

# Y-Chromosome Polymorphisms in 12 Native, Karagül, Karacabey Merino Breeds from Turkey and Anatolian Mouflon (*Ovis gmelinii anatolica*)<sup>[1]</sup>

Arif PARMAKSIZ<sup>1,a</sup> Ahmet OYMAK<sup>1</sup> Eren YÜNCÜ<sup>2</sup> Sevgin DEMİRCİ<sup>2</sup>  
Evren KOBAN BAŞTANLAR<sup>3,b</sup> Emel ÖZKAN ÜNAL<sup>4,c</sup> İnci TOGAN<sup>2</sup> Füsün ÖZER<sup>2,d</sup>

<sup>[1]</sup> The present study was supported by both Harran University [project number: 1176] and Middle East Technical University [project number: BAP-01-08-2012-014]

<sup>1</sup> The Department of Biology, Faculty of Science-Literature, Harran University, TR-63290 Şanlıurfa - TURKEY

<sup>2</sup> The Department of Biological Sciences, Middle East Technical University, TR-06800 Ankara - TURKEY

<sup>3</sup> The Department of Biology, Faculty of Science, Ege University, TR-35100 İzmir - TURKEY

<sup>4</sup> The Department of Animal Science, Faculty of Agriculture, Namık Kemal University, TR-59030 Tekirdağ - TURKEY

<sup>a</sup> ORCID: 0000-0003-0321-8198 <sup>b</sup> ORCID: 0000-0002-6296-9728 <sup>c</sup> ORCID: 0000-0001-7460-4040 <sup>d</sup> ORCID: 0000-0003-0443-5805

Article Code: KVFD-2018-19962 Received: 18.04.2018 Accepted: 25.08.2018 Published Online: 26.08.2018

## How to Cite This Article

**Parmaksız A, Oymak A, Yüncü E, Demirci S, Koban Baştanlar E, Özkan Ünal E, Togan İ, Özer F:** Y-chromosome polymorphisms in 12 native, Karagül, Karacabey Merino breeds from Turkey and Anatolian Mouflon (*Ovis gmelinii anatolica*). *Kafkas Univ Vet Fak Derg*, 24 (6): 821-828, 2018. DOI: 10.9775/kvfd.2018.19962

## Abstract

In this study, 182 male animals from 12 native sheep breeds, as well as Karacabey Merino and Karagül breeds of Anatolia, wild sheep Anatolian Mouflon (*Ovis gmelinii anatolica*) were used as the study material. Based on SRY and SRYM18 regions on the Y-chromosome, haplotypes of the populations were analyzed using DNA sequence analyses. The SRY region, A-oY1 allele was observed in all of the individuals studied. On the other hand, four different alleles corresponding to four Y-chromosome haplotypes were detected at the SRYM18 microsatellite region. Among native Anatolian breeds (n=143), H6 haplotype (80.41%), H4 haplotype (9.09%), H8 haplotype (8.40%) and H12 haplotype (2.1%) were identified. H6 haplotype was observed in all 16 individuals of *Ovis gmelinii anatolica*. Pairwise  $F_{ST}$  values based on haplotype frequencies were calculated for domestic sheep, and the highest  $F_{ST}$  value was observed between Karagül and Kıvrıkcık along with Karagül and *Ovis gmelinii anatolica* with pairwise  $F_{ST}$  value of 0.43202 ( $P<0.01$ ). Y chromosome polymorphism of sheep from Turkey were examined comparatively with the accumulated data in the literature. Out of seven haplotypes (H4, H5, H6, H7, H8, H12, H19) observed in Europe and Asia, 4 haplotypes (H4, H6, H8 and H12) were observed in Anatolia. H12 was a private haplotype of Sakız, H6 seems to be the predominant haplotype of domestic sheep (79.51%) as well as being the only haplotype observed in *Ovis gmelinii anatolica*. H4 haplotype seemed to be associated with fat tailed sheep migrating to Turkey, entering from south east of Turkey, which may be related with the arrival of nomadic Turks.

**Keywords:** Y-Chromosome haplotypes, SRY, SRYM18, *Ovis gmelinii anatolica*, Native sheep breeds of Turkey

## Türkiye'den 12 Yerli, Karagül, Karacabey Merinosu ve Anadolu Yaban Koyununda (*Ovis gmelinii anatolica*) Y-Kromozom Polimorfizmleri

## Öz

Bu çalışmada, Anadolu'dan yerli evcil koyun (*Ovis aries*) ırklarından 13 ırk ile kökeni Avrupa'dan olan Bandırma Merinosu koyun ırkı ve Anadolu Yaban Koyunu (*Ovis gmelinii anatolica*)'na ait olmak üzere toplam 182 erkek birey materyal olarak kullanılmıştır. Y kromozomunda bulunan SRY 5'promotor bölgesi ve Y kromozomuna özel bir mikrosatellit olan SRYM18 bölgeleri DNA dizi analizi yöntemleriyle incelenmiştir. SRY bölgesine ait analizlerde tüm bireylerde SNP A-oY1 alleline rastlanmıştır. SRYM18 mikrosatellit bölgesinde ise 4 farklı allel görülmüş ve toplamda 4 farklı babasal soy, haplotip, tespit edilmiştir. Yerli Anadolu ırklarında (n=143), H6 haplotipi (%80.41), H4 haplotipi (%9.09), H8 haplotipi (%8.40) ve H12 haplotipi (%2.1) belirlenmiştir. *Ovis gmelinii anatolica*'nın 16 bireyinde de sadece H6 haplotipi gözlenmiştir. Evcil koyunlar için haplotip frekanslarına dayalı  $F_{ST}$  değerleri hesaplanmış ve en yüksek  $F_{ST}$  değeri Karagül-Kıvrıkcık ve Karagül-*Ovis gmelinii anatolica* arasında  $F_{ST}$  değeri 0.43202 ( $P<0.01$ ) gözlenmiştir. Türkiye koyunlarının Y kromozomu polimorfizmi literatürdeki birikmiş verilerle karşılaştırmalı olarak incelenmiştir. Avrupa ve Asya'da gözlenen yedi haplotipten (H4, H5, H6, H7, H8, H12, H19) dördü Anadolu'da (H4, H6, H8 ve H12) gözlenmiştir. H12, Sakız'ın özel bir haplotipi, H6 ise evcil koyunların baskın haplotipi (%79.51) ve *Ovis gmelinii anatolica*'nın tek haplotipi olarak görülmektedir. H4 haplotipinin Türkiye'ye güneydoğudan girmiş olabileceği düşünülen yağlı kuyruklu koyunla ilişkili olduğu görülmüştür ve bu koyunların göçebe Türklerin gelişi ile ilişkili olabileceği düşünülmektedir.

