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RESEARCH ARTICLE

Analysis of Genetic Diversity in Indigenous Çine Çaparı Sheep under Conservation by Microsatellite Markers [1]

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Summary

In this study, 123 animals from 3 different flocks (ADU-ÇÇKP conservation flock and two breeders' flocks) were genotyped with 10 microsatellite markers to investigate genetic diversity in the endangered native Çine Çaparı sheep breed. The number of alleles per microsatellite marker ranged from 7 (OarCP34) to 17 (OarFCB193), with an average of 11.5 alleles per locus. Total number of alleles for the investigated 10 microsatellite markers was found to be 104, 72 and 45 for ADÜ-ÇÇKP, EA and MV flocks, respectively. The considerable difference in the allele numbers of the flocks indicates high genetic variability in the ADÜ-ÇÇKP flock versus the other two breeders' flocks. The observed alleles have different size from other shows the existence of private alleles for flocks. According to the polymorphism information content (PIC) values, the highest and lowest polymorphism was detected to be 0.875 and 0.699 for OarFCB193 and OarFCB304 markers, respectively. The mean expected heterozygosity (He) and the mean observed heterozygosity (Ho) for the whole Çine Çaparı population under conservation were 0.727 and 0.789, respectively. The highest genetic similarity (0.8262) was observed between the ADU-ÇÇKP conservation flock and Erdoğan Aktürk's flock. The results obtained in the present study will help to interpret the genetic structure of indigenous Çine Çaparı sheep and will be of benefit to the efforts for conservation of this breed.

Keywords: *Genetic resources, Sheep, Çine Çaparı, Diversity, Microsatellites*

Koruma Altındaki Çine Çaparı Koyunlarda Genetik Çeşitliliğin Mikrosatellit İşaretleyiciler İle Analizi

Özet

Çalışmada, Adnan Menderes Üniversitesi Çine Çaparı Koruma Programı (ADÜ-ÇÇKP) kapsamında oluşturulan koruma sürüsü ile 2 yetiştiricide bulunan toplam 123 baş hayvanda 10 mikrosatellit lokusu kullanılarak genotipleme yapılmıştır. Sürüler arası genetik benzerlik ve uzaklıklar incelenmiştir. Mikrosatellit işaretleyici başına elde edilen allel sayısı 7 (OarCP34) ile 17 (OarFCB193) arasında değişmekle birlikte lokus başına ortalama allel sayısı 11.5'tur. Populasyon bütününü oluşturan ADÜ-ÇÇKP, EA ve MV sürülerinde belirlenen toplam allel sayıları sırasıyla 104, 72 ve 45 olarak belirlenmiştir. Allel sayıları bakımından sürüler arası gözlenen bu ciddi fark iki yetiştirici sürüsüne oranla ADÜ-ÇÇKP koruma sürüsünde yüksek genetik çeşitliliğe işaret etmektedir. Diğer allelere oranla farklı büyüklükte allelerin gözlenmesi sürülere özgün özel allellerin varlığına işaret etmektedir. Polimorfik bilgi içeriği (PIC) değerleri dikkate alındığında en yüksek ve en düşük polimorfizm değerleri OarFCB193 ve OarFCB304 lokusları için sırasıyla 0.875 and 0.699 olarak belirlenmiştir. Ele alınan tüm lokuslara dayalı değerlendirme sonucunda gözlenen (Ho) ve beklenen (He) heterozigotluk ortalamaları sırasıyla 0.727 ve 0.789 olarak elde edilmiştir. En yüksek genetik benzerlik (0.8262) ADÜ-ÇÇKP koruma sürüsü ile Erdoğan Aktürk'e ait sürü arasında ortaya çıkmıştır. Bu çalışmadan elde edilen sonuçlar yerli Çine Çaparı koyunlarda genetik yapının tanımlanmasına yardımcı olmakla birlikte gelecekte bu ırkın korunmasına yönelik çabalara fayda sağlayacaktır.

