



## Analysis of genetic variability within and among Italian sheep breeds reveals population stratification and suggests the presence of a phylogeographic gradient

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### ABSTRACT

Italy has a long history of sheep breeding and, despite a dramatic number contraction occurred in the last century, still counts several local sheep breeds that may represent a unique source of genetic diversity. Here we present the results from the genetic characterization of seven Italian dairy sheep breeds by 19 STR markers. High levels of polymorphism were generally detected, with 16.2 average alleles per locus and an average gene diversity of 0.79. The within-breed analysis highlighted, in six out of the seven breeds, a remarkable proportion of loci displaying significant heterozygote deficit. Moreover, within-breed percentages of locus pairs with significant genotypic disequilibrium were, for all breeds, markedly higher than expected by chance thus suggesting the presence of population stratification. The subdivision was particularly evident for the Lecce sheep breed and was confirmed by the STRUCTURE analysis. On the contrary, results generally highlighted Sarda as the most homogeneous, but less inbred, population, consistently with its large census size and longstanding effective genetic management practices. Both STRUCTURE analysis and measures of genetic distance, with the exception of those based on allele sharing, suggested the presence of a phylogeographic gradient, with Sarda as the most differentiated breed and continental breeds from the same region tending to group closer to each other. Taken together, the results highlighted that all the Italian breeds display a genetic variability content comparable to other European breeds, thus demonstrating their importance as genetic reservoir for future selection options. However, management actions should be more effectively implemented in local endangered breeds in order to reduce inbreeding and within-breed sub-structuring.

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### 1. Introduction

Sheep have traditionally been reared in Italy since ancient times, being in the past the main livestock species in a great part of the country. In more recent times the importance of sheep has decreased, mainly due to interest

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in intensive farming of cattle and pig and to the loss of competitiveness of the national wool production system. To date, the national consistency is more than 8 million of heads and the population is concentrated in Sardinia and in southern and central regions.

In the centuries many breeds have been selected or imported in the country. Today about sixty different breeds (the vast majority of them being of autochthonous origin) are listed to be reared in the country and some of these breeds are exploited to produce typical products in marginal rural areas. The existence of a significant sheep biodiversity is of pivotal importance, not only acting as genetic reservoir to meet future breeding options but also to preserve the environment, the landscape and the human presence in marginal areas (Ajmone-Marsan, 2010).

Knowledge about genetic variability is essential to preserve and exploit biodiversity thus investigations at the molecular level are needed. Microsatellite polymorphisms (Short Tandem Repeats, STRs) are by far the most employed loci to investigate biodiversity at the DNA level in livestock, including sheep (Arranz et al., 2001; Stahlberger-Saitbekova et al., 2001; Peter et al., 2007; Lawson Handley et al., 2007; Tapio et al., 2010), even if, more recently, SNPs have been introduced as well (Kijas et al., 2009; Hoda et al., 2011; Kijas et al., 2012). Here we present the results from the genetic characterization of seven Italian sheep breeds performed using 19 STRs in order to assess within-breed diversity and reconstruct relationship among breeds.

## 2. Materials and methods

### 2.1. Animals and breeds

A total of 470 animals, representative of seven breeds from southern and insular Italy (Sarda, SAR: 97; Comisana, COM: 67; Altamura, ALT: 83; Leccese, LEC: 86 Gentile di Puglia, GEN: 74; Bagnolese, BAG: 33; Laticauda, LAT: 30) were sampled from different flocks trying to avoid closely related individuals.

Sarda is a high milk-yielding breed indigenous of the Sardinian island, whose origin is still a matter of debate. The breed seems to have been influenced, over centuries, by mutual introgression of Sardinian mouflon (*Ovis aries musimon*) which is thought to be among the remnants of the first domesticated sheep readapted to feral life (Chessa et al., 2009). After a long-lasting period of very strict isolation, half a century ago it began to spread out over the Italian peninsula replacing low-producing local sheep breeds; it counts at present more than 3 million heads, mainly located in Sardinia, Tuscany and Latium.

Comisana originated at the beginning of the XX century in southeast Sicily by crossbreeding local ewes with rams from Malta and North Africa. The breed is nowadays spread throughout the peninsula and particularly in Southern Italy where it is preferred to Sarda for its rusticity and excellent adaptation to harsh and semi-arid climate conditions. Moreover, the breed display a valuable milk productivity also when reared under extensive or semi-extensive systems.

