

## Y chromosome haplotype characterization of Tunisian sheep breeds

Samia KDIDI<sup>1,2,\*</sup>, Mohamed Habib YAHYAOUTI<sup>1</sup>, Belén GARCÍA-MANRIQUE<sup>3</sup>, Pilar SARTO<sup>3</sup>,  
Mohsen BEN SASSI<sup>4</sup>, Touhami KHORCHANI<sup>1</sup>, Jorge Hugo CALVO<sup>3,5</sup>

<sup>1</sup>Livestock and Wildlife Laboratory, Arid Lands Institute, Medenine, Tunisia

<sup>2</sup>Laboratory of Genetics, Immunology, and Human Pathology, Faculty of Science, El-Manar University, Tunis, Tunisia

<sup>3</sup>Animal Production Technology Unit, Aragon Center for Food Technology and Investigation (CITA), Zaragoza, Spain

<sup>4</sup>Livestock and Pasture Office, Tunis, Tunisia

<sup>5</sup>Aragon Foundation for Research and Development (ARAID), Zaragoza, Spain.

Received: 29.05.2014

Accepted/Published Online: 31.12.2014

Printed: 10.06.2015

**Abstract:** This study aimed to investigate Y chromosomal haplotypes in the main Tunisian sheep breeds. For this purpose, we sequenced 2 regions of *SRY* (549-bp and 598-bp fragments) and 1 region in each of the *AMELY* and *DBY* genes in Barbarin, Western Thin Tail, Sicilo Sarde, and Black Thibar breeds. In addition, we analyzed the diversity at the *SRYM18* microsatellite locus and detected 4 alleles of 139, 141, 143, and 145 bp. The sequencing analysis did not reveal any polymorphism in the *AMELY* or *DBY* regions; however, 2 samples carried the G allele in the 549-bp fragment of *SRY*. A total of 5 haplotypes, H4, H5, H6, H8, and H12, were found in the 4 breeds. The most common haplotype was H8 (55.5%), followed by H6 (34.5%), while other haplotypes were observed at low frequencies. H4 and H5 haplotypes were observed only in Western Thin Tail and Black Thibar breeds, respectively. In spite of the small area of Tunisia, a high Y chromosome diversity was observed. The results underlined the genetic relation of Tunisian sheep breeds with Middle Eastern, African, and European sheep breeds.

**Key words:** Y chromosome, haplotype, Tunisian sheep breeds

### 1. Introduction

The genetic diversity of farm animals has long been a subject of interest, and it has important implications for genetic improvement, breeding management, and effective genetic conservation programs. Microsatellites are the most common markers used for genetic characterization of sheep breeds (1–7) while mitochondrial DNA and Y-chromosomal DNA have been used to study maternal and paternal lineages. The genetic history of the domestication of sheep has been investigated by analyzing maternally inherited mitochondrial DNA in modern sheep breeds (8). The unipaternal and haploid inheritance of Y-specific microsatellites and SNPs make them extremely sensitive for detecting genetic history, the domestication process of breeds, population relationships, and male-bias gene flow (9,10). Thus, these markers have been used to study male-specific gene flow to gain insight into the species' history, particularly admixture between populations, migration, and other events (11). Several studies have investigated this type of genetic system in sheep (12–14).

In Tunisia, sheep farming is an important economic and social activity contributing to more than 40% of the total red meat production (<http://www.oep.nat.tn/index.php/fr/donnees-sectorielles/41-productions>). There are more than 7 million sheep heads, and 4 million breeding ewes (<http://www.onagri.tn/statistiques>) that belong to 4 different breeds: Barbarin (60.3% of the total), Western Thin Tail (34.6%), Black Thibar (2.1%), and Sicilo Sarde (0.7%). Available information on genetic biodiversity and the origin of Tunisian sheep breeds is still very limited (15,16) and the Y-chromosome diversity has not been studied yet.

