



Contribution of microsatellites markers in the clarification of the origin, genetic risk factors, and implications for conservation of Tunisian native sheep breeds

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ABSTRACT. The genetic diversity and genetic relationship of the two main groups of African sheep, thin-tailed and fat-tailed sheep, represented by the indigenous Tunisian sheep breeds “Barbarine” (BAR, fat-tailed) and “Queue Fine de l’Ouest” (QFO, thin-tailed) were investigated. The genotypes of 110 animals belonging to these two breeds and their crossbreed (CRO) were assessed using 17 microsatellite markers. The results showed high levels of genetic diversity and a total of 256 alleles were identified in the whole population. The mean values of observed and expected heterozygosity were 0.719 and 0.789, respectively, and the mean allelic richness estimate was 10.89. The average F_{IS} (0.112) and F_{IT} (0.118)

values over all loci indicated a notable level of inbreeding within the whole population. However, the F_{ST} value (0.007) showed a low level of genetic differentiation between these two native breeds. The high level of both gene flow and molecular coancestry coefficient detected between the two breeds and their CRO revealed an old miscegenation between the BAR and QFO breeds. The clustering analysis performed with the STRUCTURE software confirmed gene flow between these two breeds. Results arising from this study provide evidence regarding the genetic structure and variability of the two main local sheep breeds, and the implications of their actual management, which indicates the need for an urgent conservation strategy in order to prevent significant gene flow and preserve the remaining breed specificity for future generations.

Key words: Tunisian indigenous sheep; Microsatellite markers; Genetic variability; Population structure

INTRODUCTION

In developing countries, livestock genetic resources greatly contribute to sustainable development and to livelihood security and food safety by reducing hunger and poverty. In Tunisia, sheep is the most important livestock species, representing 77% of the total number of reared animals. Sheep production in Tunisia holds an important place in the economy, contributing to almost 42% of total red meat production (OEP, 2009). The sheep breeding sector is largely dominated (94%) by indigenous meat breeds: the “Barbarine” (BAR) and the “Queue Fine de l'Ouest” (QFO). According to the morphology of the tail and their geographic origins, these two sheep have been classified as belonging to two different African sheep groups: BAR to the fat-tailed group and QFO to the thin-tailed group (Muigai and Hanotte, 2013).

In Tunisia, the BAR and the QFO are classified as two different breeds that may have different ancestral origins. BAR is the most important native breed, representing 64% of the total sheep reared in Tunisia (Agriculture Ministry, 2006). This breed is found in large parts of North Africa and it is the dominant breed in Tunisia and Libya, and is also widespread in Algeria. The BAR breed is thought to be derived from the Egyptian fat-tailed sheep (Ben Salem et al., 2011; Muigai and Hanotte, 2013). Originating from the Asiatic steppes (Mason, 1967), the BAR breed is a medium sized meat-type sheep, characterized essentially by its bi-lobed fat tail resulting from the accumulation of fat reserves on each side of the coccygeal vertebra. This fat is considered to be an effective tool for resistance to severe climate conditions. The BAR breed has developed tolerance to both warm and cold climates, a remarkable mothering ability, resistance to parasites, and the ability to use a wide range of low quality feed resources (Ben Salem et al., 2011). Furthermore, BAR had an important socio-cultural role as the dominant sacrificial animal, and consumers still prefer BAR meat, mainly for its tenderness, flavor, and smell (Bedhiaf-Romdhani et al., 2008). QFO is a thin-tailed sheep, and represents 30% of the total sheep breeds reared in Tunisia (Agriculture Ministry, 2006). This meat-type breed, derived from the Algerian Ouled Djellal breed, is adapted to harsh dry conditions but not as well adapted to warmth as the BAR breed. The QFO breed has been gaining importance over the BAR breed during the last few decades; the proportion of BAR ewes has decreased from approximately 85% in the seventies to only 60.3% in 2011 (Agriculture Ministry, 2012), while QFO ewes have increased from 9 to 34.6% over the same period.

