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# Genetic Diversity and Bottleneck Analysis of Endangered Güney Karaman Sheep

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**ABSTRACT**

The present study was conducted to investigate the genetic variability and genetic bottleneck of native Güney Karaman sheep breed, which is conserved as a genetic resource. Animal material for the study was consisted of 119 Güney Karaman sheep, raised in Bahri Dagdas International Agricultural Research Institute, were genotyped with sixteen microsatellites marker recommended by FAO. A total of 277 alleles were detected from studied sixteen microsatellite markers. Although total population size is very limited, the mean number of alleles (17.31), observed heterozygosity (0.81) and polymorphic information content (0.82) findings indicated that noticeable genetic variability found in the Güney Karaman sheep population. Ten out of the sixteen microsatellite markers studied had a positive $F_{IS}$ value. The mean value of $F_{IS}$ was 0.042. The infinite allele model (IAM), two-phase mutation model (TPM) and stepwise mutation model (SMM) in the Bottleneck software were used to check genetic bottleneck. The L-shaped curve obtained from analyze indicates absence of bottleneck in Güney Karaman sheep population. These results will help to develop conservation strategies for the Güney Karaman sheep population.

# Yok Olma Tehlikesi Altındaki Güney Karaman Koyunlarda Genetik Çeşitlilik ve Genetik Darboğaz Analizleri

**MAKALE BİLGİSİ**

**Araştırma Makalesi**

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**ÖZET**

Bu çalışma, genetik kaynak olarak korunan yerli Güney Karaman koyun ırkındaki genetik çeşitlilik ve genetik darboğazların tanımlanması amacıyla gerçekleştirilmiştir. Çalışmada için hayvan materyali olarak Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsünde yetiştirilen 119 Güney Karaman koyun kullanılmıştır. Çalışmada kullanılan hayvan materyali FAO tarafından önerilen on altı mikrosatellit marker ile genotiplenmiştir. Çalışılan on altı mikrosatellit marköründen toplam 277 allel tespit edilmiştir. Çalışılan

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ırkın populasyon büyüklüğü sınırlı olmasına rağmen elde edilen ortalama allel sayısı (17.31), gözlemlenen heterozigotluk (0.81) ve polimorfik bilgi içeriği (0.82) değerleri Güney Karaman koyun populasyonunda önemli düzeyde bir genetik çeşitlilik varlığına işaret etmektedir. İncelenen on altı mikrosatellit işaretleyicinin onunda pozitif $F_{IS}$ değerleri gözlemlenmiştir. $F_{IS}$ değerlerinin ortalaması 0.042 olmuştur. Çalışılan ırktaki genetik darboğaz durumunun kontrolünü sağlamak Bottleneck programındaki sonsuz alel modeli (IAM), iki fazlı mutasyon modeli (TPM) ve aşamalı mutasyon modeli (SMM) kullanılmıştır. Analizden elde edilen mode-shift grafiğindeki L şeklindeki eğri, Güney Karaman koyun populasyonunun yakın zamanlarda herhangi bir genetik darboğaza göstermiştir. Elde edilen sonuçlar, Güney Karaman koyun popülasyonu için sürdürülen koruma stratejilerine önemli katkı sağlayacaktır.

## Introduction

Native livestock breeds that are very well adapted to the ecological and economic conditions of different geographies all over the world, is one of the most important cornerstones of livestock breeding. The change of consumer habits and the increasing population needs caused the domestic breeds to be threatened with extinction and leave their places to culture breeds. Indigenous breeds, which are very well adapted to the area where they are raised, are defined as animals with unique properties, resistant to adverse environmental conditions and diseases, even if their productivity is low. Extinction of native breeds leads to the loss of the original features of these animals that may be needed in the future (Cemal et al., 2013; FAO, 2015; Rege and Gibson, 2003; Wollny, 2003). It is noteworthy that the genetic variation which is very important for biological systems with each passing day is decreasing due to many factors. Therefore, the activities on the conservation of animal genetic resources have become a very important phenomenon in the world today (Bruford et al., 2015; Hoffmann et al., 2011). In this scope, an international consensus has been achieved to conserve biodiversity, including livestock genetic resources with the acceptance of the Convention on Biological Diversity at the Nairobi Conference in 1992 (UNE, 1992).

Over the last few centuries, the dramatic increase in world populations has had a negative impact on natural habitats, causing some species and breeds to decrease or disappear. Rapid reduction in the population or occurrence of bottlenecks can have a deep impact on the effective population size and preservation of genetic diversity in farm animal as well as other animal population. Accordingly, it is generally accepted matter that bottlenecks should be avoided in threatened species conserved as genetic resources (Cornuet and Luikart, 1996).

