



## MOLECULAR POLYMORPHISM OF THE SICILIAN-SARDINIAN DAIRY SHEEP IN THE NAGACHIA REGION IN TUNISIA

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**ABSTRACT:** Microsatellite markers or SSR (Simple Sequence Repeats) were used to study molecular polymorphism in Sicilian-Sardinian sheep born and bred in Tunisia in the Nagachia region (Beja North Delegation). 28 samples, taken from two farms, were analyzed using two SSR primers. A total of 30 bands were amplified, 26 of which were polymorphic (86.67%). Within the two farms and in all the studied animals, the average rates of expected heterozygosity ( $H_e$ ) are 0.1039, 0.1336 and 0.2212 respectively. The gene flow ( $N_m$ ) between the animals of the two farms is 0.5795 while the Nei's genetic distance between herds of the two studied farms is 0.2595.

**Key words:** Molecular polymorphism, SSR, sheep, Sicilian-Sardinian, Tunisia

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### INTRODUCTION

In Tunisia, sheep farming plays a very important socio-economic role. Sheep farms account for 53% of total farms [1]. There are four indigenous breeds: Barbarin (58%), Western thin tail (37%), Black of thibar (2%) and Sicilian-Sardinian (1%) [2]. Sicilian-Sardinian is the only dairy breed in North Africa [3], its livestock is concentrated in the north of the country in the subhumid bioclimatic zone (Beja and Bizerte) where climatic conditions and forage production are advantageous. Currently, the Sicilian-Sardinian breed is very threatened by genetic erosion due to the dramatic decline in its numbers and the consanguinity that characterizes it [4]. On the other hand, we noticed during our field survey that there is no genetic exchange between the animals of the different farms. This would make the consanguinity within the herds more dramatic than in the overall Tunisian population of the Sicilian-Sardinian breed. It is within this frame work that the present work focuses on the study of intra-farm genetic diversity, the quantification of the gene flow between the two studied Sicilian-Sardinian herds and the estimation of Nei's genetic distance between the animals of each of the two farms.

### MATERIALS AND METHODS

#### Samples collection and DNA extraction

Blood samples were taken by a veterinarian in accordance with animal ethics. The sampling was done from two farms, noted here (KB and ZBY), located in the region of Beja Nord and distant of five kilometers. The DNA was extracted from blood from 28 Sicilian-Sardinian sheep (07 males and 07 females from each farm). Individuals were classified into four groups (G1, G2, G3 and G4) according to gender and farm. The blood was taken by a veterinarian from the jugular vein of the animals on EDTA tubes (Figure. 1).

The samples were stored at  $-20^{\circ}\text{C}$  until DNA was extracted. Total genomic DNA was extracted using the Invitrogen iPrep machine and its Kits. After extraction, the quality and quantity of the DNA were evaluated by horizontal electrophoresis on an agarose gel (0.8%). DNA standards of known concentration were used as a reference to determine the concentration of the DNA. All DNA samples were diluted to a concentration of  $50\text{ ng} / \mu\text{l}$ .



**Figure-1: Blood Collection**

### SSR-PCR reaction conditions

The PCR amplifications were carried out in a reaction volume of  $50\ \mu\text{l}$  containing  $50\text{ ng}$  of genomic DNA,  $0.2\ \mu\text{M}$  of each primer (forward and reverse),  $200\ \mu\text{M}$  of dNTP (Invitrogen),  $2\ \text{mM}$  of  $\text{MgCl}_2$ ,  $0.8$  units of Taq DNA polymerase (Invitrogen) and  $10\ \mu\text{l}$  of Taq buffer (5X). In order to detect any contamination, control reactions not containing the genomic DNA were carried out at each amplification. The SSR-PCR reactions were conducted in a thermocycler (Bio-Rad, C1000) programmed to perform 40 cycles of  $95^{\circ}\text{C}$  for 45 seconds,  $60^{\circ}\text{C}$  for 45 seconds and  $72^{\circ}\text{C}$  for one minute. An initial denaturation step of 5 minutes at  $95^{\circ}\text{C}$  and a final ten minute extension step at  $72^{\circ}\text{C}$  were included in the first and last cycles, respectively. The amplification products were separated by 3% agarose gel electrophoresis containing ethidium bromide in Tris Borate EDTA buffer and visualized under ultraviolet light. Two microsatellite primers were used in this study: McM 527 and OarFCB 11 (Table 1).

**Table-1: SSR primers used, their sequences and their melting temperatures ( $T_m$ )**

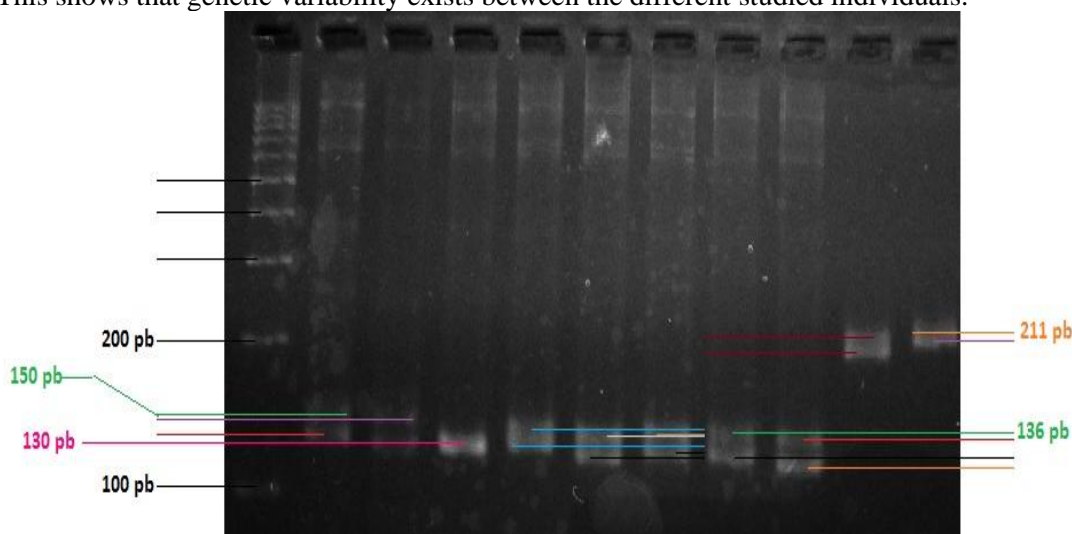
Primer	Sequence 5'.....3'	$T_m$ of the primers
<b>McM 527</b>	<b>Forward:</b> GAC CAC AAA GGG ATT TGC ACA A <b>Reverse:</b> AAA CCA CTT GAC TAC TCC CCA A	$62^{\circ}\text{C}$
<b>Oar FCB 11</b>	<b>Forward:</b> GCA AGC AGG TTC TTT ACC ACT <b>Reverse:</b> GGC CTG AAC TCA CAA GTT GAT ATA	$63^{\circ}\text{C}$