**Anahtar sözcükler:** Y-Kromozom haplotipleri, SRY, SRYM18, *Ovis gmelinii anatolica*, Türkiye'nin yerli koyun ırkları



## İletişim (Correspondence)



+90 414 318 3562



aprmksz@gmail.com

## INTRODUCTION

Sheep, goat, cattle and pigs, which are farm animals of great importance for human beings, were domesticated in the area spanning between Central Anatolia and North of Zagros Mountains<sup>[1]</sup> and domesticated sheep spread to Asia, Africa and Europe in the following few millennia. During the spread, in accordance with the multiple founder effects, only subset of the variation that was present in the center of domestication must have reached to the peripheries of the domestic sheep distribution. Thus, at least part of the native breeds and wild sheep of Turkey distributed in or close to the center of domestication may have unique genetic variations of special value<sup>[2]</sup>.

While husbandry of farm animals can improve the desired characteristics, these attempts can also cause deterioration in some other characteristics, such as adaptation to environmental conditions and resistance against diseases. Native breeds from Anatolia deserve special conservation efforts due to being free from systematic selection and isolation and their endurance against challenging environmental conditions (e.g. surviving under extreme temperatures with low-quality feed, covering long distances for grazing, resisting parasites)<sup>[3]</sup>. However, since there are many candidates of native breeds and wild stocks to be conserved, for a sensible and sustainable conservation plan there must be a prioritization scheme<sup>[4]</sup>. Genetics-based information will reveal the evolutionary history of breeds and will also provide information on their genetic composition, thus will contribute for the prioritization in their conservation.

Sheep is one of the first domesticated farm animals<sup>[5]</sup>. Various genetic markers of Y chromosome<sup>[6-9]</sup>, mitochondrial DNA (mtDNA)<sup>[10-17]</sup> and autosomal markers<sup>[5,15,18,19]</sup> of domestic sheep are used to understand the evolutionary history of the domestic sheep genome.

Previous studies examined domestic sheep and wild sheep individuals from *Ovis canadensis*, *Ovis dalli* spp., *Ovis vignei*, *Ovis ammon*, *Ovis musimon*, *Ammantragus lervia*<sup>[6-8]</sup>. These studies revealed that sex-determining gene (SRY) from the male specific region of the sheep Y chromosome had 8 polymorphic sites (oY1-oY8). It was observed that SNP site oY1 is polymorphic within the domestic sheep as A/G with A being the predominant one<sup>[7]</sup>. Other 7 SNP sites were polymorphic between the wild sheep species but not within the species<sup>[8]</sup>. When another region, SRYM18 microsatellite region, was sequenced from the male specific region of the sheep Y chromosome three polymorphic sites were detected: a pentanucleotide (TTTTG), an indel and a dinucleotide (TG) sites (8). Based on two regions 18 Y chromosome haplotypes (H1-H18) were defined in sheep<sup>[8]</sup>.

Regarding the wild sheep, mtDNA based evidence indicated that *Ovis orientalis* (*Ovis gmelini* by the new nomenclature)<sup>[20]</sup> was suggested as the ancestral source of domestic sheep.

While *Ovis gmelini musimon* was accepted as the early feral forms of domesticated sheep, wild *Ovis gmelini* populations were found in Anatolia, Iran, Azerbaijan and Armenia<sup>[21]</sup>.

*Ovis gmelini musimon* samples: 10 individuals from Spain<sup>[7]</sup> and 19 individuals from Ukraine<sup>[8]</sup> were studied previously based on the Y chromosome markers. However, none of the wild *Ovis gmelini anatolica* samples were examined based on these markers.

An Anatolian wild sheep, *Ovis gmelinii anatolica*, populations is located in the 42.000 km<sup>2</sup> Bozdağ protection area in Konya province of Central Anatolia. *Ovis gmelinii anatolica* went through a recent bottleneck with only 15-20 individuals left at 1970s<sup>[22]</sup>. Currently, their population size is approximately 500 individuals<sup>[16]</sup>.

The present research aims to determine the genetic diversity of native sheep breeds and the wild sheep (*Ovis gmelinii anatolica*) of Turkey in terms of Y chromosome based genetic markers. It is believed that results of the present study will contribute to the understanding of evolutionary history of domestic sheep and sheep domestication in Turkey.

## MATERIAL and METHODS

### Ethics Statement

Blood samples from modern sheep were collected with the approval of Istanbul University Veterinary Faculty Ethics Committee (permit number: 2006/172). *Ovis gmelinii anatolica* blood samples were collected with the approval of both Selçuk University Veterinary Faculty Ethics Committee (permit number: 2009/041) and the General Directorate of Nature Conservation and National Parks, Turkish Republic Ministry of Forestry and Hydraulic Works. The samples were studied with the approval of the Middle East Technical University Ethics Committee (permit number: 2011/02). Thus, all necessary permits were obtained for the described study, which fulfilled all of the regulations.

### Samples and Sampling

In our study, selection of native sheep breeds (for each breed more than 3 flocks were sampled), their blood sampling and DNA isolation were carried out within the scope of the national project TÜRKHAYGEN-I (<http://www.turkhaygen.gov.tr>). Karacabey Merino, samples were collected by Sheep Breeding Research Institute of Bandırma district. Blood samples of Anatolian Mouflon (*Ovis gmelinii anatolica*) individuals were collected by Ministry of Forestry and Water Affairs of the Republic of Turkey, Directorate General for Nature Protection and National Parks.