Anahtar sözcükler: *Genetik kaynaklar, Koyun, Çine Çaparı, Çeşitlilik, Mikrosatellit*

INTRODUCTION

Agricultural biodiversity includes the diversity of the cultivated plants and domestic animals utilized by humankind for the production of food and other goods and services. The livestock species (over 40 species) contributing

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to today's agriculture and food production are shaped by a long history of domestication and development. The term animal genetic resources (AnGR) is used to include all animal species, breeds and strains (and their wild relatives) that are of economic, scientific and cultural interest to humankind in terms of food and agricultural production for the present or in the future [1-3].

Being part of the Fertile Crescent, Turkey has an important position for domestication. Therefore, native breeds of sheep, goat and cattle have an important level of diversity. Sheep in Turkey's animal husbandry have great genetic potential together with high numbers and with wide breeds and local type diversity which is formed according to different ecological conditions. The large genetic changes observed in sheep populations in Western Anatolia with the intensification of agriculture have threatened the existence of native Turkish sheep breeds in last decades [4]. In a short period of time including the last two to three decades, some breeds have become extinct (the Ödemiş breed) or endangered (the Dağlıç, Çine Çaparı, Sakız, Kıvırcık breeds).

The fat-tailed Çine Çaparı is a local native sheep breed originating in the mountains (especially in mountain Madran) of the Aydın province in Turkey. In the last 20 to 30 years, the number of purebred Çine Çaparı sheep has declined very much due to the backcrossing of the breed with rams of the Kıvırcık and prolific Sakız (Chios) breeds. Consequently, this process has put them in danger of extinction. A conservation program termed the "Adnan Menderes University - Çine Çaparı Conservation Program (ADÜ-ÇÇKP)" was established in 1996 to characterize and conserve this endangered breed. Studies to establish a conservation flock at Adnan Menderes University were also initiated in the same year. In mountainous villages only two flocks including purebred animals remained. Members of all other flocks were turned to a synthetic form, namely Karya. The main reasons for breeders to keep this breed are the easiness of management and its resistance to diseases and high temperatures.

Studies for the characterization and conservation of indigenous breeds in Turkey in the last decade are encouraging. The efforts made to characterize and conserve the Çine Çaparı sheep breed are a good example of conservation activities in Turkey. The trend of a decrease in the animal number was changed to a trend of an increase with the efforts of researchers at Adnan Menderes University and the support of the Ministry of Food, Agriculture and Animal Husbandry. The ongoing studies have stopped the process of extinction of the breed and have guaranteed the future of the breed.

Microsatellites are valuable genetic markers due to their dense distribution in the genome, great variation, codominant inheritance and easy genotyping. In recent years, they have been extensively used in parentage testing, linkage analyses, population genetics and other genetic studies [5,6].

The aim of this study was to determine the within-breed genetic diversity in endangered Çine Çaparı sheep using 10 microsatellite markers and to obtain the genetic similarities and distances among animals within and between flocks.

MATERIAL and METHODS

DNA samples of 123 animals from 3 existing flocks were genotyped with 10 microsatellite markers (OarCP34, OarFCB193, OarFCB304, OarJMP29, OarFCB128, BM8125, OarJMP58, OarVH72, MAF65, and DYMS1) that selected from the list recommended by FAO [7]. Forward markers were marked with three fluorescent dyes (D2, D3, D4). Three multiplex groups were formed with 8 out of the 10 microsatellites. Annealing temperatures of MAF65 and DYMS1 were not appropriate for the other 3 multiplex groups. Therefore, these two microsatellites were amplified by Polymerase Chain Reaction (PCR) separately. *Table 1* shows details for the considered microsatellites.

DNA was isolated from blood samples using a DNA extraction kit. Specific genomic regions were amplified by Polymerase Chain Reaction (PCR) in accordance with the touchdown PCR technique. The thermal cycling conditions are given in the *Table 2*.

For every microsatellite locus, the amplification reaction took place in a total volume of 25 µl and contained the following constituents in the final concentrations indicated in brackets; dNTP's (0·2 mM for each one), $MgCl_2$ (2.0 mM), primers (0.25 mM for each one), and *Taq* DNA polymerase (1 unit reaction^{-1}). Approximately 100 ng of genomic DNA was used as template for each of PCR amplification. Fragment analysis was achieved using the automatic laser-induced fluorescence DNA sequencer (Beckman Coulter CEQ 8000 Genetic Analysis System). Obtained data was analyzed by the Beckman Coulter CEQ Fragment Analysis Software.