Out of the three autochthonous Apulian breeds, two (Altamura and Leccese) are considered as belonging to the Zackel group of sheep and are reared mainly for milk production while Gentile di Puglia, also known as Apulian Merino, is a fine-wool sheep whose true origin is still object of debate (Altobella and Muscio, 1995). All these breeds have suffered a severe numerical reduction in the last fifty years, more pronounced for Altamura, whose population size dropped from 140,000 heads in 1960s to very few hundred heads at the present days.

Laticauda and Bagnolese, both autochthonous of Campania, are usually reared by small family farms under semi-extensive systems and are thought to derive by crossbreeding local sheep from the Apennines with fat-tailed North African sheep, likely imported under the Bourbonns dynasty in the XVIII century. Laticauda has a prevailing attitude toward meat production, associated to a good prolificacy (Matassino et al., 2000),

while Bagnolese is a dual-purpose breed, particularly valued for the excellent quality of dairy products derived from its milk.

### 2.2. STR analysis

Genomic DNA was extracted from peripheral blood samples following standard protocols. Animals were genotyped using 19 ovine microsatellites belonging to the recommended ISAG-FAO panel (Hoffmann et al., 2004). The list of loci, together with further details, is provided in Table S1.

Number of alleles ( $N_a$ ) and allelic frequencies were estimated using FSTAT v2.9.3 (Goudet, 2001). In order to correct for different breed sample-sizes, allele richness (AR) was calculated using the software HP-Rare (Kalinowski, 2005) by rarefying breed samples to 25 individuals each. Locus by locus  $F_{ST}$  and deviations from the Hardy-Weinberg equilibrium (HWE) were evaluated using the ARLEQUIN package v. 3.11 (Excoffier et al., 2005). Pair-wise gametic disequilibrium among microsatellite loci was estimated using PowerMarker v. 3.25 (Liu and Muse, 2005). The FSTAT v2.9.3 (Goudet, 2001) software was adopted to estimate differentiation among breeds in terms of population pair-wise  $F_{ST}$ . In order to take into account complex evolutionary relationships (reticulated evolution) among the studied breeds, network analysis using Reynolds (Reynolds et al., 1983) and Nei (1972) genetic distances was carried out by the software Neighbor-Net (Bryant and Moulton, 2004). Molecular coancestry coefficients ( $f_{ij}$ ), kinship distance ( $D_k$ ), Wright's  $F_{IS}$  and inbreeding coefficients ( $F$ ) were computed at the population level using the program Molkin (Gutiérrez et al., 2005). Breed differentiation was also investigated using the Bayesian clustering algorithm implemented in the STRUCTURE software v. 2.2 (Falush et al., 2007). We adopted the "admixture model", without providing a priori information on population membership. Ten independent runs were performed for each  $K$  value from 2 to 10 (where  $K$  is the number of cluster to be tested), adopting a burn-in period of 100,000 generations, followed by 100,000 iterations. The algorithm of Evanno et al. (2005) was adopted in order to evaluate the most probable value of  $K$ .

## 3. Results

### 3.1. Within-breed genetic diversity

All markers were polymorphic across the seven analyzed populations (Table S1), with a mean number of 16.2 alleles per locus, ranging from 8 (BM1824) to 28 (MAF214). By rarefying each breed sample to a sample size of 25 animals (Table 1 and Table S2), ALT and SAR displayed the lowest average values of allele richness (6.8 and 6.9, respectively) while GEN displayed the highest value (8.0).

In the total sample, the average expected heterozygosity ( $H_e$ ) was 0.79 while the average observed heterozygosity ( $H_o$ ) was 0.69 (data not shown). SAR showed the lowest (0.71) while GEN and LEC the highest gene diversity (0.77; Table 1). Average within-breed  $H_o$  values found in our study varied from a minimum of 0.65 in ALT to a maximum of 0.72 in COM (Table 1). Within the seven breeds, zero (BAG) to seven (COM) loci were in significant ( $P < 0.01$ ) Hardy-Weinberg disequilibrium (Table S3). Since dealing with a population dataset of varying breed sample sizes, we also repeated the analysis on a randomly drawn subsample of 30 animals for each breed in order to exclude that this pattern was biased by the unequally distributed population samples but a similar trend was observed (data not shown). Hardy-Weinberg deviation was mainly due to heterozygote deficiency (Table S3); in particular, for the locus OarAE129, a significant heterozygote deficiency was observed in all breeds but one (BAG).

In the total population, a significant ( $P < 0.01$ ) gametic disequilibrium, estimated using the parameter  $r^2$  (Hill

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