models tested, and that this efficiency is comparable to that of an exhaustive search for multiple QTL. The genetic algorithm is thus a powerful and computationally tractable alternative to the exhaustive enumerative search for simultaneous mapping of multiple interacting QTL. The use of genetic algorithms for simultaneous mapping of more than two QTL, and for determining empirical significance thresholds using permutation tests, are also discussed.

A011

Genetic analysis of X-linked anhidrotic ectodermal dysplasia (EDA) in Cattle

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In a family of German Holstein Cattle three male, maternal half-sibs with congenital hypotrichosis and abnormal sweat glands were observed. Furthermore, the affected calves almost completely lacked teeth. They had no incisors and only very few and deformed molars. The pedigree of the investigated Holstein family suggested X-linked inheritance of the genetic defect as only male animals were affected. In order to further characterize the genetic defect responsible for the observed phenotype, 20 microsatellite loci on the X-chromosome were genotyped in 10 members of the family. Haplotype analysis showed that the mutation is located on the chromosomal segment flanked by the proximal marker BMS513 and the distal marker BMS2798. Syntenic regions on the human and mouse X-chromosomes were derived from the respective comparative maps and analyzed for the presence of candidate genes, which might be causative for skin and tooth abnormalities in cattle. Interestingly, a similar phenotype was already described for human patients with anhidrotic ectodermal dysplasia (EDA) as well as the mouse mutant Tabby. In human and mouse it was shown that defects in the X-chromosomal ectodysplasin A gene (*EDA*) are causative for this disease. Therefore, the bovine homolog of the human *EDA* gene seems to be a strong candidate for the affected gene in this hereditary disease.

A012

Detection and parameter estimation for dominance effects of quantitative trait loci

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Detection of dominance effect is important for the detection of a quantitative trait locus (QTL), because methods to detect additive effects would fail to detect the QTL when dominance is the primary QTL effect. Detection of dominance effect is also important to understand the QTL functions. Dominance effect of a QTL can be detected using a marker contrast between the heterozygous and the homozygous marker genotypes. Once a QTL is determined to be present, its location can be determined by estimating the marker-QTL recombination frequency. The estimation of the QTL location needs to consider three cases: (1) the chromosome contains only one QTL; (2) additional linked QTLs exist to one side of the target QTL, and (3) additional QTLs exist on both sides of the target QTL. Formulations to estimate the marker-QTL recombination frequency for these three cases are as follows

$$\theta_{Aq} = \text{‰}\{1-[(1 \bullet 2\theta_{AB})\ w_1]^{¼}\}$$

for case (1)

$$\theta_{Aq} = \text{‰}\{1 \bullet \{1 \bullet \frac{(1 \bullet 2\theta_{AB})^2}{(1 \bullet 2\theta_{AB})^2 + [1 \bullet (1 \bullet 2\theta_{AB})^4] w_2}\}^{¼}\}$$

for case (2)

$$\theta_{Aq} = \text{‰}\{1-[\frac{w_3 (1 - 2\theta_{AB})^2 + (1 - 2\theta_{AB})^4}{1 + w_3 (1 - 2\theta_{AB})^4}]^{¼}\}$$

for case (3)

where θ_{Aq} = recombination frequency between flanking marker *A* and the target QTL, θ_{AB} = recombination frequency between flanking markers *A* and *B*, and w_1, w_2 and w_3 are ratios of partial regression coefficients for cases (1-3) respectively. Dominance effect of each QTL is then estimated based on the estimated marker-QTL recombination frequency and the marker contrast that was used to detect the dominance effect.

A013

Development and characterization of Japanese quail microsatellite markers and their utility in chicken

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Japanese quail (*Coturnix japonica*) is a member of the family Phasianidae. It is valued for its egg and meat and also used widely as a laboratory research animal. In order to promote the construction of a quail genetic map and the construction of a comparative genetic map in Phasianidae, this study was conducted with the aim of isolating original microsatellite markers in Japanese quail and determining their utility as cross-reactive markers between chicken and quail. A Japanese quail genomic library enriched for (CA/GT)n simple sequence repeats was screened, and then positive clones were sequenced. PCR primer-pairs complementary to unique DNA sequences flanking microsatellite repeats were designed so as to amplify DNA fragments. Optimal conditions for PCR were determined for quail and the markers were also tested on chicken using White Leghorn and Fayoumi DNA as templates. Out of a total of 368 positive clones that were sequenced and characterized, 50 original microsatellite sequences were isolated. Amplification products were obtained in 14 (28%) of the markers tested on chicken DNA at the annealing temperature optimized for quail. Mapping of markers by quail and chicken reference families is underway for the development of a genetic map for Japanese quail and the eventual construction of a comparative genetic map in Phasianidae.

A014

Microsatellite-based population structures within and between five Finnish dog breeds

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Variabilities within and between Finnish populations of Golden Retrievers, German Shepherds, Wirehaired Daschunds, Pembroke Welsh Corgis, and Bedlington Terriers were quantified with ten microsatellite loci and a sample of 50 individuals from each breed. Highest genetic diversity was exhibited in the Wirehaired Daschunds (mean allele number = 8.0; mean H_E = 0.72) and lowest in the Bedlington Terriers (mean allele number = 5.2; mean H_E = 0.56). Although statistically significant deviations from H–W equilibrium were observed, they occurred at an unexpectedly low frequency. Interestingly, the extremely small Bedlington Terrier population displayed genotypes in H–W proportions in all investigated loci. Genetic differentiation between the breeds was very large (F_{ST} = 0.182-0.266; D_A = 0.365-0.466). These estimates markedly exceed those estimated between some livestock populations. Exemplifying the level of differentiation, the highest D_A distances were only slightly lower than the lowest values inferred between humans and chimpanzees. The present data imply severe bottlenecks, genetic isolation and intense artificial selection in the history of these breeds of dogs.

A015

Mitochondrial diversity and the origins of North East Asian cattle

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The origins of North East Asian domesticated cattle are unclear. The earliest domestic cattle in the region were *Bos taurus*, which may have been domesticated from local aurochsen or perhaps had an origin in migrants from the early domestic center of the Near East. In this study, complete mitochondrial DNA displacement loop sequences from 30 Korean and 44 Mongolian native cattle were sequenced. Korean native cattle revealed altogether 32 sites of base substitution and 23 haplotypes, and all were taurine (*Bos taurus*) mitochondrial haplotypes. Mongolian native cattle showed two subspecies mitochondrial haplotypes, i.e. taurine and zebu types. Nine Mongolian animals showed five sites of base substitution and four zebu haplotypes, and the rest of the animals exhibited 40 sites of base substitution and 32 taurine haplotypes. The data of taurine haplotypes were analyzed with published sequences of taurine mtDNA from African, European and Japanese animals. In phylogenetic analysis with 76 haplotypes, taurine sequences form at least five clusters. The average sequence divergences among them were from 0.5 % to 0.9%. These clusters may represent different strains of ancestral aurochs, adopted at geographically and temporally separate stages of domestication in the old world.

A016

Association between blood group markers and first milk yield in the GIR breed

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Blood typing results of 138 cows from a Gir breed herd (a zebu breed), closed by approximately 30 years and submitted to controlled matings, were studied to verify association among the B, F, J, L and Z blood factors or phenogroups and the first milk yield. Significant results were found in first lactation between the animals presenting the Z blood factor (3634.43 kg, P<0.001), compared with the ones lacking factor Z (3074.62 kg, P<0.05). For the B blood group system, through means contrast, significant results were observed comparing the animals homozygous for the I1O1Y2A'B'E'3(J'K')P'Q' (4202.86 kg, P<0.05) and heterozygous B(P)QTE'3G'P'/ I1O1Y2A'B'E'3(J'K')P'Q' (3493.33 kg, P<0.05) and BQTA'B'I'(P')/ I1O1Y2A'B'E'3(J'K')P'Q' (3630.36 kg, P<0.05).

A017

Development of a Bovine Whole Genome Radiation Hybrid Map for Comparative Mapping Across Species and the Identification of Positional Candidate Genes for Genetically Mapped Traits.

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Positional candidate gene cloning is currently the most successful method of identifying trait genes. This project is establishing a whole genome radiation hybrid (WGRH) map in cattle. A framework map is being constructed from large number of microsatellite markers, and this will tie the WGRH map to existing linkage maps. High-resolution comparative links to maps in other species will be achieved by placing a large number of genes and ESTs on the map and will provide positional candidates genes for QTL. Two WGRH panels have been created from a primary bovine fibroblast line fused to hamster fibroblast lines deficient in either HPRT of TK (Wg3H and A23 respectively). For one panel, bovine fibroblasts were irradiated at 3000 rad dose and for the other a 10,000 rad dose was used. The 3,000 rad dose used in Cambridge has already produced RH panels in other species with resolutions ranging from 1Mb to 145Kbp.

The 3,000 rad Bovine panel used the Wg3H recipient and was initially comprised of 224 hybrid cells. These were characterised by PCR and FISH and 94 cells were selected to give the final panel (designated Tm112). On primary characterisation with 33 markers average retention of the Tm112 panel was 28% (15-30%). The Tm112 panel has been subjected to large scale culture and DNA extraction and will be available to the research community from Research Genetics Inc. Collaborators will have access to RH-mapping data via a bovine RH-database hosted at Roslin and INRA.

