

Genetic characterization of Appenninica sheep breed by microsatellites

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ABSTRACT - The conservation of genetic variability is one of the main objectives in the field of genetics applied to domestic livestock. A strong input in that direction was made by molecular biology techniques. Among molecular markers nowadays available, microsatellites are particularly appreciated and widely utilized for the study of animal genome. In this study, a genetic characterization of Appenninica sheep breed was carried out with thirty microsatellite markers; the genetic relationships between Appenninica and three Italian merinos-derived breeds were also investigated. The results show a good genetic variability of Appenninica and all the other studied breeds. At the same time, the genetic identity of each breed is confirmed. These molecular data can be utilized to improve the present selection schemes and the plans to preserve the genetic diversity.

Key words: Molecular markers, Appenninica, Italian merinos-derived breeds, Genetic variability.

Introduction - The genetic variability is the most important tool to improve the productive performance of reared animals. Therefore, it is important to quantify the genetic variability and a lot of phylogenetic studies have been performed to identify the populations that need assistance because endangered and to direct the efforts of conservation in the right way (Medrano, 2000). The genetic characterization is necessary for these purposes: in fact, if the real situation of the genetic resources in the populations are well known, it will be easier to organize a plan to maintain the genetic diversity, that means to preserve the largest number of allelic variants particularly for the genes involved in the quality of production and in the environmental adaptability. In livestock, between molecular markers nowadays available, microsatellites are particularly appreciated and widely utilized for the study of animal genome. They are right now used to map the genomes of different species, for the analysis of kinship and parentage control and to calculate the genetic distances between breeds (Diez-Tascón *et al.*, 2000).

The purpose of this study was to investigate the genetic variability of the Appenninica (APP) sheep breed and its genetic relationships with three other different breeds (Sopravissana (SOP), Gentile di Puglia (GDP) and Spanish Merinos (SM)) using microsatellite DNA polymorphisms.

Material and methods - A total of 30 samples of Appenninica breed were collected in the following Italian provinces: Perugia (1 farm), Forlì (6 farms), Grosseto (3 farms), Pisa (1 farm), and Arezzo (1 farm). DNA was extracted from blood using GenElute Blood Genomic DNA kit (Sigma Aldrich). Thirty sets of primers (Table 1), included in the lists of recommended primers for sheep analysis suggested by the FAO organisation (FAO, 2004), were chosen on the basis of their position in the sheep genome. The markers were subjected to a multiplex PCR amplification using a Biometra TGradient 96 at the following conditions: initial denaturation step of 5 min at 94°C, 35 cycles of 30 s at 95°C, 45 s at the annealing T of each multiplex PCR, 30 sec at 72°C, and a final extension of 15 min at 72°C. A reaction volume of 10 µl contained 40 ng of genomic DNA, 2.5 mM MgCl₂, 1 µl of PCR buffer 10X, 0.5 U Hot

start Taq (Sigma Aldrich), 200 μ M dNTPs, and 0.2 pmol of each primer. The multiplex PCR products were pooled in order to analyze more microsatellites in each electrophoresis. Analyses of fragments were performed using an automated DNA sequencer (ABI PRISM 3130xl - Applied Biosystems) and a computer software (GeneMapper version 4.0 - Applied Biosystems).

In the statistical analysis the results of SOP (44 samples), GDP (30 samples), and SM (40 samples) were included, because the genotypes at the same markers were already available. The first two breeds were used to determine their distances and genetic relation with APP. In fact, probably, they derived from the cross of APP breed with the SM. The SM was included because it represents the Spanish ancestor (male line) of SOP and GDP, and can provide further information on the phylogenetic analysis.

Alleles were designated according to PCR product size and total number of alleles was estimated by software Fstat 2.9.3.2 (Goudet, 2002). The heterozygosity (expected and observed) and the F_{IS} according to the method proposed by Weir and Cockerham (1984) were calculated with the software Genetix version 4.05 (Belkhir, 2001). The graphic package of the same software allowed to perform the Correspondence Analysis in order to analyze each population through various variables and the differentiation between breeds.

