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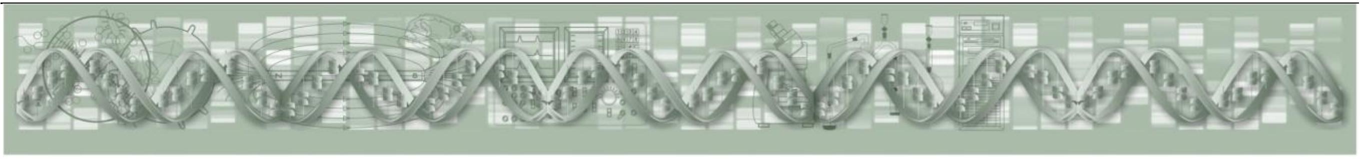

PHYLOGENETIC ORIGIN OF FIVE ALGERIAN SHEEP BREEDS

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ABSTRACT

In order to perform a phylogeny study of five Algerian sheep breeds, a sample of 26 animals belonging to Algerian sheep breeds was analysed. Multiple alignment of mitochondrial sequences was performed after the introduction of other highly similar mitochondrial sequences from different regions of the world. 20 different haplotypes were observed, with a nucleotide diversity of 0.60863 and a haplotype diversity of 0.932. The evolutionary history deduced from the study of genetic distances, and phylogeny based on the maximum likelihood model, showed that these five local breeds belong to the mixture of the two world haplogroups (A and B).

KEY WORDS: Mitochondrial sequences, genetic diversity, preservation

INTRODUCTION

The sheep was, along with the goat, one of the first domesticated ungulates. It was then transferred by man to the whole planet. The first traces of domestication date back to 10300 years B.C. Generally, the history of sheep domestication is poorly known. The existence of the first independent domestication events is suggested by the presence of numerous strongly divergent mitochondrial haplogroups in the domestic species. However, the origin of the domestic sheep is subject to controversy (Rezaei, 2007).

The study of molecular phylogeny makes it possible to reconstruct relationships between nucleotide or amino acid sequences. The reliability of phylogenetic reconstruction methods is based on an understanding of the mechanisms of sequence evolution, a field that has made great progress in recent years. This has led to an increasingly correct vision of the universal tree of life (Lopez, 2002).

The polymorphism of mtDNA is less marked than that of nuclear DNA and the analysis made of it is therefore less discriminatory. In fact, mitochondrial DNA does not allow 100% identification of an individual, but does allow the exclusion of a hypothesis. There is about 1 chance in 2000 that two unrelated individuals have the same mtDNA (Lemonnier, De Reguardati, 2000).

In Algeria, the wealth of variability in sheep genetic resources is poorly exploited. The presence of 12 Algerian sheep breeds that are well adapted to environmental conditions has been noted. There is a dominance according to the number of certain so-called primary breeds: Ouled-Djellal, Hamra, Ifilène and Sidaoun, at the expense of other so-called secondary breeds with limited numbers: Rembi, D'man, Barbarine, Berber, Taâdmit, Tazegzawt, Srandi and Darâa (Gaouar et al., 2009., 2015 and Djaout et al., 2017).

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Unfortunately, anarchic cross-breeding leads to a dispersion and erosion of the genetic capital of the "breeds", an increase in inbreeding in flocks and a drop in livestock yields. The medium-term risk is the absorption of certain "breeds" by others, and the loss of certain characteristics that make our "breeds" special (Gaouar et al., 2005, 2009, 2011, 2014, 2015a, 2015b, Ameur Ameur et al., 2017, 2020 and Djaout et al., 2017).

Local sheep populations are constantly subject to the diversity of the environment (harsh climate, food constraints) and are characterised by remarkable hardiness; however, they present heterogeneous production yields and diverse morphological characteristics that seem to have a different genetic origin (S.P.A, 2006).

Preserving these breeds means safeguarding and characterising them so that they can maintain all their qualities and avoid problems of drifting and inbreeding. Knowing their histories and the phylogenetic relationships that link these different breeds to the world heritage is another aspect that is gaining importance in breed preservation and conservation strategies.

In this context, the present work consists of a genetic characterisation of five Algerian sheep breeds ((Ouled-Djellal, Hamra, Rembi, Dmen and Berber), based on the sequencing of mitochondrial DNA of maternal origin, with the aim of carrying out a phylogenetic study and proposing an origin of our local sheep breeds.