A018

EST mapping in the pig – a new dimension to comparative mapping

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Radiation hybrid (RH) mapping has come of age in the pig. This is evident from the mapping of over 1300 markers using this approach. Among the various markers hitherto mapped in the INRA-Minnesota RH panel (IMpRH), expressed sequence tagged sites (ESTs) are of specific significance. These markers align the porcine gene map to the human gene map, thus adding to the comparative status between the two species. A total of over 120 ESTs generated from a porcine large intestine cDNA library were mapped to different pig chromosomes using the somatic cell hybrid (SCH) and IMpRH panel. Prior to the mapping efforts, *in silico* analysis of the 5'sequences from the ESTs indicated that they represent porcine genes that are orthologous to genes already mapped in humans. The findings together with the available cytogenetic mapping data helped locating the markers to specific chromosomal regions in the pig. The mapping information was used to verify and strengthen correspondence between the pig and human genomes. Further, the data were also related to the available Zoo-FISH results between the two species. A genome-wide overview of the mapped ESTs will be presented along with comparative status with the humans.

A019

A complete exon is missing in the L-gulono-gamma-lactone oxidase gene (*GULO*) causing vitamin C deficiency in the pig : a DNA-based test for the diagnosis of the deficient allele

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Some years ago, a mutant strain of Danish pigs lacking the ability to produce L-ascorbic acid (L-AscA) was discovered, and clinical cases with scurvy were described in swine for the first time. This trait was shown to be controlled by a single autosomal recessive allele designated *od* (osteogenic disorder). In a previous study we demonstrated a close linkage of *od* to microsatellites on pig chromosome 14. In addition, the L-gulono-gamma-lactone oxidase gene (*GULO*), a candidate gene for the defect, was mapped to the same chromosome region by *in situ* hybridization (FISH). *GULO* is the critical enzyme which catalyses the terminal step of the biosynthesis of L-AscA in the liver of a variety of mammals. The objective of this study is to find a molecular defect in *GULO* that is associated with vitamin C deficiency. A cDNA clone for this enzyme was isolated by screening a pig liver cDNA library using a 545-base pairs (bp) pig *GULO*-specific probe obtained by cross-species PCR from rat and guinea pig sequence information. The cDNA clone contained 1838 bp with an open reading frame of 1320 bp similar to the rat sequence. We found that at the cDNA level of the *GULO* gene vitamin C-deficient animals were devoid of exon VIII. At the genomic level, sequencing analysis indicated that the last 382 bp of intron VII, the complete exon VIII and the first 182 bp of intron VIII were missing, as a result of an insertion of a porcine nucleotide sequence of about 2.5 kbp preceded by a 61-bp SINE sequence. These findings allowed us to perform a PCR-based test to discriminate normal *OD*/*OD*, *OD*/*od* and deficient *od*/*od* pigs.

A020

Comparative mapping of a cattle trypanotolerance QTL region on *Bta* 7

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Quantitative trait loci (QTL) influencing trypanotolerance have recently been mapped in a broad region on bovine chromosome 7 (*Bta* 7). Given the limited resolution of available livestock maps, data from the marker-rich human or mouse genomes was exploited to improve the mapping resolution of the identified QTLs. Two whole genome (WG) radiation hybrid (RH) panels were used for high-resolution comparative mapping. A total of 37 DNA markers, comprising 17 genes (6 previously unmapped), 17 microsatellites and 3 STS (generated by microdissection) were mapped by PCR against the 5000rad RH panel. In addition, a higher resolution radiation map is presented for 22 of these markers using the 12000rad RH panel. These maps are compared to published linkage maps of *Bta* 7. Comparative mapping confirms the regions of conserved synteny between cattle, human and mouse in the QTL region. However, the linear order of genes appears to differ; gene orders between cattle and human being more conserved than between cattle and mouse. Also, a previously unknown small region of conserved synteny between *Bta* 7 and a major trypanotolerance QTL region on *Mmu* 17 is revealed. The linkage maps and RH maps are generally in good agreement. The refined comparative map should allow more accurate selection of candidate genes and the narrowing down of the *Bta* 7 trypanotolerance QTL region to a region small enough to facilitate marker assisted selection or introgression of trypanotolerance.

A021

Improvement of the comparative map of Chicken linkage group E48C28W13W27 to Human chromosome segment 5q23-q35

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Six genes mapped on chicken linkage group E48C28W13W27 (74 cM) are homologous to genes on the human chromosome (HSA) 5q23-q35 region. To improve the chicken-human comparative map of linkage group E48C28W13W27, the Wageningen BAC library has been screened for BAC clones of these 6 genes (*MSX2, SPARC, POU4F3, SPOCK, CDX1* and *CAML*). In addition, 14 microsatellite markers located on E48C28W13W27 were used for screening the BAC library. So far, 54 different BAC clones have been isolated and are used to build a contig. Two genes (*SPARC* and *SPOCK*) are located on the same BAC clone. Eleven BAC clones have been sub-cloned and will be used for shutgun sequencing in order to perform sequence comparison to other species. In addition, a BLAST search was performed with genes from HSA 5q31 to find homologous chicken sequences. Primers of these sequences have been designed and were used to screen the chicken BAC library. BAC clones have been isolated for three chicken genes (*FGF1, DTR* and *UBE2*). Our results improve the chicken-human comparative map of linkage group E48C28W13W27 and HSA 5.

A022

Milk protein genetic variation in Southwestern European cattle breeds

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The first milk protein genetic variants in cattle were detected in the 1960's, and since then many new variants have been detected in local cattle breeds. Presently, many of the world local cattle breeds are threatened with extinction and thus the study of their genetic diversity is an urgent conservation priority. As a result of many years of artificial selection, Iberian cattle breeds became well adapted to the constraints of the Mediterranean environment. However, changing commercial demands have driven the majority of these breeds to the verge of extinction. We have analysed the genetic variation at milk protein loci from several breeds of western Iberian cattle and compared our data with those observed in other breeds. Genetic variation at the αS_1-Casein (CSN1S1), β-casein (CSN2), αS2-casein (CSN1S2), k-casein (CSN3, α-lactoalbumin (ALA) and β-lactoglobulin (BLG) *loci* was identified by isoelectric focusing techniques. The observed gene frequencies were statiscally analysed with Principal Component Analysis and Reynold's genetic distances were used to construct Neighbour Joining and UPGMA unrooted trees. We observed i) an inversion of allele frequencies in certain breeds, ii) a small genetic differentiation between beef cattle and dairy cattle breeds, and iii) a similarity between African breeds from *B. indicus* and *B. taurus* and Southern Iberian cattle. It is suggested that genetic drift was the main factor shaping the genetic structure of these breeds, whereas the crossing between neighbouring breeds contributes to their genetic homogenisation. The genetic similarity between Southern Iberian and African breeds, needs to be clarified by further analysis.

A023

PCR-RFLPs of four genes (*OTC*, *TBG*, *ANT2* and *FMR1*) and their linkage mapping on porcine chromosome X

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Positional candidate gene cloning of genes accounting for significant genetic variation of a QTL ultimately requires alignment of the human and swine maps for which linkage mapping of comparative markers is essential. PCR fragments were amplified and sequenced, and RFLPs found within the porcine *OTC* (ornithine carbamoyltransferase), *TBG* (thyroxine-binding globulin), *ANT2* (adenine nucleotide translocator 2, fibroblast), and *FMR1* (fragile X mental retardation syndrome protein 1) genes. Multi-point linkage analyses were performed in the USDA-MARC backcross pedigree (Rohrer *et al.* 1994 Genetics 136, 231) using CRI-MAP, version 2.4. Gene location on the USDA-MARC linkage map (Rohrer *et al.* 1996 Genome Res. 6, 371) of SSCX follows: *OTC* at position 44 cM, final order *SW2126* - 8.4 cM - *OTC* - 2.3 cM - *SW2470*; *TBG* at position 76 cM, final order *SW1346* - 1 .2 cM - *TBG* - 2.3 cM - SW2476; *ANT2* at position 90 cM, final order *SW1943* - 3.0 cM - *ANT2* - 8.0 cM -S0501; and *FMR1* at position 124 cM, final order *SW2059* - 5.0 cM - *FMR1* - 4.0 cM -*SW2588*. Even after the addition of these data there is no evidence of rearrangements in gene order between porcine and human X chromosomes.

A024

Genetic differentiation within a new Polish horse breed

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During the 1970s and 1980s, horse breeders in Poland used Trakehner stallions from East Germany crossed to Polish mares to produce sport horses. The success of this program lead to other breeding efforts involving crosses of stallions from western countries with Wielkopolska mares. This lead to the establishment of the Polish Warm Blood Horse (PWBH). In this study we examine genetic variation based upon seven blood group and ten biochemical genetic loci in the PWBH. We also examine genetic differentiation within the breeds based upon differences in the breed of stallion used to establish lines within the PWBH. Within the breed, genetic variation as estimated by expected heterozygosity was somewhat below the mean for domestic horses within each different stallion breed line. This was unexpected as crosses between different breeds would be predicted to produce higher variation. The lowest variation was within the line derived from Sella Francais stallions with $He = 0.29$. For the other six stallion breed lines He ranged from 0.32 to 0.36. The greatest degree of differentiation (as shown by genetic distance and Fst) was among individuals derived from Sella Francais stallions as compared to all other stallion breed lines. Overall, there was little differentiation seen among stallion breed lines, probably due to the use of the same mare types in the creation of this breed.