Results and conclusions - All the microsatellites examined resulted polymorphic; a total of 358 alleles were detected and the average number of alleles per locus was 11.93 (Table 1).

Table 1. Microsatellite markers, chromosomes involved (Chr.) and alleles detected.

Locus	Chr.	Alleles	Length (bp)	Locus	Chr.	Alleles	Length (bp)
BM1824	1	6	180-192	CRSD247	14*	18	218-244
BM6506	1	8	192-208	INRA63	14	15	165-199
INRA006	1	18	116-141	SPS115	15	8	185-205
OarFCB11	2	15	121-143	MAF65	15	8	123-135
OarFCB20	2	14	92-112	TGLA126	16	15	110-240
OarCP34	3	9	112-126	MAF214	16	9	182-230
McM527	5	10	165-175	MAF209	17	13	109-135
D5S2	5*	8	190-210	BM8125	17	9	116-122
ETH10	5*	4	208-208	OarFCB48	17	12	143-167
RM006	5	11	119-130	TGLA122	18	15	133-153
ETH225	9	11	136-156	OarFCB304	19	16	150-188
CSSM66	9	18	180-202	HSC	20	16	268-300
ILSTS11	9	11	268-282	BM1818	20	15	258-284
INRA35	12	12	120-140	OarCP20	21	11	71-87
TGLA53	12	11	121-147	BM6526	26	12	161-175

*: relatives to cattle linkage map (not mapped in *Ovis aries*).

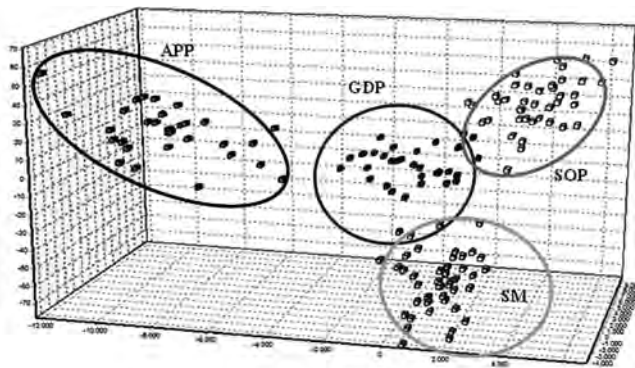
Expected and observed H values are reported in Table 2. The APP breed showed the highest value of expected heterozygosity and also the other three studied breeds have very high values for H exp and H obs. These data are similar to the values observed by Peter *et al.* (2007) in other European sheep breeds. The comparison of H exp and obs values shows a high deficit of heterozygotes in APP

Table 2. Average values of expected (H exp) and observed (H obs) heterozygosity and inbreeding values (F_{IS}).

Breed	H exp	H obs	P	F_{IS}
SOP	0.74	0.70	*	0.048 (0.007 – 0.064)
GDP	0.74	0.69	ns	0.070 (-0.010 – 0.106)
SM	0.71	0.65	*	0.086 (0.034 – 0.108)
APP	0.76	0.67	*	0.118 (0.050 – 0.130)

*= $P < 0.05$; ns=not significant.

Figure 1. Spatial representation of the individuals of the studied breeds as defined by Correspondence Analysis.



breed, like confirmed by the high F_{IS} value (Table 2). We can suppose that this disequilibrium could be determined by the little sample size that, probably, does not permit to detect all the alleles in the population, like reported by B-Rao (2001).

In Figure 1 the spatial distribution of all the samples according to the results of the Correspondence Analysis is shown: the clusters of the four breeds are well defined and this fact confirms their genetic identity. In fact, as expected, the Merinos derived breeds are very close for their common origin. Furthermore, because the APP breed is closer to GDP, it can be assumed that this breed has contributed more to its constitution compared to the SOP that is close to the Spanish ancestor. The molecular information confirms the bibliographic data (Sarti, 1996) about the common origin of some Italian breeds derived from APP (SOP and GDP).

In conclusion, the results show the importance of a possible employment of the molecular data in the selection programs in order to preserve the genetic variability and therefore the possibility to improve APP breed productive performance.

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