The numbers of individuals examined in each breed and their tail types were as follows: Thin-tailed sheep breeds; Karayaka (n=15), Gökçeada (n=16), Kıvrıkcık (n=16), Karacabey

Merino (n=12); Semi-fat-tailed sheep breeds; Sakız (n=15) which is similar to Chios breed of Greece, Hemşin (n=14), Herik (n=11); Fat-tailed sheep breeds; Akkaraman (n=11), Dağlıç (n=10), Çineçaparı (n=12), İvesi (n=7) which is similar to Awasi breed of Middle East, Morkaraman (n=9), Norduz (n=7), Karagül (n=11). Herik and Hemşin were hybrids of the breeds from Turkey. Karagül was brought from Russia in 1926, Karacabey Merino was a hybrid between German meat Merino (rams) and Kivircik (ewes) and it has been in Anatolia since 1935. Although, all of the breeds were in Turkey for many generations the last two breeds were not considered as native breeds of Turkey. Additionally, 16 individuals of Anatolian Mouflon (*Ovis gmelinii anatolica*) were examined. Sites of the breeds and *Ovis gmelinii anatolica* were indicated on the map presented in Fig. 1. Blood samples (10 cc) were drawn by specialized veterinarians from the jugular vein into K3 EDTA tubes. DNA isolations were performed by using phenol-chloroform isoamyl alcohol (25:24:1) extraction method<sup>[23]</sup>.

### Y-Chromosome Markers

In the present study, 5' promoter region of SRY (Gene Bank No: AY604734) and SRYM18 (EU980105.1) loci of Y chromosome were amplified by PCR. Primer sequences for amplification of the SRY region were as follows: Forward; 5'-TCA GTA GCT TAG GTA CAT TCA-3' and Reverse: 5'-GTG CTA CAT AAA TAT GAT CTG C-3'<sup>[6]</sup>. Primer sequences for the amplification of SRYM18 microsatellite locus were; Forward; 5'-GGC ATC ACA AAC AGG ATC AGC AAT-3' and Reverse: 5'-GTG ATG GCA GTT CTC ACA ATC TCC T-3'<sup>[7]</sup>.

All PCR amplifications were performed in a volume of 25 µL containing 10 pmol/µL of each primer, 0.2 mM of each dNTP, 1X Taq buffer, 2.5 mM MgCl<sub>2</sub>, 1 unit Taq polymerase and approximately 90 ng of template DNA. The PCR thermo-cycling conditions were as follow: initial denaturation at 94°C for 3 min, and 35 cycles of denaturation at 95°C for 25 s, annealing at 56°C for 35 s, extension at 72°C for 50 s, and a final extension at 72°C for 10 min. PCR products were purified using FavorPrep Gel/PCR purification kit (Favorgen) prior to Sanger sequencing, which was performed by REFGEN Gene Research and Biotechnology Limited Inc. (Ankara, Turkey).

Raw sequences were assembled by using Chromas Pro version 1.5 (<http://www.technelysium.com.au/ChromasPro.html>) and consensus sequences were aligned by ClustalW algorithm<sup>[24]</sup>, implemented in Bioedit version 7.1.3<sup>[25]</sup>.

Haplotypes were determined in accordance with the Table 1 of Meadows and Kijas's<sup>[8]</sup> study.

### Statistical Data Analyses

In order to display relationships between haplotypes based on mutations, a median joining network (MJ)<sup>[26]</sup> was constructed with NETWORK 4.6.1.0 ([\[engineering.com\]\(http://www.fluxus-engineering.com\)\), by using the default settings. Genetic distances between sub-populations were obtained by calculating pairwise  \$F\_{ST}\$  values based on haplotype frequencies using Arlequin 3.11<sup>\[27\]</sup>. Significance of the results was tested with 1000 permutations.](http://www.fluxus-</a></p>
</div>
<div data-bbox=)

## RESULTS

In the present study, SRY and SRYM18 sequences were analysed for a total of 182 sheep including 166 individuals from native sheep breeds (including a breed imported from Russia and a hybrid of a merino breed) and 16 individuals from *Ovis gmelinii anatolica*. The analysis of SRY showed that all individuals had allele A in terms of SNP oY1 (A/G). The whole SRY region was reanalyzed in all of the individuals but no polymorphism was found. In SRYM18 microsatellite locus no polymorphism was observed between individuals in terms of pentanucleotide (TTTTG) repeat number. Furthermore, the presence of G nucleotide between TTTTG and dinucleotide (TG) repeats was the same in all individuals. Considering TG repeat numbers, 13 TG, 14 TG, 15 TG and 16 TG repeats were found. The classification of haplotypes was performed in accordance with Meadows et al.<sup>[7]</sup> study using the oY1 SNP in the SRY gene and the SRYM18 microsatellite locus.

In this study, H4, H6, H8 and H12 haplotypes were found as shown in Table 1. All individuals of *Ovis gmelinii anatolica* and Karacabey Merino had H6 haplotype.

Among the 14 domestic breeds (n=166), H6 was the most common haplotype observed in 132 individuals (79.51%) from Turkey. H12 haplotype was found in only 3 individuals (1.81%) of Sakız breed. H4 and H8 haplotypes were found in 17 (10.24%) and 14 individuals (8.44%), respectively. Haplotypes H4 and H8 were observed in various breeds with different tail types. Similarly, among 12 Anatolian native domestic sheep breeds (n=143), the most frequent haplotype was H6 haplotype (80.41%) followed by H4 haplotype (9.09%), H8 haplotype (8.40%) and H12 haplotype (2.1%).

Y-chromosome haplotypes found in native breeds, Karacabey Merino, Karagül breeds and *Ovis gmelinii anatolica* from Turkey are shown in Fig. 1. It is observed that embedded in H6 haplotype distribution the frequency of H4 haplotype seemed to decrease from east to west, whereas, H8 haplotype is mainly in the central parts of Anatolia.

