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RESEARCH ARTICLE

Analysis of Genetic Polymorphism with Microsatellite Method in Turkey Local Sheep Breeds

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Summary

In this study, genetic relationships within and between the sheep breeds of Turkey called Bafra, İvesi, Karayaka, Morkaraman, Sakız and Turkish Merino was determined by microsatellite method. All breeds were screened for OarCP34, MAF65, MAF209 and DYMS1 loci by using ABI PRISM 310 sequencer. The obtained data were evaluated by using "Genetix 4.05" and " Populations 1.0" statistics programs. It was determined that the Turkish Merino breed had the highest heterozygosity. The shortest genetic distance was found between Sakız and Morkaraman breeds and the longest genetic distance was observed between Turkish Merinos and İvesi sheep breeds. As a result, in this study, genetic characterization in Turkey Local Sheep Breeds called Bafra, Sakız, Karayaka, İvesi and Turkish Merino breeds were made by using microsatellite markers.

Keywords: *Microsatellite, Sheep, PCR, Polymorphism, DNA*

Türkiye Yerli Koyun Irklarında Genetik Polimorfizmin Mikrosatelit Yöntemi ile Analizi

Özet

Bu çalışmada, Türkiye'nin Bafra, İvesi, Karayaka, Morkaraman, Sakız ve Türk Merinosu olarak adlandırılan 6 yerli koyun ırkında ırk içi ve ırklar arasındaki genetik ilişki mikrosatellit analizi ile belirlendi. Tüm ırklar OarCP34, MAF65, MAF209 and DYMS1 lokusları için ABI PRISM 310 sekans cihazı kullanılarak tarandı. Elde edilen bütün veriler "Genetix 4.05" ve "Populations 1.0" istatistik programları kullanılarak değerlendirildi. Türk Merinosu ırkının en yüksek heterozigotluğa sahip olduğu belirlendi. En kısa genetik mesafe Sakız ve Morkaraman ırkları arasında gözlendi ve en uzak genetik mesafe ise Türk Merinosu ve İvesi ırkları arasında gözlendi. Sonuç olarak, bu çalışmada Bafra, Sakız, Karayaka, İvesi ve Türk Merinosu isimli Türkiye yerli koyun ırklarında mikrosatellit belirleyicileri kullanılarak genetik karakterizasyon yapıldı.

Anahtar sözcükler: *Mikrosatellit, Koyun, PCR, Polimorfizm, DNA*

INTRODUCTION

Turkey which has seven different regions in terms of geographical location and climatic characteristics is a rich country in biological diversity. This biodiversity is threatened with disappearance. All over the world for the protection of genetic resources in gene bank was created under the leadership of FAO (Food and Agriculture Organization). To make these studies based on genetic characterization and to protect animal genetic resources, it requires to follow and apply the rapidly developing technology and implementation of needs to be closely monitored. This type of breed studies to determine priorities for protection has a great importance.

There are different methods for the identification of the genetic structure of animal breeds. In the literature, analysis of microsatellites (STRs, short tandem repeats) is one of the effective methods to determine the genetic characterization of a population [1,2]. It has been used in molecular genetic analysis of farm animals and in the population genetics studies because of having codominant inheritance model and being highly polymorphic [3]. In recent years, these determinants have been used frequently in analysis of genetic resources also in Turkey. However, there are very limited data from molecular analysis about local sheep breeds, which have a great importance for Turkish

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economy. Microsatellite markers used in the present study are highly polymorphic for the molecular genetic analysis of some sheep breeds in Turkey.

The aim of this study is to determine the genetic structure and the genetic variation of the breeds. Also aimed to identified the presence of breed-specific alleles that can be used in characterization of the breeds.

MATERIAL and METHODS

In the presented study, use of microsatellite markers for six local sheep breeds of Turkey, genetic diversity within and among breeds and distances between each other were investigated genetically.

Samples

In collection of samples, the selection of animals that reflect features of breed mostly in terms of morphological and have non-relations and kinship were cared to be selected. Blood samples were taken into vacuum-EDTA tubes by veterinarians. Samples has been reached to the laboratory environment with cold chain in 48 hours. Distribution of breeds according to their numbers and the regions taken from was given in *Table 1*.

DNA Isolation

DNA isolation procedure was performed with Fermentas Genomic DNA Purification Kit ® by taking blood samples as belonging to racial. The resulting DNA samples were then stored in -20°C.

