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Molecular tests for horse coat color determination

Maria Cristina Cozzi, Marta Bottagisio, Erika Frigo, Maria Giuseppina Strillacci, Fausta Schiavini, Raphaelle Teresa Matilde Maria Prinsen, Alessandro Bagnato

Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare, Università degli Studi di Milano, Italy

Corresponding author: cristina.cozzi@unimi.it

Wild animals often have muted colors that allow them to blend into the background. During the domestication, people delight in selecting for color variants, so that domestic animals, including cats, dogs, cows, sheep, horses and goats exhibit a wide range of color patterns, even though they belong to the same species. The horse basic coat colors (chestnut, bay and black) are controlled by the interaction between two genes: Melanocortin-1-Receptor [MC1R; Extension (E,e)] and Agouti Signaling Protein [ASIP; Agouti (A,a)]. The Extension gene (red factor) controls the production of red and black pigment. Agouti controls the distribution of black pigment either to a point pattern (mane, tail, lower legs, ear rims) or uniformly over the body. Ten other genes may modify the distribution, production and quantity of these pigments and are responsible for the large variety of horse coat colors. Most of coat colors may be detected based on physical appearance or phenotype alone. The aim of this study is to develop molecular tests in order to define phenotypes that are visually ambiguous and identify the correct coat color phenotype for the four dilute phenotypes palomino, buckskin, cremello and perlino due to the effects of Membrane Associated Transporter Protein gene [MATP/SLC45A2; Cream (Cr,cr)] on basic colors. We demonstrate the effect of polymorphisms of MC1R, ASIP and MATP/SLC45A2 genes on 26 Akhal-Tekè horses bred in Italy with defined phenotypes bay, chestnut, black, buckskin, cremello and perlino. The 38.46% of horses tested, show a discrepancy between the phenotype reported on the certificate and the result of analyses carried out. On 11,54 % of the horses certificate is indicated any color. With the genetic analysis we can determine the correct genetic basis of the coat color and thus complete in a correct manner the certificate with the phenotype description.

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Genetic diversity in three Italian donkey populations assessed by microsatellite markers

Enrico D'Alessandro[1], Antonino Siclari[2], Luigi Liotta[1]

[1]*Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, Italy*

[2]*Ente Parco Nazionale dell'Aspromonte, Santo Stefano in Aspromonte, Gambarie (RC), Italy*

Corresponding author: edalessandro@unime.it

We analysed the genetic variability of three Italian donkey populations, Ragusano (RAG), Grigio Siciliano (GSI) and Calabrese (CAL). Calabrese breed is mainly reared in the Natural Park of Aspromonte (Calabria) and represents a rare example of semiferal autochthonous donkey. The genetic diversity of these populations has been investigated using 12 microsattelite loci approved for identification and parentage test by International Society for Animal Genetics (ISAG). We tested 100 unrelated animals: 40 Ragusano, 20 Grigio Siciliano and 40 Calabrese. Genomic DNA was amplified for 12 microsatellite loci (HTG10, VHL20, HTG7, HTG4, AHT5, AHT4, HMS3, HMS6, HMS7, HMS2, HTG6 and ASB17) in two multiplex PCR reactions. The PCR products were mixed with GeneScan 350 ROX internal size standard. Gel electrophoresis and genotype determination were performed on an 3500 Genetic Analyzer. We calculated: - the allele number and the allelic frequency; - the Polymorphism Information Content (PIC); - the heterozygosity; - the deviation from Hardy-Weinberg equilibrium; - the Inbreeding coefficients (FIS); - The Wright diversification (FST) according to Weir and Cockerham, using the computer packages GENEPOP and FSTAT. Data were analysed using different software (MICROSATELLITES ANALYSER, FSTAT and MICROSAT) to calculate basic population parameters, including genetic distances among breeds/populations. GENETIX and STRUCTURE software were used to evidence potential population structures among the Calabrese population and the other considered breeds. All microsatellites were polymorphic in each breed. The total number of alleles was 118. Considering the three tested donkey populations, allele number varied from 4 for AHT4 locus to 16 HTG7 and HTG5 loci; PIC varied from 0.809 for AHT5 to 0.174 for HTG6. The mean heterozygosity for each population was 0.436 (Ragusano), 0.545 (Grigio siciliano) and 0.503 (Calabrese). The inbreeding coefficient (FIS) was 0.0576±0.02, while the Wright diversification (FST) was 0.0862±0.02. Most loci did not show a significant deviation from HWE. We observed a considerable genetic variability even if the three populations are similar and show reciprocal influences, considering all tested parameters.