Turkey has a great genetic diversity that can be characterized by numerous breeds belong to cattle, sheep and buffalo due to geographical and intercontinental position. Güney Karaman sheep breed which is one of these breeds and especially raised in Taurus Mountains located in Mediterranean region, is a fat

tail native sheep breed. It was reported that the hides of this breed, which is very similar to the Karagül breed, can be used in making inner fur because of having curly pattern of fleece (Canatan et al., 2014a, Canatan et al., 2014b; Ertuğrul et al., 2009; Kiraz et al., 2014). In the last 15-20 years, non-systematic cross-breeding practices and changes in consumer habits have triggered a quantitative reduction of this breed and quickly faced the risk of extinction threat. The Güney Karaman sheep breed was included in the national genetic resource conservation program in 2001 by the Republic of Turkey, Ministry of Agriculture and Forestry to transfer existing genetic diversity to future generations with minimal loss. There are total of 119 animal consisted of 52 male and 67 female within the scope of the genetic conservation program shaped at Bahri Dagdas International Agricultural Research Institute in Konya (Canatan et al., 2014a, Canatan et al., 2014b).

The aims of the present study were to describe genetic diversity, structure of population and potential genetic bottleneck in Güney Karaman sheep breed by using microsatellite markers.

## Material and Methods

### *Animal Resources and DNA Isolation*

The study was carried out on 119 (52 male and 67 female) Güney Karaman sheep (Figure 1) is raised in Bahri Dagdas International Agricultural Research Institute genetic conservation flock.


Figure 1. Güney Karaman sheep breed
*Şekil .Güney Karaman koyun ırkı*

Blood samples were collected from *Vena jugularis* into containing K3EDTA tubes and stored at -20°C until DNA extraction. Salting-out technique, reported by Miller et al. (1988) and Montgomery and Sise (1990), was used for DNA extraction from whole blood. Subsequently, the quality and quantity of the DNA sample were checked using NanoDrop 2000 (Thermo Scientific, Waltham, MA).

### *PCR procedure and microsatellites genotyping*

In the present study, sixteen microsatellite markers, recommended by

FAO (2011), was used to reveal intra-breed genetic diversity, population structure and bottleneck test. Microsatellites used, grouped as two multiplexes, were amplified with Touchdown PCR method reported by Hecker and Roux, (1996) (Table 1).

Table 1. Amplification conditions for touchdown PCR protocol
*Tablo 1. Touchdown PCR protokolü için amplifikasyon koşulları*

| Multiplex Group | Loci (Fluorescent label) | First Denat. | Denat. | Annealing | Ext. | Cycles | Final Ext. |
|---|---|---|---|---|---|---|---|
| M1 | OARFCB193 (D3)<br>OARFCB304 (D3)<br>INRA0023 (D3)<br>OARFCB20 (D2)<br>OARAE0129 (D2)<br>OARCP34 (D4)<br>INRA0132 (D4)<br>BM1818 (D4) | 95 °C<br>(5 min) | 95 °C<br>(40 s) | 60-50 °C<br>(40 s) | 72 °C<br>(60 s) | 30 | 72 °C<br>(10 min) |
| M2 | BM8125 (D3)<br>MCM0527 (D3)<br>CSRD0247 (D3)<br>OARFCB128 (D2)<br>BM1329 (D2)<br>HSC (D2)<br>OARJMP29 (D4)<br>MAF214 (D4) | 95 °C<br>(5 min) | 95 °C<br>(40 s) | 60-50 °C<br>(40 s) | 72 °C<br>(60 s) | 30 | 72 °C<br>(10 min) |

Polymerase chain reaction (PCR) were implemented in a total of 20 µl volume including 0.10 µM for each forward and reverse primers, 0.20 mM dNTPs, 2.0 mM $MgCl_2$, 1X PCR buffer, 1 U of Taq DNA polymerase (Applied Biological Materials Inc., Canada) and ~50 ng of genomic DNA. Afterward, PCR fragments was separated with Beckman GeXP fragment analyzer (Beckman Coulter, Inc., USA). GenomeLab™ DNA Size Standard Kit 400 was used to determine fragment size belong to microsatellite markers.