The BAR and the QFO breeds assume a national importance as they will continue to be the main meat providers to Tunisian commercial channels, and they significantly contribute to meeting the objectives of the national strategy aimed at red meat self-sufficiency. Crossbreeding between these two breeds is currently being practiced by breeders in central and northern Tunisia, creating a new population named "Chirki" (CRO). Avoiding the large BAR fat tail, which represents an obstacle to free mating, and the difficulties encountered by butchers in selling this fat, which can reach 15% of the carcass weight, are the reasons for these crossbreeding trends, as mentioned by Bedhiaf-Romdhani et al. (2008). Identification of the genetic relationships and the patterns of gene flow among the BAR and QFO breeds could provide important clarification, especially regarding the extent of their crossbreeding. Microsatellite markers are widely used for genetic analyses in livestock and are of fundamental importance in supporting the establishment of conservation programs for the genetic resources. In this study, microsatellites may improve the understanding of the present population structure of these native Tunisian sheep breeds and could help to clarify the results of this crossbreeding. Hence, the aim of the present study is to assess the level of genetic differentiation and gene flow between BAR, QFO, and their crossbreed (CRO), using microsatellite markers to support the decision-making process in crossbreeding activities.

MATERIAL AND METHODS

Animal sampling and microsatellite analysis

A total of 110 individual blood samples were collected from unrelated animals belonging to BAR, QFO, and CRO individuals from 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 information provided by the farmer to avoid sampling of closely related individuals. DNA extraction was carried out from whole blood using the Wizard Genomic DNA Extraction kit (Promega, USA) following the manufacturer protocol. A panel of 17 microsatellite markers (Table 1) was established, including some markers from the ISAG/FAO recommended microsatellite markers (FAO, 2011) and others from previous studies (Baumung et al., 2006; Dalvit et al., 2009; Ben Sassi-Zaidy et al., 2014a). Genotypes for all 17 microsatellite markers were identified as described in Ben Sassi-Zaidy et al. (2014b).

Statistical analysis

The number of alleles per locus (N_A), allelic frequencies, observed (H_O) and expected (H_E) heterozygosity, and gene flow (N_m) were calculated using GENETIX version 4.05.2 (Belkhir et al., 1996-2004). The number of private alleles (PA) in the different breeds was counted using the CONVERT software (Glaubitz, 2004). Exact tests for deviations from Hardy-Weinberg Equilibrium (HWE) were applied using GENEPOP version 4.3 (Raymond and Rousset, 1995). The MSA software (Dieringer and Schlötterer, 2003) was used to calculate allelic richness (AR, the mean number of alleles per locus corrected by sample size), and Wright's fixation indices (F_{IS} , F_{IT} , and F_{ST} ; Weir and Cockerham, 1984). Polymorphism information content (PIC) and relatedness among individuals estimated by measuring within-breed molecular coancestry (f_{ij}) were measured using Molkin 3.0 (Gutiérrez et al., 2005). Nei's (1978) genetic distance (D_A) among populations was estimated and a neighbor-joining (NJ) tree was constructed using the PHYLIP package (Felsenstein, 1989). Bootstraps of 1000 replicates were performed to test the robustness of the

tree topology. The dendrogram was depicted using MEGA 5 (Tamura et al., 2011). Moreover, a factorial correspondence analysis was performed based on individual multilocus genotypes using GENETIX version 4.03 (Belkhir et al., 1996-2004). The analysis of molecular variance (AMOVA) was performed by the ARLEQUIN software (Excoffier et al., 2005) using the codominant allelic distance matrix with 1000 permutations. To analyze the population structure and to detect the most likely number of clusters (K) in the dataset, the STRUCTURE version 2.3.4 software (Pritchard et al., 2000) was used. To choose the appropriate number of inferred clusters to model the data, 50 independent runs were performed for each K ($2 < K < 7$). All analyses used a burn-in period of 30,000 and 150,000 iterations for data collection. The optimum number of clusters fitting the data was established by plotting $\ln \Pr(X|K)$ over the 50 independent runs for each K, as suggested by Pritchard et al. (2000). The output obtained was used directly as input data in the cluster visualization program DISTRUCT (Rosenberg, 2004).