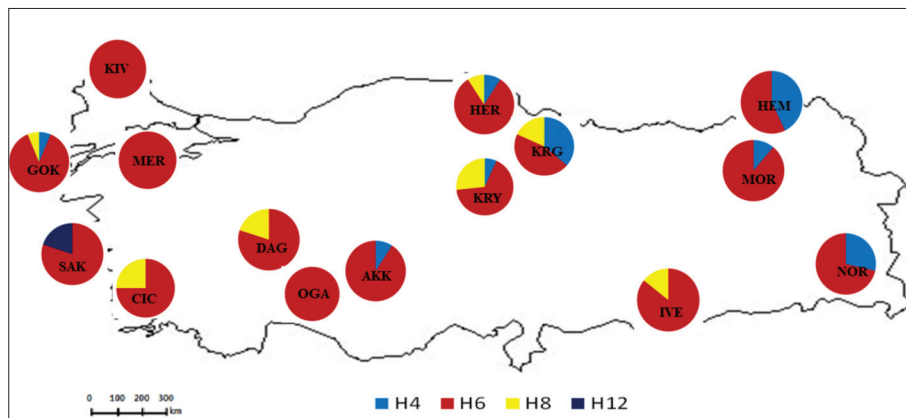
### Statistical Analyses

**Median-joining network analysis based on Y chromosome polymorphisms:** The molecular proximity (mutational differences between haplotypes) of the haplotypes we observed was visualized in Fig. 2. In this figure, the relationship between haplotypes as well as tail types (fat-tailed, semi fat-tailed and thin-tailed) of the sheep breeds are displayed.

**Table 1.** Y-chromosome haplotypes observed in this study in each of the sheep breeds

Sheep Breeds		Chromosome				
		H4	H6	H8	H12	Total
Thin-tailed sheep breeds	Karayaka	1	10	4	-	15
	Gökçeada	1	14	1	-	16
	Kıvrıcık	-	16	-	-	16
	Karacabey Merino	-	12	-	-	12
Semi-fat-tailed sheep breeds	Sakız	-	12	-	3	15
	Hemşin	6	8	-	-	14
	Herik	1	9	1	-	11
Fat-tailed sheep breeds	Akkaraman	1	10	-	-	11
	Dağlıç	-	8	2	-	10
	Çineçaparı	-	9	3	-	12
	İvesi	-	6	1	-	7
	Morkaraman	1	8	-	-	9
	Norduz	2	5	-	-	7
	Karagül	4	5	2	-	11
Total		17	132	14	3	166

Sheep breeds were classified based on their tail types

**Fig 1.** Distribution of Y chromosome haplotypes of domestic sheep breeds and Anatolian Mouflon in Turkey. Abbreviations: Karayaka (KRY), Akkaraman (AKK), Gökçeada (GOK), Dağlıç (DAG), Morkaraman (MOR), Kıvrıcık (KIV), İvesi (IVE), Herik (HER), Karagül (KRG), Hemşin (HEM), Çineçaparı (CIC), Sakız (SAK), Norduz (NOR), Karacabey Merino (MER), *Ovis gmelinii anatolica* (OGA)

In Fig. 2, it can be seen that H4, H6 and H8 haplotypes were found in sheep breeds with all types of tails, while H12 was found only in a semi fat-tailed sheep breed (Sakız). Thin tail breeds seemed to display H4 relatively less than breeds with other tail types. Furthermore, based on Fig. 2 it is possible to suggest that H4 and H8 haplotypes were genetically related to H6 haplotype and evolved from H6 independent of each other. Similarly, the H12 haplotype could have been evolved from H8.

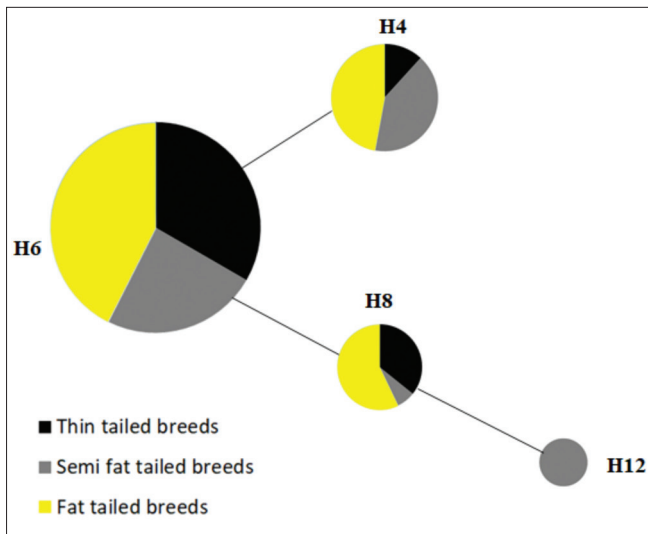
**Pairwise  $F_{ST}$  values between the breeds:** Pairwise  $F_{ST}$  values between the breeds based on haplotype frequencies are presented in Table 2. According to the results, Hemşin is significantly different from Karayaka, Gökçeada, Kıvrıcık, Sakız, Çineçaparı, OGA and Merinos. Karagül is also significantly different from Gökçeada, Kıvrıcık, Sakız, OGA and Merinos. Most distant breeds on the basis of their Y chromosome haplotype frequencies are Karagül

and Kıvrıcık as well as Karagül and OGA with pairwise  $F_{ST}=0.43202$  ( $P<0.01$ ). The most similar breeds, however, seemed to be the Akkaraman and Morkaraman with pairwise  $F_{ST}$  value of - 0.10984 (non-significant).

**Turkey, Asia and Europe in the range of the Y chromosome haplotype distribution:** Y chromosome haplo-type distributions of domestic sheep from different countries in Europe and Asia along with different breeds within Turkey (the present study) are shown in Fig. 3.