***Statistical analyses***

Genetic variation analysis was revealed using GenAlEx (Peakall and Smouse, 2006, Peakall and Smouse, 2012) and POPGENE (Yeh et al., 1997) to compute number of alleles per locus (Na), effective number of alleles (Ne),

observed heterozygosity (Ho), expected heterozygosity (He), $F_{IS}$ value, known as inbreeding coefficient, from Wright's F-statistics (Weir and Cockerham, 1984; Wright, 1990) and Hardy–Weinberg equilibrium. CERVUS 3.0.3 (Kalinowski et al., 2007; Marshall et al., 1998) was used to calculate polymorphic information content (PIC) and null allele frequencies.

Bottleneck events were tested with Sign, Standardized differences and Wilcoxon sign–rank tests under the different mutation models such as Infinite Allele Model (IAM), Stepwise Mutation Model (SMM), and Two Phase Model of Mutation (TPM) model in Bottleneck software version 1.2.02 (1 000 simulation) (Piry et al., 1999).

## Results and Discussion

A total 277 alleles were observed from sixteen microsatellites used in this study. Computed genetic diversity statistics was given in Table 2.

The highest number of alleles and effective number of alleles were obtained from OarFCB20 (24) and BM1329 (13.65), respectively. When the PIC values ranging from 0.74 (MCM0527) to 0.92 (BM1329) were examined, it was determined that all microsatellites were highly informative for this studied population. Overall mean of observed heterozygosity value was lower than the expected heterozygosity value. Obtained genetic diversity parameters such as mean number of allele and effective allele were considerably higher than those of earlier studies conducted in sheep breeds raised in different countries (Arora and Bhatia, 2006; Ben Sassi-Zaidy et al., 2016 ; Cemal et al., 2013; Guang-Xin et al.,2016; Kdidi et al., 2015 ; Ocampo et al.,2016 ; Oner et al.,2014 ; Yilmaz et al., 2014), while these values and polymorphic information content were lower than those of earlier studies (Abdelkader et al., 2018; Hoda and Marsan, 2012; Yilmaz et al., 2015).

On the other hand, obtained high heterozygosity and polymorphic information content values supported high genetic variability in Güney Karaman sheep breeds studied. Although the Güney Karaman sheep breed, which have very limited population size, are protected as a genetic resource, the high genetic variation revealed in the present study is quite an important finding. The findings related allele numbers indicated that a high level of allelic richness in breed studied.

The average of $F_{IS}$ value, also known as inbreeding coefficient and described as Wright' F statistics, was 0.042. $F_{IS}$ values, which is a measure of the deviation of genotypic frequencies from panmixia in populations in terms of heterozygous deficiency or excess, showed that loss of heterozygosity at six microsatellite loci (OarFCB20, OarCP34, INRA0132, BM1818, BM8125 and CSRD0247). Similar findings have been expressed in the previous literature conducted in different sheep breeds (Loukovitis et al., 2016; Salamon et al., 2015; Vahidi et al., 2016; Yilmaz et al., 2014; Yılmaz et al., 2015).

Table 2. Genetic diversity statistics of sixteen microsatellite markers in Güney Karaman sheep breed

*Tablo 2. Güney Karaman koyun ırkındaki 16 mikrosatellite ait genetic çeşitlilik istatistikleri*

| Loci | Na | Ne | Ho | He | PIC | $F_{IS}$ | HWE | F(Null) |
|---|---|---|---|---|---|---|---|---|
| OarFCB193 | 23 | 9.16 | 0.84 | 0.89 | 0.88 | 0.056* | *** | 0.028 |
| OarFCB304 | 23 | 5.59 | 0.81 | 0.82 | 0.80 | 0.015 | *** | 0.004 |
| INRA0023 | 20 | 5.70 | 0.81 | 0.82 | 0.81 | 0.025 | *** | -0.005 |
| OarFCB20 | 24 | 10.88 | 0.93 | 0.91 | 0.90 | -0.023 | ns | -0.015 |
| OarAE0129 | 19 | 6.21 | 0.71 | 0.84 | 0.82 | 0.157*** | ns | 0.089 |
| OarCP34 | 13 | 6.88 | 0.95 | 0.85 | 0.84 | -0.106 | *** | -0.057 |
| INRA0132 | 17 | 4.94 | 0.87 | 0.80 | 0.77 | -0.092 | ** | -0.056 |
| BM1818 | 17 | 8.18 | 0.93 | 0.88 | 0.87 | -0.050 | *** | -0.029 |
| BM8125 | 13 | 4.86 | 0.83 | 0.79 | 0.77 | -0.045 | *** | -0.036 |
| MCM0527 | 9 | 4.44 | 0.64 | 0.77 | 0.74 | 0.181*** | ** | 0.105 |
| CSRD0247 | 15 | 4.80 | 0.82 | 0.79 | 0.77 | -0.028 | *** | -0.026 |
| OarFCB128 | 13 | 6.01 | 0.71 | 0.83 | 0.81 | 0.156*** | * | 0.084 |
| BM1329 | 20 | 13.65 | 0.83 | 0.93 | 0.92 | 0.107*** | ns | 0.053 |
| HSC | 19 | 7.07 | 0.84 | 0.86 | 0.85 | 0.023 | ns | 0.010 |
| OarJMP29 | 14 | 5.37 | 0.64 | 0.81 | 0.79 | 0.220*** | *** | 0.119 |
| MAF214 | 18 | 5.90 | 0.78 | 0.83 | 0.81 | 0.070* | *** | 0.032 |
| **Overall** | **17.31** | **6.85** | **0.81** | **0.84** | **0.82** | | | |

