Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

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To cite this article: R. Ciampolini, F. Cecchi, E. Mazzanti, E. Ciani, M. Tancredi & B. De Sanctis (2007) The genetic variability analysis of the Amiata donkey breed by molecular data, Italian Journal of Animal Science, 6:sup1, 78-80, DOI: 10.4081/ijas.2007.1s.78

To link to this article: https://doi.org/10.4081/ijas.2007.1s.78

The genetic variability analysis of the Amiata donkey breed by molecular data

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ABSTRACT: This study presents the results of the genetic characterization of the Amiata donkey breed using STR markers. A total of 18 microsatellite loci were analysed in 50 unrelated individuals reared in Tuscany and in Lazio (34 and 16 animals respectively). The average number of alleles per locus was moderate (5.61±2.893), ranging from 2 (ASB02) to 13 (HTG7). Mean observed heterozygosity was 0.579, whereas mean expected heterozygosity was 0.609. Six markers showed a significant (P<0.01) deviation from the Hardy-Weinberg proportions. The average similarity values within the two groups were 0.523 ± 0.020 for animals reared in Tuscany and 0.458 ± 0.016 for those reared in Lazio (total average similarity 0.489 ± 0.019) while inbreeding coefficients were respectively 0.440 and 0.390.

Key words: Microsatellites, Amiata Donkey, Genetic variability, Heterozygosis.

INTRODUCTION – The donkey breeds that have suffered a substantial decline in population size may present elevated levels of inbreeding, resulting in inbreeding depression and risk of extinction. One of the first stages in the conservation programme of endangered breeds is represented by the evaluation of their variability. In a previous study we have evaluated demographic and genetic parameters of the Amiata donkey breed by analysing the available pedigree information (Cecchi *et al*., 2006). The aim of this research was to evaluate the genetic variability of the Amiata breed using microsatellite markers.

MATERIALS AND METHODS – *Animals.* We sampled 50 Amiata donkey reared in Tuscany and in Lazio (34 and 16 animals respectively). Subjects were selected based on genealogical data in order to minimize relationship between animals (this was particularly difficult due to the general lack of accurate genealogical information). *DNA extraction*. Donkey DNA was extracted from whole blood according to standard methods involving lysates of the washed white-cells and phenol-chloroform-isoamylalcohol (25:24:1) extraction (Sambrook *et al*., 1989). *Microsatellite analysis.* The locus name, chromosome location, dye label, and size range of the amplified products for all markers are presented in table 1. Out of 18 selected STR markers, thirteen belonged to the ISAG-FAO panel recommended for diversity studies in equine species (HTG6, HMS6, HTG7, HMS2, AHT4, ASB23, COR58, HTG10, HMS7, VHL209, ASB2, HMS3 and SGCV28) The other markers, originally developed for the equine species were selected based on literature information on this species. Markers were amplified in four multiplex PCR reactions using fluorescently-labelled primers (primer sequences and PCR conditions are available upon requests). PCR products were detected by capillary electrophoresis on an Applied Biosystems 310 DNA Sequencer, using the ROX 350 bp internal-size standard. Data analysis was performed using the GENEMAPPER software (ABI, v. 4.0, 2005). *Statistical analysis.* Allelic frequencies were estimated by direct counting. Exact tests for deviation from the Hardy-Weinberg equilibrium (HWE) were performed using the ARLEQUIN package (Schneider *et al.*, 2000). Genetic similarities (Ciampolini *et al.*, 1995) within and among animals grouped according to their geographical origin (group 1: animals from Tuscany; group 2: animals from Lazio) were calculated by performing pair-wise comparisons between all possible individual multilocus genotype pairs. Genetic similarity is defined as P=A/2L, where P is the

proportion of common alleles (A) in relation to the 2L possibilities (L= number of considered loci). The similarities between each pair of individuals were then averaged over the whole population. F-statistics, molecular coancestry coefficients, Kinship distance and inbreeding coefficients were obtained using MolKin v.2.0 (Gutièrrez *et al.*, 2005).