MATERIAL AND METHODS

The objective of the present work is to present the results of analyses of an original genetic marker which is the sequence of the control region (D-Loop) of the mitochondrial DNA studied in the Algerian sheep population.

For this phylogenetic study of mitochondrial DNA, 26 samples of sheep from 5 Algerian sheep breeds were analysed: 5 samples from the Ouled Djellal breed, 5 from the Rumbi breed, 5 from the Hamra breed, 5 from the Berber breed, and 6 from the D'men breed, previously dosed with nano-drop and reduced to a concentration of (25µ/.ml).

A volume of blood varying from 7 ml to 25 ml was taken from each animal on anticoagulant (tri-sodium citrate 3.8%) and stored at -20°C, the animals selected were unrelated. This sampling was carried out according to Gaouar et al (2004, 2005, 2009).

DNA extraction is performed using the NaCl technique (Miller et al., 1889), followed by PCR amplification with 1X Master mix kits, PCRMastermix ready to use (Promega Madison, WI), in a final volume of 20µl containing 0.4µM of each primer (Forward (CR3F) and Reverse (CR2R) specific to segment 1 and 2 of the control region (D-Loop) with a final quantity of 25ng of DNA.

Primers for the amplification of the region (D-Loop) were designed using Primer3 software (at primer3.ut.ee), starting from the complete mtDNA sequence of the reference Ovisaries in Genbank (NC_001941.1) (Table 1). Given the size of the control region, approximately 1200 bp, two runs were performed, each segment being approximately 600 bp.

Table I: Sequences of primers used

Name of primer	**Sequence of primer**	**Tm °C**	**Taux GC**
Mtovin 1F (Forward)	5'-CCCCACTATCAACACCCAAAG-3' (21)	59.8	52.4%
Mtovin 1R (Reverse)	5'-GACAGGATACGCATGTTGACTAGA-3' (24)	61.0	45.8%
Mtovin 2F (Forward)	5'-TCTAGTCAACATGCGTATCCTGTC-3' (24)	61.0	45.8%
Mtovin 2R (Reverse)	5'-CGTTATGTATGTGACCCAGGTG-3' (22)	60.3	50%

The amplification PCR conditions consist of denaturation at 94ºC for 3min, 35 denaturation cycles (90ºC/30s), hybridization (60ºC/300s), and elongation (72ºC/1min), and a final 10min cycle at 72ºC. A purification with the enzyme "ExoSAP" (Affymetrix, (Santa Clara, CA) is performed, in order to remove any residual genetic particles, PCR sequencing was performed with Big Dye Kits (Terminator v1. 1) (Life Technologies, Karlsruhe, Germany), with conditions consisting of a denaturation step at 94ºC for 3 min, 25 cycles of denaturation (96ºC/10s), hybridization (60ºC/05s), and elongation (60ºC/4min).

After sequencing with the ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, CA), the sequences were corrected using Sequencher 4.1.4 (Gene Codes Corporation, Ann Arbor, MI USA).

The resulting sequences are presented in the FASTA format and then submitted to the Multiple Sequence Alignment Software (MEGA6). All positions containing gaps have been eliminated. No deletions or insertions were detected. The determination of the different haplotypes was performed using the DnaSP6 software. In order to identify sequences highly similar to our mitochondrial sequences of local sheep breeds, different banks were queried using BLAST tools set up on the website (NCBI).

Our phylogenetic analysis is done according to the Minimum Evolution statistical method, and is based on the Maximum Likelihood Model. To estimate the statistical reliability of the phylogenetic tree, the non-parametric bootstrap method was applied using 1000 iterations

RESULTS AND DISCUSSION

The study of substitution rates is important in order to understand the dynamics of sequence evolution. In general, and especially at the mitochondrial DNA level, substitutions of the transition type, purine (A-G) and pyrimidine (T-C), appear more often than substitutions of the transversion type, that means purine-pyrimidine (A-C), (A-T), (G-C) and (G-T) (Gillespie ,1991), which is confirmed in this study.

Substitution rates were estimated according to the Kimura two-parameter model using the Maximum Likelihood method, giving transition type substitution rates of (8.99), and transverse type substitution rates of (8.01) (Kimura ,1980).

However (Koseniuk and Slota, 2016) have shown that in Polish sheep breeds the rate of transversion-type substitution is much higher (10%) than the rate of transition-type substitution (4%). Similarly (Meadows et al., 2006), studying Turkish breeds, showed a very high transversion-type substitution rate (20%) compared to the transition-type substitution rate (10%).