### Statistical analysis

In this study we considered four groups of animals: males of the farm KB (G1), females of the farm KB (G2), males of the farm ZBY (G3) and females of the farm ZBY (G4). The data was recorded as a binary matrix by assigning the value 1 when the band of a given level is present and 0 when it is absent. To estimate genetic diversity within and between farms, we calculated the expected heterozygosity ( $H_e$ ) [5] under the equilibrium conditions of Hardy-Weinberg [6], the unbiased biological distance of Nei [7] between animal groups, the genetic differentiation coefficient ( $G_{st}$ ) and the gene flow ( $N_m$ ) using the Popgen program (Population Genetic Analysis) version 1.31 [8]. This program was also used to develop a dendrogram from the genetic distances of Nei representing the four animals groups.

**RESULTS AND DISCUSSION**

A total of 30 bands were generated using Oar FCB11 and MCM527 primers. 16 bands were amplified by the Oar FCB11 primer having sizes ranging from 112 to 211 bp (Figure 2). 14 bands were amplified by the MCM527 primer having sizes ranging from 174 to 198 bp. Each of these bands may be present or absent depending on the individual. This shows that genetic variability exists between the different studied individuals.



**Figure-2: Primer amplification profiles By FCB 11 on agarose gel (3%) stained with ethidium bromide**

In all animals, the average rate of expected heterozygosity ( $H_e$ ), the Shannon index ( $I$ ) and the percentage of polymorphism ( $P$ ) are respectively 0.2212, 0.3543 and 86.67% (Table 2). The indices of genetic diversity for each group of animals are given in Table 2. Nei, 1987 [9] considers that the calculated or expected heterozygosity ( $H_e$ ) is a good indicator of the genetic variability of populations. This study shows that  $H_e$  is higher in our sample of the ZBY farm than in the KB farm and is relatively low compared to values found by other authors working at the national or regional level. Kdidi et al. (2015) [10] revealed in the Sicilian-Sardinian breed an expected heterozygosity of 0.769. Ben Sassi-Zaidy et al. (2014) [4] studied genetic variability in two Sicilian-Sardinian populations and in the Comisana exotic dairy breed using 17 SSR markers. They reported expected heterozygosity rates of 0.795; 0.785 and 0.793 respectively in the three populations. Using microsatellite markers, Ould Ahmed et al., 2010 [11] and Sharma et al., 2016 [12] reported an expected heterozygosity of about 0.6 in the Tunisian cameline population and the Tibetan sheep population in India. The unbiased Nei's genetic distance [7] is lower between males and females on the same farm than between same-sex animals from different farms (Table 3). The genetic differentiation coefficient ( $G_{st}$ ) which translates the proportion of total genetic diversity due to inter-firm variability is 0.4632. The inter-firm gene flow ( $N_m$ ) which represents the number of migrants per generation is 0.5795. This reflects the absence of migratory flows between the two farms. A UPGMA dendrogram, regrouping all the studied groups, was constructed from the Nei's genetic distances. It shows two well-differentiated branches, the first includes the two groups of the KB farm and the second, those of the ZBY farm (Figure 3).



**Figure-3: UPGMA dendrogram of the four studied groups, constructed from Nei's genetic distances**

**Table-2 : Nei's genetic diversity ( $H$ ), Shannon index ( $I$ ) and percentage of polymorphic loci ( $P$ ) at the level of all animals and intra-farm animals for 28 analyzed samples.**

Group Of Animals	N <sup>(*)</sup>	H	I	P
All animals	28	0,2212	0,3543	86,67
Farm KB	14	0,1039	0,1613	33,33
Farm ZBY	14	0,1336	0,2083	43,33

(\*): Number of studied animals

**Table 3: Distances and unbiased genetic identities of Nei (Nei, 1978) between males of the farm KB (G1), females of the farm KB (G2), males of the farm ZBY (G3) and females of the farm ZBY (G4)**

	G 1	G 2	G 3	G 4
G 1	-	0,8823	0,7510	0,6886
G 2	0,1252	-	0,6543	0,6110
G 3	0,2864	0,4241	-	0,8624
G 4	0,3731	0,4926	0,1481	-

Nei's genetic distance (Below the diagonal) and genetic identity (Above the diagonal)

## CONCLUSION

This study shows the high level of inbreeding within farms even though genetic diversity is notable at the level of the total Tunisian population. It is therefore important to consider the allelic richness within each farm because in our example we have shown that sheep populations are genetically isolated although they are geographically close. This study should be supplemented by considering several farms and using a larger number of animals and SSR markers.

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