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A023

Genetic polymorphism at the Calpastatin, K-Casein, Leptin and BoLA-DRB3 loci in Iranian Sistani cattle (*Bos Indicus*)

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The genotypes for Calpastatin, K-Casein, Leptin and BoLA-DRB3 Loci were determined by polymerase chain reaction and restriction enzyme digestion method in native Iranian breed cattle, Sistani. Blood samples were collected from Sistani breeding station located in Zehak, Zabol. The extraction of genomic DNA was based on Guanidin Thiocyanate-Silica gel method. After PCR reaction, amplicons were digested with restriction enzymes. The Calpastatin locus had 3 genotypes with frequencies of 0.62, 0.29 and 0.09 for MM, MN and NN, respectively; K-Casein and Leptin had 3 genotypes with frequencies of 0.27, 0.57 and 0.16 for K-Casein, 0.77, 0.22 and 0.01 for Leptin for AA, AB and BB genotypes, respectively. For *BoLA-DRB3* we identified 19 alleles and *DRB3.2*8* had the highest allelic frequency (22.4%). One of the 19 allele had a new pattern. Average heterozygosity value for all loci was low. χ^2 test did not confirm the Hardy-Weinberg equilibrium for Calpastatin in this population. These data provide evidence that Iranian's Sistani breed have a variability, which opens interesting prospects for future selection programs, especially marker-assistant selection.

A070

Novel Y chromosomal haplotypes reveal wild and domestic sheep diversity

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The development of commercial sheep flocks is a male mediated process that to date has been measured using autosomal microsatellites or variation in the maternally inherited mitochondrion. This study presents the first analysis of both domestic and wild sheep genetic diversity by analysing molecular markers residing on the ovine Y chromosome. Analysis of the SNP oY1 revealed allele A-oY1 was present in all wild bighorn sheep (*Ovis canadensis*), two subspecies of thinhorn sheep (*O. dalli*), European Mouflon (*O. musimon*) and Barbary (*Ammontragis lervia*). A-oY1 also had the highest frequency (71.4%) within 458 domestic sheep drawn from 65 breeds sampled from Africa, Asia, Australia, the Caribbean, Europe, the Middle East and Central Asia. Sequence analysis of a second locus, microsatellite SRYM18, revealed a compound repeat array displaying fixed differences that identified bighorn and thinhorn sheep as distinct from the European Mouflon and domestic animals. Combining genotypic data resulted in the identification of 11 male specific haplotypes that represent at least two separate lineages. Investigation of the geographic distribution of each haplotype revealed one (H6) was both very common and widespread in the global sample of domestic breeds. The remaining haplotypes each displayed more restricted and informative distributions such as H5 which was likely founded following the domestication of European breeds and was used to trace the recent transportation of animals to both the Caribbean and Australia. A high rate of Y chromosomal dispersal appears to have taken place during the development of domestic sheep as only 12.9% of the total observed variation was partitioned between major geographic regions.

A088

Cost-effective parentage verification with 17plex PCR for goat and 19plex PCR for sheep

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Today microsatellite markers are well established in routine typing services as markers of choice for verifying the parentage and identification of individual animals since they are numerous, highly polymorphic and relatively easy to score. Multiplex polymerase chain reaction (MPCR) is a powerful technique typically used in genotyping applications where the simultaneous analysis of multiple markers is required. In parentage testing and individual identification easy and cost-effective MPCR is routinely used. MPCRs with at least 10 microsatellite markers were developed for cattle, horses, dogs and cats; commercial kits are also available (Applied Biosystems, Finnzymes Diagnostics). In small ruminants multiplex systems are not generally known up to now. In this study we propose a set of 17 markers for goat (BM1258; BM1329; BOBT24A; CSRD247; ETH10; HSC; ILSTS005; INRA005; INRA040; INRA063; INRA231; OarFCB20; OarFCB128; SPS113; SPS115; SRCRSP01; SRCRSP08) and a set of 19 markers for sheep (CSRD247; ETH10; HSC; ILSTS005; ILSTS011; INRA040; INRA063; INRA231; MAF65; MAF209; McM527; OarCP49; OarFCB20; OarFCB128; OarFCB304; SPS113; SPS115; TCRGC4; TCRVB6) which can be co-amplified simultaneously and meet the needs for parentage testing and individual identification. Markers were amplified using the QIAGEN Multiplex PCR Kit, PCR products were separated on a ABI PRISM® 3100. A total of 165 and 249 alleles were found in 11 goat breeds (426 samples tested) and 13 sheep breeds respectively (344 samples). Assuming one known parent the individual exclusion power ranged between 0.20 and 0.84 for the 17 markers tested in goat and between 0.48 and 0.81 for 18 of the 19 makers tested in sheep. The cumulative exclusion probability reached 0.999993 in goat and 0.999999 in sheep. The range of gene differentiation coefficients of the markers (G_{ST}) was 0.10-0.26 for goats and 0.09-0.20 for sheep. Both MPCRs may therefore also be used to distinguish between breeds isolated for a longer time and without introgression. For breeds with a recent common history or living in the same region, more markers will be necessary in order to determine breed differences.

A095

Analysis of polymorphisms at candidate genes in chicken associated to genetic resistance in avian viral diseases.

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The objective of this study is to assess polymorphisms at candidate genes associated with genetic resistance to viral diseases in chicken, such as Marek's disease and avian influenza. Two candidate genes were shortlisted from literature and their sequences obtained from Ensembl. BLBII (class II) major gene is present in chicken within the MHC region on chromosome 16. It has been shown to be associated with resistance to avian viral infection, including Marek's disease. Mx gene on chromosome 1 is a putative candidate gene for genetic resistance to avian influenza. Analysis of the ChickVD database revealed polymorphism present in both genes. Species homologs of these two genes were obtained from the NCBI BLAST database and analysed with the PAML package for detection of signature of positive adaptive selection (dN/dS ratio). For the Mx gene, in chicken, the results indicated a dN/dS ratio of 0.64: a result in agreement with high level of sequence conservation observed in this gene in both mammalian and other bird species. PCR-sequencing was carried out for both genes to detect novel SNPs and to validate the SNPs in the ChickVD database in both indigenous and commercial chicken populations. Polymorphism analysis at the BLBII gene exon 2 revealed that this gene is highly polymorphic with new polymorphisms detected in indigenous chicken populations. Analysis of exon 13 at the Mx gene revealed two SNPs, one of which is present in codon 631. This SNP is a G/A transition which leads to a non-synonymous amino-acid substitution from serine to asparagine believed to be associated with avian influenza resistance. Analysis of this exon 13 Mx SNP in different chicken populations reveals distinct allelic frequencies among the indigenous rural and commercial chicken populations examined.