The estimation of genetic distances is based on the number of nucleotide substitutions present in the sequences to be analysed. Each substitution can be the translation of one or a series of modifications. In fact, the substitution rate is the result of a multifactorial process involving mutagen sensitivity, the reliability of the DNA polymerase and the efficiency of the DNA repair system (Gillespie, 1991).

Another phenomenon that needs to be considered is the saturation of transitions with high degrees of genetic divergence (Brown et al., 1982), (Moritz et al., 1987).

Therefore it seems that the estimation of substitution processes is better when it is carried out on very close sequences, than when it takes into account more divergent sequences (Purvis et al., 1997).

Table II: Estimation of the genetic diversity of the different Algerian sheep breeds

N = number of analysed samples ; π = nucleotide diversity ; nh = number of unique haplotypes ; Hd = haplotype diversity ; S = number of polymorphic sites

Breed	**ID**	**Origin (Region)**	**N^a**	**π**	**nh**	**Hd**	**S**
Ouled-Djellal	O	South-West Algeria (Djelfa, Beskra)	5	0.57956	5	0.889	1077
Berber	B	Eastern Algeria (El-Taref)	5	0.64201	5	0.889	544
Rembi	R	Western Algeria (Tiaret)	5	0.52027	6	0.911	982
Hamra	H	Western Algeria (Oran et Beyad)	5	0.61380	5	0.889	582
Dmen	D	South-West Algeria (Bechar et Djelfa)	6	0.22394	5	0.848	145
Total			26	0,60863	20	0.932	200

Genetic diversity is defined by the level of similarity or difference in the genetic make-up of individuals, populations and species. This genetic diversity is extremely important because it represents the basic material on which selection can act (Duminil, 2006).

For a total of 1130 sites and excluding ambiguous sites, the global sequence alignment revealed 200 polymorphic sites (S), all represented by single nuclear polymorphisms (SNPS), with a global nucleotide diversity (π) of 0.60863. The number of nucleotide differences between two randomly selected sequences was (K=121,725). (Table II).

A total of (Eta=530) mutations and 20 different haplotypes were identified, with a total haplotype diversity (Hd) of 0.932.

The Ouled-Djellal breed showed the highest number of polymorphic sites (S=1077), followed by the Rembi breed (S=982), while the Dmen breed showed the lowest number of polymorphic sites (S=154).

When comparing the indices of nucleotide diversity, no significant differences were observed between the breeds (Ouled-Djellal, Berber, Hamra and Rembi) ($\pi \approx 0.60$), while the Dmen breed had the lowest nucleotide diversity ($\pi=0.22394$), while the highest nucleotide diversity was observed in the Berber breed ($\pi = 0.64201$).

Hiendleder et al (2002) presented the first complete sequencing of the mitochondrial DNA control region of 63 sheep representing five wild taxa (O. musimon, O. gmelini, O. vignei, O. ammon, O. canadensis) and domestic lines from Asia, Europe and New Zealand, and demonstrated the presence of two haplogroups, A and B, within the domestic lineages.

Further work was subsequently carried out: (Pedrosa et al., 2005) studied D-loop and the cytochrome B gene in the mitochondrial DNA of 79 domestic sheep from Turkey, (Meadows *et al.*, 2007) analysed the same mitochondrial DNA in 197 domestic sheep from Turkey and Palestine. This work added three new haplogroups (C, D and E) to the mitochondrial genetic diversity of sheep:

-Haplogroup A; This is the most widely distributed in the world and includes many European breeds.

-Haplogroup B; is dispersed throughout northern Europe and the continents of the central Mediterranean (southern Italy and Spain, Portugal, western Balkans) according to the work of (Lancioni et al., 2013) on Italian breeds derived from the Spanish Merino. It is also dominant in Africa, demonstrated by the work of (Brahi et al., 2015) on the djallonke breed, in Mauritania (Alvarez et al., 2013), Somalia and Kenya (Resende et al., 2016) and in Egypt by analysing the Ossimi and Barki breeds by (Othman et al., 2014).

In addition, a study of Neolithic sheep remains from a South African cave has demonstrated their attribution to haplogroup B (Horsburgh et al., 2010).

-Haplogroups C, D, and E mainly cover the Near East (Syria, Palestine, Turkey and Egypt) (Demirci et al., 2013).