In this study, the distribution of Y-chromosome haplotypes was analyzed using variations in 2 fragments of the 5'-flanking region of the *SRY* gene (AY604734 and AY604735) and the *SRYM18* microsatellite locus in 4 native Tunisian sheep breeds. In addition, polymorphisms in the 3'-untranslated region of the *DBY* gene (171 bp) and a 182-bp region from the *AMELY* gene (DQ469593) were also examined.

\* Correspondence: [kdidi\\_samia@yahoo.fr](mailto:kdidi_samia@yahoo.fr)

## 2. Materials and methods

### 2.1. Animals

Blood samples used for DNA isolation were collected from 52 unrelated animals from different flocks belonging to 4 native sheep breeds of Tunisia, including Barbarin (n = 23), Western Thin Tail (n = 18), Sicilo Sarde (n = 8), and Black Thibar (n = 3). A total of 3 and 6 flocks were sampled from the northern regions of the country for the Black Thibar and Sicilo Sarde breeds, respectively. Barbarin and Western Thin Tail are reared throughout the whole country, and 21 and 18 flocks were sampled, respectively (Figure 1). The genomic DNA was extracted using standard phenol-chloroform method (17).

### 2.2. PCR amplification and sequence analysis

Amplification of the *SRYM18* microsatellite locus was performed according to Meadows et al. (12) using a fluorescently labeled forward primer, and the polymerase chain reaction (PCR) products were visualized on an ABI 310 (Applied Biosystems, Madrid, Spain). To accurately determine the allele size, a sample from the Spanish Rasa Aragonesa breed, previously validated as 141 bp by Meadows et al. (12), was also included in each run. PCR amplifications of two 5'-upstream regions (549 and 598 bp in length) of the *SRY* gene and a fragment of the 3'-untranslated region of the *DBY* gene (171 bp) were carried out as described by Meadows et al. (18). The *AMELY* fragment (182 bp) was also analyzed as described by Oner et al. (14). The PCR fragments were purified using the Macherey-Nagel Extract II purification kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. The purified PCR products were then sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) on an automated ABI 3100 genetic analyzer (Applied Biosystems). Sequences were aligned using Clustal X2 software (19) and were compared against the GenBank and EMBL databases using the NCBI BLAST tool. The unweighted pair group method with arithmetic mean (UPGMA) tree was made following Nei's standard genetic distance (20) using Populations 1.2.32 (21) and TreeView program 1.6.6 (22).

### 3. Results

No polymorphisms were found in the *DBY* and *AMELY* genes or the 598-bp fragment of the *SRY* gene. However, 2 samples carried the G allele in oY1 *SRY* (the 549-bp fragment) while the rest of animals carried the A allele. Haplotypes were characterized according to Meadows et al. (12) by using the oY1 SNP in the *SRY* gene and the genotype of the *SRYM18* microsatellite locus. In this regard, 5 paternal haplotypes were observed in the 4 sheep breeds. H6, H8, and H12 haplotypes were distinguished by their allele sizes of 143, 141, and 139 bp at the *SRYM18* locus, respectively, whereas haplotypes H4 and H5 were

characterized by an allele size of 145 bp at the *SRYM18* locus and the A or G allele at oY1 *SRY*, respectively. The distribution of the 5 haplotypes in all breeds is given in Table 1 and Figure 1. The most frequent haplotype was H8 (55.5%), while H6 was found with a frequency of 34.5%. H4, H5, and H12 haplotypes were present at low frequencies of less than 4%. Western Thin Tail and Barbarin breeds showed 4 and 3 haplotypes, respectively (Table 2). H8 is the most common haplotype in the Sicilo Sarde and the Barbarin, while H6 and H5 were the most frequent haplotypes observed in Western Thin Tail and Black Thibar, respectively. Two animals with the H12 haplotype were found in the Barbarin and Western Thin Tail breeds. H4 and H5 haplotypes were observed only in the Western Thin Tail and Black Thibar breeds, respectively.

