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Short communication

Genetic diversity, structure, and breed relationships in Tunisian sheep

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ABSTRACT

The aim of this research was (i) to quantify the levels of genetic variability in Tunisian sheep breeds and (ii) to investigate their population structure and the extent of admixture, to provide information for their conservation. Blood samples from 249 sheep, belonging to 5 breeds and 1 crossbred population were collected across different agroecological zones in Tunisia. Tunisian sheep and 31 Appenninica Italian sheep (APP), used as an out-group, were genotyped at 17 microsatellite loci. All the sheep breeds investigated were genetically diverse, as evidenced by the high allele (>7) and gene (>0.7) diversity values. The average F_{IS} (0.105) and F_{IT} (0.132) values indicated significant levels of inbreeding within these breeds. The average F_{ST} estimate (0.030) between all breeds and Reynolds' genetic distance revealed the close relationship among native Tunisian breeds and their clear distinction from the D'man exotic breed and APP. The clustering analysis performed with STRUCTURE also evidenced that native Tunisian breeds could be considered as subpopulations belonging to one breed. The high level of admixture found also reflects the sheep breeding history of Tunisia. Application of this knowledge may be useful in breeding programs and genetic management of breeds. These findings represent a starting point for the characterization of Tunisian sheep breeds suggesting the need to set up accurate conservation measures aiming to safeguard and monitor their genetic variability.

1. Introduction

Appropriate measures should be taken for stopping the genetic erosion of animal genetic resources, especially in breeds with high genetic diversity which have the ability to adapt to environmental changes (FAO, 2006). Before taking measures, data covering molecular genetic diversity of breeds is an absolute requirement as already done for Alpine sheep breeds (Dalvit et al., 2008) and Italian native sheep breeds (Dalvit et al., 2009). Tunisian sheep breeds were recently investigated using RAPD-PCR markers (Khaldi et al., 2010; El Hentati et al., 2012) and microsatellites (Ben Sassi-Zaidy et al., 2014) but information on genetic relationships and population structure among all Tunisian breeds is absent. The Tunisian sheep breeding sector is largely dominated, in terms of animal numbers, by the indigenous fat tailed Barbarine (BAR) breed (64%) while the remaining thin tail breeds are: "Queue Fine de l'Ouest" (QFO, 30%), Noire de Thibar (NTH, 2%) and Sicilo-Sarde (SS, 0.5%). The most significant introduction of an exogenous sheep breed in Tunisia is that of

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Table 1
Number of analyzed samples (N), allelic richness (N_{AR}) obtained with rarefaction method, private allelic richness (P_{AR}) expected (H_e) and observed (H_o) heterozygosity, within-population heterozygote deficiency (F_{IS}), number of loci deviated from Hardy–Weinberg equilibrium (HWE).

Breed[a]	N	N_{AR} (28)[b]	P_{AR} (28)[b]	H_e	H_o	F_{IS}	HWE[c]
BAR	64	8.68	0.52	0.806 ± 0.095	0.747 ± 0.107	0.073 ± 0.062	0
QFO	41	8.82	0.46	0.823 ± 0.075	0.739 ± 0.143	0.101 ± 0.143	2
CRO	30	8.32	0.35	0.797 ± 0.089	0.682 ± 0.167	0.139 ± 0.192	3
NTH	41	8.08	0.27	0.806 ± 0.094	0.748 ± 0.149	0.071 ± 0.121	2
SS	45	8.18	0.67	0.811 ± 0.106	0.726 ± 0.133	0.102 ± 0.103	2
DM	28	8.94	0.71	0.815 ± 0.112	0.716 ± 0.165	0.123 ± 0.138	1
APP	31	6.84	0.38	0.762 ± 0.075	0.663 ± 0.141	0.119 ± 0.184	4

[a] Breed name: BAR, Barbarine; QFO, Queue Fine de l'Ouest; CRO, Crossbred (BAR × QFO); NTH, Noire de Thibar; SS, Sicilo-Sarde; DM, D'man; APP, Appenninica.
[b] Number of observations in each breed
[c] $P < 0.05$ after Bonferroni correction.

the D'man (DM) Moroccan breed which represent 0.25% of the total number of sheep reared in Tunisia (OEP, 2009).

