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Genetic differentiation and gene flow between the Tunisian ovine breeds Barbarine and Western thin tail using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis

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Sheep is an important livestock species of Tunisia. They contribute greatly to the food safety of the country and in the livelihood of a large number of small and marginal farmers and landless labourers engaged in sheep rearing. In this study, random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis was used to assess the genetic difference and gene flow among two Tunisian sheep breeds (the Barbarine and the Western thin tail). A total of 62 bands were detected with an average of 7.75 bands per primer. The unweighted pair-group method with arithmetic average (UPGMA) and principal component analysis (PCA) showed a clear differentiation between the two studied breeds. Genetic differentiation coefficient (Gst) over all loci was 0.1922, the fixation index [Fst by Analysis of molecular variance (AMOVA)] was 0.308 (P<0.001), and the gene flow value (Nm) was 1.3102. It is clear from this study that Barbarine and Western thin tail breeds are completely distinct and that they show high and significant genetic differentiation. However, the level of gene migration between them is quite high and may lead eventually to the loss of breed's purity.

Key words: Sheep, genetic differentiation, gene flow, RAPD-PCR.

INTRODUCTION

In Tunisia, there are three important livestock species: Sheep, goats and cattle which represent 77, 14.9 and 8.1%, respectively of the farm animals effective (Rekik et al., 2005). With a population of over four million female sheep, domestic sheep (*Ovis aries*) species has an important economic role in Tunisia. In fact, it constitutes the main source of meat and provides more than 41% of the country's total red meat production. Tunisian flock consists of four breed, mainly the Barbarine (B) (60.3%) and the Western thin tail (W) (34.6%) which are reared across all the territory, but also the Black of thibar (2.1%)

and the Sicilo Sarde (0.7%) which are solely concentrated in the north of the country (Rekik et al., 2005). The Barbarine is fat-tailed, while the other breeds are thin-tailed. To improve performance of flocks, breeders make use of two practices: selection and crossbreeding. Bedhiaf-Romdhani et al. (2008) reported that farmers are crossing the local Barbarin with thin tailed breeds (W and Black of Thibar) due to the difficulty of selling the fat of the tail that represents up to 15% of the carcass weight in the B breed. Certainly crossbreeding allows genetic progress but could lead to a dilution of genetic structure of the breed. It is therefore essential to determine the level of differentiation and gene flow among Tunisian breeds. Indeed intermixing of breeds may pose a danger of maintaining breed purity in

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the long run. Previous studies on genetic improvement of Tunisian sheep population were focused on quantification and characterization of genetic characters of growth and reproduction (Ben Hamouda, 1985; Lassoued and Rekik, 2001, Lassoued et al., 2004). The objective of this study was to evaluate gene flow, genetic structure and differentiation of B and W breeds using random amplified polymorphic DNA (RAPD) markers.

MATERIALS AND METHODS

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

Blood samples were collected on ethylenediaminetetraacetic acid (EDTA) tubes from the jugular vein of animals. All together, eight animals of each sex of six herds were sampled. The 96 samples were obtained from herds in two areas: the northern dorsal (Beja, Bizerte, Tunis) characterized by a humid climate where annual rainfall exceeds 400 mm and the southern dorsal (Sousse, Sfax and Gabes) with an arid and semi-arid climate where annual rainfall is less than 400 mm (Figure 1). Therefore, 48 samples were obtained from each breed in all six regions. DNA extraction was carried out using the blood DNA preparation kit™ (Jena Bioscience). In order to improve the quality and quantity of extracted DNA, some modifications to the standard protocol were conducted. The red blood cell lyses step was followed by additional washes by adding 900 µl of bidistilled sterilized water to the sample, vortexing vigorously for 5 min, centrifuging at 13000 rotations per minute for 5 min and removing the supernatant. This stage was repeated until obtaining a clean pellet rid of any trace of hemoglobin. The DNA hydration time was prolonged to three days and conducted in dark at room temperature. According to the kit manual, a 300 µl sample of whole blood yields 10 to 20 µg of DNA. The DNA quality and quantity were assessed using agarose gel 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 $MgCl_2$, 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

Analysis of the results of RAPD polymorphism was based on the phenotypic interpretation of revealed electrophoretic profiles. First, amplified fragments by all primers and in all individuals were identified Then, a contingency table with n rows (individuals) and p columns (amplified band) was established by assigning the value (1) when the band of a given level (molecular weight in bp) is present and (0) when it is absent and served as a data matrix for the calculation of various statistical parameters. Since RAPD markers are dominants, it was assumed that each band represented the phenotype at a single biallelic locus (Williams et al., 1990). An unweighted pair-group method with arithmetic average

(UPGMA) dendrogram and a principal component analysis (PCA) containing the 96 studied animals 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.