The frequency of particular alleles of the microsatellite sequences were used to calculate the number of alleles (n_A), the number of effective alleles (n_E), the polymorphic information content (PIC), the observed heterozygosity (Ho), the expected heterozygosity (He) and average heterozygosity ($\hat{H}$) [8-11]. Nei's genetic similarities and genetic distances between flocks [12] and F statistics [8] were also estimated. A dendrogram based on Nei's genetic distances was obtained using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method. We used the microsatellite loci analyzed to find out if the examined herd of Çine Caparı was in a genetic equilibrium conformable to the Hardy-Weinberg principle. Propriety of allele frequencies Hardy-Weinberg Equilibrium (HWE) was checked by χ^2 test ($P<0.01$), which estimated observed genotype frequencies and expected numeric values of the same genotypes. These parameters were calculated using GenAlEx [13], PowerStatsV12 [14], MEGA 4 [15], Arlequin 3.5 [16] and POPGENE [17] softwares.

Table 1. *Details of considered microsatellites*
Tablo 1. *Çalışmada kullanılan mikrosatelitlere ait bilgiler*

Locus Name	Primers	Base Pair (bp)	Multiplex Group
OarCP34	**F:** GCTGAACAATGTGATATGTTCAGG **R:** GGGACAATACTGTCTTAGATGCTGC	112-130	1
OarFCB304	**F:** CCCTAGGAGCTTTCAATAAAGAATCGG **R:** CGCTGCTGTCAACTGGGTCAGGG	150-188	
OarFCB193	**F:** TTCATCTCAGACTGGGATTCAGAAAGGC **R:** GCTTGGAAATAACCCTCCTGCATCCC	96-136	
OarJMP29	**F:** GTATACACGTGGACACCGCTTTGTAC **R:** GAAGTGGCAAGATTCAGAGGGGAAG	96-150	2
OarFCB128	**F:** ATTAAAGCATCTTCTCTTTATTTCCTCGC **R:** CAGCTGAGCAACTAAGACATACATGCG	96-130	
BM8125	**F:** CTCTATCTGTGGAAAAGGTGGG **R:** GGGGGTTAGACTTCAACATACG	116-122	
OarJMP58	**F:** GAAGTCATTGAGGGGTCGCTAACC **R:** CTTCATGTTCACAGGACTTTCTCTG	145-169	3
OarVH72	**F:** GGCCTCTCAAGGGGCAAGAGCAGG **R:** CTCTAGAGGATCTGGAATGCAAAGCTC	121-135	
DYMS1	**F:** AACAACATCAAACAGTAAGAG **R:** CATAGTAACAGATCTTCCTACA	159-211	Individual
MAF65	**F:** AAAGGCCAGAGTATGCAATTAGGAG **R:** CCACTCCTCCTGAGAATATAACATG	123-135	Individual

Table 2. *Thermal cycling conditions according to Touchdown PCR*
Tablo 2. *Touchdown PZR koşulları*

Loci	Denaturation (°C)	Time (sec)	Annealing (°C)	Time (sec)	Extension (°C)	Time (sec)
OarCP34	95	45	60-58	45	72	45
OarFCB193						
OarFCB304						
OarJMP29	95	45	61-57	45	72	45
OarFCB128						
BM8125						
OarJMP58	95	45	60-56	45	72	45
OarVH72						
MAF65	95	45	59-57	45	72	45
DYMS1	95	45	52-50	45	72	45

RESULTS

One hundred and fifteen alleles were determined from 10 microsatellites markers. Total number of alleles for the investigated 10 microsatellite markers was found to be 104, 72 and 45 for ADÜ-ÇÇKP, EA and MV flocks, respectively. In the all loci, the number of alleles ranged from 7 (OarCP34) to 17 (OarFCB193), with an average of 11.5 alleles per locus. The frequency of alleles varied according to locus; the allele size range (ASR), number of allele (n_A), number of effective allele (n_E), the polymorphic information content (PIC), the observed heterozygosity (Ho), the expected heterozygosity (He) and average heterozygosity (Ĥ) are given in *Table 3* according to all flocks.