RESULTS AND DISCUSSION

A total of 256 alleles were detected across the 17 microsatellite loci assessed in the 110 genotyped animals and all markers were highly informative, having PIC values >0.5 , confirming their usefulness in genetic diversity studies. Descriptive statistics on the variability of the investigated loci are reported in Table 1. The highest number of alleles was found at locus *OarCP49* (26) and the lowest at locus *OarAE129* (7). Mean N_A was 15.06 ± 5.02 , and the mean AR was 11.28 ± 3.17 . The mean PIC across all loci was 0.791 ± 0.091 , ranging from 0.546 (*OarAE129*) to 0.905 (*OarCP49*). These values are comparable to those recently recorded by Ben Sassi-Zaidy et al. (2014a,b) in the first investigation of Tunisian sheep and higher than those estimated in the Zulu South African sheep (Kunene et al., 2014) and in the Kenyan sheep populations (Mukhongo et al., 2014). In the overall population, the homozygote excess (F_{IT}) of 0.118 ± 0.091 was mainly due to a significant homozygote excess within breeds ($F_{IS} = 0.112 \pm 0.091$) rather than genetic differentiation among them since the F_{ST} index was limited to 0.007 ± 0.008 ($P < 0.001$).

Table 1. Characteristics of the microsatellite loci used to genotype individuals from three Tunisian sheep breeds: Barbarine, Queue Fine de l'Ouest, and a crossbred population Chirki.

Locus	Chr	Fragment size (bp)	N_A	AR	PIC	F_{IS}	F_{IT}	F_{ST}
Inra023	1	195-221	13	11.62	0.882	0.013	0.013	0.000
Inra063	14	168-206	19	13.29	0.839	0.142	0.153	0.012
OarCP49	17	71-137	26	18.86	0.905	0.076	0.080	0.004
OarFCB304	19	145-219	19	13.64	0.820	0.117	0.131	0.016
OarFCB20	2	87-117	15	11.26	0.863	0.083	0.091	0.009
MAF65	15	119-139	12	8.830	0.735	0.032	0.048	0.018
ILST087	6	142-178	22	15.40	0.879	0.206	0.205	-0.002
OarAE119	19	141-183	11	9.390	0.759	0.043	0.047	0.004
MCM527	5	164-188	12	9.610	0.804	0.191	0.206	0.020
MAF214	16	176-262	14	8.510	0.656	0.319	0.318	0.001
OarAE129	5	135-163	7	6.180	0.546	0.256	0.265	0.011
OarCP34	3	101-117	8	6.460	0.737	-0.002	0.007	0.009
OarAE54	25	124-148	13	10.84	0.730	0.037	0.035	-0.001
TGLA53	12	139-167	11	10.16	0.820	0.122	0.136	0.016
URB058	13	159-211	18	13.31	0.801	0.069	0.070	0.000
CSRD247	14	214-262	19	12.30	0.809	0.086	0.082	-0.003
HSC	20	260-296	17	12.11	0.858	0.042	0.066	0.026
Mean	-	-	15.06	11.28	0.791	0.112	0.118	0.007
S.D.	-	-	5.02	3.170	0.091	0.091	0.091	0.008

Chr = chromosome; N_A = number of alleles; AR = allelic richness; PIC = polymorphic information component; F_{IS} , F_{IT} and F_{ST} = fixation indices according to Weir and Cockerham (1984); S.D. = standard deviation.

The genetic variability of the two breeds and their crossbreed was initially studied in terms of N_A , PA, and AR, as shown in Table 2. The BAR breed had the highest N_A , while CRO had the lowest. A total of 70 (27.34%) PA were detected among the studied groups. However, only 16 PA were present in frequencies of >0.025 . The highest number of PA (35) was found in the BAR breed, with 9 of them were present at a frequency >0.025 . In this breed, the marker (*OarAE129*) exhibited only one PA at a frequency of 0.05, while three and four PA at a frequency >0.025 were found in QFO and CRO, respectively.

Table 2. Genetic variability of two Tunisian sheep breeds, Barbarine (BAR) and Queue Fine de l'Ouest (QFO), and their crossbred population, Chirki (CRO).