RESULTS AND DISCUSSION

Eight primers were used for typing the 96 studied animals. The number of fragments detected by each primer varied from six for OPA06 and OPA12 to 11 for the OPA10 (Table 1), with a mean of 7.75 fragments Elmaci et al. (2007) reported that the number of fragments revealed by each primer varied from three to 14 in Turkish sheep breeds. Tariq et al. (2012) cited that the number of fragments amplified in four sheep breeds in Pakistan varied from two to 10 per primer and a total of 92 RAPD fragments were obtained using 17 primers with a mean of 5.41. Mahfouz et al. (2008) also studied the genetic diversity of Egyptian sheep breeds and had identified 57 fragments amplified using five primers (11.4 bands/primer). An example of RAPDs banding pattern is shown in Figure 2.

From the binary matrix (0/1) on all analysed individuals, we first estimated the Nei and Li (1979) similarity coefficients between pairs of individuals and then established a UPGMA dendrogram grouping all animals (Figure 3). Individuals could be grouped into two clusters; the first one with animals of the W breed and the second group with the individuals of the B breed.

To better visualize the genetic relationships among the two breeds, we determined the PCA (Figure 4). In agreement with what was found in the cluster analysis, the individuals from each breed formed separated plot. It was evident from both the dendrogram and the PCA presentations that the two breeds are well differentiated. The W breed showed the greatest spatial representation of relative genetic distances and this showed that diversity in W breed is highest than in B breed.

The coefficient of gene differentiation (Gst) was 0.1922, which could mean that 80.88% of the total variation is within breeds, whereas the variability between the two studied breeds is 19.22%. The population structure was also tested by analysis of molecular variance (AMOVA). There was a genetic variation of 30.80% (Fst= 0.308) among the breeds and within them 69.20% (P<0.001). Literature review of works performed in sheep breeds in different countries reveals that Balcioglu et al. (2008) detected a Gst value of 0.5117 in eight breeds in Turkey by using RAPD-PCR method. Gene flow (Nm) represents the number of effective migrants per generation. The

Table 1. Percentage of polymorphism for each primer and for all primers.

Primer	Number of amplified bands	Number of polymorphic bands	Percentage of polymorphism
0PA02	9	3	33.33
OPA06	6	6	100
OPA07	7	3	42.86
OPA10	11	8	72.73
OPA12	6	5	83.33
OPA15	7	7	100
OPA16	9	7	77.77
OPA18	7	5	71.43
Total	62	44	70.96

