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Genetic relationship and admixture in four Tunisian sheep breeds revealed by microsatellite markers

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ABSTRACT

The aim of this study was to assess the genetic diversity and differentiation patterns of four Tunisian sheep breeds. A total of 186 animals belonging to Barbarin ($n = 59$), Western Thin Tail ($n = 46$), Black Thibar ($n = 40$), and Sicilo Sarde ($n = 41$) breeds were genotyped for a panel of 30 microsatellite markers. In addition, a sample of 29 Spanish Merino sheep breed was used as an outgroup for tree topology. All markers were highly informative with PIC values ranging from 0.588 to 0.885 and a mean number of private alleles of 0.016. In all breeds, inbreeding was indicated by heterozygosity deficiency with estimated F_{IS} values varying from 0.134 (Barbarin) to 0.165 (Western Thin Tail), and a highly significant departure ($p < 0.01$) from Hardy–Weinberg proportions was observed. Wright's F_{ST} index indicated low genetic differentiation between Tunisian breeds (0.017). The lack of clear genetic differentiation was also evidenced by cluster analysis using STRUCTURE as well as by the significant gene flow observed between breeds, especially Barbarin and Western Thin Tail, on the basis of the estimated Reynolds' genetic distances (D_R) and the number of migrants (N_m). The highest D_R values were observed between Sicilo Sarde and the three other breeds, reflecting different phylogenetic origins of this breed. The study represents a first step towards further genetic characterization of Tunisian sheep breeds and results presented here are useful for better management and conservation of sheep resources.

1. Introduction

The North African region, which includes Morocco, Algeria, Tunisia, Libya and Egypt, raises more than 100 million sheep (FAOSTAT, 2012), and is ranked among the top sheep production areas in the world (FAO, 1981). These sheep are mainly reared for meat and leather production, and to a small extent for wool and milk. In Tunisia, sheep farming is an important economic and social activity contributing to 39 % of the total red meat production (OEP, 2014). More than 6.8 million sheep heads are raised in Tunisia, including about 3.9 million ewes (ONAGRI, 2012) that belong to four different breeds: Barbarin (60.3%), Western Thin Tail (34.6%), Black Thibar (2.1%) and Sicilo Sarde (0.7%).

The fat-tailed Barbarin (BB) is the most representative of Tunisian sheep and is commonly found throughout the country. It is a rustic breed well adapted to the arid and semi-arid environments characterised by the prevalence of low input production systems (Djemali, 2000). Western Thin Tail (WTT) is a common breed in Tunisia and Algeria—where it is known as "Ouled Jellal" (Iñiguez, 2006) and is mainly found in the steppes of central Tunisia. The breed shows a relatively good adaptation to harsh and dry conditions but it is more sensitive to high temperatures than the Barbarin breed (Djemali, 2000). The Black Thibar (BT) breed is a composite black coat sheep found in the sub-humid northern region of Tunisia with meat production vocation. Its roots can be traced from the beginning of the 20th century where native Algerian thin tail sheep were crossed with the French Merinos d'Arles (Chalh et al., 2007) with an aim to obtain uniformly black animals. The breed was rescued in mid 1980s by introducing Brown Swiss rams after appearance of white fleeces and fertility problems due to an increasing level of inbreeding (Chalh et al., 2007). The Sicilo-

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Sarde (SS) is the only dairy sheep breed native to North Africa. SS is derived from a cross between two imported Italian dairy breeds (Sarde and Sicilian) from Sicily and is currently restricted to limited areas of the North-western Tunisia (Djemali, 2000).

Genetic characterisation of livestock species provides useful information about the magnitude of genetic structure and relationships between populations as well as their diversity and conservation status (Arranz et al., 1998, 2001; Pariset et al., 2003; Alvarez et al., 2004; Uzun et al., 2006; Peter et al., 2007; Ligda et al., 2009; Tapio et al., 2010; Calvo et al., 2011; Tolone et al., 2012; Ciani et al., 2013). Genetic variability and relationships among Tunisian sheep breeds using only 17 microsatellites has been published (Ben Sassi-Zaidy et al., 2014). The aim of our study is to investigate the genetic diversity and admixture in four major breeds of sheep raised in Tunisia by use of 30 microsatellites.

2. Materials and methods

2.1. Blood samples

A total of 186 blood samples were collected from the four Tunisian sheep breeds (Barbarin ($n = 59$), Western Thin Tail ($n = 46$), Black Thibar ($n = 40$) and Sicilo Sarde ($n = 41$)). Sampling was carried out in 2011, and was obtained from different flocks located in the north, center and south, and representing all geographic regions of the country. A total of 23 and 17 flocks were sampled from the northern parts of the country for the Black Thibar and Sicilo Sarde breeds, respectively. Barbarin and Western Thin Tail are reared throughout the whole country, and then, 49 and 29 flocks were sampled, respectively (Fig. S1, Table S0). Information about relatedness between animals was obtained from farmers when pedigree data is not recorded and a maximum of three samples from unrelated animals were taken per flock. DNA extraction was performed from blood according to the standard phenol–chloroform protocol (Sambrook et al., 1989).

