



Genetic diversity and relationships among Italian Merino derived breeds assessed 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. Among molecular markers, microsatellites are particularly appreciated and widely utilized for the study of animal genome. In this study a genetic characterization of three Italian Merino derived sheep breeds was carried out by 30 microsatellites markers; the genetic relationships between these breeds and the Spanish ancestors were also investigated. All the microsatellites examined resulted polymorphic and a total of 375 alleles were detected. F_{IS} values of the three Italian Merinos demonstrate a low level of inbreeding. The results show a good genetic variability of all the 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 genetic diversity.

Key words: Molecular markers, Spanish Merino, Italian Merino-derived breeds, Genetic variability.

Introduction – Several studies on livestock biodiversity have been performed in order to identify the endangered populations and to optimize the efforts of conservation (Medrano, 2000; Dalvit *et al.*, 2008). If the real situation of the genetic resources in the populations is well known, it is easier to organize a plan to maintain the genetic diversity that means to preserve the largest number of allelic variants. Microsatellites are particularly appreciated and widely utilized in livestock to quantify genetic variation within and among breeds and are also useful for the conservation management of animal populations (Diez-Tascón *et al.*, 2000).

The purpose of this study was to investigate the genetic variability of the three Italian Merino sheep breeds Gentile di Puglia (GDP), Merinizzata Italiana (MIT) and Sopravvisana (SOP) and their genetic relationships with Appenninica (APP), Spanish Merino (SMR) and Merino Precoce (MPZ), using microsatellites DNA polymorphisms. It could be useful to underline that SOP and GDP are at present recognized as endangered breeds.

Material and methods – The animals used for this study were: 30 GDP, 30 MIT, 44 SOP. Moreover, MPZ (30 samples), SMR (40 samples) and APP (30 samples) were also included as reference breeds. DNA was extracted from blood. Thirty markers (*BM1824*, *BM6506*, *INRA006*, *OarFCB11*, *OarFCB20*, *OarCP34*, *McM527*, *D5S2*, *ETH10*, *RM006*, *ETH225*, *CSSM66*, *ILSTS11*, *INRA35*, *TGLA53*, *CRSD247*, *INRA63*, *SPS115*, *MAF65*, *TGLA126*, *MAF214*, *MAF209*, *BM8125*, *OarFCB48*, *TGLA122*, *OarFCB304*, *HSC*, *BM1818*, *OarCP20* and *BM6526*), included in the lists of recommended markers for sheep analysis suggested by the FAO (FAO, 2004), were chosen on the basis of their position in the sheep genetic map. Laboratory techniques are described in a previous paper (Lasagna *et al.*, 2009). 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} estimated according to the Weir and Cockerham method (1984), were calculated by the software Genetix version 4.05 (Belkhir, 2001). The Correspondence Analysis aimed to analyze and to differentiate the breeds, and was performed by the graphic package of the same software.

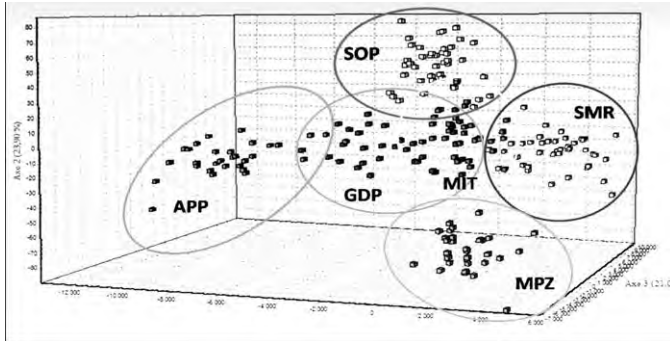
Results and conclusions – All the microsatellites resulted polymorphic in all analysed breeds; a total of 375 alleles were detected and the average number of alleles per *locus* was 8.4 in SOP, 7.8 in GDP and 8.2 in MIT (table 1). The three Italian Merinos breeds showed the highest average values of observed heterozygosity, probably because these breeds were crossed with other breeds to improve meat production and because, in the past, were submitted to a light selection process; these values are also higher than that in Spanish ancestor (SMR). These data are similar to the values observed by Peter *et al.* (2007) in several European sheep breeds. Furthermore, the three Italian Merinos derived breeds show F_{IS} values lower than the other Merinos breeds (table 1), underlining that, in these breeds, there is a low level of inbreeding. At this purpose, it has to be considered the gene flow caused by the introduction of genes from other breeds to improve the MIT breed as reported by Polidori *et al.* (1989). In figure 1, the spatial distribution of all the samples, according to the results of the Correspondence Analysis, is shown: the first axis accounts for 30.03% of the total variance (23.90 for the second axis and 21.03 for the third one). The image shows that the clusters of the six breeds are well defined and this fact confirms their genetic identity.