Figure 1. Map of Tunisia. Geographical distribution of analysed samples; ♦, ● and ■ indicate respectively sub-humid, semi-arid with mild winter and arid with mild winter bioclimatic zones [defined according to Emberger's classification (Emberger 1966, cited by Daget, 1977)].
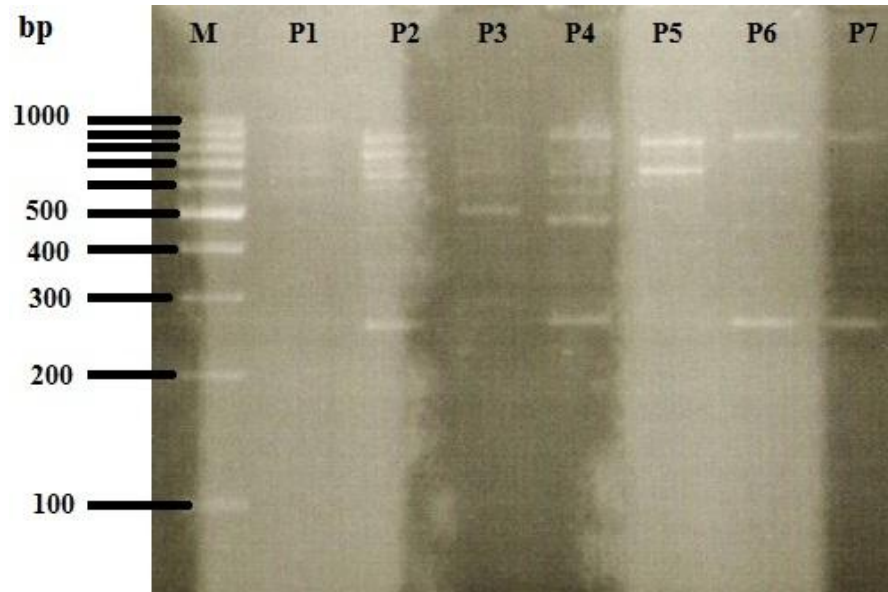
Figure 2. RAPD profiles of seven Tunisian sheep individuals generated with OPA06 primer. M, 100 bp DNA ladder (100 bp to 1 kb); P1 to P4, DNA sampled from B breed; P5 to P7, DNA sampled from W breed. RAPD, Random amplified polymorphic DNA.
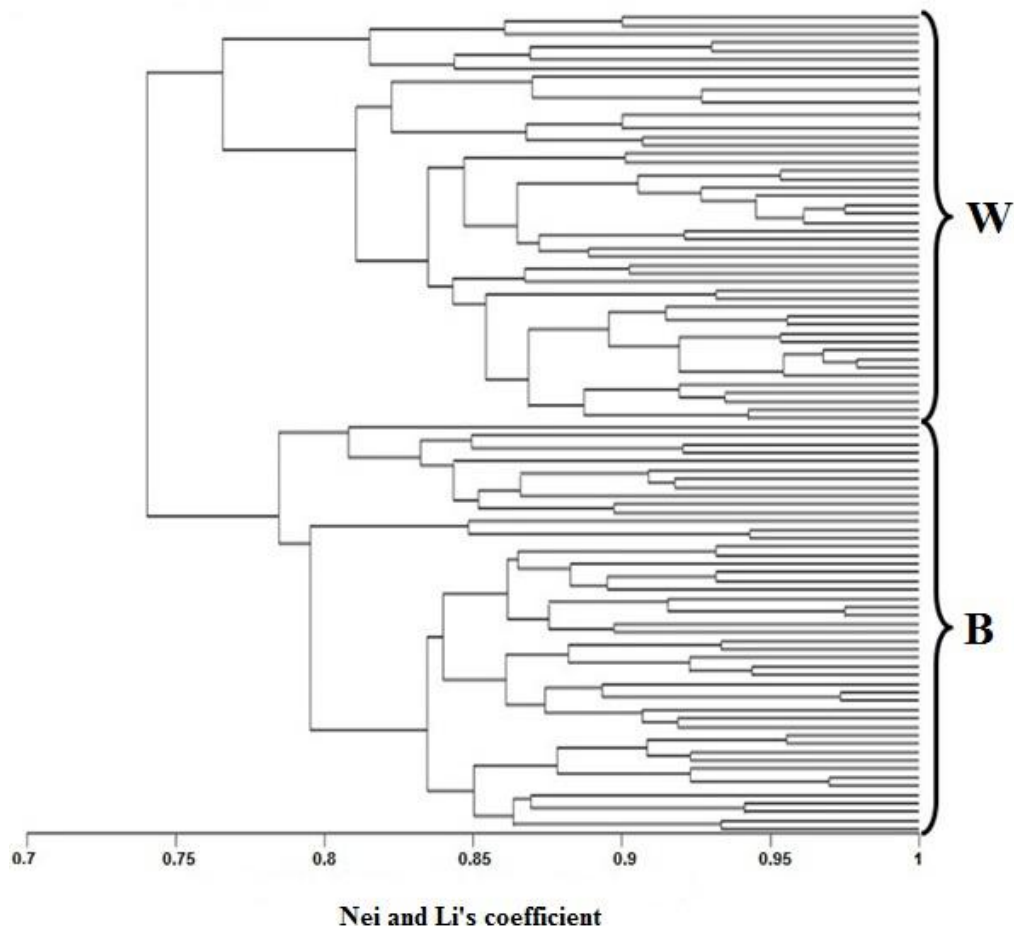