Among the ten microsatellite loci studied, the highest observed heterozygosity value was 0.950 for OarFCB193, and the lowest value was 0.450 for DYMS1. The number of alleles for the OarFCB193, DYMS1, OarJMP29, BM8125, OarJMP58, OarFCB304, OarFCB128, MAF65, OarVH72 and OarCP34 loci were found to be 7, 14, 13, 13, 11, 10, 9, 8 and 7, respectively.

Loci in all flocks were examined in terms of polymorphic information content (PIC) and the results showed that OarFCB193 (0.875), OarFCB128 (0.765) and BM8125 (0.765) loci take highest values.

The number of alleles (n_A), number of effective alleles (n_E), the polymorphism information content (PIC), the observed heterozygosity (Ho), the expected heterozygosity (He) and average heterozygosity (Ĥ) according to all three flocks are given in *Table 4*.

Considering the number of alleles, OarFCB193 showed the highest polymorphism of the three flocks. Ho and He

Table 3. *Allele size range (ASR) (bp), n_A, n_E, PIC, Ho, He and Ĥ values in considered microsatellites in all flocks*
Tablo 3. *Tüm sürüler için kullanılan mikrosatellitlere ait genel gözlenen boyut aralığı (GBA, bp), örnek sayısı (N), n_A, n_E, PIC, Ho, He ve Ĥ değerleri*

Loci	N	ASR (bp)	n_A	n_E	Ho	He	Ĥ	PIC
OarCP34	242	112/124	7	4.479	0.843	0.78	0.777	0.744
OarFCB193	242	96/136	17	8.668	0.950	0.888	0.885	0.875
OarFCB304	242	160/190	11	3.798	0.727	0.740	0.737	0.699
OarJMP29	244	116/156	13	4.289	0.820	0.770	0.767	0.738
OarFCB128	234	100/128	10	4.859	0.692	0.798	0.794	0.765
BM8125	238	108/138	13	4.775	0.782	0.794	0.791	0.765
OarJMP58	244	141/171	13	4.325	0.713	0.772	0.769	0.740
OarVH72	244	123/143	8	3.876	0.730	0.745	0.742	0.708
MAF65	222	125/141	9	4.380	0.568	0.775	0.772	0.738
DYMS1	240	169/201	14	4.312	0.450	0.771	0.768	0.739
Mean	**239**		**11.5**	**4.776**	**0.727**	**0.783**	**0.78**	
St. dev.			**3.064**	**1.407**	**0.141**	**0.041**	**0.041**	

ASR: *allele size range,* ***n_A:*** *number of alleles,* ***n_E:*** *number of effective allele,* ***PIC:*** *polymorphic information content,* ***Ho:*** *observed heterozygosity,* ***He:*** *expected heterozygosity* ***Ĥ:*** *average heterozygosity*

Table 4. *n_A, n_E, PIC, Ho, He and Ĥ values in considered microsatellites according to flocks*
Tablo 4. *Sürülere göre kullanılan mikrosatellitlere ait n_A, n_E, PIC, Ho, He ve Ĥ değerleri*

