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Estimating population structure and genetic diversity of five Moroccan sheep breeds by microsatellite markers

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ABSTRACT

Investigating the genetic variability and structure of Moroccan sheep breeds will reveal crucial information for the conservation and management of this population. This study used 22 microsatellite markers to assess the genetic diversity among and within five Moroccan sheep breeds: Sardi (N = 35), Boujaad (N = 31), Timadhite (N = 35), Beni Guil (N = 35), and D'man_Morroco (N = 35). In the whole sample, a total of 299 alleles were detected. The five breeds showed a relatively high level of gene diversity ranging between 0.725 (D'man_Morocco) and 0.764 (Timahdite). The Analysis of Molecular Variance (AMOVA) indicated that variability among populations contributed only 3.64% of the observed genetic diversity. Wilcoxon tests of excess heterozygosity under the two-phase model (TPM) did not provide strong evidence for recent bottlenecks in the five studied breeds. Unrooted neighbour joining (NJ) tree for the modified Cavalli-Sforza chord distance (D_A), pairwise multilocus estimates of an effective number of migrants (Nm) and the Bayesian clustering method cohesively revealed poor structure of genetic variation among breeds. Our results also show that in spite of the high level of phenotypic diversity in the Moroccan sheep breeds, the past breeding strategies could lead to genetic admixture occurring as a result of relatively high gene-flow among the breeds.

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1. Introduction

The economic importance of sheep production is increasing in North African countries. In Morocco, ovine meat production exceeded 35% of total red meat (Boujenane, 2006), and the population size of sheep was more than 17 million animals in total (26% males and 74% females) in 2009. Complex topography of Morocco offers diversified ecological conditions and climate types, which influenced notably sheep breed distribution (Fig. S1(a)). Successful adaptation to a particular set of environmental conditions allowed acquiring specific characteristics that vary from breed to breed. More than 10 sheep breeds have been reported in this country (Boujenane, 2005). The main breeds were Sardi, Boujaâd, Timadhite, Beni Guil and D'men. They are thin-tailed and mainly dual-purpose animals with meat being the most essential product. The wool remains the next desirable product; Milk is often produced only for family use (Bourfia, 1989). The Sardi breed is the preferred sheep in several social and religious celebrations, and this is led to its special phenotype named "Chatbi" which is characterized by a neat whiteness an open spiral-shaped horns. Bourfia (1989) reported that high demand for the Sardi could play a crucial role in increasing the area of the Sardi breed. According to Boujenane et al. (1995), Beni Guil breed includes Beni Guil, Harcha, Tounsint and Zoulay varieties, and it is adapted to different climate conditions. Timadhite breed was the result of a crossing between Berbère and Tadla Breeds, BeniGuil breed contributed also to the development of the Timahdit breed (Bourfia, 1989). Boujaâd sheep breed is with high growth rate and good conformation. D'man breed inhabits the oases of southern Morocco and the Wadi Saorua Valley of south Algeria and was known as the most prolific sheep. Different breeding strategies have been adopted in different regions of Morocco for improving wool production, quality and body weight in sheep (Boujenane et al., 1995, 2005; Boujenane, 2012a,b). Furthermore, the need to exploit the highly productive species (e.g. cattle instead of Beni Ahsen sheep breed) and breeds, some of less

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Table 1
Diversity of studied breeds of sheep obtained from the analysis of 22 microsatellite loci.

Breed	N	N_F	N_f	An	Ae	PA	Ar	Ho (SD)	He (SD)	F_{IS} (IC 95%)[f]
Tim	35	23	7	9.18	4.65	0.60	6.67	0.724 (0.035)	0.764 (0.021)	0.054*(0.00006–0.07754)
Sar	35	13	8	8.73	4.07	0.64	6.15	0.695 (0.036)	0.731 (0.021)	0.050*(−.01209–0.07970)
Bou	31	16	6	8.41	4.45	0.66	6.54	0.626 (0.038)	0.748 (0.024)	0.165*(0.10419–0.18932)
D'ma	35	26	6	8.82	4.04	0.70	6.31	0.609 (0.037)	0.725 (0.022)	0.163*(0.09078–0.20126)
Ben	35	16	4	8.96	4.78	0.70	6.55	0.656 (0.028)	0.742 (0.028)	0.132*(0.06841–0.16282)

Tim: Timahdite. Sar: Sardi. Bou: Boujaad. D'ma: D'man_Morocco. Ben: BeniGuil. N: number of individuals. N_F: number of females, N_f: number of flocks. An: mean number of alleles in each population. Ae: average number of effective alleles per locus. PA: frequence of private alleles per breed. Ar: Allelic Richness (rarefacted). H_O: observed heterozigosity. H_e: unbiased heterozygozity.

[f] 10,000 Bootstrap over FIS by population, IC 95% = confidence interval at 95%.

* Significant *p*-values ($p < 0.01$).

productive local breeds have been neglected; others have been crossed with foreigner breeds or even replaced by more productive breeds. As a result, Beni Ahsen and Atlas Mountain breeds, and perhaps the Beni Guil, have decreased tremendously in numbers (Boujenane, 2005). Molecular Genetic studies have been conducted on different breeds of sheep worldwide (Tapio et al., 2010; Calvo et al., 2011; Tolone et al., 2012; Ciani et al., 2013; Kunene et al., 2014). Northwest Africa is a major hotspot of sheep diversity, but little is known about the genetic structure of ovine populations in this region. Here, we use microsatellite DNA markers to examine patterns of genetic diversity and differentiation among Moroccan sheep breeds. Specifically, we ask: (i) Is there evidence for bottlenecks in these breeds and (ii) What are the patterns of genetic diversity within vs. among breeds in Morocco?

