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Genetic diversity of Sardinian goat population based on microsatellites

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RIASSUNTO – Diversità genetica della popolazione caprina sarda basata sull'analisi di microsatelliti. *Sono stati analizzati 331 caprini, provenienti da 35 allevamenti di diverse aree della Sardegna, con 17 microsatelliti. Gli allevamenti sono stati suddivisi in tre gruppi, maltese (M), meticcio (C) e tipo Sardo autoctono (S), sulla base dei profili fenotipici degli animali e delle informazioni fornite dall'allevatore riguardo l'origine dei riproduttori utilizzati. Le statistiche calcolate (eterozigosità, alleli privati, distanza genetica standard e di Reynolds, Gst) hanno messo in evidenza una ridotta variabilità genetica del gruppo M rispetto agli altri due, nonché, la presenza di un gruppo di allevamenti i cui individui sono assimilabili al tipo Sardo autoctono. Il gruppo meticcio ha mostrato un'elevata variabilità e distanze significative con gli altri 2 gruppi. I risultati hanno consentito di identificare il nucleo di allevamenti da cui partire per la conservazione del tipo genetico autoctono. Resta ancora da definire il ruolo del gruppo meticcio nel preservare la variabilità genetica complessiva.*

KEY WORDS: autochthonous genetic type, genetic variability, genetic distance, Gst

INTRODUCTION – During the last century, the selection for production traits of the main livestock species has led to a reduction in number of local populations with consequent loss of genetic variability. In Sardinia, the genetic improvement strategy has been based on selection for the local pure breed in sheep, whereas in the other species (cattle, swine and goat), an often unplanned crossbreeding with improved breeds has been applied. In this context, several studies on genetic diversity of these populations have been started with the final aim of recovering the autochthonous genetic types. The global approach involves different steps: - characterization of the farming system and typical products; - morphological and productive evaluation of the animals; - genetic characterization of the populations through molecular DNA analyses (microsatellites, AFLP, mitochondrial DNA, etc.); - creation of nucleus flocks in public farms and germoplasm banks; - organization of herd-books involving farmers interested in the safeguard of local breeds and valorisation of traditional products. A far as the goat population is concerned, Sardinia is the Italian region with the largest goat stock, (209.000 heads, about 23% of the total national stock). Farms are located in mountains, low hills and even plans (Macciotta *et al.*, 2002) resulting in a quite large variability (Brandano and Piras, 1978) in terms of feeding and management techniques; but extensive and semi extensive systems in marginal areas prevail (Carta *et al.*, 2001; Usai *et al.*, 2004). The current population has been constituted by crossbreeding the autochthonous animals with other improved Mediterranean breeds, mainly Maltese goat. The aim of this paper is to study the genetic variability of this population through the analysis of 17 microsatellite markers.

MATERIALS AND METHODS – A survey concerning the feeding and management, the breeding history and the reproductive and productive performances was carried out on 210 farms. Among those, 35 were chosen to represent the main areas interested by the goat farming and the overall variability of morphological

traits. In these flocks about 8 females and 2 males, were blood sampled and digital picture captured (331 heads: 270 females and 61 males). DNA was extracted from frozen white blood cells with a rapid technique (NaCl 10 mM, EDTA 10 mM and NaOH 200 mM at 65°C and TriHcl 100mM/HCl 100 mM). The 17 microsatellite markers used for the molecular analysis belong to the panel of 30 markers of the Econogene Project (http://lasig.epfl.ch/projets/econogene/). Microsatellites were selected for the polymorphism content and the good efficiency of amplification. Genotyping was carried out using an ABI PRISM 377 DNA sequencer (APPLERA, Foster City, CA). PCR primer pairs were labelled with dye TET, 6FAM or HEX and, if possible, the PCR reactions were carried out in multiplex. Data were captured using Genescan software and the allele score was carried out using Genotyper software and confirmed by visual observations of the electrophoretic runs. Successively, all the individuals belonging to the same flock were assigned to one of the three following groups on the basis of the overall phenotypic profiles and the breeding strategies of flocks. These groups were: Maltese (M, 5 flocks and 49 heads), crossbred population (C, 18 flocks and 165 heads) and local Sardinian type (S, 12 flocks and 117 heads). For each microsatellite, the actual (Na) and effective (Ne) number of alleles per locus, the expected (He) and observed (Ho) heterozygosities were calculated for both the whole and the three groups. Using specific programs developed in the SAS System Language, the coefficient of gene differentiation (Gst) (Nei, 1973), the standard genetic distance (Ds) (Nei, 1972) and Reynolds distance (Dr) (Reynolds *et al.* 1983) were calculated and the significance level assessed by 10.000 permutations of the C and S flocks.

RESULTS AND CONCLUSIONS – In the whole population, the observed Na ranged between 5 and 20, whereas Ne varied from 1.3 to 7.2. Ho was lower than He for all the microsatellites. Within group average He varied from 0.67 for M to 0.73 for C and was higher than Ho for most loci (table 1).

Table 1. Actual number of alleles (Na); effective number of alleles (Ne); expected (He) and observed eterozigosity (Ho) in the whole population and within groups.