The haplogroup B extended 6400 years ago, reached Western Europe before haplogroup A, although the divergence of the latter haplogroup may go back 20,000 years (Senna et al., 2015). Therefore, many breeds represent a mixture of two to three haplogroups, as is the case with the Algerian breeds.

Our phylogenetic tree shows that Algerian sheep breeds belong to the mixture of the two haplogroups A and B and that there are two distinct groups for our local breeds (Figure 1) :

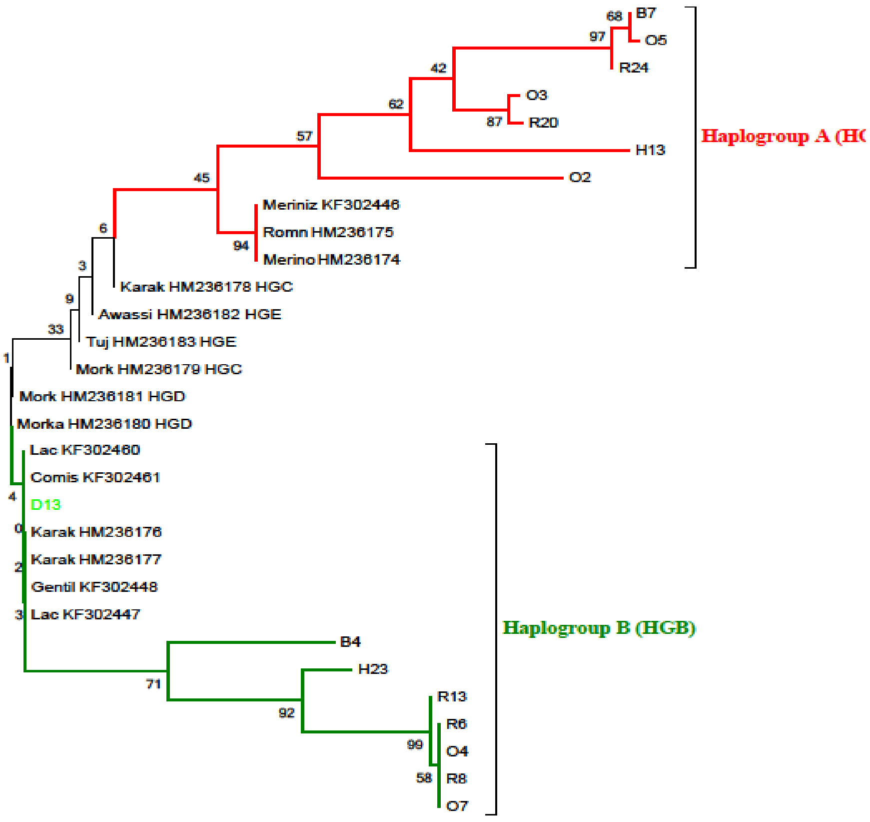

Figure 1: Phylogenetic tree of the different mitochondrial sequences of Algerian sheep breeds. The tree is constructed according to the Maximum Likelihood Method using the MEGA6 software. Haplogroups A and B are shown in red and green respectively. The bootsroop values are shown on the bottom of each branch of the tree.

-In addition to the Dmen breed which binds to haplogroup B, a strongly maintained group, which gathers the sequences of the Algerian breeds (Ouled-Djellal, Berber, Rembi and Hamra) expressed by the individuals (O4, O7, R6, R8, R13, B4, H23), is retained by a significant bootstrap of 71% to this haplogroup B.

-A second group made up of sequences of Algerian breeds (Ouled-Djellal, Berber, Rembi and Hamra) represented by the individuals (O5, O3, O2, B7, R24, R20 and H13) linked together by a significant robustness (from 42% to 97%), this group binds to haplogroup A by 45% bootstrap.

However, it should be noted that the work of (ghernouti et al., 2017) has shown that some Ouled-Djellal individuals (O1, O6, O8) belong to haplogroup C.

African sheep have been domesticated outside Africa. They share a common ancestor with Asian and European sheep. Archaeological information supports a separate introduction and a partly separate dispersal history for the fat-tailed and sharp-tailed sheep on the continent. The first sheep arrived in Africa via the Suez Isthmus and/or the southern Sinai Peninsula between 7500 and 7000 BP. They were probably of the sharp-tailed sheep type. Fat-tailed sheep entered Africa from the northeast of the continent and the Horn of Africa. Mitochondrial DNA results support a common maternal ancestral origin for all African sheep, while autosome and Y-chromosome data indicate a distinct genetic history for both fat-tailed and sharp-tailed sheep (Muigai, 2013).