**Na**: Number of alleles, **Ne**: Effective number of alleles, **Ho**: Observed heterozygosity, **He**: Expected heterozygosity, **PIC**: Polymorphic information content, **$F_{IS}$:** inbreeding coefficient, **HWE:** Significance level of Hardy-Weinberg Equilibrium, **F(Null):** Null allele frequency, *****: P<0.05, ****** : P<0.01, *******: P<0.001

Twelve microsatellite deviated from the Hardy-Weinberg equilibrium (P<0.05). It is expected results that most of the studied loci will deviate from the Hardy-Weinberg equilibrium given that the size of the population is very limited and various protection activities performed in breeds studied.

The null allele frequency values obtained from the studied microsatellite loci were below 20%. Null alleles that is defined as a non-amplifiable allele due to mutations in the PCR binding site, causing only a single allele to peek like a homozygote, thus causing erroneous reading. Observed null allele frequencies

for the all microsatellites below the critical value (20%) reported by Dakin and Avise (2004) indicated that these markers studied can be used confidently to identify genetic diversity in this native sheep breed.

It is necessary to understand the processes that cause decreasing genetic diversity such as genetic bottleneck, genetic drift and inbreeding especially in small populations. The infinite allele model (IAM) and the stepwise mutation models (SMM) generally give inconsistent results when describing the mutation in microsatellites. Therefore, it is reported that the two-phase mutation model (TPM) is the most useful model to test the heterozygosity excess in the bottleneck tests performed with microsatellites (Dirienzo et al., 1994; Luikart et al., 1998; Piry et al., 1999). On the other hand, it has been reported that the Wilcoxon test, which has high statistical confidence even in bottleneck analysis studies using a limited number of loci (<20), can be used with high confidence in bottleneck studies (Piry et al., 1999).

Genetic bottleneck analysis was performed to investigate whether there was a bottleneck in Güney Karaman sheep population conserved as a genetic resource. Since the mutation pattern of evolution and microsatellites are not clearly known, the data set obtained was tested with three different mutation models, Infinite Allele Model (IAM), Stepwise Mutation Model (SMM), and Two Phase Model of Mutation (TPM) model reported by Cornuet and Luikart (1996) , Luikart and Cornuet (1998) and Piry et al., (1999). Sign, Standardized differences and Wilcoxon sign rank tests were used to predict excess of heterozygosity (Table 3).

The expected numbers of loci with heterozygosity excess were found to be 9.75 ($P>0.05$), 9.43 ($P<0.05$) and 9.33 ($P<0.05$) in IAM, TPM and SMM in the Sign Test. It is indicated that the probability values were lower than 0.05 for the TPM and SMM except IAM. T2 statistics obtained from the Standardized difference test for IAM, TPM and SMM models were 2.032 ($P<0.05$), -3.362 ($P<0.05$) and -12.222 ($P<0.05$). On the other hand, the probability values for one tail for heterozygosity excess obtained using the Wilcoxon rank test were non-significant in two-phase mutation model and the stepwise mutation model while this value was significant the infinite allele model.

