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ABSTRACT

In the developed countries donkey has lost its main function as draft animal because of the mechanization in agriculture; as a consequence donkey population was greatly reduced. According to SAVE monitoring institute, three of the eight Italian endangered donkey breeds are native of Sicily (Ragusano, Pantesco, Grigio Siciliano). Urgent safeguard plans are required. The aim of this work is to investigate the distribution of genetic diversity and the relationships among the three Sicilian autochthonous donkey breeds using a set of microsatellite markers. A total of 116 blood samples (61 Ragusano, 39 Pantesco, 16 Grigio Siciliano) were collected in 9 herds all over Sicily. Representative samples of Ragusano and Grigio donkey populations consist of unrelated individuals, whereas the sample of Pantesco represents nearly the entire studbook-registered population managed by "Ispettorato Ripartimentale delle Foreste di Erice (TP)" in the "Azienda S. Matteo". Genomic DNA was amplified at 11 microsatellite loci (HTG10, VHL20, HTG7, HTG4, AHT5, AHT4, HMS3, HMS6, HMS7, HMS2 and HTG6), located on different chromosomes in two PCR multiplex reactions. The PCR products were mixed with GeneScan 350 ROX internal size standard and ran on an ABI Prism 377 DNA Sequencer equipped with Genescan and Genotyper softwares. MICROSATELLITES ANALYSER, FSTAT and MICROSAT softwares had been used in order to calculate the main parameters of genetic diversity and the genetic distances. Phylogeny trees were obtained using PHYLIP 3.66 and SPLITSTREE 4 4.6 software packages. Clustering analysis has been carried out using STRUCTURE 2.0 program. All microsatellites were polymorphic in each breed, generating a total of 76 alleles (from 3 to 11 in HTG4 and HTG7, respectively). The number of observed alleles was higher in Ragusano (72, 6.55 per locus), than in Grigio (55, 5 per locus) and Pantesco (45 alleli, 4.09 per locus). The number of effective alleles did not reproduce the same trend (2.62 in Grigio, 2.53 in Ragusano and 2 in Pantesco). A total of 13 private alleles (17% of the generated alleles), with frequencies always lower than 0.10, have been detected (13 in Ragusano, 2 in Pantesco, 2 in Grigio Siciliano). The genetic differentiation coefficient was low ($G_{st} = 0.065$). According to the G_{st} value (0.163) HMS3 was the most discriminating marker in the set, whereas HTG4 was the less one. The populations were not at genetic equilibrium, showing a significant deficit of heterozygotes. The average expected heterozygosity (H_e) over all population was 0.612. H_e was lower in Pantesco (0.500) than in Ragusano and Grigio (0.605 and 0.618, respectively). F_{is} value, which indicates the degree of departure from random mating, was particularly high in Pantesco (0.230), in which the sample represent the whole population, than in the other two breeds. Genetic distances estimated by different methods (D_a and D_p) and the related NJ and UPGMA dendrograms, as well as the clustering analysis, agree with the evidence that Pantesco endangered breed shows the higher degree of genetic distinctness. Ragusano and Grigio result closely related ($D_a = 0.058$), probably because of their common origin: in fact before 1953 in Sicily local donkey with bay and grey coat were usually managed as a single population.