		Na (Ne)				He				Ho			
Microsatellite	Chr	TOT	M	C	S	TOT	M	C	S	TOT	M	C	S
CSRD247	14	13 (5.1)	7 (3.8)	11 (5.3)	9 (3.5)	.80	.74	.81	.71	.68	.72	.74	.59
DRBP1	23	20 (5.4)	6 (3.8)	16 (5.7)	19 (5.2)	.82	.74	.83	.81	.40	.50	.41	.34
ETH10	5	7(2.9)	6 (3.3)	6 (2.8)	6 (2.7)	.66	.70	.65	.63	.56	.46	.56	.59
FCB48	17	9 (7.2)	7 (4.0)	9 (6.9)	9 (7.6)	.86	.75	.86	.87	.77	.63	.77	.83
INRA063	18	8 (4.4)	6 (3.6)	8 (4.7)	6 (3.3)	.77	.72	.79	.69	.60	.58	.60	.60
INRABERN172	26	14 (7.2)	11 (3.7)	14 (7.0)	10 (7.4)	.86	.73	.86	.86	.67	.55	.67	.71
MAF209	17	5 (1.3)	3 (1.2)	5 (1.4)	4 (1.3)	.25	.20	.30	.21	.21	.17	.25	.17
McM527	5	8 (4.5)	7 (3.7)	8 (4.5)	8 (3.9)	.78	.73	.78	.75	.66	.79	.63	.63
SRCRSP03	10	8 (3.1)	4 (2.4)	7 (3.0)	8 (2.7)	.67	.58	.66	.63	.43	.55	.41	.40
SRCRSP05	21	11 (6.4)	8 (3.9)	11 (6.6)	9 (5.2)	.84	.74	.85	.81	.79	.81	.83	.73
SRCRSP07	6	5 (2.6)	3 (2.0)	5 (3.0)	4 (2.4)	.62	.49	.66	.59	.47	.42	.52	.42
SRCRSP08	-	15 (4.2)	8 (3.8)	13 (3.9)	10 (4.6)	.76	.73	.75	.78	.57	.61	.57	.55
ILSTS005	10	7 (2.1)	5 (2.5)	6 (1.9)	7 (2.1)	.52	.60	.47	.53	.49	.71	.43	.50
ILSTS011	14	10 (3.2)	7 (3.4)	10 (3.6)	9 (2.4)	.69	.71	.73	.59	.47	.46	.53	.39
ILSTS029	3	14 (4.6)	8 (4.9)	12 (4.1)	10 (4.4)	.78	.79	.75	.77	.50	.67	.42	.53
ILSTS087	28	13 (5.5)	6 (2.6)	12 (6.1)	12 (5.7)	.82	.62	.84	.82	.60	.30	.64	.68
TGLA053	16	13 (4.6)	8 (3.9)	13 (5.5)	9 (2.4)	.78	.74	.82	.59	.55	.55	.60	.48
mean		10.6(4.4)	6.5(3.3)	9.8(4.5)	8.8(3.9)	.72	.67	.73	.69	.55	.56	.56	.54

The number of private alleles of C and S respect to M was very high (61 and 47 respectively), although very few of them showed frequency higher than 10%. Moreover, 28 private alleles were found in C respect to S (table 2). These results confirm, as expected, the higher genetic variability of C respect to M and S and the highest homogeneity of M. This pattern agree with previous studies (Sechi *et al.*, 2004).

Table 2. Number of alleles per group on diagonal, number of private alleles between groups out of diagonal (number of alleles having frequency >0.1-average frequency of all private alleles).

	M	C	S	All Group
M Vs	110	5 (0 - 0.019)	8 (0 - 0.032)	3 (0 – 0.016)
C Vs	61 (3 - 0.023)	166	28 (0 - 0.010)	23 (0 – 0.008)
S Vs	47 (4 - 0.031)	11 (0 - 0.010)	149	9 (0 – 0.011)

Although Gst, index of the between-groups variability, resulted of low value (0.04), it was highly significant ($P<0.0001$). Genetic distances between S and M were 0.259 and 0.099 for Ds and Dr, respectively. Whichever distance measure we used, C group showed intermediate distances and was much closer to S than M (table 3). Results of permutations demonstrated that distances were highly significant.

Table 3. Genetic distances between subpopulations: Dr (above diagonal) and Ds (below diagonal).

	M	C	S
M		0.062 P<0.0006	0.099 P<0.0001
C	0.160 P<0.0008		0.021 P<0.0003
S	0.259 P<0.0003	0.050 P<0.0005	

The comprehensive exam of the reported results confirmed that Maltese breed is easy to differentiate from the other two groups. Moreover, the autochthonous and crossbred groups, even if less distant between them, were significantly discriminated. In conclusion, the strategy of assigning whole flocks rather than individual animals to a group allowed us to identify a group of flocks (S) to start a program of safeguard of the autochthonous genetic type. However, it is still to be assessed which role the crossbred populations should assume in a strategy of preservation of the total genetic variability.

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