Molecular Coancestry And Classical Genetic Distances Depict Different Patterns Of Relationship Among Sheep Breeds From Southern Italy

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Introduction

Several molecular-based parameters, such as similarity indexes, can be adopted to optimize the management of genetic diversity in conservation programmes. From simulated data, Oliehoek *et al.* (2006) showed that molecular coancestry (Toro *et al.,* 2002) is, among the possible relatedness estimators, the one that performs better in structured populations, such as populations in need of conservation usually are. Several studies have, therefore, proposed the use of molecular coancestry coefficients as a measure of genetic variability and as a useful tool for conservation of endangered breeds (Ciampolini *et al.,* 2007; Glowatzki-Mullis *et al.,* 2009). Here we report the results obtained evaluating within- and between-breed molecular coancestry (Toro *et al.,* 2002), together with other classical genetic parameters, for two insular sheep breeds (Sarda from Sardinia and Comisana from Sicily), recently spread almost all over Italy, and for five local rare sheep breeds from Southern Italy.

Material and methods

Animal sampling and microsatellite analysis. A total of 739 individuals, representative of seven breeds from Southern Italy (Bagnolese, 100; Laticauda, 100; Comisana, 96; Sarda, 99; Gentile di Puglia, 108; Altamurana, 114; Leccese, 122) were sampled from different flocks trying to avoid closely related individuals. The following 19 ISAG/FAO microsatellites were typed on a ABI 310 DNA Genetic Analyzer, adopting a multiplex PCR protocol: OarFCB128, ILSTS11, OarAE129, ILSTS5, OarVH72, ILSTS28, MAF214, BM8125, MCM140, MAF33, MAF65, INRA063, OarJMP29, OarJMP58, OarFCB193, MAF209, OarFCB304, MAF70, and BM1824.

Statistical analyses. Molecular data were analyzed using the program Molkin v2.0 (Gutierrez *et al.,* 2005). The following parameters were computed at the breed level: number of alleles per locus (A) corrected using the Hurlbert's rarefaction method (1971), gene

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diversity (He), Wright's F_{IS} (1978), inbreeding coefficient (F) derived from self-coancestry (s_i). Additionally, the following within- and between-breeds parameters were estimated: molecular coancestry coefficients (Caballero and Toro, 2002) and kinship distance. The molecular coancestry between two individuals, *i* and *j* (f_{ij}), is the probability that two randomly sampled alleles from the same locus in two individuals are identical by state (Caballero and Toro, 2002). The molecular coancestry of an individual *i* with itself is self-coancestry (s_i), which is related to the coefficient of inbreeding of an individual *i* (F_i) by the formula $F_i = 2s_i - 1$. In turn, the kinship distance (D_k) between two individuals *i* and *j* is $D_k = [(s_i + s_j)/2] - f_{ij}$. MolKin computes within- and between-breed molecular coancestry and D_k by simply averaging the corresponding values for all the within- or between-population pairs of individuals.

Results and discussion

Some parameters characterizing genetic variability of the analyzed breeds are given in Table 1. The Sarda breed showed the lowest number of rarefacted alleles (8.88), the lowest value of expected heterozygosity (0.707) and among the highest values of molecular coancestry (0.30), highlighting a low level of genetic variation. Nevertheless, the breed displayed the lowest F_{IS} value (0.003) and a lower value for the inbreeding coefficient (0.298), suggesting that the low levels of genetic variation observed in Sarda animals have not arisen as a consequence of mating among relatives. Similarly, Moioli *et al.* (2006) observed, in a survey of the genetic diversity between Gentile di Puglia, Sopravissana and Sarda sheep breeds using 13 microsatellite markers, a low number of alleles (4.4), a low value of gene diversity (0.53) and the lowest value of F_{IS} (0.137) for the Sarda breed; however, the study was conducted on a small population sample (25 Gentile di Puglia, 20 Sopravissana and 15 Sarda animals) so possible effect of sampling bias could explain the differences observed in parameter values. In fact, values of gene diversity (0.75) and number of alleles (7.34) more similar to those observed by us had been previously reported by Pariset *et al.* (2003) who analysed 376 Sarda ewes randomly collected in 17 farms from Central Italy.

Table 1: Number of individuals (N), gene diversity (H_e), inbreeding coefficient (F), molecular coancestry (f_{ii}), heterozygote deficiency (F_{IS}), kinship distance (D_k) and average number of alleles per locus, rarefacted to 180 copies, for each breed and for the total sample.