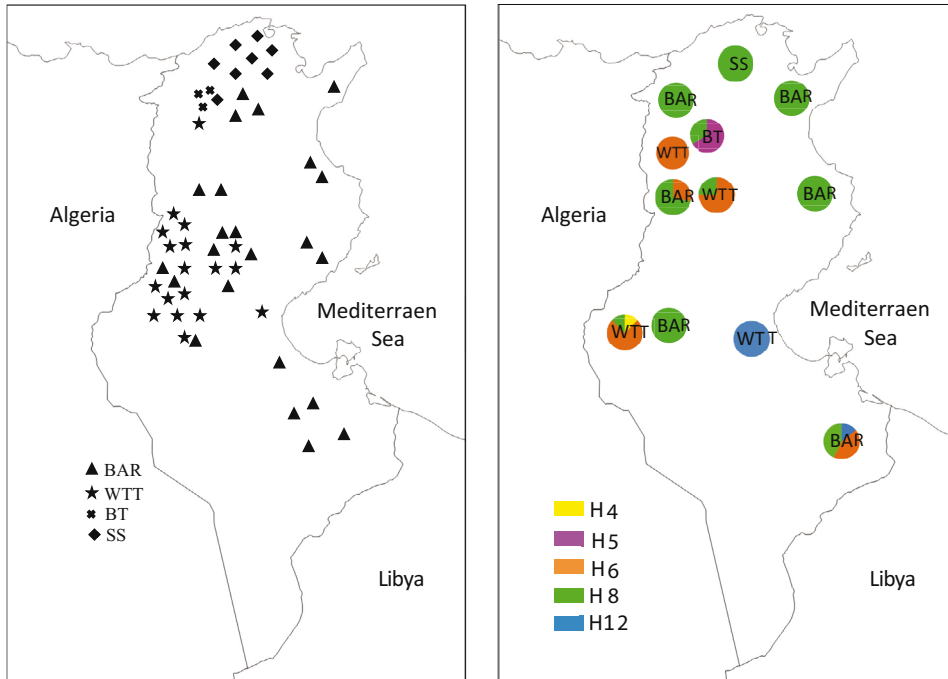
The UPGMA tree (Figure 2) indicates a close relationship between the Western Thin Tail and Barbarin breeds. On the other hand, Black Thibar seemed to be isolated from the other breeds.

### 4. Discussion

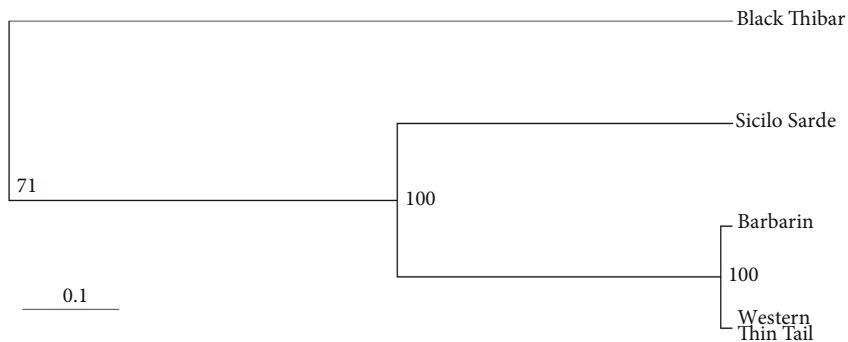
The most frequent haplotype was H8 (55.5%), while H6 was found with a frequency of 34.5%. Several studies have shown that H6 is the most predominant haplotype in different sheep breeds (12,14,23–25), except for the Tyrolean Mountain sheep (Austria) and Brown and White Mountain sheep (Germany) that exhibited only the H8 haplotype (12). In this regard, African breeds also showed a higher frequency for the H6 haplotype (66.6%) (13), while in this study the H8 haplotype is the most frequently observed (55.5%). Similar trends were observed in West Africa, where H8 was found as the most common haplotype (87.5%) in a study of 491 male individuals from 35 distinct breeds in Africa (23). However, a different distribution of haplotypes depending on the African region was found: the H6 haplotype had the highest distribution in eastern and southern African regions, with frequencies of 90.5% and 60%, respectively, while haplotype H8 was most common in West Africa

