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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Genetic differentiation and gene flow between four Tunisian sheep populations (*Ovis aries*) using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis

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Abstract

In this study, random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis was used to assess the genetic difference and gene flow among four sheep populations belonging to two native Tunisian breeds (the Barbarine and the Western thin tail). A total of 96 samples were typed using eight RAPD primers. 62 bands were scored, of which 44 bands (70.97%) were polymorphic. The unweighed pair-group method with arithmetic average (UPGMA) and principal component analysis (PCA) did not show a clear differentiation between populations within breeds. The coefficient of gene differentiation (Gst) between populations was relatively low. Analysis of molecular variance (AMOVA) showed that there was a genetic variation of 28.79% (F_{CT}= 0.2879; P<0.001) among the breeds, 4.10% among populations within breeds (F_{SC}= 0.0576; P<0.001)) and 67.10% (F_{ST}= 0.3289; P<0.001) within populations. The level of gene migration between populations within breeds is quite high to homogenizing them.

Keywords: Genetic differentiation; RAPD-PCR; sheep; geographic areas

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Introduction

Domestic sheep belongs to the genus Ovis, however, its origin remains unclear. Several studies have found that species or subspecies of wild sheep are the ancestors of domestic sheep or at least have contributed to this species, particularly the urial (*O. vignei*) and mouflon (*O. musimon* or *O. orientalis*) (Ryder, 1984). As in other species, the domestication of sheep resulted in a much greater phenotypic variations than that observed in wild species (Simm, 1988). There are between 800 and 1000 breeds of domestic sheep (Loftus and Scherf, 1993; Mason, 1996), which reflects the diversity of the species *Ovis aries*. The classification of animals into breeds is usually based on specific phenotypic characteristics, but other factors such as geographical location can be criterion for classification.

Sheep is keystone species in Tunisia with four indigenous breeds, the Barbarine (B), the Western thin tail (W), the Black of thibar and the Sicilo Sarde representing 60.3, 34.6, 2.1 and 0.7% respectively of the total population. The Dorsal, the eastern extension of the Atlas mountains runs across Tunisia in a northeasterly direction from the Algerian border in the west to the Cap Bon peninsula in the east and constitutes a natural barrier, separating the humid northern region (annual rainfall exceeding 400 mm) from the arid or semi-arid central and southern parts of Tunisia (annual rainfall less than 400 mm). The objective of this work is to estimate the variation among populations of the two most commons

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indigenous sheep breeds of Tunisia (Barbarine and Western thin tail) and to investigate the possible existence of differentiation between populations north and south of the dorsal due to geographic isolation.

Materials and Methods

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

Blood samples were collected on ethylenediamine-tetraacetic acid (EDTA) tubes from the jugular vein of both sexes belonging to two different breeds (B and W) and rearing in six regions (Beja, Bizerte, Tunis, Sousse, Sfax and Gabes). To avoid inbreeding, one sample was taken from each herd. Two study areas were considered: the northern of the dorsal including Beja, Bizerte and Tunis respectively located in the north-west, north and extreme north of the country and the southern of the represented by regions of Sousse, Sfax and Gabes located respectively in east-central, south and coastal oasis in the middle of the south-eastern Tunisia. Animals were classified into four populations (P1, P2, P3 and P4) according to their breed and geographical origin. About 24 individuals were typed by population. Blood samples were stored at -20°C until isolation of total DNA. 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. DNA quality and quantity were controlled using analysis on agarose gels and spectrophotometry. DNA of each animal was amplified using eight primers (OPA02, OPA06, OPA07, OPA10, OPA12, OPA15, OPA16 and OPA18; Operon technologies).

PCR amplifications were performed in 50 µl reaction mixtures containing 30 ng of genomic DNA, 0.8 µM of the arbitrary primer, 100 µM of dNTP (dNTP Mix, Jena Bioscience), 3 mM of MgCl2, 1.25 unit of Taq DNA polymerase (ULTRATOOLS DNA Polymerase, Biotools) and 5 µl of 10X Taq DNA polymerase buffer. In order to detect any DNA contamination, control reactions were set up without genomic DNA. Amplifications were performed using a thermal cycler (Eppendorf, Mastercycler gradient) programmed for 40 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min and extension at 72°C for 2 min. An initial denaturation step of 2 min at 94°C and a final extension step of 2 min at 72°C were included in the first and last cycles, respectively.