A025

A mixed model method for QTL detection and marker assisted selection

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A mixed model method is developed for QTL detection and marker assisted selection. In this mixed model method, marker effects are treated as uncorrelated random effects, providing a convenient framework for marker assisted selection using BLUP (best linear unbiased prediction) procedures. This mixed model approach is computationally efficient because it generates only a small number of equations to include marker effects in the model. The total genetic merit of QTL and polygenes is obtained as the sum of the predicted marker effects and the polygene effects. Each marker variance component required by the BLUP procedure is estimated using a maximum likelihood approach. Numerical results show that estimates of the variance components were close to the true parameters. Once marker variance components are obtained, marker-QTL recombination frequencies, QTL variances and effects can be obtained based on marker variance components.

A026

Genetic variation in seven native horse breeds from Greece

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Genetic variation at 29 loci (7 blood group, 10 biochemical and 12 microsatellite) was examined in seven indigenous Greek horse breeds. The breeds were the Andravidas (n=12), Crete Horse (n=40), Pindos (n=15), Pinias (n=30), Skyros Pony (n=126), Thessalias (n=4), and Zakynthos (n=5). Sample size for the latter two were too small for meaningful analysis. Variability values ranged from extremely low for a domestic horse breed for the Pinias to relatively high in the Skyros Pony. The high variation in the Skyros Pony was unexpected, as this is an island population with a small population size. There was no close correlation of variability measures of different types of loci within breeds. In general, the Greek horse breeds showed closest resemblance to Oriental type horse breeds although some of the breeds showed clear influence of Anglo-Norman type breeds.

A027

The genetic variability of six Merino populations determined by microsatellites

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Fine wool Merino sheep arose in the South of Spain in prehistoric times. Protected by the kingdom for centuries the breed did not leave the Iberian Peninsula until 18th Century, spreading through Europe and beyond, and giving origin to different breeds in the host countries. Microsatellite markers have been widely used in assessing genetic relationships among populations. We have analysed the genetic variability among 6 Merino breeds using 18 microsatellites in order to determine their usefulness in tracking differences among populations derived from the same breed in a relatively short timescale. The populations studied (N=253) include Spanish, French Mutton, German Mutton, Portuguese Black, Portuguese White and New Zealand Merino. Genetic relationships were determined using the simple allele sharing statistic and treating each animal as a taxonomic entity. The neighbor-joining method was used to construct an unrooted tree. Genetic variation was highest amongst the Spanish and Portuguese populations although genetic diversity within the other populations was also high. The French Mutton, German Mutton and New Zealand Merino populations could be differentiated from each other and from the Iberian Merinos, indicating that microsatellites are able to track relatively recent changes in the population structure of sheep breeds. Dendrograms constructed on the basis of microsatellite allelic frequencies suggest that populations that have shared selection criteria (meat *versus* wool) tend to cluster together.

A028

A high-resolution comparative gene map of pig chromosome 14

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Analysis of the human-porcine comparative gene map shows that porcine chromosome 14 (SSC14) has undergone many evolutionary rearrangements, corresponding to at least 6 different human chromosomal segments. Effective utilization of the comparative map for positional candidate cloning of QTLs in swine will require the construction of high-resolution comparative maps, particularly for chromosomes with such complex evolutionary history. A comparative mapping by annotation and sequence similarity (COMPASS) approach was used to select type I loci for mapping. Over 10,000 porcine database sequences were analyzed by COMPASS to identify their corresponding human orthologs with high-resolution RH mapping data. Eighty-four sequence clusters that were predicted to map to SSC14 in addition to resolving evolutionary breakpoints between chromosomal segments were selected. Initially, the INRA somatic cell hybrid panel (SCHP) was used to map genes to their respective chromosomes, followed by WG-RH mapping of SSC14 loci using the INRA-Minnesota porcine radiation hybrid panel (IMpRH). Of 52 loci screened, 71% were successfully amplified and yielded porcine specific products. Currently, 17 new assignments have been made to SSC14 and additional assignments to SSC5 (5), SSC4 (4), SSC10, (4), SSC9 (3), SSC6 (2), SSC3 (2), and SSC13 (1). Thirty-two additional loci are being screened to increase the resolution of the map and further refine evolutionary breakpoints on SSC14.

A029

Parentage control in the Latxa sheep breed using microsatellites

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PCR multiplexes developed for automated fluorescence genotyping were evaluated for parentage testing in the Latxa sheep breed. Multiplexes contain 11 primer pairs recommended by the ISAG Sheep and Goat Comparison Test 1998 (plex2, 5 loci) or by the LABOGENA Lab, France (plex1, 6 loci). The results showed a wide range of variability among the 11 microsatellites. In the Latxa sheep breed the number of alleles per microsatellite locus varied from 6 to 16 (mean = 9.7, n = 145 unrelated ewes) and the polymorphic information content from 0.611 to 0.848 (mean PIC = 0.725). The average exclusion probabilities are >0.9999 and >0.9970 whit and without one parent already known, respectively. In addition, in this paper we compare the results obtained from the analysis of more than 200 families (sire-dam-offspring) using the two PCR multiplexes and the biochemical polymorphism system applied since 1987 at our laboratory. Finally, we see the need for standarization of results from different laboratories, it is necessary to stablish an international panel of microsatellites for sheep paternity control and a common nomenclature.

A030

Linkage mapping ESTs in pigs using single nucleotide polymorphisms (SNPs): SNP discovery

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Comparative mapping between human and swine genomes requires the localization of conserved genes in both species. Our objective is to improve the resolution of the human/swine comparative map by mapping markers within expressed pig genes. SNPs associated with porcine expressed sequence tags (ESTs) orthologous to genes with known human map positions will be mapped by linkage analysis. Single-pass porcine EST sequence derived from two normalized libraries is subjected to automated PCR primer design aimed at developing primer-pairs that span intron/exon junctions. The results of PCR amplification from porcine, bovine, and ovine DNAs are entered into a relational database (MARCDB). Successful primer-pairs are used to generate amplicons from nine parents of the MARC porcine reference population, and seven animals likely to harbor breed-specific alleles present in mapping animals. Amplicons are purified and subject to fluorescent di-deoxy sequencing. Chromatograms are imported into MARCDB, assembled into contigs, and assessed for SNPs using Polyphred. Potential polymorphisms are interactively evaluated and tagged using Consed. Validated SNPs are exported to MARCDB for automated genotyping assay design. Preliminary assessment of our strategy for SNP-discovery reveals it to be quite efficient. Up to 85% of the amplicons sequenced harbor SNPs. To date polymorphisms have been detected in over 100 porcine genes.

A031

A panel of microsatellites for establishing parentage in domestic sheep

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The Australian sheep industry uses extensive production systems. Hence, even in stud flocks, pedigrees are often unknown or unreliable, resulting in suboptimal rates of genetic gain and unknown genetic relationships among animals selected to parent the next generation. There is a strong demand for reliable and cost effective DNA-based pedigreeing and, in response, we developed several panels of microsatellites that can be multiplexed as part of a DNA parenting system. Our primary pedigreeing set comprises 16 microsatellites, amplified in three PCR reactions, co-loaded and optimised for scoring using ABI's proprietary genotyping software. This panel has been tested extensively in Merino lines. While based on published microsatellites, largely those developed within CSIRO, all primer pairs have been redesigned to ensure high quality peaks and non-overlapping size ranges. Almost all markers are highly polymorphic; 50% have heterozygosities in excess of 80%. In a closed flock of fine-wool Merinos, the average number of alleles per marker was 9.25, and the mean PIC value of the set was 0.73. This set is adequate to assign parentage unambiguously even when the putative parents are highly related. We have, in addition, a supplementary set of 14 markers (in two PCR reactions); together these provide an even coverage of the genome and can be used to estimate genetic relationships where parents are unknown. We believe that these panels should be suitable for use with breeds other than the Merino.

A032

Discovery of a genomic clone with homology to centromeric and telomeric regions of chromosomes among species of *Equus*.

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A lambda phage clone selected for homology to s*omatostatin* was found to hybridize to the telomeric and centromeric regions of equid chromosomes. The clone hybridized to chromosomes of the following species as detected by fluorescence *in situ* hybridization (FISH): Prezwalski's horse (*Equus przewalskii),* domestic horse *(E. caballus),* donkey *(E. asinus),* Kulan *(E. hemionus kulan),* Grevy's zebra *(E. grevyi),* and Hartmann's zebra *(E. zebra hartmannae)*. For each of these species hybridization was observed at centromeres and telomeres of some chromosomes. To some extent, the patterns of hybridization reflected phylogenetic expectations. In the horses, hybridization was predominantly at acrocentric centromeres. In the zebras, hybridization was at acrocentric and metacentric centromeres and telomeres. In the kulan, hybridization was observed at only one acrocentric centromere. No hybridization was observed to chromosomes of the Burchell's zebra (E. burchelli) or the black rhinoceros (*Diceros bicornis)*. A BLAST search of a portion of the sequence reveals homology to a flanking sequence of a horse microsatellite (*UM007*). This clone appears to contain an equid specific satellite element that appeared after the divergence of the perissodactyls. Currently, sequencing and further characterization of this clone is underway.