Loci	ADU-CCKP							EA Flock							MV Flock						
	N	n_A	n_E	Ho	He	Ĥ	PIC	N	n_A	n_E	Ho	He	Ĥ	PIC	N	n_A	n_E	Ho	He	Ĥ	PIC
OarCP34	154	7	3.80	0.857	0.741	0.737	0.69	62	6	4.23	0.839	0.776	0.764	0.730	26	3	2.30	0.769	0.588	0.565	0.471
OarFCB193	154	15	6.84	0.935	0.859	0.854	0.84	62	10	5.70	0.968	0.838	0.825	0.802	26	7	4.28	1.000	0.797	0.766	0.732
OarFCB304	154	11	4.69	0.766	0.792	0.787	0.76	62	6	2.50	0.581	0.609	0.599	0.520	26	4	2.50	0.846	0.625	0.601	0.534
OarJMP29	154	9	4.01	0.805	0.756	0.751	0.72	62	7	3.62	0.807	0.736	0.724	0.691	28	6	3.32	0.929	0.725	0.699	0.649
OarFCB128	150	9	4.82	0.653	0.798	0.793	0.77	62	8	3.59	0.742	0.734	0.722	0.680	22	3	2.31	0.818	0.593	0.566	0.504
BM8125	152	10	4.71	0.790	0.793	0.788	0.77	62	9	4.65	0.839	0.798	0.785	0.752	24	4	2.97	0.583	0.692	0.663	0.600
OarJMP58	154	13	3.98	0.753	0.754	0.749	0.71	62	6	2.86	0.677	0.661	0.650	0.618	28	7	4.78	0.571	0.82	0.791	0.760
OarVH72	154	7	4.60	0.753	0.788	0.782	0.75	62	7	2.43	0.677	0.598	0.588	0.536	28	4	2.78	0.714	0.664	0.640	0.595
MAF65	142	9	4.84	0.563	0.799	0.794	0.77	62	6	3.59	0.645	0.733	0.721	0.679	18	3	2.16	0.333	0.569	0.537	0.468
DYMS1	148	14	4.42	0.514	0.779	0.774	0.75	64	7	4.50	0.438	0.790	0.778	0.746	28	4	2.82	0.143	0.669	0.645	0.577
Mean	152	10.4	4.67	0.739	0.786	0.781		62	7.2	3.77	0.721	0.727	0.716		25	4.5	3.02	0.671	0.674	0.647	
St.dev.		2.797	0.851	0.129	0.033	0.033			1.398	1.03	0.151	0.081	0.08			1.58	0.876	0.269	0.086	0.085	

n_A: *number of alleles,* ***n_E:*** *number of effective allele,* ***PIC:*** *polymorphic information content,* ***Ho:*** *observed heterozygosity,* ***He:*** *expected heterozygosity* ***Ĥ:*** *average heterozygosity*

values obtained from the study were seen to vary between 0.950 and 0.740 with 0.450 and 0.788, respectively in all populations. The average heterozygosity value was 0.780 for all loci. The observed alleles with different sizes indicate the existence of unique or private alleles for flocks within this breed. 44 out of the 115 alleles were observed in only one of three flocks as private alleles. Determined private alleles are given in *Table 5*. In general, these private alleles are found at either end of the allelic range with a low frequency (between 0.06 and 0.143), were observed to be 33 in ADÜ-ÇÇKP, 7 in EA and 4 in MV flock.

In particular, null allele frequencies were found to be remarkable for OarFCB128, OarJMP58, MAF65 and DYMS1 loci *(Table 6)*.

The high (>0.05) null allele frequencies observed for OARFCB128, OARJMP58, MAF65 and DYMS1 loci indicated the presence of null alleles and loss of heterozygosity. In spite of higher null allele frequencies of MAF65 and DYMS1 loci, the frequencies very close to 0.05 for OarFCB128 and OarJMP58 loci could be tolerated.

The Hardy-Weinberg equilibrium (HWE) was tested for all populations and results are given in *Table 7*.

The assessment of genetic equilibrium in the examined herd, based on the analysis of observed and expected frequencies of individual genotypes, showed highly significant differences between the expected and observed values for the OarFCB304, OarFCB128, OarJMP58, MAF65 and DYMS1

Table 5. *Number and frequency of common alleles in whole population and private alleles in three flocks*
Tablo 5. *Ortak allelerin tüm populasyondaki ve özgün allellerin sürülerdeki sayı ve frekansları*