Table 3. Test for null hypothesis under three microsatellite evolution models for bottleneck analysis
*Tablo 3. Darboğaz analizi için üç mikrosatellit evrim modeli analizi*

| Mutation Models | Sign test | | | | Standardized differences test | | Wilcoxon rank test (one tail for H excess) |
|---|---|---|---|---|---|---|---|
| | Hee | Hed | He | P | T2 | P | P |
| IAM | 9.75 | 3 | 13 | 0.07532 | 2.032 | 0.02107 | 0.00258 |
| TPM | 9.43 | 11 | 5 | 0.02355 | -3.362 | 0.00039 | 0.98323 |
| SMM | 9.33 | 15 | 1 | 0.00002 | -12.222 | 0.00000 | 0.99998 |

**IAM:** The infinite allele model, **TPM:** Two-phase mutation model, **SMM:** The stepwise mutation model, **Hee:** Expected number of loci with heterozygosity excess, **Hed:** heterozygosity deficiency, **He:** heterozygosity excess

The population studied was found to be bottlenecked by the Wilcoxon test according to the infinite allele model (IAM). But it should not be forgotten that the most suitable model for microsatellites in the Wilcoxon test is the TPM model. In this context, it can be said that serious demographic bottlenecks have not been experienced in the Güney Karaman sheep population, which is conserved as a genetic resource, given that considering the TPM model of Wilcoxon test results.

As a second method, a mode-shift graph was obtained using allele frequency classes of 16 microsatellite to identify potential bottlenecks in the studied population (Figure 2). The mode shift graph method, which is a qualitative graphical representation of the allele frequency distribution, was first proposed by Luikart et al. (1998). The "L-shaped distribution" of the allele frequency distribution graph is used as a criterion for the bottleneck.

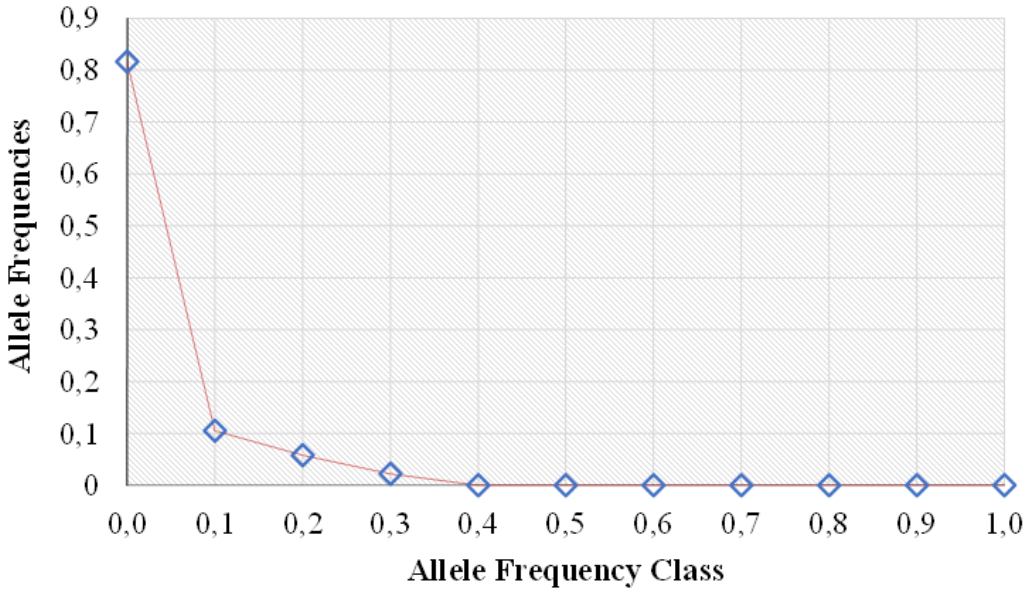


Figure 2. Mode-shift graph for bottleneck in the Güney Karaman sheep breed
*Şekil 2. Güney Karaman koyun ırkındaki darboğaz için mode-shift grafiği*

As it can be seen from mode-shift graph, an L-shaped chart consistent with the distribution ranges of the normal frequency class was obtained in the bottleneck test performed. If the graph shows the normal distribution (L-shaped), then the mutation-migration balance is concerned. Obtained L-shaped distribution suggests that there is not a genetic bottleneck in the studied populations that is large enough to be considered recently (last 40-80 generations).

## Conclusions

Consequently, the present study results indicated that although the Güney Karaman sheep population size is very small, genetic diversity was significantly high in the gene pool. Our findings revealed that the microsatellite markers used in this study that can be successfully used in genetic diversity and bottleneck studies for this breed. On the other hand, obtained results will help to interpret the genetic structure of indigenous Güney Karaman sheep and will be of benefit to the efforts for conservation of this breed. The strong inference that the Güney Karaman has not undergone major bottlenecks is also important for sheep breeders and other conservation programs.

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