A033

A comparative ruminant genetic linkage map based on the deer interspecies hybrid pedigree

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High levels of synteny observed in sub-chromosomal regions between human and other mammalian genomes allow for information which is already available from human and mouse mapping and sequencing to assist gene discovery in other species. However, the links between the current human and ruminant linkage maps (sheep, cattle, goat) have consisted predominantly of anonymous microsatellite markers which are not conserved in species beyond ruminants. The deer genetic linkage map contains a higher proportion of mapped genes due to the frequency of polymorphism found between Pere David's and Red Deer in the interspecies hybrids used to generate the map (located at Roslin http://www.ri.bbsrc.ac.uk/cgi-bin/arkdb/browsers/browser.sh?species=deer). The original deer map shares 147 loci with the human map, 123 with cattle, 95 with sheep and 114 with mouse. We have used a subset of the deer interspecies backcross linkage pedigree to map new genes using a rapid screen with two restriction endonucleases to identify restriction fragment length variants. Over 100 new genes, (cDNA and ESTs) from a variety of species and tissue types have been located on deer linkage groups. The sequence information derived from these cDNAs and ESTs provides further links to the human and mouse maps. An additional 120 mainly ruminant-derived microsatellite markers (cattle, sheep, caribou, gazelle) have been screened in the mapping pedigree to strengthen links with other ruminant maps, and over 270 AFLP markers (amplified fragment length polymorphism) have been mapped to increase the map density.

A034

The nucleotide sequence of *BoLA-DOA* cDNA

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Studying the *MHC* genes of the class IIb region in cattle, the second exon of *BoLA-DOA*, the bovine homologue of the *DOA* or*DNA* locus of other mammalian species, was identified in cDNA derived from peripheral blood leukocytes. The entire coding sequence and parts of the 5´- and 3´-untranslated regions were amplified using RT-PCR and 5´-RACE with *DOA*-specific primers. A sequence of 1109 nucleotides was determined, which contained an open reading frame encoding a polypeptide of 250 amino acids. The predicted molecule was found to express all of the features expected for functional class II a-chains, as it was composed of a putative signal peptide as well as of putative a1, a2, connecting, transmembrane, and cytoplasmic domains. It also included typical a-chain sequence elements such as the conserved cysteine residues and two potential glycosylation sites. The predicted mature protein showed 93,7 % and 81.1 % identity with the orthologous genes of sheep and pig, respectively. Further studies are required to investigate the expression profile and the function of the *DOA* gene in cattle.

A035

Organization of an ovine keratin associated protein gene cluster on OAR11

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During keratinocyte differentiation in the wool follicle, the acidic type I and basic type II keratin intermediate filaments (KIF) become embedded in a matrix of keratin associated proteins (KAP). Many of the type I and type II KIF genes are tightly clustered in two separate domains in humans and mice, but the organization of the KAP genes has not been described. There is some evidence of clustering of KAP genes in sheep and it is possible that there is a relationship between the organization of these genes and their expression in the wool follicle. A 100kb ovine bacterial artificial chromosome (BAC) containing KAP1.1 was physically mapped to 11q3.1. The presence and arrangement of additional KAP genes within this BAC was investigated by long-range restriction mapping and comparative shot-gun sequence analysis. Sequences were repeat masked and aligned to a human chromosome 17 BAC. This study demonstrated that genes highly similar to KAP1.1, KAP1.3, KAP1.4, KAP2.3, KAP3.4 and one as yet unidentified type I KIF gene were all located within this 100kb BAC. A striking feature of both the ovine and human sequences for this region was the localized, high content of repetitive DNA (45% and 41%, respectively). It is speculated, herein, that since KAP genes lack introns, they require the accumulated repetitive DNA in the extragenic regions for proper regulation or function. Transgenic studies with KAP- or KIF-containing BAC clones would help unravel the complex control of keratin gene expression in the wool follicle.

A036

Radiation hybrid mapping of bovine chromosome 2, assignment of 7 previously unmapped genes

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At least 38 genes have now been positioned on *Bta*2, either by cytogenetic mapping or by linkage analysis. Most of these genes are mapped at the telomeric end or near the centromeric region, leaving a 'gap' in the middle of the chromosome (*Bta*2q21-31). Recently, we have mapped a trypanotolerance QTL region on *Bta*2. In order to identify candidate trypanotolerant genes and possible homology between cattle and mouse trypanotolerant QTLs we are building up a comparative map between *Bta*2 and the human-mouse genomes. A radiation hybrid map of *Bta*2 has been constructed using a 5000-rad bovine whole-genome radiation hybrid panel. A total of 38 markers were typed: 17 Type I markers (genes) and 21 Type II markers (microsatellites). It includes 7 previously unmapped genes: CACNB4, CTLA4, DES, DPP4, HIS1, IDH, and NCL. The average retention frequency for all markers was 11.7 %. Identical retention patterns were found between two, three and five markers. The remaining 31 markers were linked at LOD score > 3, into four groups including 13 (group I), 3 (group II), 3 (group III) and 12 (group IV) markers. Large discrepancies were found between the large and the small groups with an average retention frequency for I, II, III and IV for 13.8%, 3%, 5.3% and 13.3%, respectively. Comparison with the *Bta*2 cytogenetic map locates radiation hybrid groups I and IV on *Bta*2q12-24 and *Bta*2q35-44, respectively. Comparative mapping confirm the chromosomal homology between *Bta*2 and *Hsa*2, *Mmu*1 and *Mmu*2 as well as the complex gene order rearrangement between cattle, human and mouse for these regions.

A037

A practical program for reconstructing DNA marker haplotypes of half-sib based pedigree structure.

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Marker haplotype reconstructed from each of individual is important in order to detect population association or linkage between traits and genetic markers. It was better to reconstruct haplotypes of all individuals simultaneously, but it may take much time when the pedigree was big. In case of half-sib analysis based complex pedigree structure that was popular in cattle, we presently could able to divide it into several small pedigrees and reconstruct independently without much loss of information. We made a simple computer program, by which reconstruct haplotypes of half-sib based pedigree structured from haplotypes individual of populations as displayed in the figures.

A038

Phylogenetic analysis and temporal changes in genetic variation of 18 horse breeds

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Temporal changes in genetic variation within and between 18 horse breeds were evaluated using 7 erythrocyte antigene systems (*A, C, D, K, P, Q, U*), 8 polymorphic protein loci (*A1B, ALB, ES, GC, GPI, HBA, PGD, TF*) and thirteen microsatellite loci (*HTG6, HTG4, HMS3, HMS1, HTG10, AHT5, HMS6, ASB2, HMS2, VHL20, HTG7, AHT4, HMS7*). Blood typing data of about 50.000 and microsatellite data of nearly 19.000 samples, sent to our laboratories for routine paternity testing since the year 1988, were analysed. Intrabreed genetic variation was quantified by conventional parameters (e.g. heterozygosity, average number of alleles per locus) and migration by the effective migration rate. The neighbourjoining and UPGMA dendrogram of relationships between breeds was constructed using Nei's D_A genetic distance. In 12 of 19 breeds enough data were available to split samples in two or three generations. All animals within a breed born in a time span of ten years were defined as one generation. Each generation (subpopulation) was represented by at least 50 animals. Heterozygosity remained stable over generations in most breeds. Arabian horses and Throughbreds showed the smallest average heterozygosity values whereas Pintos and Draft horses showed the highest average heterozygosity. Both neighbourjoining and UPGMA dendrogram and non linear map construction showed three well-separated groups of breeds: (1) Icelander, Fjords and Shetland Ponies, (2) Haflinger and South-German Draft horses and (3) all other investigated breeds. Within these three groups further reasonable clustering was observed.

A039

Nucleotide sequence variability at the Horse major histocompatibility complex DQB region

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Existence of at least two major histocompatibility complex (ELA) DQB genes has been suggested (Horín et al., in preparation). PCR-SSCP and nucleotide sequencing of the second exon of the ELA-DQB gene(s) showed that the PCR-SSCP technique specifically detected single nucleotide differences. Based on the nucleotide sequence homology, two different clusters of allelic sequences were obtained, one of them being preferentially, but not exclusively, amplified by the primer pair used. The frequencies of allelic variants differed significantly among breeds. The data provide further support for the existence of two DQB genes in the horse.

A040

Preparation and characterization of bovine/hamster somatic cell hybrid panel

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Somatic cell hybrid panels have widely been used for physical mapping of genes and DNA markers. In order to locate newly developed bovine microsatellites and ESTs, we prepared a bovine/hamster somatic cell hybrid (SCH) panel and characterized it with more than 300 microsatellites mapped onto the USDA bovine linkage map.

Bovine primary cultured fibroblasts from a Wagyu bull were fused with hamster line tk^{-}-ts13neo by 50% polyethylene glycol. Our SCH panel was composed of 30 hybrid clones isolated in the presence of HAT/G418 and grown for DNA preparation (18-137 mg DNA/clone). Retention frequencies of bovine chromosomes were estimated by Genescan analysis of microsatellite loci with an ABI 377 DNA sequencer. Ten clones out of the 30 were analyzed by FISH using total bovine DNA as probe. In the SCH panel the retention frequency in average was 40%. Most of bovine chromosomes in the panel had small deleted regions where one or more successive microsatellite loci were not PCR-amplified. We conclude that mapping of a bovine locus with our SCH panel can be more precisely carried out than expected. Further characterization of the panel is reported.

