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Genetic variation and gene flow among Tunisian sheep populations using Random Amplified Polymorphic DNA (RAPD) markers

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Abstract

Random amplified polymorphic DNA (RAPD) was used to infer the magnitude and pattern of genetic differentiation among sheep populations belonging to two native Tunisian breeds (the Barbarine and the Western thin tail). Eight primers generated 62 scorable bands, of which 44 were polymorphic. Genetic differentiation between populations within breeds was detected based on the estimated F_{ST} index. The value of gene flow (Nm) was 5.5361 and 3.5763 in Barbarine and Western thin tail breeds respectively. The diversity within populations (Hs) recorded demonstrates important genetic variation within them. The values of genetic differentiation coefficient (Gst) over all loci were 0.0828 and 0.1227 respectively between populations of the Barbarine and Western thin tail breeds. The principal component analysis (PCA) did not show a clear differentiation between populations within breeds. The results show that studied sheep populations maintain a high genetic diversity in the range of 0.21-0.23 and their genetic differentiation is consistent with their geographic origin. It is therefore necessary to preserve the purity of the Tunisian local breeds that are well adapted to country's bioclimatic conditions.

Keywords: Genetic variation; gene flow; RAPD; bioclimatic zones; Tunisian sheep

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Introduction

Tunisia is a Mediterranean country located east of North Africa between Algeria (west), Libya (south) and the Mediterranean Sea to the north and east. The climate is temperate Mediterranean. The country is divided into five bioclimatic zones: the Saharan, arid, semi-arid, sub-humid and humid where annual rainfall is in the range of 20-100, 100-400, 400-600, 600-800 and 800-1200 mm per year respectively (Daget, 1977). However, rainfall is not the only bioclimatic determining factor. Winter temperatures are also important. These do not depend only on the altitude but also on the continental situation. Thus, inland areas have hotter summers and colder winters than those benefiting from the soothing effects of sea. On the arid and semi-arid bioclimatic zones, the country is therefore, divided in areas with mild or cold winters.

Four main sheep breeds are found in the country: the Barbarine (B), Western thin tail (W), Black of Thibar and the Sicilo Sarde which represent 60.3, 34.6, 2.1 and 0.7% of the Tunisian sheep population respectively (Rekik et al., 2005). The first three breeds are butcher vocation and the last is a dairy breed. Given the variability of weather and forage availability, different farming practices are adopted in different bioclimatic zones. The objective of this study was to investigate if there is a significant genetic differentiation among populations of B and W breeds rearing in three different bioclimatic areas exploring the RAPD polymorphism.

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Materials and Methods

Samples collection, DNA extraction and polymerase chain reaction (PCR) amplification

Samples were collected from 96 animals of the two breeds; B and W in three areas belonging to different bioclimatic zones Sub-humid (Beja, Bizerte), semi-arid with mild winter (Tunis, Sousse) and arid with mild winter (Sfax, Gabes). Blood samples from both sexes of pure B and W breeds were collected from the jugular vein in ethylenediamine tetra acetic acid (EDTA) vacutainer tubes and kept at -20°C until the isolation of total DNA. One sample was collected per herd to avoid inbreeding. Animals were classified into six populations (P1, P2, P3, P4, P5 and P6) according to their breed and bioclimatic zone origin (Table 1, Fig. 1). 16 sheep were typed by population. DNA extraction was carried out using a genomic purification kit (blood DNA preparation kit, Jena Bioscience), with some modifications. According to the kit manual, a 300 µl sample of whole blood yields 10 to 20 µg of DNA. The DNA quality was assessed on agarose gel, quantified using spectrophotometry and diluted to 6 ng/µl for PCR amplification.

Fig. 1: Bioclimatic zones of Tunisia (adopted from Emberger (1930, 1955 and 1971)

Eight 10-mer oligonucleotide RAPD primers (OPA02, OPA06, OPA07, OPA10, OPA12, OPA15, OPA16 and OPA18; Operon technologies) were used to amplify DNA of each animal. The PCR reactions were accomplished in a 50 µl reaction mixture containing 1X assay buffer, 1.25 unit of Taq DNA polymerase (ULTRATOOLS DNA Polymerase, Biotools), 100 µM of dNTP (dNTP Mix, Jena Bioscience), 0.8 µM primers and 30 ng of template DNA. The PCR reaction was carried out in DNA thermal cycler (Eppendorf, Mastercycler gradient). The PCR amplification conditions for RAPD consisted of initial extended step of denaturation at 94°C for 2 minutes followed by 40 cycles of denaturation at 94°C for 1 min, primer annealing at 35°C for 1 min and elongation at 72°C for 2 min. The PCR products were visualized on 1.2% agarose gel.