Statistical analyses

Data were recorded as binary matrix by assigning the value (1) when the band of a given level is present and (0) when it is absent. An unweighed pair-group method with arithmetic average (UPGMA) dendrogram and a principal component analysis (PCA) containing the 48 typed animals of each breed 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 coefficient of gene differentiation (Gst) (Nei, 1973) which reflects the proportion of total genetic diversity due to variability between breeds 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.

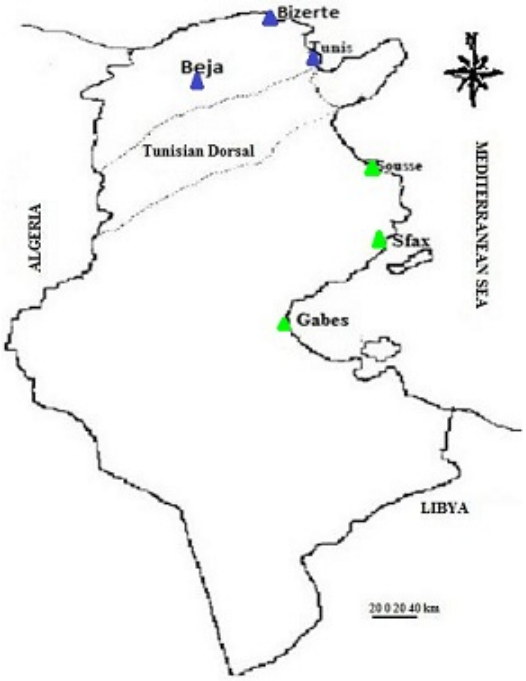

Fig. 1: Map of Tunisia. Geographical distribution of analysed samples; ▲ and ▲ indicate respectively northern dorsal and southern dorsal geographical zones

Results and Discussion

Eight primers were used for typing the 96 studied animals. A total of 62 bands were detected, of which 44 bands (70.97%) were polymorphic. From the binary matrix (0/1) on all analysed individuals, we first estimated the Jaccard similarity coefficients between pairs of individuals of each breed and then established a UPGMA dendrogram grouping the 48 animals (Figures 2 and 3). There is no clear separation between

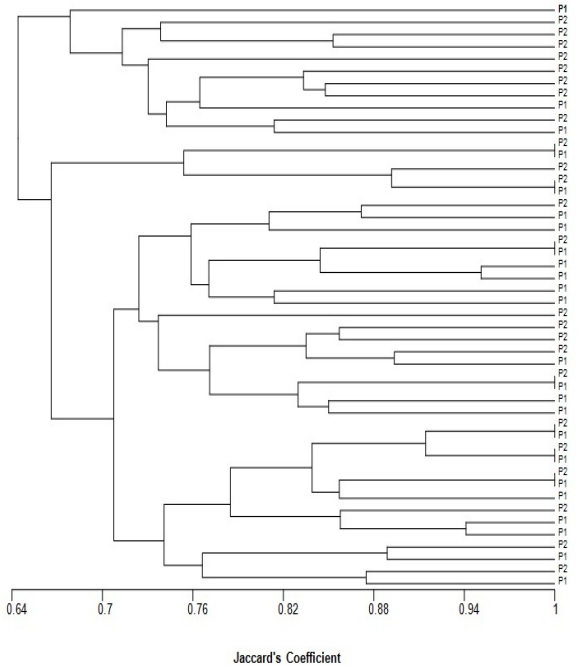

Fig. 2: UPGMA dendrogram of 48 Barbarine sheep animals based on Jaccard's similarity coefficients. UPGMA, Unweighted pair-group method with arithmetic average

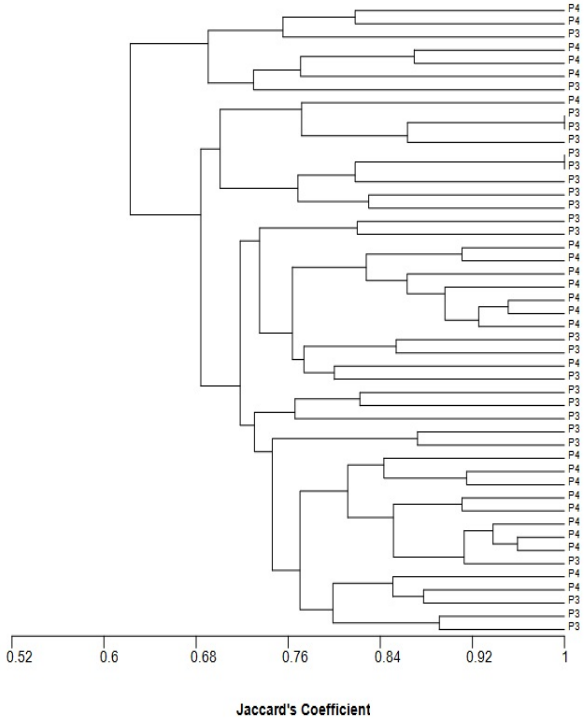

Fig. 3: UPGMA dendrogram of 48 Western thin tail sheep animals based on Jaccard's similarity coefficients. UPGMA, Unweighted pair-group method with arithmetic average