A041

Comparative mapping of livestock genomes: constructions of cytogenetic banding block counterpart (CBBC) maps

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The objectives of this study are to exploit the large databases of genes and ESTs that have been mapped and sequenced in human, rodents and other mammalian species to construct Cytogenetic Banding Block Counterpart (CBBC) maps of farm animals in order to facility gene mapping, QTL mapping and comparative mapping in livestock. Human chromosome 4 has been used in a pilot study. Of 294 gene loci that have been recorded in GDBdatabase on the chromosome, 214 designated genes with complete coding sequences were selected to search for homologous gene and EST sequences in livestock species using BLAST. 121 gene loci were found to have the homologous sequences (designated genes or ESTs) in livestock species, including 93 genes of homologous sequences for cattle, 63 for pig, 20 for sheep, 13 for dog, 6 for horse, 6 for cat and 2 for goat, respectively. Primers can be designed to amplify at least 120 fragments of genes on the basis of conserved regions of sequences between human and livestock species (87.5%) or between human and rodents (12.5%). These 120 genes cover 47 cytogenetic banding blocks of human chromosome 4, ranging from one to six genes per block. Once these genes are mapped on the genome of a specific species using RH mapping, their locations of the counterpart cytogenetic banding blocks in the species will be identified. Based on this pilot study and availability of database in GenBank, one can expect that the construction of CBBC maps with 3000 - 3500 gene loci in livestock species is currently possible. The applications of CBBC maps and challenges in their constructions are discussed.

A042

A comprehensive comparative map of pig chromosome 13

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Pig chromosome 13 (SSC13) habours the locus responsible for *E. coli* F4ab/ac resistance and QTLs for growth and fat deposition. These trait loci make mapping of SSC13 genes particularly interesting. In order to strengthen the possibility of using the positional candidate gene cloning strategy in the hunt for pig genes the resolution of the human-pig comparative map for SSC13 was improved. Eighteen different human chromosome 3 genes with a known human chromosomal localisation were selected and mapped in the pig. Through analysis of somatic cell hybrids, radiation hybrids and dual-colour fluorescence *in situ* hybridisation the genes were physically orientated on SSC13. Our comparison of SSC13 and HSA3 confirms observations from previous studies (Sun et al., 1999 Cytogenet Cell Genet 85, 273; Van Poucke et al., 1999 Cytogenet Cell Genet 85, 279), namely extensive rearrangements in gene order between the two syntenic chromosomes. The new mapping data makes the alignment of SSC13 and HSA3 more precise and enables localisation of the likely chromosomal break-points that have occurred since the divergence between human and pig. The results facilitates the identification of candidate genes from the human chromosome 3.

A043

Genetic diversity and genetic distances in threatened horse breeds from western Pyrenees by microsatellites

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As a part of a larger work on Cantabrian-Pyrenean breeds diversity, the genetic polymorphism of four autochtonous horse breeds has been investigated to characterize their genetic structure and phylogenetics. Two of these breeds, Euskal Herriko Mendiko Zaldia and Jaca Navarra, are genetically analyzed for the first time. In total, samples of more than 300 animals have been collected. A strict sampling strategy was employed: individuals sampled were chosen avoiding relatedness and taking into account their morphological characteristics.
Twelve microsatellite DNA markers included in the set of the ABI StockMark have been chosen for the analysis. The PCR products have been tested automatically by an ABI310 Genetic Analyzer and their fragment size by Genescan software. These microsatellites are quite polymorphic in these breeds with a number of alleles ranging 4 and 12.
For each locus heterozigosity, polymorphic information content and allelic frequencies have been estimated. The levels of genetic diversity observed in the breeds under study are relatively high (mean heterozigosity above 0.65 in all of them). These results suggest that they possess amounts of genetic variation similar to the more common breeds.
The allele frequencies have been used to estimate the genetic distances and to construct phylogenetic trees using different models. This information can provide baseline data for genetic conservation and improvement plans.

A044

The GENETPIG object-oriented environment

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The GENETPIG project is a european project to accelerate the mapping and to identify GENes controling Economic Traits in PIG, coordinated by J. Gellin (BIO4-CT98-0237). The bioinformatics of GENETPIG is managed by Infobiogen in a custom objet-oriented environment, which is able to cope with the diversity of genomic data and their relationship; it is based on the EyeDB OODBMS (http://www.infobiogen.fr/services/eyedb/). Among others, the database integrates the experimental details (PCR for location, sequencing, different studies), data on the obtention of ESTs, comparative mapping, bibliography, and results of sequence comparison, localization by various methods (SCH, RH, ISH..), polymorphism studies, ZooPCR ... Users have the possibility to fill and update data, make various queries and obtain different synoptic or complete views of the data, consult the experimental details, statistics, results of sequences comparison research against several private and public databases, and visualize cytogenetic and RH maps with a web interface (http, cgi-bin and java). The database also provides a lot of links to relevant other databases and data from other mammalians are being integrated. Finally, an email alert service informs users of new sequence matchs, is used for a redundancy survey and contributes to the exchanges between partners.

A045

A comparative radiation hybrid map of Bovine chromosome 24 with Human chromosome 18

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Radiation hybrid (RH) mapping technology has been proven to be a powerful approach for gene mapping and genome comparisons between species. A 5000-rad bovine whole-genome radiation hybrid panel was utilized to map 28 markers on bovine chromosome (BTA) 24. The BTA24 RH map integrates 24 Type II loci previously mapped on other bovine linkage maps and 4 Type I loci. Of the Type II loci, six are microsatellites associated with genes. Twenty seven out of 28 loci were ordered with odds of at least 1000:1 in a comprehensive framework map. The observed locus order is generally consistent with results from previously reported BTA24 linkage maps. The observed BTA24 RH map information was used for comparison with human chromosome (HSA) 18 cytogenetic and RH maps. The linear order of genes was not conserved between BTA24 and HSA18. The findings revealed four conserved regions between BTA24 and HSA18.

A046

Bioinformatics resources for genome analysis in farm animals

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The Bioinformatics group at the Roslin Institute is developing bioinformatics tools and resources for scientists engaged in genome analysis in farmed animals. The resources developed encompass both the databases and the associated analytical and display tools required for genetic and physical mapping of the complex genomes of farm animals. The World Wide Web (WWW) is used to deliver the resources to this user community. The ARKdb genome database: Our ARKdb genome database model has now been fully implemented for pigs, chickens, sheep, cattle, Tilapia, horses, cats, turkey, salmonids and deer genome data. The databases are mounted on the primary node at the Roslin Institute (http://www.ri.bbsrc.ac.uk/bioinformatics/) and subsets are also mounted on nodes Texas A&M University and Iowa State University in the United States. The Comparative Animal genome database (TCAGdb): We have also developed a comparative genome database - The Comparative Animal Genome database (TCAGdb) to capture statement that specific pairs of genes are homologous. We are developing automated methods using Artificial Intelligence to evaluate homology data. Genetic diversity: We have developed a database for genetic diversity data for cattle and are currently implementing this model for data from other domesticated animals. Linkage and QTL databases: We have also developed and operate resource databases to handle raw experimental data for linkage mapping in animals. This resSpecies database also handles trait / performance data and can be used for quantitative trait locus (QTL) mapping experiments.

A047

An ordered comparative map of the cattle and human genomes

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A cattle-human whole-genome comparative map was constructed using parallel radiation hybrid (RH) mapping in conjunction with EST sequencing, database mining for unmapped cattle genes, and a predictive bioinformatics approach (COMPASS) for targeting specific homologous regions. A total of 767 genes were placed on the RH map in addition to 320 microsatellites used as anchor markers. Of these, 638 had human orthologs with mapping data, thus permitting construction of an ordered comparative map. The large number of ordered loci revealed at least 105 conserved segments between the two genomes. The comparative map suggests that 41 translocation events, a minimum of 54 internal rearrangements, and repositioning of all but one centromere can account for the observed organizations of the cattle and human genomes. In addition, the COMPASS *in silico* mapping tool was shown to be 95% accurate in its ability to predict cattle chromosome location from random sequence data, demonstrating this tool to be valuable for efficient targeting of specific regions for detailed mapping. The comparative map generated will be a cornerstone for elucidating mammalian chromosome phylogeny and the identification of genes of economic importance to the dairy and beef industries.

A048

Replicator network arrays for applications in genomic informatics

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We are developing replicator network arrays to automate the classification and analysis of large complex datasets that have proven resistant to traditional artificial intelligence, neural network, and data mining approaches. We present validated categorized reference datasets to basis vector extractors, which extract custom basis sets for each individual data category. These basis sets are used to construct arrays of associated replicator networks, which we have named Adaptive Focused Replicator Networks or AFRNs. The extracted basis vectors are frozen, so that each AFRN has its own custom-tailored basis vector set for its reference category. In production use, arrays of AFRNs are presented with new data and each AFRN attempts to reconstruct a faithful copy of the new exemplar using its own basis set. A higher-level informatic critic reviews the replication fidelity of the AFRN arrays and decides which AFRN has most faithfully reproduced the novel exemplar. If none of the AFRNs has performed adequately or if multiple AFRNs have inappropriately performed well, the exemplar is tagged for human interpretation and possible inclusion into an auxiliary reference database that can itself be used to build additional AFRNs in future. We demonstrate the method with two applications in genotyping and sequencing. Our genotyping pilot studies indicate that greater than 90% of our STR electropherograms can be scored accurately by AFRN arrays – completely bypassing the manual double-checking step required by other scoring methods in current use. Sequencing pilot studies indicate that we may be able to extend read length and raise the quality scores of distant base calls.