Breed	N	N_A	PA		AR \pm SD	$H_O \pm$ SD	$H_E \pm$ SD	$F_{IS} \pm$ SD	HWE	$f_{ij} \pm$ SD
			t	f						
BAR	50	12.18	35	9	10.88 \pm 3.73	0.739 \pm 0.116	0.796 \pm 0.093	0.082 \pm 0.146	1***	0.20 \pm 0.02
QFO	30	11.35	19	3	11.29 \pm 3.24	0.736 \pm 0.145	0.812 \pm 0.068	0.110 \pm 0.117	1***	0.18 \pm 0.02
CRO	30	10.65	16	4	10.50 \pm 3.26	0.682 \pm 0.168	0.787 \pm 0.078	0.150 \pm 0.144	3***	0.20 \pm 0.02

N = number of analyzed samples; N_A = mean number of alleles; PA = private alleles (t = total number of PA; f = number of PA with frequency >0.025); AR = allelic richness; H_O = observed heterozygosity; H_E = expected heterozygosity; F_{IS} = within-population heterozygote deficiency; HWE = number of loci deviating from Hardy-Weinberg equilibrium; f_{ij} = within-population molecular coancestry coefficient. ***P < 0.001. Lowest heterozygosity and greatest inbreeding coefficient (F_{IS}) values exhibited by CRO population are shown in bold font.

In general, the analyzed populations showed reasonably high allelic richness and heterozygosity values compared to some African (Soma et al., 2012; Kunene et al., 2014; Mukhongo et al., 2014), European (Salamon et al., 2014), Asian (Al-Barzinji et al., 2011; Blackburn et al., 2011b), and American (Blackburn, et al., 2011a; Souza et al., 2012; Ferreira et al., 2014) sheep breeds. The lowest values of AR and heterozygosity were observed in the crossbred population in which H_O was notably lower than the H_E (Table 2). These findings show that both native breeds, BAR and QFO, are characterized by high genetic variability, highlighting the importance of safeguarding their genetic variability in the context of preserving the Tunisian global sheep genetic diversity. All loci in the BAR and QFO breeds were in HWE (Table 2), with the exception of *ILST087* and *OarAE129*, respectively. In CRO, 3 loci (*Inra063*, *MCM527*, and *MAF214*) departed from HWE. The F_{IS} values for each population were low for BAR but relatively high for QFO and CRO, indicating a strong presence of inbreeding in these two populations. This difference in F_{IS} values could be due to the morphological specificity of the BAR breed, which confers a paternity control-like system during mating. In fact, in BAR herds, mating relatives is generally avoided by shepherds by lifting the fat tail during copulation. This reproductive behavior could explain the low level of inbreeding found in BAR. This mating management is absent in QFO herds and in the CRO population (mainly derived from BAR males and QFO females with thin tails), where shepherd assistance is not required. Furthermore, exchange of rams between neighboring small breeders, practiced in the western-center of Tunisia (center of origin of CRO), probably allows for frequent mating between relatives. The relatedness among individuals (f_{ij}) was considerable in BAR and CRO (0.20 \pm 0.02), reflecting the within-breed diversity level (Table 2). Moreover, f_{ij} detected between QFO and CRO was large (0.46), confirming the high genetic identity of these two populations. The BAR breed shares a slightly lower f_{ij} coefficient (0.45) with both QFO and CRO. The molecular coancestry coefficient between breeds reflects the genetic relationship in the founder population (Alvarez et al., 2005). Thus, the high f_{ij} between the BAR and QFO breeds may be evidence of previous N_m between these populations in the past. Lower values of f_{ij} were revealed by Alvarez et al. (2005) and Bozzi et al. (2009). N_m and D_A are provided in Table 3. N_m between the three populations was

positive and considerably high. The number of migrant individuals estimated between BAR and CRO (27.47) was notably lower than between QFO and CRO (42.16), revealing dilution of BAR genes in the crossbred population.

Table 3. Nei's (1978) genetic distance (D_A), below the diagonal, and gene flow (N_m), above diagonal, between three Tunisian sheep populations.