Table 1: Description of analysed animals

Acronym	N	Breed	Bioclimatic zone
P1	16	Barbarine	Sub-humid
P2	16	Barbarine	semi-arid with mild winter
P3	16	Barbarine	arid with mild winter
P4	16	Western thin tail	Sub-humid
P5	16	Western thin tail	semi-arid with mild winter
P6	16	Western thin tail	arid with mild winter

N: number of analysed animals

Table 2: Analysis of Nei's gene diversity among three populations of B breed

	H_T	Hs	Gst	Nm
Average (over all loci)	0.2109	0.1935	0.0828	5.5361
Standard deviation	0.2240	0.0455		

Table 3: Analysis of Nei's gene diversity among three populations of W breed

	H_T	Hs	Gst	Nm
Average (over all loci)	0.2395	0.2101	0.1227	3.5763
Standard deviation	0.2265	0.0442		

Statistical analyses

Bands obtained were treated as binary characters and coded accordingly (presence =1, absence = 0). A principal component analysis (PCA) containing the 16 typed animals of each population were constructed on the basis of the matrix of genetic distance using Multi-Variate Statistical Package for Windows (MVSP) Version 3.1 (Kovach, 2003). In addition, the total genetic diversity (H_T), genetic diversity within populations (Hs), coefficient of gene differentiation (Gst) (Nei, 1973) and gene flow (Nm) (McDermott et al., 1993) were estimated using Popgene (Population Genetic Analysis) version 1.32 (Yeh and Boyle, 1997) software. Analysis of molecular variation (AMOVA) was conducted using Arlequin program ver. 3.0 (Excoffier et al., 2005), significance of genetic structure indices was evaluated after 1000 random permutations.

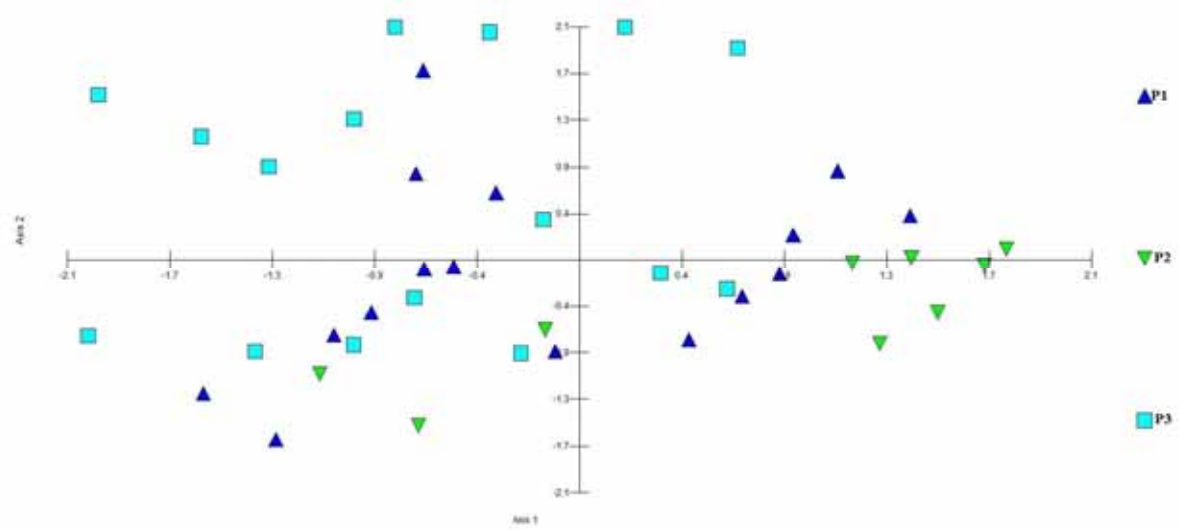

Fig. 2: Principal component analysis showing the relationships among the three populations of Barbarine breed