A049

Characterization of the LOSINO HORSE and Phylogenetic Proximity with the Principal American Equine Breeds

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The Losino Horse (*pony sp.*) receives its denomination from the original breeding area, the Valley of Losa, in the north of the province of Burgos (Spain). Is the only autochthonous horse breed of Castilla-León, found to be related geographically and historically to other autochthonous breeds derived from the Cantabrian-Pyrenean branch: Portuguese Garrano, Galician Faco, Asturcón, Pottoka and Meren's Horse. During the Middle Ages it occupied an important place in the Reconquest of Spain and later it participated in the American Conquest. As of now, it is a species in danger of extinction with only 200 animals left.

The study of genetic markers has contributed to the genetic characterization. In order to determine intrapopulation genetic variability between the 2 nuclei of the Losino equine breed (Pancorbo and Quincoces) it was estimated the expected average Heterozigosity Index. It presented an average value for both nuclei of 0.412 for 10 biochemical polymorphism loci, and of 0.733 for 10 DNA microsatellite; being Wright's Fixation Index = 0.25, result that permits to affirm that the Losino Horse has a medium-high genetic variability. It was also established its genetic relation with those equine breeds related geographically as much as historically by using Nei's Genetic Distance. The contribution of the Losino Horse in the Conquest and Colonization of America remains reflected by its genetic proximity to American breeds such as the Paso Fino, the Quarter Horse or the Chilote Horse.

A050

Polymorphic markers within the promotor region of the Horse *NRAMP1 (SLC 11A1)* gene

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Based on inter-species sequence homology, several pairs of primers identifying parts of equine *NRAMP1 (SLC 11A1)* gene were designed. PCR products obtained were cloned and sequenced. The nucleotide sequence of the 5´UTR was examined for the existence of restriction sites which were subsequently tested for polymorphisms. Three polymorphic markers (Nla III, Taq I, Msp I) were identified either by direct sequencing or by PCR-RFLP. Allele frequencies in two bi-allelic PCR-RFLP polymorphisms (Taq I, Msp I) in two horse breeds were: Old Kladruber: Taq I + 0,66; Taq I – 0.34;Msp I + 0.99; Msp I – 0.01 (N= 81); Akhal-Teke: Taq I + 0,83; Taq I – 0.17; Msp I + 0.37; Msp I – 0.63 (N= 49).

A051

Genetic relationships among genus *Gallus* based on mitochondrial and genome DNA polymorphisms

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The genealogical origin of the domestic chicken and its relation to four species of jungle fowls, Red Jungle Fowl (*Gallus gallus*), Green Jungle Fowl (*G. various*), Gray Jungle Fowl (*G. sonneratii*), and Ceylon Jungle Fowl (*G. lafayettei*), were explored using DNA polymorphisms of mitochondrial D-loop gene, MHC genes (*B-L*βII and *Y-L*βIII) and AFLP as genetic markers. In addition, Japanese Quail (*Coturnix japonica*) was analyzed as an outgroup of genus *Gallus*. Among four species of jungle fowls, the Red Jungle Fowl showed closest relationship to the domestic chicken. Grey and Ceylon Jungle Fowls were closely related to each other, but most distantly related to the domestic chicken. These results supported the hypothesis that the Red Jungle Fowl is a monophyletic ancestor of all domestic chickens. Among Red Jungle Fowls, that collected in Laos, which is thought to be *G. g. spadiceus*, was relatively close to the domestic chicken in genetic distance. On the other hands, the White Leghorn (inbred CB line) was very far from both native chickens and jungle fowls. This unique genetic position of the White Leghorn might result from intensive inbreeding in this line and/or high selection for egg production ability and immunological characters in this breed.

A052

Genetic characterization of the South American camelids using microsatellite markers

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The South American camelid (SAC) species are not completely reproductively isolated, as fertile hybrids of any combination of taxa can be produced. This has led to the general belief that in this group a process of speciation is still in progress, being also the origin of domestic species under debate. The main aim of this work was to help to define the genetic diversity and relationships among populations representing these species, alpaca (*Lama pacos*), llama (*L. glama*), guanaco (*L. guanicoe*) and vicuña (*Vicugna vicugna*). This work was done using a set of 23 microsatellite markers developed in our laboratory.

Phenetic analysis based on 38 alpacas, 14 llamas, 16 guanacos and 16 vicuñas showed that each species are clustered separately, with the only exception of some alpacas that were excluded of the alpaca group; these animals correspond morphologically to hybrids ("guarizos"). Interestingly, alpaca and vicuña clusters formed a major group, clearly separated from the llama and guanaco clusters, which were closer among them. This comes to support new evidence based on mithocondial DNA analysis, that suggests the alpacas as descendants from vicuñas (E. Palma, personal communication). Finally, principal component analysis (PCA) based on 193 polymorphic alleles showed that wild species (guanaco and vicuña) had more narrow genetic background than domestic species (alpacas and llamas). The domestic SAC clusters are more heterogeneous, what could be explained by the management of mixed herds, facilitating the generation of guarizos.

A053

Genetic diversity analysis of the 3 Portuguese native horse breeds inferred from microsatellite data

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The study of genetic diversity of autochthonous breeds is very important for programmes related with the conservation of biodiversity in livestock species. Therefore, genetic polymorphism of the 3 Portuguese native horse breeds (Lusitano, Garrano and Sorraia) was investigated in order to characterize their genetic structure. Nine horse microsatellites (ASB2, HMS2, HMS3, HMS7, HTG4, HTG6, HTG10, LEX023 and VHL20), chosen for being highly polymorphic in other Iberian breeds, were used in this analysis. DNA extracted from blood samples was amplified by PCR and the products were separated in 6% polyacrylamide gels using a fluorescence 4200S Li-Cor automated sequencer. For each locus, heterozygosity, allele frequencies and polymorphic information content (PIC) were estimated. The genetic equilibrium according to Hardy-Weinberg, the usual estimators for differentiation between populations (F-statistic) and the genetic distances based on allelic frequencies and multivariate analysis were also calculated. Microsatellites were highly polymorphic for Lusitano and Garrano breeds with the number of alleles ranging from 6 to 11. For Sorraia horse, a rare and very inbred breed, the number of alleles ranged from 2 to 5.

A054

Two major mitochondrial DNA types in the New Zealand Jersey cattle

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Mitochondrial DNA (mtDNA) was used to verify whether a particular cow was mother to a Jersey bull which had been selected as a donor in artificial insemination. The method used was to PCR amplify and sequence the mitochondrial D-loop region of the two test animals to check whether their sequences were identical. An additional fifteen Jersey animals were included in the test to increase the degree of confidence in the result. In total mtDNA D-loop sequences of seventeen NZ Jersey animals were generated in this exercise. Analysis of the D-loop data revealed the existence of two major mtDNA types in the NZ Jersey animals. When compared to the published mtDNA data of cattle breeds from Europe, India, East and West Africa in our analysis, the two NZ Jersey mtDNA types belong to either the *Bos taurus* group (10 animals) or the *Bos indicus* group (7 animals).

A055

Sequencing and mutation analysis in exon 1 of Horse Tyrosinase gene

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The tyrosinase gene (*Tyr)* is essentially involved in melanogenesis and coat colour as well as in hormone production. Here we first report the sequence of 765 bp of exon 1 in the horse tyrosinase gene (*Tyr)* amplified by PCR using the following primers: AAT GCT CCT GGC TGT TTT GTA (upper primer) and CTG CCA GGA GGA GAA GAA GGA TGC T (lower primer). The horse tyrosinase sequence shows a high sequence identity to the corresponding known sequences in humans, mouse, dog and pig both at the nucleotide and amino acid level. Mutation screening in the horse *Tyr* gene was carried out in 250 individuals of different coat colour and different breeds. One mutation with a simple Mendelian inheritance causing an amino acid substitution (Pro -> Ser) was detected. A possible association of the detected allele with different phenotypes will be elucidated. The results will be shown.

A056

Homology of bovine tyrosinase with human, mouse, and chicken sequences.

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The cattle tyrosinase gene sequence was obtained by designing PCR primers from human exons (M63235, M63236, M63237, M63238, M63239). Each exon product was amplified separately since the introns in this gene are so large. For the 80% of the cattle tyrosinase sequenced, amino acid homology between cattle and human is 95% with 87% homology in the nucleic acid sequence. Protein homology of 93% was found between mouse and cattle, with a nucleic acid homology of 82%. Lower homologies of 89% for amino acid and 72% for nucleic acid sequences were found between cattle and chicken tyrosinase. Through the course of sequencing the gene a SNP was found, from which a purposeful mismatch PCR-RFLP will be useful in linkage mapping the gene. The tyrosinase gene is implicated in albinism and oculocutaneous albinism in humans and various forms of partial albinism in mice and is therefore of interest in the study of cattle coat color.

A057

High-resolution physical mapping confirms similar gene order with an inversion between human 17 (Hsap17) and porcine chromosome 12 (Sscr12).