## DISCUSSION

In this study, 182 male individuals from 12 native sheep breeds as well as Karacabey Merino, and Karagül breeds from Anatolia and male sheep from Anatolian Mouflon (*Ovis gmelinii anatolica*) were subjected to sequence analysis of SRY and SRYM18 loci of Y chromosome. Previously in Öner



**Fig 2.** Relationship between haplotypes based on median joining network, distribution of haplotypes and tail types of native sheep breeds of Turkey by using pie charts

*gmelinii anatolica* individuals examined in this study. Early feral forms of domestic sheep from Ukraine (n=19)<sup>[8]</sup> and Spain (n=10)<sup>[7]</sup> also displayed only A-oY1 allele. In fact, all other wild sheep samples exhibited A-oY1 allele<sup>[8]</sup>. Thus, the results proposes that A-oY1 allele is the ancestral form of whereas G-oY1 allele that is observed in domestic sheep with H5 and H7 haplotypes may be, the derived form. However, this hypothesis must also be confirmed by examining wild *Ovis gmelinii* populations from Armenia, Iran and Cyprus<sup>[21]</sup> which have not been studied yet.

SRYM18 microsatellite region sequences revealed no polymorphism among *Ovis gmelinii anatolica* individuals When analyzed along with the SRY locus, they displayed H6 haplotype which was also observed uniformly in *Ovis musimon*<sup>[7,8]</sup>. Absence of polymorphism in *Ovis gmelinii* might be an ancestral state. If this is the case, then haplotypes observed in domestic sheep breeds emerged after the sheep domestication. Alternatively, wild populations in the Middle East<sup>[21]</sup> might be exhibiting polymorphism(s)

**Table 2.** Pairwise  $F_{ST}$  values and their significances between the breeds on the basis of their Y-chromosome haplotype frequencies

	KRY	GOK	KIV	SAK	HEM	HER	AKK	DAG	CIC	IVE	MOR	NOR	KRG	OGA	ME
KRY	0.00000														
GOK	0.04709	0.00000													
KIV	0.23742*	0.03333	0.00000												
SAK	0.08163	0.02666	0.15044	0.00000											
HEM	0.11796*	0.19376*	0.40584**	0.20031*	0.00000										
HER	-0.01779	-0.07314	0.09315	0.00386	0.10961	0.00000									
AKK	0.08661	-0.06748	0.03562	0.02941	0.18107	-0.06333	0.00000								
DAG	-0.05691	-0.03226	0.17949	0.03069	0.17768	-0.07089	0.01811	0.00000							
CIC	-0.06671	0.01437	0.22372	0.06203	0.17792*	-0.03806	0.06764	-0.09310	0.00000						
IVE	-0.04371	-0.08893	0.12840	-0.00707	0.17323	-0.10811	-0.04804	-0.12544	-0.08896	0.00000					
MOR	0.05764	-0.07881	0.06796	0.01172	0.13332	-0.08400	-0.10984	-0.00269	0.04327	-0.06305	0.00000				
NOR	0.01566	0.02553	0.33214	0.05987	-0.07207	-0.04671	0.01282	0.03686	0.05204	0.01754	-0.03616	0.00000			
KRG	0.03903	0.20482*	0.43202**	0.20249*	-0.04017	0.10429	0.21194	0.12378	0.10650	0.13612	0.16301	-0.03047	0.00000		
OGA	0.23742*	0.03333	0.00000	0.15044	0.40584**	0.09315	0.03562	0.17949	0.22372	0.12840	0.06796	0.33214	0.43202**	0.00000	
MER	0.19941	0.01101	0.00000	0.11765	0.36122*	0.05979	0.00826	0.13669	0.18182	0.08197	0.03356	0.27147	0.38128**	0.00000	0.0000

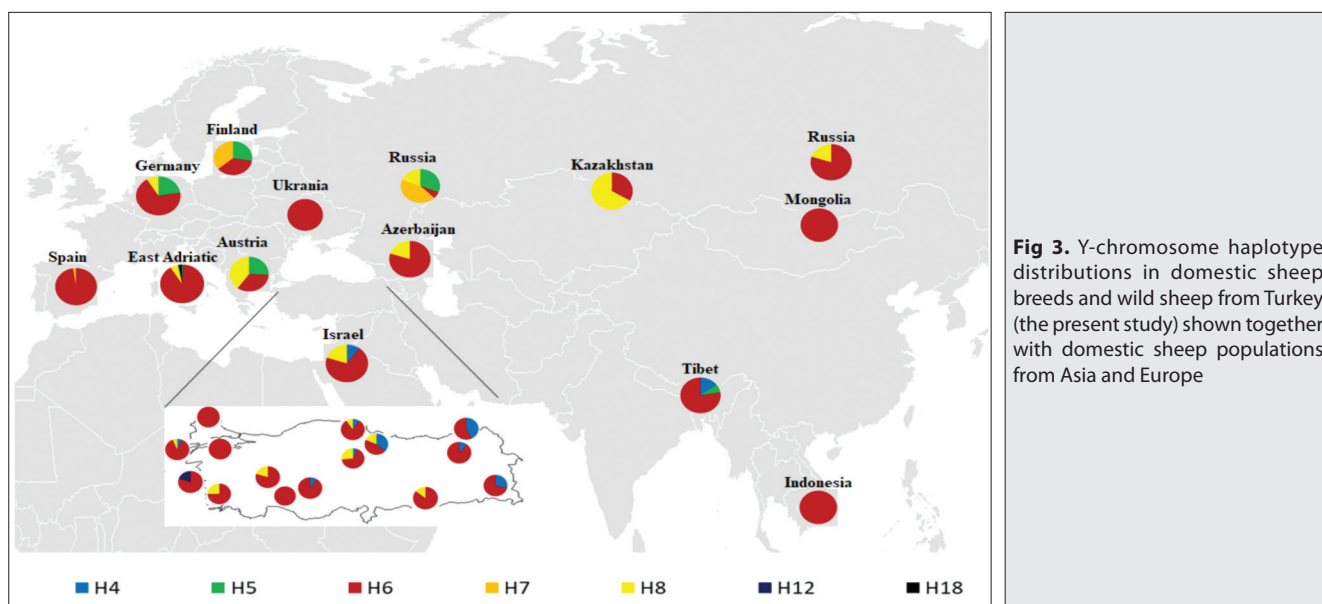
\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$