2. Materials and methods

2.1.Samples and amplification

In the present study, a total of 171 blood samples of sheep were collected from Morroco. Samples came from 5 native breeds (Fig. S1(a)):Sardi (N = 35), Boujaâd (N = 31), Timadhite (N = 35), Beni Guil (N = 35), and D'men (N = 35). Sampling was carried out in 2006, and was obtained from different flocks. Information about relatedness between animals was obtained from breeders and farmers when pedigree data is not available and unrelated animals were taken per flock. The genomic DNA purification from blood was performed according to the Salting out protocol (Miller et al., 1988).

The 171 samples were genotyped at 22microsatellite loci (Table S1). Moreover, a sample of 30 Merino de Rambouillet sheep breed was used as an outgroup for tree topology. The microsatellite analysis and detection of amplified products were performed as described in Gaouar et al. (2015).

2.1. Data analysis

Significant deviations from Hardy-Weinberg equilibrium expectations were evaluated by Fisher's exact tests, with unbiased P-values (10,000 dememorizations, 100 batches, 1000 iterations per batch) as implemented in GENEPOP 3.4 program (Raymond and Rousset, 1995). We used GENALEX 6.1 (Peakall and Smouse, 2006) to estimate, in the whole sample, mean number of alleles (MNA) and effective allele number (Ne) by locus, and for each breed, mean number of alleles (An), observed and expected unbiased heterozygosity (H_o and H_e respectively) and average number of effective alleles per locus (Ae). Rarefaction approach as developed in HP-RARE (Kalinowski, 2005) was used for allelic and private allelic richness estimation. We calculated for each locus number of individuals typed (N), number of alleles (Na) observed (H_o) and expected unbiased (H_e) using CERVUS 3.0.3 (Marshall et al., 1998). GENETIX 4.05 (Belkhir and Borsa, 1996–2004) was used to determinate the F_{ST} values for pairwise comparisons of the breeds, to compute Wright's inbreeding estimator (F_{IS}; Weir and Cockerham, 1984) and to assess F_{IS} significance using 1000 random permutations of alleles in each breed. To test for bottlenecks, we used the program BOTTLENECK version 1.2 (Cornuet and Luikart, 1996) utilizing the Wilcoxon test for heterozygote excess, as well as the two-phase model (TPM) recommended by Piry et al. (1999) and Peery et al. (2012). The significance of fixation indices determined using permutation tests (1000 permutations) and the estimation of the variance within and between breeds, and analysis of molecular variance (AMOVA) were performed with ARLEQUIN 3.5.1.2 (Excoffier et al., 2005). The population structure was analysed by cluster techniques with the software STRUCTURE 2.3.4 (Pritchard et al., 2000) with K ranging from 1 to 8. The most probable K was determined using STRUCTURE HARVESTER Web version 0.6.93 (Earl, 2012). Modified Cavalli-Sforza chord distance (D_A; Nei et al., 1983) were estimated using POPULATIONS v 1.2.32 (Langella, 1999) and dendrograms were constructed according to the neighbour-joining algorithm, with the French sheep breed Merino de Rambouillet as an outgroup (data not published). 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 unrooted tree was viewed in FIGTREE version 1.4 (http://tree.bio.ed.ac.uk/software/figtree/). To assess the genetic relationship of Algerian (data from Gaouar et al., 2015) sheep breeds, a standard Nei genetic distance (D_s; Nei, 1972) matrix between all pairs of populations was calculated using French Merino de Rambouillet sheep breed as outgroup in the program POPULATIONS v 1.2.32 (Langella, 1999). Bootstrap values were calculated over all loci and neighbour-joining (NJ) data were exported to FIGTREE program version 1.4 (http://tree.bio.ed.ac.uk/software/figtree/) for graphing.

3. Results and discussion

Genetic diversity statistics are summarised in Table S1. Twenty-two microsatellite markers resulted in a total number of 299 alleles. Twenty-one markers showed high informative (Polymorphic Information Content (PIC) > 0.5), only one locus (*OarAE129*) showed moderate PIC (0.25 < PIC < 0.5). Numbers of alleles per locus ranged from 6 (*BM182*and*OarAE129*) to 23 (*HUJ616*). The result of HPRARE program revealed a low number of private alleles (between 0.60 and 0.70 per breed) as well as medium allele richness (between 6.15 and 6.67). Allelic richness values were lower than those observed in Turkish sheep breeds (9.40–13.75; Yilmaz et al., 2014) and Tunisian sheep breeds (8.08–8.94; Sassi-Zaidy et al., 2014), but higher than those reported in Algerian sheep breeds (4.97–6.16; Gaouar et al., 2015).

The mean value of H_e (Table 1) was between 0.725 (Moroccan D'man) and 0.764 (Timahdite), and that of H_o between 0.609 (D'man_Morocco) and 0.724 (Timahdite). Previous studies on D'man breeds showed similar values to D'man breed of Algeria (H_o = 0.64; H_e = 0.68; Gaouar et al., 2015) and D'man breed of Tunisia (H_o = 0.716; *He* = 0.815; Sassi-Zaidy et al., 2014). The gene