	BAR	QFO	CRO
BAR	-	34.50	27.47
QFO	0.036	-	42.16
CRO	0.044	0.034	-

BAR, Barbarine breed; QFO, Queue Fine de l'Ouest breed; CRO, crossbreed (BAR x QFO).

Using random amplified polymorphic DNA (RAPD) analysis, El Hentati et al. (2013), found lower but considerable N_m values (1.31) between the BAR and QFO breeds. Both investigations of these two native breeds, using RAPD and microsatellite markers, have revealed that a level of N_m greater than one (Wright, 1931) leads to homogenize BAR and QFO breeds. Consequently, the high N_m between these two native Tunisian breeds is the main reason for the limited genetic differentiation observed between them. Very low values of N_m were revealed by Salamon et al. (2014). In the current study, Nei's D_A was low between the studied groups, revealing that CRO is closer to QFO than to BAR (Table 3). The NJ consensus tree, based on D_A distances (Figure 1), showed a high bootstrap value (100%) for the BAR-CRO node. Variation between populations, analyzed using AMOVA, was 0.8% between both the BAR and QFO and the BAR and CRO, while it was only 0.31% between the QFO and CRO. The majority of genetic variation (99.38%) was found within breeds.

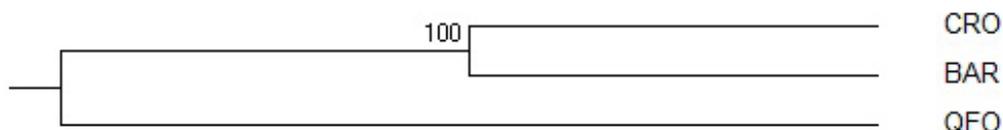


Figure 1. Neighbor-joining consensus tree showing the genetic relationship between the native Tunisian sheep breeds based on D_A distances. BAR = Barbarine; QFO = Queue Fine de l'Ouest; CRO = crossbreed (BAR x QFO). Bootstrap value is indicated on the tree node.

Factorial correspondence analysis was performed including all populations and loci, and using the corresponding allele frequencies (Figure 2). The first three components explained only 6.91% of the total variation and no clear separation was found between the analyzed groups.

The optimum number of clusters, $K = 3$, derived using the STRUCTURE analysis is shown in Figure 3. Individuals from the analyzed breeds were split into three different clusters. Admixed individuals were differently distributed between the clusters and the average membership coefficient for BAR, QFO, and their crossbreed CRO is depicted in Table 4. The proportion of membership in the different clusters was moderate and varied among the analyzed groups. BAR exhibited the highest value (45.7%) of membership into the third cluster (pink color in Figure 3) whereas QFO exhibited the highest proportion of membership (46.9%) into the first cluster (yellow color in Figure 3). The lowest value of assignment was found for the CRO population, which showed similar proportions of membership in the three different clusters.