Karayaka (KRY), Akkaraman (AKK), Gökçeada (GOK), Dağlıç (DAG), Morkaraman (MOR), Kıvrıkcık (KIV), İvesi (IVE), Herik (HER), Karagül (KRG), Hemşin (HEM), Çineçaparı (CIC), Sakız (SAK), Norduz (NOR), Karacabey Merino (MER), *Ovis gmelinii anatolica* (OGA)

et al.'s<sup>[9]</sup> study 10 native breeds from Turkey were examined and only A-oY1 allele in the SRY gene region was observed. In conformity to the Öner et al.'s<sup>[9]</sup> result, only A-oY1 allele was observed among the domestic breeds we studied. However, previously, in a single breed (Karakas) from Turkey, G-oY1 allele was observed in two individuals<sup>[8]</sup>. Karakas was not examined in the present study and in Öner et al.'s<sup>[9]</sup> study and it was not reported as one of the native sheep breeds of Turkey<sup>[28]</sup>.

*Ovis gmelinii anatolica*, was not previously studied in terms of SRY locus. A-oY1 allele was found in all (n=16) *Ovis*

while, *Ovis gmelinii anatolica* might have lost its variability in the previous century due to a severe bottleneck<sup>[22]</sup>. However, the frequency of most common Y chromosome haplotype(s) found in different countries were as follows: 72.8% H6<sup>[29]</sup> in African breeds; 60.7% H6<sup>[7,8]</sup> in sheep breeds from all over the world; 65.0%<sup>[30]</sup> in Northern Eurasian breeds; 55.5% H8 and 34.5% H6<sup>[31]</sup> in Tunisian breeds; 43.4% H6<sup>[32]</sup> in Chinese breeds. Thus, it seems that H6 haplotype is the most frequent haplotype (except in Tunisia) in most of the places of the old continents. These results also support the proposal that H6 might be the



**Fig 3.** Y-chromosome haplotype distributions in domestic sheep breeds and wild sheep from Turkey (the present study) shown together with domestic sheep populations from Asia and Europe

ancestral haplotype. Similarly, among the haplotypes found in Anatolian native domestic sheep breeds, the most frequent was H6 haplotype (80.41%). Then followed by haplotypes H4 (9.09%), H8 (8.40%) and H12 (2.1%) among 143 individuals. All individuals of *Ovis gmelinii anatolica* had H6 haplotype. Central location of H6 in the present study and in the previous study<sup>[8]</sup> also supports the proposition.

Haplotypes H6 and H5 were observed in German Merino but only H6 in Merino Long wool from Germany<sup>[7]</sup>. H6 was the only haplotype observed in merino breeds from Australia and Mexico<sup>[8]</sup>. Despite the fact that rams of German meat merino were used for the Karacabey merino, only H6 haplotype was found in our study in 12 individuals of Karacabey Merino breed in Turkey.

To our knowledge, Herik and Karagül breeds were analyzed for the first time in our study and H4, H6 and H8 haplotypes were found in both breeds. In our study, only 1 individual from each of thin-tailed Karayaka and Gökçeada breeds exhibited H4 haplotype. However, this haplotype was not observed in other studies that focused on thin-tailed sheep breeds in Turkey<sup>[7,9]</sup>. Presence of H4 in thin-tailed breeds can be taken as the evidence of the weak isolation between the breeds. Differences between the haplotype frequencies of the same breeds in different studies for instance presence of private haplotype H12 in Sakız only in the present study can be attributed to the sampling effect. Since more than 3 flocks were visited during the sampling of native breeds in the present study, results could be used in further comparisons.

Studies on Y chromosome haplotype distributions in domestic sheep breeds and wild sheep from Turkey (the present study) in Europe and Asia show in the Fig. 3<sup>[7,8,33]</sup>. According to Fig. 3, it can be seen that sheep

samples examined were scattered and scarce over the vast geography. Therefore, conclusions based on the haplotype distributions should be interpreted cautiously. Nevertheless, it is seen again that H6 haplotype is the most prominent haplotype, in every breed or country except in the western Russia, Finland, Kazakhstan and Austria. However, H5 and H7 (both having G-oY1 allele) seemed to be common in Northern Europe (Russia and Finland) and in Austria. H7 seemed to be associated with H5 in Russia and Finland. Just 2 samples out of 19 sheep from Tibet also displayed H5 haplotype (H5 may be reached to Tibet by sheep trading). Therefore, as a first approximation it can be suggested that G-oY1 allele and thus H5 and H7 emerged later in the spread of domestic sheep and may be confined in the northern and central Europe. H8 existing from east to west in the northern Asia and Europe is also seen in Azerbaijan, Austria, East Adriatic and in Israel as well as in the breeds of Anatolia. However, H8 is neither present in the most eastern and most western breeds (except in GOK with a low frequency, 6.25%) of Turkey. The emergence and direction of spread of H8 cannot be determined by the distribution of the haplotype. Haplotype H4 is seen in Tibet and Israel and in Eastern breeds of Turkey as well as in Akkaraman, a central Anatolian breed. Furthermore, these breeds do not have haplotype H8 (Fig. 3). This observation may suggest that, there was a massive wave of male sheep migration partly harboring H4 arrived to Turkey from the south of Caspian Sea (since sheep of Azerbaijan is not exhibiting H4). This migration might be relatively recent because although they were surrounded by breeds having H8 haplotype they did not exhibit H8. These sheep might be represented by Morkaraman, Hemsin, Norduz and Akkaraman breeds of Turkey. The Norduz is believed to be a variety of Akkaraman breed<sup>[34]</sup>. Indeed, these two breeds have non-significant pairwise  $F_{ST}$  value ( $F_{ST}=0.01282$ ). Also Morkaraman and Akkaraman have non-significant pairwise  $F_{ST}$  value ( $F_{ST}=0.10984$ ). These observations support