Breed	N	H_e	F	f_{ii}	F_{IS}	D_k	A
Bagnolese	100	0.774	0.290	0.30	0.078	0.415	10.91
Laticauda	100	0.763	0.267	0.24	0.035	0.393	11.43
Comisana	96	0.762	0.304	0.24	0.082	0.410	11.22
Sarda	99	0.707	0.298	0.30	0.003	0.353	8.88
Gentile di Puglia	108	0.784	0.331	0.22	0.140	0.446	11.61
Altamurana	114	0.742	0.356	0.26	0.130	0.416	9.99
Leccese	122	0.781	0.316	0.22	0.120	0.436	11.21
Total Sample	739	0.793	0.310	0.21	0.090	0.395	17.98

On the other hand, Gentile di Puglia seemed to show the highest level of genetic variability, as suggested by the highest number of rarefacted alleles (11.61) and the highest value of gene diversity (0.784). Gentile di Puglia resulted to be the most variable breed also in the study of Moioli *et al.* (2006), when compared to Sarda and Sopravissana breeds. Similar findings have been reported also by d'Angelo *et al*. (2009) who observed high values of gene diversity (0.767) and heterozygote deficiency for the Gentile di Puglia breed, as highlighted in our study (F_{IS}=0.14). Together with the low molecular coancestry, these results suggest a possible stratification of the breed into genetically distinct subpopulations, probably derived from the lack of rams exchange among flocks and/or divergent management strategies within each flock. A similar hypothesis may be formulated for the Leccese breed as well. This hypothesis is supported by the higher molecular coancestry observed within-flocks than within the whole breed sample. For example, in the Leccese breed, we observed a within-breed molecular coancestry of 0.220 while within-flocks values ranged from 0.296 to 0.360; data not shown). The patterns of genetic variability observed for the other breeds appear less evident.

The studied breeds showed a low but significant genetic differentiation (F_{ST}=0.049, data not shown). Such results are in accordance with other studies on European and Middle-Eastern sheep breeds (F_{ST}=0.057, Peter *et al.,* 2007) and Ethiopian sheep populations (F_{ST}=0.046, Gizaw *et al.,* 2007) though other authors observed slightly higher F_{ST} value, such as Dalvit *et al.* (2008) on Alpine sheep breeds (F_{ST}=0.064), Baumung *et al.* (2006) on Austrian sheep breeds (F_{ST}=0.08), Alvarez *et al.* (2005) on Spanish sheep breeds (F_{ST}=0.070). The between-breed molecular coancestry (f_{ij}) and kinship distance (D_k) matrices are given in table 2.

Table 2: Between-breeds molecular coancestry (below diagonal) and between-breeds kinship distance (above diagonal).

Breed	1	2	3	4	5	6	7
1. Bagnolese		0.404	0.413	0.384	0.431	0.416	0.426
2. Laticauda	0.225		0.401	0.373	0.421	0.405	0.417
3. Comisana	0.224	0.220		0.381	0.429	0.413	0.424
4. Sarda	0.238	0.235	0.247		0.402	0.387	0.399
5. Gentile di Puglia	0.208	0.209	0.211	0.223		0.431	0.440
6. Altamurana	0.223	0.226	0.228	0.242	0.225		0.426
7. Leccese	0.208	0.206	0.213	0.224	0.208	0.227	

The high correlation between D_k and f_{ij} (roughly −0.80) showed that they almost completely offer the same information. The lower molecular coancestry values were found for Gentile di Puglia *vs.* Bagnolese, *vs.* Laticauda and *vs.* Leccese and for Leccese *vs.* Bagnolese and *vs.* Laticauda, showing that there exists lower genetic identity between these breeds. The higher f_{ij} values were observed among Sarda and all the other breeds; this result is in contrast with that observed using the pair-wise F_{ST} distance and the Nei's (1972) standard distance (D_s), which highlighted the Sarda as the most differentiated breed (data not shown). Considering that the between-population coancestry would represent the between-breed genetic relationships at the moment of separation (Alvarez *et al.,* 2005), we could suppose that the Sarda genetic make-up reflects more closely the genetic composition of the ancestral

population before the breed differentiation, which could be consistent with the phylogeographic history of this native insular breed. In addition, the long-lasting isolation experienced by the breed may also explain the higher differentiation observed using distance measures (F_{ST} and D_s) highly dependent on the observed allele frequencies, which are in turn highly dependent on recent evolutionary processes such as genetic drift.

Conclusion

Both within- and between-breed parameters highlighted native breeds as the most variable breeds; these results suggest the possible introgression of alleles from past crossbreeding practices with improved breeds and highlight the importance of identifying and recovering original genotypes to start conservation programmes. Molecular coancestry analysis suggests that the Sarda breed might be considered as a relic of the ancestral sheep population before breed separation.

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