RESULTS AND CONCLUSIONS – Allelic size range and number of alleles per locus are presented in Table 1. The mean number of alleles per locus in the whole population was 5.61 ± 2.893, ranging from 2 (ASB02) to 13 (HTG7). This value was lower with respect to that observed by Jordana et al (2001) in the Catalonian donkey using 10 loci, and by Arangures-Mendez et. al. (2001) in five Spanish donkey breeds, using 15 loci. Microsatellites AHT4, HTG10 and VHL20 showed a number of alleles higher than reported in literature for horses (Sun-young Lee and Gil-jae Cho, 2006), while ASB02, HMS3 and HMS6 showed a lower number of alleles. Microsatellites AHT4, HMS7 and VHL20 showed a number of alleles higher than reported in literature for Catalonian donkey breed (Jordana *et al*, 2001); microsatellites HMS2 and HMS6 showed the same number of alleles, while microsatellites HMS3 and HTG6 showed a lower number of alleles. Microsatellite HMS1 showed four alleles, while resulting to be monomorphic in three Italian donkey population (Romagnolo, Ragusano and Martina Franca) studied by Blasi *et al*. (2005) and in five Spanish donkey breeds (Arangures-Mendez et. Al., 2001). Observed heterozygosity, averaged over loci (0.579) was higher than observed by Blasi *et al*. (2005), whereas expected heterozygosity (0.609) was lower than observed by Jordana *et al.* (2001) and by Arangures-Mendez *et al.* (2001). Six microsatellites (ASB02, ASB23, HMS1, HMS2, HTG10 and SGCV28) were in Hardy-Weinberg disequilibrium (P<0.05 for SGCV28 and P<0.01 for all the others); significant deviations from Hardy-Weinberg proportions were mostly associated with heterozygote deficiency.

Table 1. Microsatellite information. Observed (H_{obs}) and expected (H_{exp}) heterozygosity from the total sample are also reported.

	Chromosome	Dye	N. of alleles	Size range (bp)	H_{obs}	H_{exp}	P value	s.d.
AHT4	24	6-FAM	10	138-170	0.620	0.719	0.449	0.005
ASB02	15	NED	2	222-256	0.640	0.465	0.006	0.003
ASB23	3	6-FAM	6	176-212	0.755	0.784	0.000	0.000
COR58	12	NED	5	210-230	0.620	0.664	0.928	0.007
COR71	26	NED	7	190-202	0.700	0.726	0.456	0.009
HMS1	15	HEX	4	166-178	0.460	0.615	0.000	0.000
HMS2	10	NED	8	215-236	0.640	0.653	0.000	0.000
HMS3	9	NED	5	150-174	0.700	0.691	0.808	0.010
HMS45	27	6-FAM	3	185-197	0.580	0.573	0.729	0.015
HMS6	4	NED	3	153-171	0.540	0.500	0.508	0.010
HMS7	1	6-FAM	6	167-189	0.400	0.427	0.381	0.004
HTG10	21	NED	9	89-171	0.580	0.758	0.000	0.000
HTG4	9	HEX	3	127-141	0.490	0.505	0.143	0.008
HTG6	15	6-FAM	4	74-103	0.680	0.704	0.084	0.006
HTG7	4	NED	13	114-126	0.840	0.874	0.770	0.004
SGCV28	7	6-FAM	4	151-163	0.440	0.538	0.020	0.002
VHL20	30	6-FAM	6	89-107	0.420	0.419	0.955	0.006
VHL209	14	NED	3	84-96	0.326	0.335	0.262	0.012

Table 2 describes the genetic variability of the analyzed populations. Genetic differentiation between group 1 and group 2 (F_{st} = 0.043; P<0.001) was moderate. F_{is} and F_{it} for the whole population were respectively 0.004 and 0.033. Some alleles were observed in only one group (16 and 13 alleles "private", for group 1 and 2, respectively). Observed heterozygosity was higher in the group 2 (0.424) compared to group 1 (0.392). Genetic similarity within the whole population was representative of a relatively low genetic variation. A possible interpretation of the high genetic similarity is that Amiata population has suffered a severe bottleneck in recent years and has, con-

sequently, undergone an increase in the inbreeding degree. These hypothesis seems to be inconsistent with our previous data based on genealogical analysis (Cecchi *et al.*, 2006); however, this is probably due to the lack of genealogical records for a substantial number of individuals that may have caused a possible underestimation of the inbreeding coefficient. Nevertheless, coancestry coefficients obtained from molecular information has been shown to be useful for conservation purposes so the individuals that have the lowest average molecular coancestry values, should be selected for this aim. In addition animals reared in Lazio showed an higher genetic variability characterized by a lower f_{ij}, a lower coefficient of inbreeding and a lower genetic similarity. The differences between the two groups could be ascribed to a more accurate breeding management of stallions by the breeders from Lazio with respect to those from Tuscany.

Table 2. Inbreeding coefficient, molecular coancestry (f_{ij}), Kinship distance (D_k) and average genetic similarities for each population and for the entire population.

	Animals of Tuscany	Animals of Lazio	Entire population
Inbreeding	0.440	0.390	0.424
Molecular coancestry (f_{ij})	0.435	0.391	0.420
Mean Kinship distance (D_k)	0.284	0.303	0.290
Average genetic similarities	0.523	0.469	0.489

The research was supported by MIUR (PRIN 2004).

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