Nei and Li's coefficient

Figure 3. UPGMA dendrogram of 96 Tunisian sheep animals based on Nei and Li (1979) similarity coefficients. UPGMA, Unweighted pair-group method with arithmetic average.
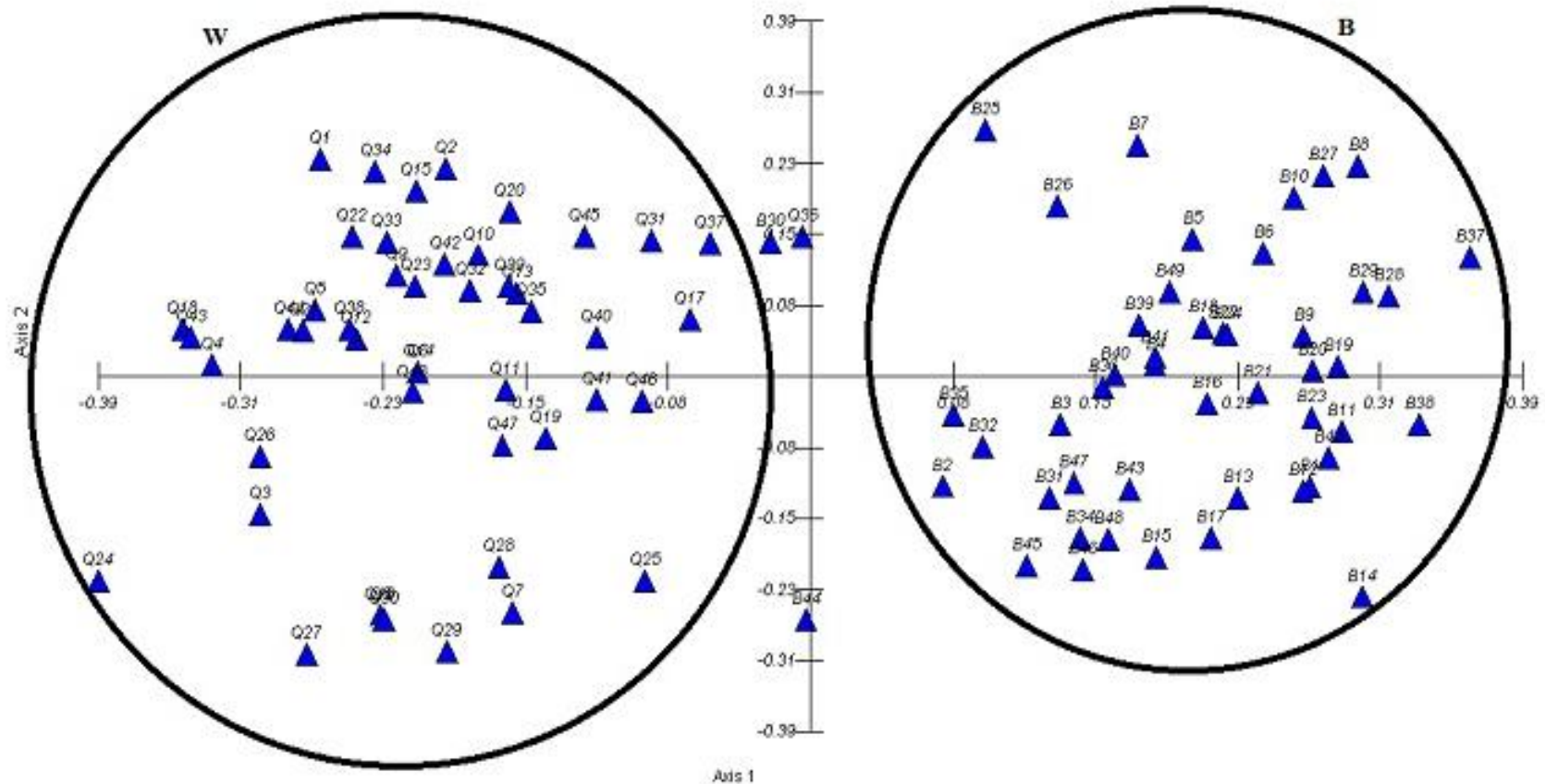
Figure 4. Principal component analysis showing the relationships among the two breeds.

main effect of gene flow is the homogenization of allele frequencies between populations; more gene flow between them is important, moreso they are expected similar. The estimation of gene flow between the two studied breeds from Gst value showed that Nm is equal to 1.3102. This level of gene flow can have negative consequences by intermixing the two breeds. Indeed, according to Wright (1931), a gene flow value greater than one, leads to homogenization of populations. Geng et al. (2008) have used microsatellite markers to study genetic diversity in six sheep populations in China and found values of gene flow ranged from 2.74 to 44.39 with a mean value equal to 11.25. Missohou et al. (2006) found values of gene flow ranging from 0.46 to 6.21 in seven West African goat breeds using microsatellite markers. Mao et al. (2007), studying three Chinese cattle populations, reported that values of gene flow between pairs of populations are 0.509 and 1.149.

Conclusion

This study helps to estimate the level of divergence between the two most common sheep breeds in Tunisia and showed that the RAPD-PCR applied to DNA sheep produced a sufficient level of polymorphism which can be a useful method to evaluate genetic structure in this species. The estimation of average gene differentiation (Gst) and fixation index (Fst) showed that the two breeds are well differentiated and confirms different characteristics which are definitively fixed in each breed. The gene flow value (1.3102) detected in the present study showed that breeders are practicing crosses between the two breeds. These crossings must be controlled to preserve the purity of breeds and the diversity within each breed. These crossings should not lead to a decrease in the relative importance of one of the two breeds over time.

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