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Comparative mapping between livestock and human provides a powerful tool for exploiting gene map information from the well-studied human genome. Although a large number of conserved chromosome fragments have been identified between human and pig, little is known about the precise gene order between the two species. To better understand the evolutionary relationship of human and pig chromosomes and predict the location of homologous pig genes, we physically mapped 6 genes/ESTs of Hsap17 to Sscr12 and Sscr2, using both somatic cell hybrid panel (SCHP) and radiation hybrid panel (RH) analysis. The primers were designed for the 6 genes/ESTs of human Hsap17 based on pig embryo EST library sequences. The genes/ESTs are *RBP56, H3F3B, PLI, PSA, P311 and EST15*. SCHP analysis located 5 genes/ESTs *(RBP56, H3F3B, PLI, PSA* and *EST15*) on Sscr12. The mapping result indicated an inversion of gene order between Hsap17 and Sscr12 for gene *PLI*. This gene was located on distal Hsap17q, but was assigned to distal Sscr12p. Gene order by RH analysis is *H3F3B-EST15-PSA-RBP56-PLI* on Ssc12, the exact inverse of the order of these genes on Hsap17. By using SCHP and RH mapping, gene *P311* was mapped to Sscr2. Mapping of these six genes confirmed the conserved synteny relationships observed with bidirectional chromosome painting. Furthermore, RH mapping in the present study reduced the number of Sscr12 RH linkage groups from five to four.

A058

The use of bovine microsatellites in the genetic study of African antelopes in European captive populations.

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The genetic structure of the African antelope European captive population of *Aepyceros melampus* (impala), *Hippotragus niger* (sable antelope), *H. equinus* (roan antelope), *Kobus ellipsiprymnus* (waterbuck) and *Kobus leche* (lechwe) have been studied using bovine microsatellite markers, mapping to 19 chromosomes. The aims of the study are to quantify the genetic variability and to test the usefulness of these markers for parentage testing. The data generated will allow the development of a genetically based management plan for these captive populations. From an initial set of 22 bovine microsatellites tested only 12 were chosen, as the others did not amplify, were difficult to score or were monomorphic. DNA was extracted from blood, tissue, hair and faeces using phenol/chloroform and commercial kits. DNA was amplified by PCR and the products were separated in 6% polyacrylamide gels using Li-Cor 4200 automated sequencer. Preliminary results showed the number of alleles ranging from 2 to 7 and the analysis of a well-characterised impala captive group (n=24) suggest that those microsatellite markers can be used for parentage assignment.

A059

Characterization of a normalized cDNA library constructed from bovine mammary gland tissues.

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Characterization of mammary-specific gene expression will aid the discovery of biological factors that influence production and udder health in dairy cattle. Expressed sequence tags (ESTs) are a critical component of this investigation, serving as a resource for gene discovery, mapping, and expression profiling. As a resource to generate 10,000 unique bovine mammary ESTs, we constructed a Cot_{500} normalized cDNA library. To encompass a larger repertoire of gene expression, mRNA was isolated from udder biopsies performed on animals from eight different stages of mammary growth, development, and health. Equimolar amounts of mRNA from each stage were combined and used to synthesize cDNA. To make the library amenable for automated high-throughput sequence analysis, 50,000 clones were picked and arrayed into 384 well plates. DNA template for sequencing was generated by PCR amplification of cDNA inserts from bacterial culture. A preliminary analysis of the sequencing data from 4,224 clones was performed using BLAST against GenBank nr and dbEST to assess inter- and intra-library EST redundancy. Clonal redundancy within the library was 35%. Of the uniquely identified ESTs, 61% had no similarity to bovine sequence, and 16% had no identity to any sequence. For the ESTs (39%) with similarity to bovine sequence, 62% of these were redundant with ESTs generated from the four bovine cDNA libraries at ARS-USDA MARC. The EST sequence data indicate ~16,000 clones need to be sequenced to reach our goal of 10,000 ESTs.

A060

Genetic relationships in Swiss sheep breeds based on microsatellite analysis

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The present investigation makes use of a combined set of thirty-one microsatellite markers developed from the bovine, ovine and caprine genomes to characterize the genetic relationships between seven Swiss sheep breeds. Most of these microsatellites are currently in use in an European sheep and goat biodiversity project. The populations studied were the four main Swiss breeds White Alpine, Oxford Down, Black-Brown Mountain, Valais Blacknose and three local Swiss breeds Valais Red, Spiegel Sheep, Engadine Red. The latter three are considered endangered. In addition the wild type Mouflon was included in this study. PCR amplification of 31 bovine, ovine and caprine microsatellites was performed in a total of 307 animals. The average heterozygosity within each population was high in the domestic breeds (0.60 to 0.71) and lower in Mouflon 0.45. The average coefficient of gene differentiation over all loci was 0.17; i.e. only a small part of the variability at the 31 microsatellite loci can be ascribed to the between-breed variability. Cavalli-Sforza's chord measure was used to calculate genetic distances and to build a neighbor-joining tree. The first three components of the principal component analysis could explain 52% of the total variation. Microsatellites developed from the closely related species cattle and goat were useful for estimating genetic relationships among sheep breeds.

A061

Precise mapping of breakpoints in conserved syteny between human chromosome 1 and pig chromosomes 4, 6, and 9.

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Previous comparative mapping suggested at least five pig chromosomes (Sscr4, 6, 9, 10 and 14) share homology with human chromosome 1 (Hsap 1). Eleven genes, including *Janus kinase 1 (JAK1), prostaglandin E receptor 3 (PTGER3), urate osidoase (UOX), coagulation factor 3 (F3), vascular cell adhension molecule 1 (POU2F1), coagulation factor 5 (F5), prostagladin endoperoxide synthase-2 (PTGS2), myosin binding protein H (MYBPH)* and *antithrombin III (AT3),* were selected to refine the synteic boundaries between Hsap1 and pig chromosomes. Pig STSs were developed from heterologous sequence, then sequenced and physically mapped using a somatic cell hybrid panel. Five genes were also amped by using fluorescent in situ hybridization (FISH) to improve map resolution. This study located *JAK1, PTGER3,* and *UOX* to pig chromosome 6; *F3, RPL5, VCAMI, F5,* and *POU2F1* to *AT3, PTGS2,* and *MYBPH* to Sscr9. Using the GB4 human radiation hybrid information for *PRL5* (258 cR), and for *PRKACB* (237cR) (which maps to Sscr6; Marklund et. al., 1992), we propose the pig Sscr4/9 and Hsap1 we used a heterologous FISH approach using pooled human YACs. The FISH analysis demonstrated that Hsap1q22-24 YAC clones map to Sscr4q15-16 clones map to Sscr9q25, and Hsap1q44 YAC clones map to Sscr14q22. These results suggest the precise breakpoints coreesponding to Sscr4/9 on Hsap1 are most likely on Hsap1q24-25.

A062

New Microsatellite Markers in Chicken

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About 1000 chicken microsatellite markers have been reported in the last decade. However, many more microsatellite markers are still needed for efficient detection of QTL. Here we report 678 new microsatellite markers. A $(CA)_n$-enriched library was constructed according to Takahashi *et al.* (1996) with modifications. CA-positive clones were selected by colony hybridization screening and the DNA sequences were determined. 37.5% of the clones from the library were CA-positive. To date, 1800 CA-positive clones have been sequenced. Of these clones, 1031 clones were subjected to DNA database homology search (DDBJ, GenBank and EMBL). Of the 1031 clones, 678 clones (65.8%) were unique and judged to be suitable for developing PCR primer pairs to detect $(CA)_n$ repeat length polymorphism. Microsatellite markers identified at the National Institute of Agrobiological Resources have reached 800. These markers will be applied to QTL analysis of a Japanese resource family based on Oh-Shamo (Japanese Large Game) and White Leghorn.

A063

Large scale bovine cDNA sequencing toward the construction of the bovine/human comparative map

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To facilitate the cloning of economic trait loci, we have started to construct a bovine high-density gene map and a precise bovine/human comparative map. We constructed poly A-deleted cDNA libraries from various bovine fetal tissues to overcome the difficulties of sequencing cDNA from the 3' end owing to poly A. We determined cDNA sequences from both 5' and 3' ends, producing 5' and 3' ESTs. Those ESTs were submitted to our database system in which redundancy was checked among our ESTs, followed by blast search against GenBank DB. The ESTs were applied to be located on a human gene map, utilizing the similarity to human EST. Each EST is being assigned to bovine chromosome using a somatic cell hybrid panel. We have determined 35,700 EST sequences in total which are grouped into 7,337 and 6,571 of 5' and 3' ESTs, respectively. 34% of 5' ESTs and 22% of 3' ESTs showed high similarity to the genes or ESTs registered in GenBank DB. About 650 of ESTs were located on a human gene map and fifty of them were assigned to bovine chromosomes by SCH panel.

A064

Rejection of *KIT* as the gene responsible for appaloosa coat color spotting patterns in horses

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The coat color pattern of the Appaloosa horse appears similar to that caused by the *rump-white* gene in the mouse. Rump-white color pattern in the mouse is the result of an inversion within the *proto-oncogene c-kit* (*KIT*). Therefore, we investigated whether or not *KIT* encodes the appaloosa coat color gene (*Lp*) in horses. *KIT* encodes a transmembrane tyrosine kinase receptor that belongs to the PDGF/CSF-1/c-kit receptor subfamily. *KIT* plays a critical role in hematopoiesis, gametogenesis, and melanogenesis. *KIT* has limited, known genetic variation but is located on horse Chromosome 3 close to *albumin* (*ALB*), *esterase* (*Es*), *Vitamin D binding protein* (*GC*) and microsatellite markers *ASB23, LEX07, LEX57,* and *UCDEQ437*. Family studies were conducted to investigate linkage of *Lp* to these markers using 8 half-sib families in which Appaloosa stallions were mated to solid colored mares. None of the markers demonstrated linkage with the *Lp* gene as indicated by lod scores of 3.0 or greater. Based on cytogenetic studies, ASB23 is likely to be most closely linked to *KIT*. *ASB23* was informative in 7 out of 8 families and lod score analysis rejected linkage at $\Theta = 0.1$ (Lod= -2.16). Therefore, it is concluded that *Appaloosa (Lp*) is not linked to *ASB23*, and thus Appaloosa is unlikely to be the product of *KIT*.