the implication that Akkaraman, Morkaraman, Norduz and Hemşin might be sharing a common evolutionary history. These 4 breeds (fat tailed or semi fat tailed) seemed to have a different evolutionary history than those of İvesi, Dağlıç, Çineçaparı and Karagül (fat tailed or semi fat tailed), Gökçeada (thin tailed) breeds all harboring H6 and H8 but rarely H4 (1 individuals from each of Karagül and Gökçeada). It is well known that nomadic Turks arrived to Anatolia in the 11<sup>th</sup> century, originally spreading from East of Aral Sea<sup>[35]</sup> travelling through Iran. Together with their sheep they settled mostly in Eastern and Central Anatolia<sup>[36]</sup> and migration of nomadic Turks continued for two hundred centuries<sup>[36,37]</sup>. Perhaps their sheep were the ones with H4 and without Y Chromosome H8 haplotype. In this case, for conservation studies of native breeds of Turkey, 'sheep of nomadic Turks' must also be considered as a group from which breed(s) must be chosen for conservation.

In the present study, we focused on domestic breeds and wild sheep of Turkey and studied their Y chromosome haplotypes in order to contribute to the paternal evolutionary history of sheep by combining the available data from the literature. Further genetic studies utilizing higher resolution genetic markers on ancient as well as modern samples of *Ovis gmelini* and domestic sheep from different regions and time periods of Anatolia, the Middle East and other regions of the old continents will expand the understanding of both the early stages of the domestication process and the evolutionary history of domestic sheep.

## ACKNOWLEDGMENT

The present study was supported by both Harran University Scientific Research Committee with the project number 1176 and Middle East Technical University project number BAP-01-08-2012-014.

## REFERENCES

- Zeder MA:** Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. *Proc Natl Acad Sci U S A*, 105, 11597-11604, 2008. DOI: 10.1073/pnas.0801317105
- Bruford MW, Bradley DG, Luikart G:** DNA markers reveal the complexity of livestock domestication. *Nat Rev Genet*, 4, 900-910, 2003. DOI: 10.1038/nrg1203
- Soysal MI, Tuna YT, Ozkan E, Gurcan EK, Togan I, Altunok V:** A study on the wool characteristics of several Turkish sheep breeds according to the microsatellite DNA types. *Pak J Biol Sci*, 8, 186-189, 2005. DOI: 10.3923/pjbs.2005.186.189
- Boettcher P:** Priority Setting for Conservation of Animal Genetic Resources. GLOBALDIV Final International Workshop. February 8 and 9, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, pp.16-18. 2010.
- Kijas JW, Lenstra JA, Hayes B, Boitard S, Porto Neto LR, San Cristobal M, Servin B, McCulloch R, Whan V, Gietzen K, Paiva S, Barendse W, Ciani E, Raadsma H, McEwan J, Dalrymple B, members of the International Sheep Genomics Consortium:** Genome-wide analysis of the world's sheep breeds reveals high levels of historic mixture and strong recent selection. *PLoS Biol*, 10 (2): e1001258, 2012. DOI: 10.1371/journal.pbio.1001258
- Meadows JRS, Hawken RJ, Kijas JW:** Nucleotide diversity on the ovine Y chromosome. *Anim Genet*, 35, 379-385, 2004. DOI: 10.1111/j.1365-2052.2004.01180.x
- Meadows JRS, Hanotte O, Drogemuller 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*, 37, 444-453, 2006. DOI: 10.1111/j.1365-2052.2006.01496.x
- Meadows JRS, Kijas JW:** Re-sequencing regions of the ovine Y chromosome in domestic and wild sheep reveals novel paternal haplotypes. *Anim Genet*, 40, 119-23, 2009. DOI: 10.1111/j.1365-2052.2008.01799.x
- Oner Y, Calvo JH, Elmacı C:** Y chromosomal characterization of Turkish native sheep breeds. *Livest Sci*, 136, 277-280, 2011. DOI: 10.1016/j.livsci.2010.08.015
- Wood NJ, Phua SH:** Variation in the control region sequence of the sheep mitochondrial genome. *Anim Genet*, 27, 25-33, 1996. DOI: 10.1111/j.1365-2052.1996.tb01173.x
- Hiendleder S, Mainz K, Plante Y, Lewalski H:** Analysis of mitochondrial DNA indicates that domestic sheep are derived from two different ancestral maternal sources: no evidence for contributions from urial and argali sheep. *J Hered*, 89, 113-120, 1998.
- Pedrosa S, Uzun M, Arranz JJ, Gutierrez-Gil B, San Primitivo F, Bayon Y:** Evidence of three maternal lineages in Near Eastern sheep supporting multiple domestication events. *Proc Biol Sci*, 272, 2211-2217, 2005. DOI: 10.1098/rspb.2005.3204
- Bruford MW, Townsend SJ:** Mitochondrial DNA diversity in modern sheep: Implications for domestication. In, Zeder MA, Bradley DG, Emshwiller E, Smith BD (Eds): Documenting Domestication: New Genetic and Archaeological Paradigms. 307-317, CA, USA: University of California Press, 2006.
- Meadows JRS, Cemal I, Karaca O, Gootwine E, Kijas JW:** Five ovine mitochondrial lineages identified from sheep breeds of the Near East. *Genetics*, 175, 1371-1379, 2007. DOI: 10.1534/genetics.106.068353
- Tapio M, Ozerov M, Tapio I, Toro MA, Marzanov N, Cinkulov M, Goncharenko G, Kiselyova T, Murawski M, Kantanen J:** Microsatellite-based genetic diversity and population structure of domestic sheep in northern Eurasia. *BMC Genet*, 11 (76): 1-11, 2010. DOI: 10.1186/1471-2156-11-76
- Demirci S, Koban Baştanlar E, Dağtas ND, Pişkin E, Engin A, Özer F, Yüncü E, Doğan ŞA, Togan İ:** Mitochondrial DNA diversity of modern, ancient and wild sheep (*Ovis gmelinii anatolica*) from Turkey: New insights on the evolutionary history of sheep. *PLoS ONE*, 8, e81952, 2013. DOI: 10.1371/journal.pone.0081952
- Lv FH, Peng WF, Yang J, Zhao YX, Li WR, Liu MJ, Ma YH, Zhao QJ, Yang GL, Wang F, Li JQ, Liu YG, Shen ZQ, Zhao SG, Hehua E, Gorkhali NA, Vahidi SMF, Muladno M, Naqvi AN, Tabell J, Isotouru T, Bruford MW, Kantanen J, Han JL, Li MH:** Mitogenomic metaanalysis identifies two phases of migration in the history of eastern Eurasian sheep. *Mol Biol Evol*, 32 (10): 2515-2533, 2015. DOI: 10.1093/molbev/msv139
- Peter C, Bruford M, Perez T, Dalamitra S, Hewitt G, Erhardt G, the Econogene Consortium:** Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. *Anim Genet*, 38, 37-44, 2007. DOI: 10.1111/j.1365-2052.2007.01561.x
- Chessa B, Pereira F, Arnaud F, Amorim A, Goyache F, Mainland I, Kao RR, Pemberton JM, Beraldi D, Stear MJ, Alberti A, Pittau M, Ianuzzi L, Banabazi MH, Kazwala RR, Zhang YP, Arranz JJ, Ali BA, Wang Z, Uzun M, Dione MM, Olsaker I, Holm LE, Saarma U, Ahmad S, Marzanov N, Eythorsdottin E, Holland M J, Ajmone-Marsan P, Bruford MW, Kantanen J, Spencer TE, Palmarini M:** Revealing the history of sheep domestication using Retrovirus integrations. *Science*, 324, 532-536, 2009. DOI: 10.1126/science.1170587
- IUCN/SSC Caprine Specialist Group:** *Workshop on Caprinae Taxonomy*, Ankara, Turkey: pp.1-8, 1999.
- Ziaie H:** Iran, Middle East. In, Shackleton DM (Ed): Wild Sheep and Goats and Their Relatives: Status Survey and Conservation Action Plan for Caprinae. Switzerland: *World Conservation Union*, pp.49-55, 1997.
- Kaya MA, Bunch TD, Konuk M:** On Konya wild sheep, *Ovis orientalis anatolica*, in the Bozdag protected area. *Mammalia*, 68, 229-232, 2004.