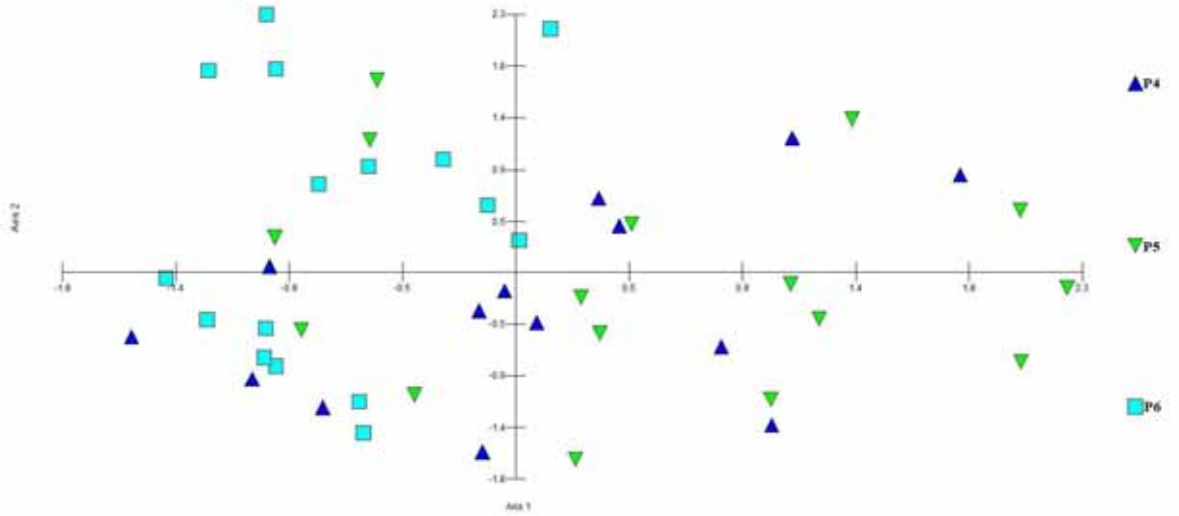

Fig. 3: Principal component analysis showing the relationships among the three populations of Western thin tail breed

Results and Discussion

This study estimated the genetic diversity between populations of the two most common sheep breeds in Tunisia (Barbarine and Western thin tail) and shows that the genetic variability within populations and breeds, estimated by the parameters Hs and H_T, is important. In effect, Kijas et al. (2012) showed that sheep have maintained a vast genetic diversity despite the domestication and selection unlike other species such as cattle and dogs.

Eight RAPD (decamer oligonucleotide) primers used produced 62 bands in total, out of which 44 bands were polymorph and 18 bands (loci) were present in all the animals studied. Statistical analysis of the obtained data was used to investigate genetic variation among populations within breeds.

The Gst value found between the three populations of B breed is 0.0828, this means that the variation between populations accounts for 8.28% of the total variability, while 91.72 of the genetic variation is found within populations. Gst values varied between typed loci from 0.0035 to 0.3842. This result indicates a low level of differentiation between populations. The Nm (Table 2) shows a sufficient migrants' exchange rate to homogenize populations of B breed. Total genetic diversity (H_T) and genetic diversity within populations (Hs) were 0.2109 and 0.1935 respectively (Table 2). To better visualize the population structure, a principal component analysis (PCA) was conducted (Fig. 2). P2

showed the smallest spatial representation of relative genetic distances and this showed that diversity in P2 is lowest than in P1 and P3 populations.

Analysis of molecular variance (AMOVA) used to assess the distribution of genetic diversity within and between populations. Quantification of genetic differences between groups is estimated by the F_{ST} fixation index calculated using the estimator of Weir and Cockerham (1984). Its value actually reflects the combined action of the two evolutionary forces that are genetic drift and migration. In this study, the F_{ST} value between population of the B breed found was 0.0606 ($P<0.01$). This result showed a significant bioclimatic subdivision between populations of B breed. Using microsatellite markers, Chem et al. (2011) and Abdullah Ghazy et al. (2013) reported values of F_{ST} estimates in the range of 0.007-0.0123 and 0.0288-0.1471 between four Chinese Tan breed populations and three Egyptian sheep breeds respectively.

The differentiation coefficient (Gst) between the three populations of W breed varied between loci from 0.0035 to 0.3415 with an average of 0.1221, which implies that 87.73% of the total variation lies within populations. Jawasreh et al. (2011) reported that the Gst coefficient varies between 104 typed loci from 0.001 to 0.3762 with an average value of 0.0962 in Jordanian sheep populations. The gene flow found value is 3.5763. This value is much lower than the value detected for the three analysed Barbarine populations (Table 3), however, this gene exchange rate is sufficient to prevent a complete isolation between populations of different bioclimatic areas. Total genetic diversity (H_T) and genetic diversity within populations (Hs) were 0.2395 and 0.2101 respectively (Table 3). Tariq et al. (2012) reported varying values from 0.0998 to 0.1474 in four sheep breeds in Pakistan. Pandey et al. (2010) reported hetrozygosity in the population ranged (0.279-0.739) with the mean (0.501±0.151) using STR markers.

To visualize differences between populations of W breed, the genetic distance matrix was used to run a Principal Component Analysis (PCA). It seems clear from this representation that the individuals from each population did not form a separated plot.

Analysis of molecular variance showed that the three bioclimatic populations of W breed are significantly differentiated with F_{ST} value of 0.1183 ($P<0.001$). However, although genetic differentiation between populations was significant, the F_{ST} value was low. This may be due to the gene flow that is carried out between them that allowed avoiding genetic drift in populations. Paiva et al. (2005) found that differences among populations within breeds contribute for 9.27% ($P<0.01$) of total variation in Brazilian sheep populations.

Using RAPD markers, Estimation of Gst and F_{ST} coefficients shows that the differentiation between populations of each breed is significant but not high enough to form completely separated groups. Indeed principal component analysis showed that the individuals from each population did not form a separated plot.

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