2.2. Genotypic data

Thirty ovine microsatellites were analysed in all the individuals. Microsatellites used in this study were selected from the list recommended by the FAO-ISAG group for biodiversity studies (www.fao.org/docrep/meeting/021/j1998e.pdf). Details of markers and PCR conditions are given in supporting information (Tables S1 and S2). Fragments were detected using an ABI Prism 310 DNA Sequencer (Applied Biosystems, Spain) and the accompanying GeneScan software, v.3.1 for the determination of allele sizes.

Genotyping data of Spanish Merino sheep -used here as an out group- was previously reported (Calvo et al., 2011).

2.3. Statistical analysis

Cervus v. 3.0.3 (Marshall et al., 1998) software was used to analyse the number of alleles, observed and expected heterozygosity (corrected for sampling bias) and polymorphic information content (PIC). The effective number of alleles and the frequencies of private alleles were calculated using Genetic Analysis in Excel (GenAlex) version 6.501 (Peakall and Smouse, 2006). Genepop v.4 (Raymond and Rousset, 1995) software was utilised to calculate the exact test for Hardy–Weinberg equilibrium, the linkage disequilibrium test between markers and the gene flow value (N_m). We used Bottleneck 1.2.02 (Cornuet and Luikart, 1996) to test significant deviation in allelic diversity and heterozygosity from mutation-drift equilibrium predictions. Arlequin 3.5.1.2 (Excoffier et al., 2005) was used to determinate the F_{ST} values for pairwise comparisons of the breeds, and for the analysis of molecular variance (AMOVA). Parameters of genetic differentiation (F statistics), mean number of alleles across populations, observed, average expected (non-biased) and average observed heterozygosities were calculated using Genetix 4.05.2 software (Belkhir and Borsa, 1998). Rarefaction approach as implemented in HP-RARE (Kalinowski, 2005) was used for allelic and private allelic richness estimation. The population structure was analysed by cluster techniques with the software STRUCTURE 2.3.4 (Pritchard et al., 2000) with K ranging from 2 to 10 (the real number of breeds plus 6). The true K was determined using Structure Harvester Web version 0.6.93 (Earl, 2012). Reynolds Genetic distances (D_R) (Reynolds et al., 1983) were estimated using Populations v 1.2.32 (Langella, 1999) and dendrograms were constructed according to the neighbour-joining algorithm, with Spanish Merino sheep used as outgroup (Calvo et al., 2011). Tree topology was constructed using Populations v 1.2.32, and the reliability of each node was estimated by 1000 resampling of the data. The Tree view program 1.6.6 (Page, 1996) was used for tree drawing.

3. Results and discussion

3.1. Microsatellite analysis

A total of 428 alleles were detected in the 30 loci studied. The number of alleles per locus ranged from 6 (*BM1824*) to 24 (*HUJ616*) with a mean number of alleles per locus of 14.27. These results are similar to those observed in several European sheep breeds (Pariset et al., 2003; Alvarez et al., 2004; Uzun et al., 2006; Peter et al., 2007; D'Angelo et al., 2009; Ligda et al., 2009), but higher than those detected in African sheep (Wafula et al., 2005; Muigai et al., 2009). Descriptive statistics of the 30 microsatellite loci in the overall population are shown in Table 1. All markers were highly informative with PIC values ranging from 0.588 (*OARAE129*) to 0.885 (*INRA063*), indicating that all the microsatellite loci were sufficiently polymorphic and thus suitable for diversity analysis. Similar PIC values were given by Ben Sassi-Zaidy et al. (2014) for the same breeds. Analysis of within-breed genetic diversity showed that all Tunisian breeds had a large mean number of alleles per breed, ranging from 9.23 for Sicilo Sarde to 11.1 for Barbarin (Table 2). On the other hand, allelic richness (A_R) varied from 7.27 (Sicilo Sarde) to 7.84 (Western Thin Tail). These values were lower than those reported by Ben Sassi-Zaidy et al. (2014). All breeds showed substantial reduction in allelic richness when using the rarefaction approach, most notably for the Barbarin and Western Thin Tail, yielding private allele estimates that were less than one per breed varying between 0.65 and 0.96 for Sicilo Sarde and Western Thin Tail, respectively (Table 2). However, the lowest (0.27) and the highest (0.67) frequency of private alleles found by Ben Sassi-Zaidy et al. (2014) were detected for the Black Thibar and the Sicilo Sarde, respectively. Ben Sassi-Zaidy et al. (2014) used 17 microsatellite markers while in the present study the animals were genotyped for 30 microsatellites. Overall, the mean frequency of private alleles and the number of migrants (N_m) after correction for size were 0.016 and 10.389, respectively, indicating a relatively high gene flow among Tunisian breeds.

Twelve microsatellites showed significant ($p < 0.05$) departures from the Hardy–Weinberg proportions in the whole population, however, when considering breeds separately, only one marker per breed was in Hardy–Weinberg disequilibrium ($p < 0.05$): OARFCB226, MAF214, MAF65, and ILSTS11 microsatellites for Barbarin, Black Thibar, Sicilo Sarde, and Western Thin Tail breeds, respectively.

The four breeds showed a strongly significant departure ($p < 0.01$) from Hardy–Weinberg proportions when considering all loci, which might be explained by the uncontrolled mating in history of the breeds. This deviation might also be caused by the presence of null alleles, however no pedigree was available to be used in the estimation of null alleles as well as for an unbiased esti-