**Table 1.** Haplotype frequencies in the 4 studied sheep breeds.

Haplotypes	Number of animals	Frequency (%)
H12	2	4
H4	1	2
H5	2	4
H6	18	34.5
H8	29	55.5
Total	52	100



**Figure 1.** Distribution of (a) collected samples and (b) Y-chromosomal haplotypes of 4 sheep breeds in Tunisia (BAR: Barbarin, WTT: Western Thin Tail, BT: Black Thibar, SS: Sicilo Sarde).



**Figure 2.** Phylogenetic relationship among the 4 studied sheep breeds constructed by the UPGMA method and using Nei's standard genetic distance. Numbers shown at the nodes are the bootstrap values after 1000 replications.

with a frequency of 87.5%. In this study, we analyzed North African sheep breeds, while in the other studies the breeds were from sub-Saharan Africa (Senegal, Mali, Botswana, Ethiopia, Nigeria, South Africa, Zimbabwe, and Tanzania). A relatively high genetic diversity was detected (Table 2), especially for the Western Thin Tail, reflecting the absence of well-structured programs of selection. The H8 haplotype was observed in the 4 breeds (Table 2) and is the major haplotype in the Barbarin, with an observed frequency of 74%. In addition, H8 is the unique haplotype detected in the analyzed samples of the Sicilo Sarde, which is a breed with European ancestry resulting from a cross between Italian Comisana

and Sarde breeds (26). H6, the main haplotype observed in the Western Thin Tail with a frequency of 72%, was not found in the genotyped samples of the Black Thibar and the Sicilo Sarde breeds. On the other hand, the H4 haplotype, previously observed only in the fat-tail breeds (14,23), was not detected in the fat-tail Barbarin samples of the present study. Interestingly, this haplotype was found in the Western Thin Tail. The H5 haplotype has been observed in European (19%) and Australian (27.2%) sheep (13), as well as in Asian and Caribbean (12,13) breeds, but it was absent in all African breeds analyzed so far. In this study, this haplotype was detected only in the Black Thibar breed, which could be explained by the

**Table 2.** Distribution of Y-chromosome haplotypes among Tunisian sheep breeds.

Breeds	H4	H5	H6	H8	H12	Total
Barbarin			5	17	1	23
Black Thibar		2		1		3
Sicilo Sarde				8		8
Western Thin Tail	1		13	3	1	18
Total	1	2	18	29	2	52

European paternal origin of this breed, resulting from a cross between native Algerian Thin Tail sheep with the French Merino d'Arles sheep breed (27). The H12 haplotype, characterized by the 139-bp allele of *SRYM18*, was observed only in 2 animals, Barbarin and Western Thin Tail, in the current study. This haplotype has been observed in the Turkish Sakiz breed in the Middle East (13) and in some Chinese sheep breeds (SishuiFur, Luzhong Mountain, and Taihang Fur) (25). This allele of the *SRYM18* microsatellite was also detected in 2 thin-tailed sheep from West Africa, the Djallonke and Maure from Senegal and Mali, respectively (23). The origin of the Tunisian Western Thin Tail is the Ouled Jellal breed from Algeria (16), and a possible genetic exchange could have occurred between Algerian sheep breeds and neighboring sub-Saharan breeds via the southern borders with Mali and Mauritania. The Barbarin breed's origin could be in the steppes of Central Asia (15). Today it is well known that the domestication of sheep occurred approximately 10,000 to 11,000 years ago in the region that spans from northern Zagros to southeastern Anatolia (28,29). In this sense, after domestication in the Middle East, gene flow between the Middle East and Northeast Africa could have occurred. Moreover, further connection with the Middle

East might have occurred during colonization of the North Africa by the Ottoman Empire in the 16th century.