Loci	n_A	Common Alleles		Private Alleles					
		No	Frequency (Min-Max)	ADÜ-ÇÇKP Flock		EA Flock		MV Flock	
				No	Size: Freqeency	No	Size: Freqeency	No	Size: Freqeency
OarCP34	7	6	0.016-0.500	1	**124:**0.013	-	-	-	-
OarFCB193	17	11	0.006-0.346	4	**96:**0.143	-	-	2	**126:**0.038
					102:0.013				**128:**0.038
					112:0.013				
					120:0.071				
OarFCB304	11	6	0.013-0.538	5	**160:**0.013	-	-	-	-
					166:0.104				
					184:0.019				
					188:0.045				
					190:0.032				
OarJMP29	13	6	0.019-0.452	3	**116:**0.019	2	**122:**0.016	2	**142:**0.036
					146:0.032		**132:**0.016		**154:**0.107
					148:0.026				
BM8125	13	6	0.016-0.417	4	**108:**0.039	3	**110:**0.016	-	-
					114:0.020		**124:**0.032		
					130:0.033		**134:**0.016		
					138:0.007				
OarJMP58	13	9	0.006-0.548	4	**153:**0.026	-	-	-	-
					155:0.006				
					169:0.006				
					171:0.006				
OarVH72	8	6	0.006-0.581	1	**137:**0.019	1	**143:**0.016	-	-
MAF65	9	6	0.016-0.611	3	**133:**0.056	-	-	-	-
					135:0.014				
					139:0.042				
DYMS1	14	8	0.007-0.429	6	**169:**0.007	-	-	-	-
					189:0.034				
					193:0.020				
					195:0.014				
					199:0.041				
					201:0.014				
OarFCB128	10	7	0.013-0.591	2	**100:**0.053	1	**108:**0.016	-	-
					120:0.007				

n_A: *number of alleles*

(P<0.001) in the ADÜ-ÇÇKP flock; OarJM29, OarFCB128, BM8125 and DYMS1 (P<0.001) in the EA flock; OarJMP58, DYMS1 (P<0.001) and MAF65 (P<0.05). The other markers were in the Hardy-Weinberg (HW) equilibrium in all 3 flocks.

Genetic similarity and genetic distance values are presented in *Table 8*. A dendrogram of genetic distances between the three flocks is given in *Fig. 1*.

Both *Table 1* and *Fig. 1* show that the highest and lowest genetic similarities were found between ADÜ-ÇÇKP and EA (0.826) and EA and MV flocks (0.710), respectively. When the drawn dendrogram was examined, it was found that the MV flock was in a separate group from ADÜ-ÇÇKP and EA flocks.

A Factorial Correspondence Analysis (FCA) chart was drawn in order to demonstrate how much individuals in the three flocks were separated. Factorial correspondence analysis of the Çine Caparı sheep in the three flocks based on the 10 microsatellite loci is given in *Fig. 2*.

Table 6. *Null allele frequencies obtained from 10 STR loci*
Tablo 6. *İncelenen 10 mikrosatellit lokusuna ait null allel frekansları*

Loci	Null Allele Frequency
OARCP34	0.0000
OARFCB193	0.0000
OARFCB304	0.0290
OARJMP29	0.0000
OARFCB128	0.0532*
BM8125	0.0262
OARJMP58	0.0503*
OARVH72	0.0178
MAF65	0.1375*
DYMS1	0.1731*

** null alleles with high frequency*

F statistics are widely used for defining population structure. Fis, Fit and Fst values of between the flocks averaged over ten microsatellites are shown in *Table 9*.

Fis values were found to be between -0.194 and 0.502 in general. High values of Fis for all loci in the MV flock indicate for the existence of high inbreeding. It is mainly stem from small size of this flock. The results *(Table 9)* indicated that using the microsatellites in this population was useful for the planned objectives. When ADÜ-ÇÇKP, EA and MV flocks are evaluated for this parameter, five loci in the ADÜ-ÇÇKP flock, seven loci in the EA flock and six loci in the MV flock showed high heterozigosity rates. Low Fst values imply that flocks are similar due to some gene flow between them. This result is strongly supported with efforts are given within ADÜ-ÇÇKP at past years to change rams between flocks to limit inbreeding.