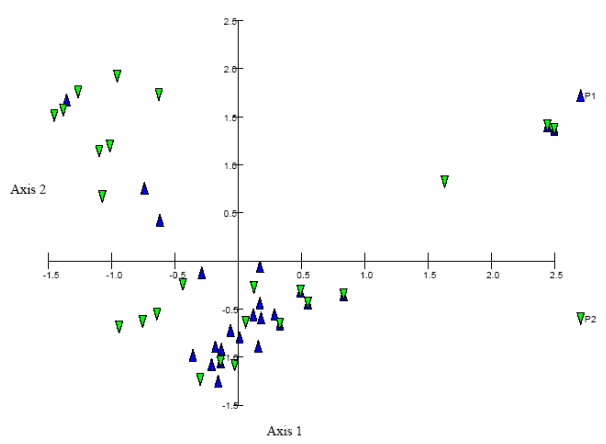

Fig. 4: Principal component analysis showing the relationships among the two populations of Barbarine breed

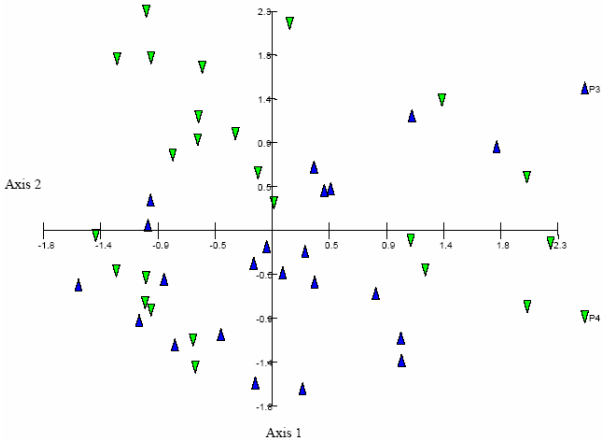

Fig. 5: Principal component analysis showing the relationships among the two populations of Western thin tail breed.

Table 1: Analysis of molecular variance (AMOVA) in B and W breeds

Source of variation	Df	SS	VC	PV
Among breeds	1	159.458	2.96658 Va	28.79
Among populations within breeds	2	34.125	0.42286 Vb	4.10
Within populations	92	636.083	6.91395Vc	67.10

populations of same breed. To better visualize the genetic relationships among populations, we determined the PCA (Fig. 4 & 5) for populations of each breed. In agreement with what was found in the cluster analysis, the individuals from each population did not form a separated plot.

The coefficient of gene differentiation (Gst) was 0.0272, 0.0718 and 0.2333 respectively between the B populations, W populations and all populations. Jawasreh et al. (2011) reported that the coefficient of

differentiation (Gst) varies between 104 typed loci from 0.001 to 0.3762 and the average Gst value is 0.0962 in three populations of Awassi and Najdi breed using RAPD markers. The population structure was also tested by analysis of molecular variance (AMOVA) (Table 1). There was a genetic variation of 28.79% (F_{CT}= 0.2879; P<0.001) among the breeds, 4.10% among populations within breeds (F_{SC}= 0.0576; P<0.001) and 67.10% (F_{ST}= 0.3289; P<0.001) within populations. Gene flow (Nm) represents the number of effective migrants per generation. The estimation of gene flow from Gst value showed that Nm is equal to 17.87, 6.46 and 1.64 respectively between the B populations, W populations and all studied populations. Gene flow is one of the evolutionary forces that significantly influence the genetic structure of the population. In the absence of gene flow, genetic drift causes the appearance of isolated populations and fixing of different alleles at neutral locus, which leads to differentiation of populations.

In conclusion, it appears from this study that there is no clear differentiation of populations within breeds. The detected F_{SC} value although significant, is small and does not justify the conclusion that there is a separation between populations of the same breed due to genetic drift. This argues rather in favor of a local adaptation developed by the populations of different geographical areas and shows that the effect of natural selection allowed each population to develop features that will facilitate adaptation to local environmental conditions.

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