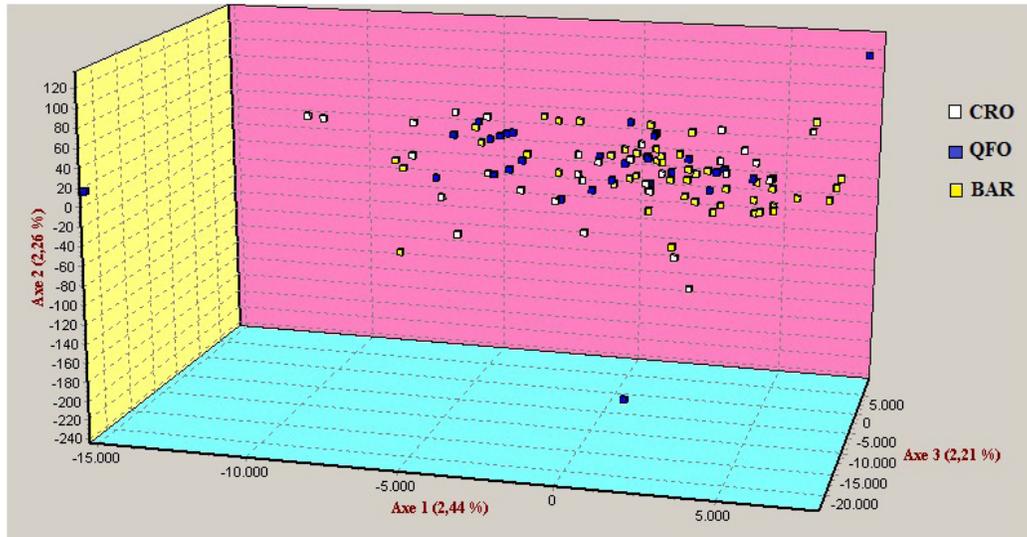


Figure 2. Spatial representation of two native Tunisian sheep breeds, Barbarine (BAR) and Queue Fine de l'Ouest (QFO), and their crossbred (CRO = BAR x QFO), as defined by the factorial correspondence analysis based on all microsatellite loci and corresponding allele frequencies. The first three axis explained 6.91% of the total variation; the share of each axis is indicated by the value in parentheses.



Figure 3. Estimated subdivision of three native Tunisian sheep breeds, Barbarine (BAR), Queue Fine de l'Ouest (QFO), and their crossbred (CRO = BAR x QFO), into three clusters, inferred from STRUCTURE analysis. Mean membership of each breed to each cluster is provided in Table 4.

Table 4. Proportion of membership of each of the analyzed Tunisian sheep breeds in the 3 inferred clusters derived using the STRUCTURE software (maximal contribution in bold).

Breed/cluster	1	2	3
BAR	0.349	0.194	0.457
QFO	0.469	0.286	0.246
CRO	0.324	0.381	0.295

BAR = Barbarine breed; QFO = Queue Fine de l'Ouest breed; CRO = crossbred (BAR x QFO).

This result revealed that, aside from the same pattern of miscegenation taking place in the past during the development of the BAR and QFO breeds, specific genes of these two breeds are diluted in the CRO population. It should be noted that these results are in accordance with the narrative history of sheep in Tunisia and North Africa. In fact, Phoenician and Roman monuments depict that a very long, thin-tailed breed replaced a fat-tailed breed previously brought

by the Phoenicians approximately 400 B.C. After the miscegenation of these two sheep, a second reintroduction of a fat-tailed sheep by the Arab tribes was maintained until 900 A.D. (Sarson, 1973). Furthermore, Muigai and Hanotte (2013), summarizing the history of African sheep, indicate that the first sheep coming to North Africa from the domestication center at approximately 6500 BP were thin-tailed, then at approximately 3000 BP, a second wave of fat-tailed sheep selected in the domestication center entered North Africa and replaced and/or were subjected to introgression with the local thin-tailed sheep. After these waves of migrations and miscegenations, isolation of each breed would have occurred. In fact, two evolution trends would have taken place depending on the new environment: a) evolution to a thin-tailed sheep in the eastern highlands of Algeria, where the fat originally stored in the tail was no longer needed and the result was the Ouled Djellal Algerian breed from which was derived the recent QFO Tunisian breed; b) In Tunisia, the evolution to the recent fat-tailed BAR breed would have occurred, especially since the last wave of sheep introduced by the Arab tribes (900 AD) from North Africa brought only fat-tailed sheep and concerned mainly Tunisia and Libya. Consequently, the fat-tail phenotype reappeared and was dominated in Tunisia, despite the presence of the thin-tailed genes in the miscegenated genetic background of the Tunisian sheep.