Table 7. *Chi-Square test values belong to 10 microsatellites in all population*
Tablo 7. *Tüm sürülerde incelenen 10 mikrosatellit lokusuna ait Ki-kare test değerleri*

Loci	ADÜ-ÇÇKP				EA				MV			
	DF	χ^2	Prob	Sig.	DF	χ^2	Prob	Sig.	DF	χ^2	Prob	Sig.
OarCP34	21	18.690	0.605	NS	15	5.985	0.980	NS	3	2.479	0.479	NS
OarFCB193	105	80.181	0.966	NS	45	55.314	0.139	NS	21	19.810	0.533	NS
OarFCB304	55	125.283	0.000	P<0.001	15	8.900	0.883	NS	6	8.258	0.220	NS
OarJMP29	36	36.581	0.442	NS	21	72.412	0.000	P<0.001	15	8.226	0.914	NS
OarFCB128	36	91.898	0.000	P<0.001	28	88.696	0.000	P<0.001	3	5.272	0.153	NS
BM8125	45	51.902	0.223	NS	36	104.634	0.000	P<0.001	6	4.113	0.661	NS
OarJMP58	78	158.569	0.000	P<0.001	15	10.073	0.815	NS	21	48.351	0.001	P<0.001
OarVH72	21	16.749	0.726	NS	21	7.223	0.998	NS	6	4.542	0.604	NS
MAF65	36	140.760	0.000	P<0.001	15	11.218	0.737	NS	3	9.146	0.027	P<0.05
DYMS1	91	204.811	0.000	P<0.001	21	55.388	0.000	P<0.001	6	28.505	0.000	P<0.001

DF: *Degree of freedom,* ***NS:*** *Non-significance*

Table 8. *Genetic similarity (above diagonal) and genetic distance (below diagonal) in three flocks*
Tablo 8. *Üç sürüdeki genetik benzerlik (diyagonal üzeri) ve genetik mesafe (diyagonal altı)*

Flocks	ADÜ-ÇÇKP	EA	MV
ADÜ-ÇÇKP	****	0.8262	0.7612
EA	0.1909	****	0.7107
MV	0.2729	0.3415	****

DISCUSSION

The considerable differences in allele numbers in flocks indicate high genetic variability in the ADÜ-ÇÇKP flock versus the other two breeders' flocks (EA and MV). The observed allele numbers show the existence of unique alleles for flocks.

The results show that number of alleles, which was observed in three small Çine Çaparı sheep flocks, was higher than some reported studies [18,19] and relatively lower than

Fig 1. Dendrogram based on Nei's genetic distances among three flocks
Şekil 1. Üç sürü arası Nei'nin genetik uzaklıklarına dayalı elde edilen dendrogram

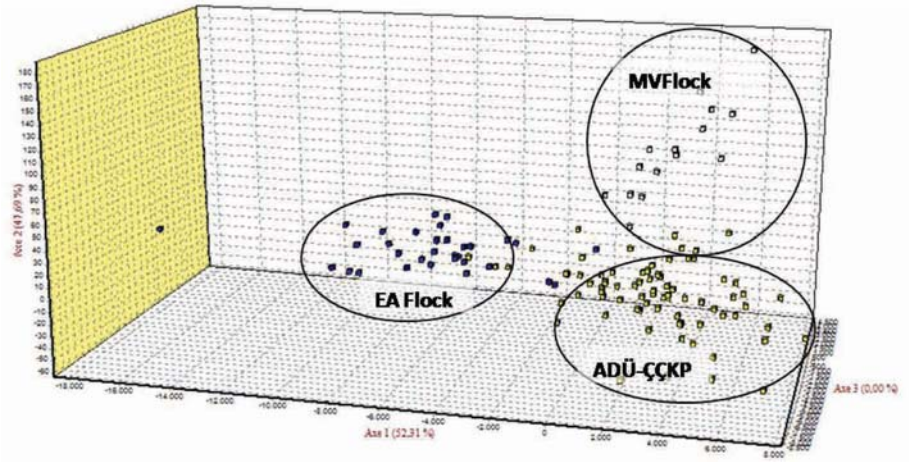

Fig 2. Factorial correspondence analysis of Cine Capari sheep in three flocks

Şekil 2. Üç sürüdeki Çine Çaparı koyunlara ait faktöriyel birleştirici analizine ait grafik

Table 9. *Fis, Fit and Fst values for whole Çine Çaparı population and for each flock*
Tablo 9. *Çine Çaparı populasyonunun tamamı ile her biri sürüye ait Fis, Fit ve Fst değerleri*