A065

Development of new placental and fetal ESTs for gene discovery in pig reproduction.

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One major problem that has high economic impact on pig reproduction is the unexplained loss of potential porcine conceptuses during the first month of gestation. To better understand when and how these losses occur, it is imperative to investigate the underlying genetic regulatory mechanisms. We have recently initiated a large-scale cDNA sequencing project to provide molecular information regarding the genes expressed in female reproductive tissues. cDNA libraries are planned for ovary, hypothalamus, pituitary, placenta, uterus, and several stages of embryonic development. Sequence information will also be highly useful in developing sequence-tagged sites for physical mapping and developing comparative links between the human, mouse, and pig genome maps. We have previously reported the creation of 2 cDNA libraries, porcine fetal (day 20), and conceptus (day 17). Sequencing of these libraries produced 220 ESTs, with 180 sequences analyzed by clustering algorithms, and 139 clusters being identified within these sequences. We now report the creation of 2 more libraries from porcine fetal (day 45) and placental tissues. The day 45 fetal library has 971,150 independent clones (average insert: 1.4 kb), while the placental library has 1,320,000 independent clones. Initial sequencing of the fetal library has produced 98 ESTs (81 clusters), while we have obtained 1446 ESTs (1056 clusters) from the placental library. After clustering all sequences thus far obtained, we have identified 1240 unique clusters. Sequences obtained in this project will be deposited into Genbank dbEST, and all comparative homology information will be summarized on a public website.

A066

Refined genetic and comparative physical mapping of the canine copper toxicosis locus

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Copper is an essential trace element for the survival of all organisms, although it is highly toxic above a certain threshold. A well-regulated copper metabolism is required to ensure a cellular process for copper homeostasis. Only two genetic defects of copper metabolism in man, Menkes and Wilson's diseases have been described. A copper overload disorder, copper toxicosis (CT), is also seen in the Bedlington terrier. CT in Bedlington terriers is an autosomal recessive disorder characterised by an accumulation of copper in the liver leading to chronic hepatitis and, ultimately, cirrhosis. Recently, we assigned the CT locus in Bedlington terriers to the canine chromosome region CFA 10q26, which is homologous to HSA 2p13-21. A radiation hybrid map of the CFA 10q21-26 region was constructed containing 10 DNA markers and 6 genes, to facilitate positional cloning of the CT gene. Using homozygosity mapping, the CT locus was confined to a 42.3 cR_{3000} region estimated to be about 9 Mb. The homologous region of the CT region in man is about 30 cR_{3000} (± 8 Mb). Isolating of a gene for CT will be an important addition to the limited knowledge of the genetic regulation of copper metabolism in mammals.

A067

Microsatellite polymorphism in dog breeds-the AKC Parent Club study

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The American Kennel Club (AKC) registers 151 breeds of dog, approximately half of all breeds worldwide. Since 1997, 75 breeds have be evaluated with a panel of 17 microsatellite markers to determine 1) the polymorphism at each locus in each breed, 2) the effectiveness of each locus for identity and parentage testing, and 3) the genetic diversity within and between breeds. Herein we report results on the 56 breeds tested in 1998 and 1999 and include data from the previously reported 19 breeds when appropriate. Buccal swab samples (average n=120) were collected at breed specialty shows by AKC personnel and, thus, are not randomly selected but instead tend to include a large number related individuals. All samples were analyzed with a primary and secondary battery of multiplexed, fluorescently-labeled microsatellites containing 10 and 7 loci, respectively. Labeled fragments were electrophoresed on an ABI 377 automated sequencer using an in-lane internal size standard. Overall, the expected heterozygosity for individual loci ranged from 0.01 to 0.92. The mean expected heterozygosity over 17 loci for each breed ranged from 0.45 to 0.74. The number of loci required for a Combined Power of Exclusion to attain 99% in different breeds ranged from 6-14 loci. While the nonrandom structure of the populations tested does not allow absolute assessments of locus informativeness, it is evident that 1) a single battery of 10 microsatellite loci can accurately and efficiently determine identity and parentage in many dog breeds, and 2) a second battery of loci can assist in more inbred populations.

A068

Low genetic differentiation among South American population of the Wood Stork (*Mycteria americana*)

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The Wood Stork (*Mycteria americana*) is a colonial wading bird of the tropical and lower subtropical zones that breeds in North, Central and South America. We assessed genetic structure within and among six colonies of Wood Stork from the Brazilian Pantanal. Samples of 224 individuals were studied using protein electrophoresis to evaluate their genetic variability and differentiation. Of 23 loci examined, 7 were polymorphic (mean heterozygosity = 0.065), indicating low levels of allelic diversity and low genetic divergence among colonies. The low value of Fst indicates that 0.3% of the total variance can be explained by differences among colonies. Estimated number of migrants per generation based on F-statistics (Nm = 83.1) suggest high levels of gene flow. Values of Nei's genetic distance among South American colonies ranged from 0.000. Values of genetic distance between South and North America colonies according to Nei ranged from 0.000 – 0.007, while according to Rogers they ranged from 0.010 – 0.049. Our data indicated high levels of gene flow between populations of North and South America, intermediated by a probable interbreeding population in Central America. FAPESP (98/06160-8, 98/16359-6)

A069

RAPD-Analysis of the Genetic Divergence of Nuclear DNA in Ducks (ANAS PLATYRHYNCHOS) in the Course of Selection

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The efficiency of using polymorphic DNA-markers as marker trait which reflect gene pool of duck breeds (Anas Platyrhynchos) and intrabreed lines is shown with a view to determine the degree of intrabreed differentiation conditioned by lineage belonging and the degree of af interlinear intrabreed differentiatin conditioned by selection work and to express this differentiation degree as a quantitative criterion-indices of the genetic distance. The opinion is put forward that the genetic differentiation revealed is an important factor in obtaining heterosis by line hybridization and in gene pool preservation.
DNA-polymorphism of duck intrabreed lineages was demostrated using the method of polymerase chain reaction with 5 different random primers (RAPD-PCR). Genetic distances were calculated by the methods of Nei and Cavalli-Sforza.
Analysis of the segregation of random amplified DNA-markers in F1 progeny was done. It is shown that the parameters of genetic distances calculated on the basis of the RAPD-PCR patterns, objectively reflects even small alterations in the genetical structure of intrabreed lineages in the course of transformation of the initial parental forms.
The results obtained may de used in further genetic improvement of the existing forms and breeding of the new high-yield lineages.

A070

Breeding and biological characteristic of the White Siberian cattle

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The White (black-and-red-eared) Siberian cattle was developed by breeder N.E. Kosov in the state farm of 'Gutovskiy', Novosibirsk Region, in the 1940s - 1960s. The breeding was performed on the basis of the local Siberian cattle with the addition of blood of the Dutch and Friesian breeds. The three leading lines were the following: Lebed 147, Margo 86 and Lobach 122. The herd numbered more than 2000 animals, the average productivity being 4000 kgs per lactation, fat content 4.0 - 4.1%. The milk had wonderful dietary indices, that is the high content of protein, calcium and vitamin D. The animals were distinguished by their excellent adaptability characteristic and the resistance to parching heat and Siberian midges. The analysis of antigen indices of nine blood systems allowed to reveal gene pool of the White Siberian cattle and to estimate the indices of genetic affinity with regard to the animals of other breeds. As for the Black-and-White cattle of the Siberian and European areas, the Dutch and Friesian breeds, the indices were 0.8646, 0.8519, 0.8586 and 0.8405 respectively. The investigation results witness a considerable difference of cattle developed from the animals of initial breeds.

A071

Genetic analysis in two populations of Peruvian Horse: Peruvian Paso and Blood Horse Race

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Today, our breed of Peruvian Paso Horse is considered a national treasure. The study was realized in order to kwon and compair the genetic variability between two breed of Peruvian Horses.
Two populations, which are males, were analyzed using seven bloods group systems: *A,C,D,K,P,Q,U* and eight serums proteins systems: *Pi, Tf, A1B, Es, GC, Es, PGD, ALB,* and *HBA.* Blood samples were collecte from 329 peruvian horses (116 Peruvian Paso and 213 Pure Blood Race) of diferents peruvian cities and study by polyacrilamide gel electroforesis (PAGE) at pH 7.9, Isoelectric focusing (IEF) pH 4.0 – 6.5 and starch gel electrophoresis (STAGE) al pH 4.6
International recognized alleles were detected in the fifteen systems studied and own variants were founded in every one populations in the systems *Pi, Tf, Es and A1B.*
Allelic frequencies in that groups were compared and our results indicate that of thirteen allelic variants identified in the *Pi* systems, five of them: *H, J, L3, R, S** were detected only in PP Horses (the new unrecognized variant [S*] was similar to S) and the allelic variant *I* and *L2* was identified only in Blood Horse Race. Also in *A1B* and *Es* systems the allele *S* (f=0.349) and *G* (f=0.3157) respectively were observed only in Peruvian Paso Horse.
Finally, in the *Tf* system was detected only one individual for allele *F1* in Peruvian Paso Horse in comparison whit nineteen individuals in Blood Horse Race.