This situation seems to be similar to the history of the two groups of Moroccan sheep breeds, the group of the plateau and the group of Atlantic coast, where a fat-tailed breed originated from Asia has evolved to the recent thin-tailed sheep breeds of these two regions (Guessous et al., 1989). The STRUCTURE results from the current study suggest similarity of the BAR and QFO native breeds, highlighting their common genetic background. The study of Muigai and Hanotte (2013) revealed a closer relationship between the North African fat-tailed sheep and the thin-tailed West African sheep than with any of the East and South African fat-tailed sheep, which corroborates the hypothesis of a common genetic background for the fat-tailed BAR and the thin-tailed QFO Tunisian native breeds. However, in the current study, differences in the admixture distribution of the three clusters identified between BAR and QFO during the STRUCTURE analysis reveal the existence of genetic material specific to each breed (QFO has the highest proportion of membership in the first cluster and BAR has the highest proportion of membership in the third cluster), which emphasizes the uniqueness of each breed. According to the STRUCTURE analysis and the estimates of N_m , which reveal high N_m between the studied populations, genetic material specific to each breed is gradually being eroded in the crossbred CRO population. Indeed, since CRO is more distant from the BAR breed than the QFO breed, and N_m between QFO and CRO is higher than N_m between BAR and CRO, the dilution of BAR genes following this crossbreeding practice has become strongly established. Thus, specific genetic characters of BAR, which are absent in QFO, especially high rusticity, adaptability to both cold and warm climates, high mothering ability, resistance to external and internal parasites, and superior meat quality, have been compromised by this crossbreeding. The situation for sheep livestock in Tunisia is therefore precarious since mutations within Tunisian production systems induced mainly by climate change, which will be warmer and drier, are in favor of breeding very adapted and rustic breeds like BAR (Ben Salem et al., 2011). This crossbreeding trend should consequently be stopped immediately to prevent a complete loss of specifically adapted genes, mainly present in the BAR breed.

Conservation of indigenous Tunisian meat sheep through breeding strategy

Characterization of the genetic variability of local breeds is one of the global priorities of scientific research and it is dictated both by the re-evaluation of practices in livestock breeding

and by the conservation of genetic resources. In Tunisia, despite traditional quantitative genetic improvement methods of growth traits being in place for many decades, no clear genetic improvement has been obtained. In fact, despite a national strategy in red meat self-sufficiency, ovine meat imports grew from zero tons in 2003 to 1800 tons in 2012 (FAOSTAT, 2014). The contribution of sheep meat to the total supply of meat has steadily declined over the past three decades of the twentieth century [from approximately 40% in 1978 to 33% in 1986 and 25% in 1996, with an average consumption per capita decreasing from 7 kg in 1980 to 4 kg in 1996 (Hammami et al., 2007)]. The contribution of sheep meat to the total supply of meat reached 40% again during this decade and it stabilized at this level, despite efforts made to increase it. Moreover, the major socio-cultural role of using Tunisian local sheep as a sacrificial animal in the religious ceremony of “Aid al-Adha” is no longer carried out as sheep have been imported from Romania and Spain for this ceremony by the Tunisian government in the last couple of years. Consequently, complementary actions should urgently be planned in both public and private sectors to improve productive traits without losing the breed integrity and the high genetic variability found in these autochthones Tunisian breeds.

Several measures need to be taken, starting with the establishment of an adequate organizational structure of breeders and stakeholders for the implementation of policies for genetic resources, maintaining, as well as implementing, breeding goals and strategies. Subsequently, the establishment and reconstruction of a pedigree, also with the aid of molecular tools, is fundamental for the implementation of both functional and productive traits, for controlling crossbreeding, and to implement a conservation strategy.

All these actions require a strong collaboration between breeders, associations, universities, and research centers that needs to be established. Actions of these collaborators should be under national agricultural development goals, which must include Tunisian economic variables, accommodate ethics, and other social aspects of human well-being, as indicated by the FAO (2007).

The lack of information on the genetic variability and population structure of local sheep genetic resources will be a barrier for future sustainable sheep industry developments. The current study shows that microsatellite markers can be successfully used to investigate the genetic variability and structure of Tunisian local sheep breeds, providing important and useful tools for improving breeding programs of local breeds that are highly adapted to hard environmental conditions. The BAR and QFO breeds can be efficiently used for increasing the production chain if they are subject to suitable breeding programs to improve within-breed production traits in order to increase their genetic ability for both productivity and profitability. In contrast, this investigation highlighted that the BAR breed shows stronger signs of genetic erosion than the QFO breed and its gene pool is being diluted in the CRO population, which has been expanding recently. The BAR breed is numerically the most important and its products are in demand with local needs. This clearly indicates that urgent measures of conservation and sustainable management of the BAR gene pool must be undertaken.

Conflicts of interest

The authors declare no conflict of interest.

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