Loci	General				ADÜ-ÇÇKP			EA			MV		
	N	Fis	Fit	Fst	N	Fis	Fit	N	Fis	Fit	N	Fis	Fit
OarCP34	242	-0.194	-0.075	0.100	154	-0.164	-0.164	62	-0.098	-0.098	26	-0.361	-0.361
OarFCB193	242	-0.187	-0.084	0.087	154	-0.095	-0.095	62	-0.174	-0.174	26	-0.305	-0.305
OarFCB304	242	-0.104	-0.068	0.033	154	0.026	0.026	62	0.031	0.031	26	-0.409	-0.409
OarJMP29	244	-0.169	-0.109	0.051	154	-0.072	-0.072	62	-0.114	-0.114	28	-0.329	-0.329
OarFCB128	234	-0.064	0.031	0.089	150	0.176	0.176	62	-0.028	-0.028	22	-0.445	-0.445
BM8125	238	0.011	0.040	0.029	152	-0.002	-0.002	62	-0.068	-0.068	24	0.120	0.120
OarJMP58	244	0.086	0.173	0.096	154	-0.006	-0.006	62	-0.042	-0.042	28	0.277	0.277
OarVH72	244	-0.067	-0.026	0.038	154	0.037	0.037	62	-0.152	-0.152	28	-0.116	-0.116
MAF65	222	0.249	0.301	0.070	142	0.290	0.290	62	0.105	0.105	18	0.379	0.379
DYMS1	240	0.502	0.511	0.018	148	0.336	0.336	64	0.438	0.438	28	0.779	0.779
Mean	239	0.007	0.068	0.062	152	0.053	0.053	62	-0.008	-0.008	25	-0.036	-0.036

some of the other researches [20,21]. The size of Çine Çaparı population is very small, so it has led to a low number of alleles. In addition, differences between the literature and the present study are natural due to the use of different breeds and using a number of microsatellites.

In conservation studies, the most important criterion to decide the priority of the breed is the high level of heterozygosity that it exhibits. The average heterozygosity values (Ĥ) (ranging between 0.737-0.885) were higher than reported studies in other sheep breeds [22-26]. These results occurred due to high heterozygosity levels in the studied loci. In addition to the high genetic diversity in this breed is one of the supporting observations for the proximity of the breeds to the centre of domestication.

Genetic distance and genetic similarity values obtained from the study were similar to the values in a previous study conducted for the same genotype [27].

It is understood from *Fig. 2* that among all the individuals in every flock a group formed between themselves. Individuals of the MV flock seem to have a relatively higher level of decomposition of the other flocks. Although ADÜ-ÇÇKP and EA flocks show dense clustering, some of the animals involved themselves between the two clusters, some others entered into other clusters depending on the brood transfers among the flocks. The results obtained from factorial corresponding analysis indicated that clustering will become clearer if we use a large number of microsatellites for identification.

Four loci in the ADÜ-ÇÇKP flock, 4 loci in the EA flock and 3 loci in the MV flock were not in the Hardy-Weinberg (HW) equilibrium in the studied population. This situation may have occurred as a result of a controlled reproduction program for many years to prevent inbreeding in this population.

All Fis values obtained from the studies showed that pure breeding did not apply in these flocks. Although Fst values diverged from 1, this value was 0 at the basis of the flocks. This result points out that individuals came from a common ancestor in terms of these loci.

The minimum allele number was observed in the MV flock. This result is natural due to the fact that the population number has decreased dramatically in the MV flock.

When *Table 6* is examined, the MAF65 and DYMS1 loci, which had high null allele frequency, should not be used in genetic diversity studies in this population to prevent incorrect interpretation of results.

The spread of specific alleles in all populations can be ensured by the transfer of rams among flocks. In this way, unique or private alleles will be spread over all populations.

The present study results indicate that although the Çine Çaparı sheep population size is very small, genetic diversity was significantly high in the gene pool. Results show that the conservation flock founded at Adnan Menderes University has higher genetic diversity than the other 2 farmers' flocks according the results of 10 loci studied. These results stem from the establishment of the conservation flock with animals from different flocks and from implementation of planning mating for many years according to relationships between members of the flock. Maintenance of controlled breeding practices considering pedigree and molecular genetic data and transferring studs between flocks will maintain genetic diversity in this breed in the future.

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