Our phylogenetic study has enabled us to trace the phylogenetic history of Algerian sheep breeds, also based on some historical data:

Concerning the first hypothesis on the introduction of the main breed Ouled-Djellal into Algeria by the Banu Hillal, Ibn Khaldun announced in the history of the Berbers that entire Bedouin tribes lived between the Nile and the Red Sea, "Banu Hilal and Banu Sulaym", expelled from Arabia because of their disruptive actions, and unleashed on the Maghreb by the Fatimid vizier, set off with women, children, ancestors, animals and camp equipment. In the 13th Century, these Bedouin Arabs settled in the whole of North Africa, except for the main mountain ranges and certain coastal regions which remained entirely Berber. These nomads raised exclusively camels and penetrated into the desert. Later one of the Bedouin "Tawba" tribes, finding themselves too weak to visit the desert pastures, gave up their old trade of raising camels to look after sheep and oxen (Le Tourneau, 1966). As a result, the Bedouin Arabs did not bring sheep with them, but only camels, so the Ouled-Djellal is not Arab. In addition (Chellig, 1992) adds that the Eastern and Asian sheep breeds are all big-tailed Barbary breeds.

The second hypothesis supported by Dr Trouette (1933) pleads for the introduction of the Ouled-Djellal breed in Algeria by the Romans about 1900 years ago, great lovers of wool, coming from Taranto in Italy where this type of sheep exists until now, it is represented on the funerary steles of the ruins of Timgad (Batna) in Algeria (Chellig, 1992).

This hypothesis is confirmed by our results concerning the belonging of the Ouled-Djellal and the other Algerian breeds to the mixture of the two haplogroups A and B, which include many European breeds. This similarity is clearly observed phenotypically between the local Ouled-Djellal breed and the prolific French " Lacaune " breed, which has a naked belly, between the Berber woolly breed called Azoulai and the Italian Sarda and Mirinizzata breeds, also between the Dmen breed and the Turkish Morkaraman breed of haplogroup B.

Furthermore (Othman et al., 2014) have shown in a comparative study of mitochondrial sequences of Egyptian (Barki, Ossimi and Rahmani) and Italian (Sarda and Laticauda) sheep breeds that these two population groups are completely distant from each other, with very high genetic distances.

Another study based on the mitochondrial DNA of Moroccan sheep breeds demonstrated that the Beni-Guil breed belongs to haplogroup A (Kandoussi et al., 2020). This Moroccan breed has a great phenotypic resemblance with the Algerian Hamra breed (Chellig, 1992). Also in a study of mitochondrial DNA variation in several Kenyan sheep breeds, (Resende A. et al., 2016) showed that the majority of individuals of the Kenyan African breeds belonged to haplogroup B and only one individual to haplogroup A.

The phylogenetic similarity between the Algerian sheep breeds, especially the Ouled-Djellal and the Merino breed of haplogroup A, can be explained by the origin of the latter, which according to its name can come from the Merinids (Banu Marin), which constitute a dynasty of Berber Zenet origin between the 13th and 15th centuries in North Africa. These Merinids imposed their empire in 1465 on the Maghreb and a small part of the Andalusian coast (current Spain).

The rapprochement of the Algerian breeds to the two haplogroups A and B is also explained by some Neolithic historical data. This period was characterised by the contribution of the Phoenicians to Carthage. This contribution was nuanced by the coexistence of different populations such as the Berbers, Greeks, Italians or Iberians from Spain. Many mixed marriages contributed to the establishment of the Punic civilisation (Dridi, 2006).

CONCLUSION AND PROSPECTS

The relations that exist between the different Algerian breeds studied are merely the reflection of a long century of ethnic mixing due to migration and colonisation between the two shores of the Mediterranean, resulting in the Algerian breeds (Ouled-Djellal, Rembi, Berber, Hamra and Dmen) belonging to the mixture of the two world haplogroups (A, B).

Although the analysis of simple types of biomolecules is extremely useful for the understanding of biological phenomena, large-scale parallel research on DNA, RNA and proteins opens up new perspectives in the interpretation of origins. The molecular approach is at the basis of all actions for the management and genetic improvement of biological resources.

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