Historical origins of the BAR breed were described and investigated in Ben Sassi-Zaidy et al. (2014), this breed is known for its hardiness and it had a major socio-cultural importance in Tunisia. The QFO or "Algerian Thin Tail" is derived from the Ouled Djellal breed from the eastern highlands of Algeria. It is a relatively hardy breed adapted to harsh dry condition. A new population named "Chirki" or "Crossbred" (CRO), resulting from a crossbreeding between the BAR and the QFO, is nowadays spreading in central and northern Tunisia. The NTH is mainly considered a meat breed and it was created in 1924 by crossing the French Merino d'Arles with the Algerian Thin Tail to produce animals uniformly black in the sub humid North-western region of Tunisia (Thibar). Brown Swiss rams were later used to restore the black color and to introduce new gene pools into the breed (Djemali and Alhadrami, 1997) The SS breed is the only milk sheep in Tunisia and North Africa, the breed results from a crossbreeding between the Sarda and the Comisana dairy breeds originating from Sardinia and Sicily (Italy), respectively. This breed has undergone a dramatic decrease (90% of dairy ewes) during the last decades indicating that this breed is threatened by disappearance (Mohamed et al., 2008). The DM is a Moroccan prolific breed introduced in Tunisia in 1994 as a flock of two hundred breeding ewes and 12 rams. This breed characterized by its general black color has been reared in the oases of the southern country and the population accounted for 5000 breeding ewes in 2001 (Rekik et al., 2002). Appenninica (APP) is a native Italian meat sheep breed mainly reared in central Italy; in the present work, it was used as an out-group.

The objectives of this research were (i) to quantify the levels of genetic variability in Tunisian sheep breeds and (ii) to investigate their population structure and the extent of admixture by using microsatellites markers to provide information for their conservation.

2. Materials and methods

2.1. Sample acquisition, and genotyping

A total of 249 individual blood samples were collected from unrelated animals corresponding to the Tunisian sheep breeds belonging to the different agroecological zones. Because of the absence of herd books, the animals were chosen as three unrelated animals from each farm or small flock based on the information provided by the farmer to avoid sampling of closely related individuals. Number of analyzed samples for each breed is shown in Table 1. The DNA extraction was carried out from whole blood using the Wizard Genomic DNA Extraction Kit (Promega, USA) following manufacturer's protocol. A microsatellite set, including 17, markers was analyzed on all the individuals as described in Ben Sassi-Zaidy et al. (2014). Allele size was determined with a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA).

2.2. Statistical analysis

Statistical analysis were performed as described in Ben Sassi-Zaidy et al. (2014) regarding the total number of alleles per locus (TNA), allelic frequencies, observed (H_o) and expected (H_e) heterozygosity, deviation from Hardy-Weinberg equilibrium (HWE) for all locus-population combinations and linkage disequilibrium between pairs of loci. For each population allelic richness (N_{AR}) and the number of private alleles per population (P_{AR}) together with the Wright's fixation index (F_{IS}), polymorphism information content (PIC) and Reynolds' (D_R) genetic distances were also calculated. A neighbor-joining (NJ) tree was reconstructed using D_R distances in the PHYLIP package (Felsenstein, 1989). Bootstraps of 1000 replicates were performed to test the robustness of tree topologie. Moreover population structure analysis was performed using STRUCTURE (Pritchard et al., 2000) and to choose the appropriate number of inferred clusters to model the data, 50 independent runs were performed for each K cluster ($2 < K < 7$, burn-in 30,000, 150,000 iterations for data collection).

3. Results

The total number of alleles detected across the 17 microsatellites markers spread over 14 ovine chromosomes was 297. Summary of polymorphism of the genotyped markers is given in Table S1. All of the markers were highly polymorphic with PIC values larger than 0.500 (Table S1). Using the rarefaction approach the estimates of private alleles in each breed was less then one confirming the absence of breed specific alleles (Table 1). The fixation indices F_{IS}, F_{IT} and F_{ST} for each locus and across all loci are given in Table S1. The mean values of these parameters were: $F_{IS} = 0.105 \pm 0.076$, $F_{IT} = 0.132 \pm 0.079$ and the F_{ST} index was equal to 0.030 ± 0.009 ($P < 0.001$).

All the Tunisian sheep breeds showed good genetic variability among the 17 loci, with high N_{AR} (>8) and H_e (>0.79) values (Table 1). In general all Tunisian breeds showed considerable H_o values ranging from 0.682 ± 0.167 (CRO) to 0.748 ± 0.149 (NTH) but always lower than the H_e. Deviation from HWE, after Bonferroni correction ($P < 0.05$) was limited and resulted significant for 4 markers in APP and 3 in CRO. This divergence is partly reflected in the F_{IS} index which is rather high for CRO (0.139 ± 0.192), DM (0.123 ± 0.138) and APP (0.119 ± 0.184). The between