Table 1. Mean number of alleles per locus (MNA), expected (H exp), observed (H obs) heterozygosity and FIS values with confidence interval in analysed sheep breeds.

Breed	Na	H exp	H obs	P	FIS	Confidence interval
Sopravissana	8.43	0.74	0.70	*	0.048	(0.007 ÷ 0.064)
Gentile di Puglia	7.79	0.74	0.69	ns	0.070	(-0.010 ÷ 0.106)
Spanish Merino	7.7	0.71	0.65	*	0.086	(0.034 ÷ 0.108)
Appenninica	8.0	0.76	0.67	*	0.118	(0.050 ÷ 0.130)
Merino Precoce	5.17	0.64	0.61	ns	0.057	(-0.019 ÷ 0.071)
Merinizzata Italiana	8.23	0.75	0.70	*	0.069	(0.011 ÷ 0.094)

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

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



improve MIT performance and in the safeguard of the ancient Merinos derived Italian breeds (SOP and GDP).

Indeed, as expected, the three Italian Merinos derived breeds are very close for their common origin. Furthermore, the APP breed is close to GDP and this can be due to the fact of the first breed contributed to the constitution of the second. The MIT breed, because of its origin, is close to the SMR and MPZ. In conclusion, the results show the importance of a possible employment of the molecular data both in the selection programs devoted to

REFERENCES – Belkhir, K., 2001. Genetix version 4.04: Logiciel sous WindowsTM pour la génétique des populations. Laboratoire Génome, Populations, Interactions. CNRS UPR 9060. **Dalvit, C.**, Saccà, E., Cassandro, M., Gervaso, M., Pastore, E., Piasentier, E., 2008. Genetic diversity and variability in Alpine sheep breeds. *Small Ruminant Res.*, 80: 45-51. **Diez-Tascón, C.**, Littlejohn, R.P., Almeida, P.A.R., Almeida, M.C.A., 2000. Genetic variation within the Merino sheep breed: analysis of closely related populations using microsatellites. *Animal Genetics* 31: 243-251. **FAO**, 2004. Secondary Guidelines for development of natural farm animals genetic resources management plans: measurement of domestic animal diversity (MoDAD): recommended microsatellite markers. Available at: <http://dad.fao.org>. **Goudet, J.**, 2002. FSTAT (version 2.9.3.): a program for Windows to estimate and test gene diversity and fixation indices. Université de Lausanne. **Lasagna, E.**, Landi, V., Bianchi, M., Martinez Martinez, A., Sarti, F.M., 2009. Genetic characterization of Appenninica sheep breed by microsatellites. *Proceedings of the A.S.P.A. XVIII Congress, Palermo - Italy, June 9-12, 2009. Avenue Media, Bologna*, 96-98. **Medrano, J. A.**, 2000. Recursos animales locales del centro del Mexico. *Archivos zootecnico*, 49: 385-390. **Peter, C.**, Bruford, M., Perez, T., Dalamitra, S., Hewitt, G., Erhardt, G., 2007. Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. *Animal Genetics*, 38: 37-44. **Pollidori, P.**, Panella, F., Morbidini, L., 1989. Indagine conoscitiva sulla situazione delle razze ovine merinizzate. *Economia montana*, 4. **Weir, B.S.**, Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. *Evolution*, 38: 1358–1370.