The Barbarin and Western Thin Tail clustered together in the UPGMA tree (Figure 2), suggesting possible genetic admixture as a result of uncontrolled reproduction. Although the Black Thibar and Sicilo Sarde seem to be more differentiated in the tree, this should be confirmed by analyzing more samples from these 2 breeds.

In conclusion, the results of this study point out a relatively high Y-chromosome diversity of Tunisian sheep breeds, with 5 paternal haplotypes (H4, H5, H6, H8, and H12) in spite of the limited number of samples analyzed from Black Thibar and Sicilo Sarde breeds. Further investigation of more samples, and of the mitochondrial DNA diversity, will provide more information on their origin and history.

#### Acknowledgments

The authors thank the sheep owners and the Office d'Elevage et des Pâturages (central office and regional directions) in Tunisia for providing blood samples. S Kdidi was supported by a scholarship from the Tunisian Ministry of Higher Education.

#### References

1. Arranz JJ, Bayon Y, Primitivo FS. Genetic relationships among Spanish sheep using microsatellites. *Anim Genet* 1998; 29: 435–440.
2. Pariset L, Savarese MC, Cappuccio I, Valentini A. Use of microsatellites for genetic variation and inbreeding analysis in Sarda sheep flocks of central Italy. *J Anim Breed Genet* 2003; 120: 425–432.
3. Alvarez I, Royo LJ, Fernandez I, Gutiérrez JP, Gómez E, Goyache F. Genetic relationships and admixture among sheep breeds from Northern Spain assessed using microsatellites. *J Anim Sci* 2004; 82: 2246–2252.
4. Peter C, Bruford M, Perez T, Dalamitra S, Hewitt G, Erhardt G. Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. *Anim Genet* 2007; 38: 37–44.
5. Ligda CH, Altarayah J, Georgoudis A. Genetic analysis of Greek sheep breeds using microsatellite markers for setting conservation priorities. *Small Ruminant Res* 2009; 83: 42–48.
6. Tapio M, Ozerov M, Tapio I, Toro MA, Marzanov N, Činkulov M, Goncharenko G, Kiselyova T, Murawski M, Kantanen J. Microsatellite-based genetic diversity and population structure of domestic sheep in northern Eurasia. *BMC Genet* 2010; 11: 76.
7. Calvo JH, Alvarez-Rodriguez J, Marcos-Carcavilla A, Serrano M, Sanz A. Genetic diversity in the Churra tensina and Churra lebrijana endangered Spanish sheep breeds and relationship with other Churra group breeds and Spanish mouflon. *Small Ruminant Res* 2011; 95: 34–39.