DOI: 10.1515/mamm.2004.022

**23. Sambrook J, Fritsch E, Maniatis T:** Molecular Cloning: A Laboratory Manual. New York, Cold Spring Harbor Laboratory, 1989.

**24. Thompson JD, Higgins DG, Gibson TJ:** CLUSTAL-W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*, 22, 4673-4680, 1994.

**25. Hall TA:** BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98, 1999.

**26. Bandelt J, Forster P, Rohl A:** Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol*, 16, 37-48, 1999. DOI: 10.1093/oxfordjournals.molbev.a026036

**27. Excoffier L, Laval G, Schneider S:** Arlequin (ver. 3.0): An integrated software package for population genetics data analysis. *Evol Bioinform Online*, 1, 47-50, 2005.

**28. TAGEM:** Domestic Animal Genetic Resources in Turkey. Republic of Turkey Ministry of Food Agriculture and Livestock General Directorate of Agricultural Research and Policy, Ankara, 2011.

**29. 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 30<sup>th</sup> International Conference on Animal Genetics on Biodiversity, the future pass through preservation*. Belo Horizonte, Brazil: *Colegio Brasileiro de Reproducao Animal*, pp.20-25, 2006.

**30. Zhang M, Peng WF, Yang GL, Lv FH, Liu MJ, Li WR, Liu YG, Li JQ,**

**Wang F, Shen ZQ, Zhao SG, Hehua E, Marzanov N, Murawski M, Kantanen J, Li MH:** Y-chromosome haplotype diversity of domestic sheep (*Ovis aries*) in northern Eurasia. *Anim Genet*, 45, 903-907, 2014. DOI: 10.1111/age.12214

**31. Kdidi S, Yahyaoui MH, García-Manrique B, Sarto P, BEN SASSI M, Khorchani T, Calvo JH:** Y chromosome haplotype characterization of Tunisian sheep breeds. *Turk J Vet Anim Sci*, 39, 333-337, 2015. DOI: 10.3906/vet-1405-95

**32. Wang Y, Xu L, Yan W, Li S, Wang J, Liu X, Hu J, Luo Y:** Y chromosomal haplotype characteristics of domestic sheep (*Ovis aries*) in China. *Gene*, 565, 242-245, 2015. DOI: 10.1016/j.gene.2015.04.015

**33. Ferencakovic M, Curik I, Pérez-Pardal L, Royo LJ, Cubric-Curik V, Fernández I, Álvarez I, Kostelic A, Sprem N, Krapinec K, Goyache F:** Mitochondrial DNA and Y-chromosome diversity in East Adriatic sheep. *Anim Genet*, 44, 184-192, 2013. DOI: 10.1111/j.1365-2052.2012.02393.x

**34. Ocak E, Bingöl M, Gökdağ Ö:** Van yöresinde yetiştirilen Norduz koyunlarının süt bileşimi ve süt verim özellikleri. *YYÜ Tar Bil Derg*, 19, 85-89, 2009.

**35. Berkman CC, Dinç H, Sekeryapan C, Togan I:** *Alu* insertion polymorphisms and an assessment of the genetic contribution of Central Asia to Anatolia with respect to the Balkans. *Am J Phys Anthropol*, 136, 11-18, 2008. DOI: 10.1002/ajpa.20772

**36. Toynbee AJ:** The Western Question in Greece and Turkey. A Study in the Contact of Civilizations, 3<sup>rd</sup> ed., New York: Howard Fertig, Inc; 1970.

**37. Roux JP:** Türklerin Tarihi: Büyük Okyanus'tan Akdeniz'e İki Bin Yıl. 5. Baskı. AD Yayıncılık, İstanbul, 1997.