8. Tapio M, Marzanov N, Ozerov M, Cinkulov M, Gonzarenko G, Kiselyova T, Murawski M, Viinalass H, Kantanen J. Sheep mitochondrial DNA variation in European, Caucasian, and Central Asian areas. *Mol Biol Evol* 2006; 23: 1776–1783.
9. Jorde LB, Watkins WS, Bamshad MJ, Dixon ME, Ricker CE, Seielstad MT, Batzer MA. The distribution of human genetic diversity: a comparison of mitochondrial, autosomal, and Y-chromosome data. *Am J Hum Genet* 2000; 66: 979–988.
10. Hellborg L, Ellegren H. Low levels of nucleotide diversity in mammalian Y chromosomes. *Mol Biol Evol* 2004; 21: 158–163.
11. Pidancier N, Jordan S, Luikart G, Taberlet P. Evolutionary history of the genus *Capra* (Mammalia, Artiodactyla): discordance between mitochondrial DNA and Y-chromosome phylogenies. *Mol Phylogenet Evol* 2006; 40: 739–749.
12. Meadows JRS, Hanotte O, Drögemüller C, Calvo J, Godfrey R, Coltman D, Maddox JF, Marzanov N, Kantanen J, Kijas JW. Globally dispersed Y chromosomal haplotypes in wild and domestic sheep. *Anim Genet* 2006; 37: 444–453.
13. Meadows JRS, Kijas JW. Re-sequencing regions of the ovine Y chromosome in domestic and wild sheep reveals novel paternal haplotypes. *Anim Genet* 2009; 40: 119–123.
14. Oner Y, Calvo JH, Elmaci C. Y chromosomal characterization of Turkish native sheep breeds. *Livest Sci* 2011; 136: 277–280.
15. Mason IL. Sheep Breeds of the Mediterranean. Commonwealth Agricultural Bureau, Edinburgh. 1967.
16. Khaldi Z, Haddad B, Souid S, Rouissi H, Ben Gara A, Rekik B. Caractérisation phénotypique de la population ovine du Sud-Ouest de la Tunisie. *Anim Gene Resources* 2011; 49: 1–8 (in French with an English abstract).
17. Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY, USA: Cold Spring Harbor Press; 1989.
18. Meadows JRS, Hawken RJ, Kijas JW. Nucleotide diversity on the ovine Y chromosome. *Anim Genet* 2004; 35: 379–385.
19. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R et al. Clustal W and Clustal X version 2.0. *Bioinformatics* 2007; 23: 2947–2948.
20. Nei M. Genetic distance between populations. *Am Nat* 1972; 106: 283–292.
21. Langella O. Populations 1.2.32, Population Genetic Software. Gif-sur-Yvette, France: Laboratoire Evolution, Génomes et Spéciation; 1999.
22. Page RD. Tree View: An application to display phylogenetics trees on personal computers. *Comput Appl Biosci* 1996; 12: 357–358.
23. Ouna AB, Kijas JW, Meadows JRS, Limo M, Hanotte O, Mburu D. Analysis of genetic diversity and relationship between African male sheep using two Y chromosome specific markers. In: Proceedings of the 30th International Conference on Animal Genetics on Biodiversity, the future pass through preservation. Belo Horizonte, Brazil: Colégio Brasileiro de Reprodução Animal; 2006. pp. 20–25.
24. Ferencakovic M, Curik I, Royo LJ, Pérez-Pardal L, Cubric-Curik V, Fernández I, Álvarez I, Kostelic A, Sprem N, Krapinec K et al. Mitochondrial DNA and Y-chromosome diversity in East Adriatic sheep. *Anim Genet* 2013; 44: 184–192.
25. Zhang M, Peng WF, Yang GL, Lv FH, Liu MJ, Li WR, Liu YG, Li JQ, Wang F, Shen ZQ et al. Y chromosome haplotype diversity of domestic sheep (*Ovis aries*) in northern Eurasia. *Anim Genet* 2014; 45: 903–907.
26. Djemali M. Genetic improvement objectives of sheep and goats in Tunisia. Lessons learned. Analysis and definition of the objectives in genetic improvement programmes in sheep and goats. In: Options Méditerranéennes, Série A: Séminaires Méditerranéens. Paris, France: CIHEAM; 2000. pp. 121–127.
27. Chalh A, El Gazzah M, Djemali M, Chalbi N. Genetic and phenotypic characterization of the Tunisian Noire De Thibar lambs on their growth traits. *J Biol Sci* 2007; 7: 1347–1353.
28. Uerpmann HP. Animal domestication - Accident or intention? In: Harris David R, editor. *The Origins and Spread of Agriculture and Pastoralism in Eurasia*. London, UK: UCL Press; 1996. pp. 227–237.
29. Vigne JD, Buitenhuis H, Davis S. Les premiers pas de la domestication animale à l'Ouest de l'Euphrate: Chypre et l'Anatolie centrale. *Paléorient* 1999; 25: 49–62 (in French with English abstract).