Microsatellite Analysis

In selection of used primers, it was considered to be studied previously and designated as polymorphic [4,5]. Selected loci (OarCP34, MAF65, DYMS1, MAF209) were amplified using the multiplex technique. Name, origin, sequence, chromosome number and the color of these loci were also shown in *Table 2*. The reaction mixture was performed for each locus, which was consisting 1X PCR buffer, 200 mM dNTPs, 2.0 mM $MgCl_2$, 3 pmol from each primer, 0.5 U Taq DNA Polymerase for a sample, 100-200 ng genomic DNA in total volume of 15 µl. The PCR cycle conditions for DYMS1 and OarCP34 loci; 3 min denaturation step at 94°C, followed by 35 cycles of 20 s at 94°C denaturation, 20 s at 57°C, and 40 s at 72°C extension with a final extension step of 10 min at 72°C.

Obtaining of Data and Statistical Analysis

After amplified PCR products were checked in 1.7% agarose gel, was installed in the ABI Prism 310 genetic analyzer device. The loci have been marked in different colors and used to determine the lengths with the help of standard lengths marker named Tamra (Taqman® Probe Tamra TM). By using the GeneScan program, forming a portion of the device for each locus, allelic lengths were recorded. The obtained data were evaluated by Genetix 4.05 software [6] and Populations 1.0.

Table 1. *Distributions of breeds according to numbers and collected regions*
Tablo 1. *Irkların sayılarına ve alındığı bölgelere göre dağılımları*

Name of Breed	Number of Samples	Regions of Samples Collected
İvesi	18	From the herds around Urfa and region
Morkaraman	13	Van, Production Farm of Altındere
Turkish Merino	15	Bursa, Haras of Karacabey
Sakız	12	From the herds around Çeşme and region
Karayaka	14	From the herds around Giresun and region
Bafra	14	Gökhöyük Agriculture Management, Amasya
Total	86	

Table 2. *Name, origin, sequence, chromosome number and the color of used the primers*
Tablo 2. *Kullanılan primerlerin isim, orjin, dizi, kromozom sayısı ve rengi*

Primer Name	Sequences (5'-3')	Origin	Chromosome Number	Fluorochorome Dye
OarCP34	GCTGAACAATGTGATATGTTCAGG GGGACAATACTGTCTTAGATGCTGC	Bovine	11	HEX
MAF65	AAA GGC CAG AGT ATG CAA TTA GGA G CCA CTC CTC CTG AGA ATA TAA CAT G	Ovine	26	FAM
DYMS1	AACAACATCAAACAGTAAGAG CATAGTAACAGATCTTCCTACA	Bovine	20	HEX
MAF209	GATCACAAAAAGTTGGATACAACCGTGG TCATGCACTTAAGTATGTAGGATGCTG	Ovine	17	NED

RESULTS

In this study Bafra, Sakız, Karayaka, İvesi, Morkaraman and Turkish Merino breeds of Turkey were analyzed by four microsatellite loci. Totally, 21 allelles have been observed. Most of the alleles have been noted for the Turkish Merino sheep breed. Additionally, Turkish Merino sheep breed with the highest heterozygosity, includes six alleles among the specific nine alleles for MAF209 and OarCP34 loci. The other three of the specific alleles were observed in the Bafra breed for MAF65 locus.

The avarage observed (Ho) and expected (He) heterozigosity values were the highest for MAF209 locus in all of the breeds (0.961-0.596). The lowest avarage Ho and He values were observed in OarCP34 locus (0.250-0.178). The observed and expected heterozygosity values for Bafra, İvesi, Karayaka, Morkaraman, Sakız and Turkish Merino breeds for MAF209, MAF65 and OarCP34 loci are given in *Table 3*. Observed heterozygosity value could not be calculated for DYMS1 because of being single-locus allele.

In the current study, it was observed that in Bafra, İvesi, Karayaka, Morkaraman, Sakız and Turkish Merino breeds the total F_{IS}, F_{IT}, and F_{ST} values as - 0.386, - 0.098 and 0.208 respectively for MAF209, MAF65, OarCP34 and DYMS1 loci. For each breed, F_{IS} values ranged from - 0.149 to - 0.814. These values are given in *Table 4*.

Table 3. *Observed and expected heterozigosity values and mean for MAF209, MAF65, OarCP34 loci in the six sheep breeds*
Tablo 3. *Altı koyun ırkında MAF209, MAF65, OarCP34 lokusları için gözlenen ve beklenen heterozigotluk değerleri ve ortalamaları*

Breed / Locus	MAF209		MAF65		OarCP34	
	Ho	He	Ho	He	Ho	He
Bafra	1.000	0.753	0.643	0.667	0.643	0.436
İvesi	1.000	0.500	0.059	0.057	0.056	0.054
Karayaka	1.000	0.500	0.308	0.269	0.000	0.000
Morkaraman	0.900	0.495	0.600	0.420	0.000	0.000
Sakız	1.000	0.500	0.333	0.278	0.000	0.000
Turkish Merino	0.867	0.829	0.733	0.624	0.800	0.576
Mean/breed	0.961	0.596	0.446	0.386	0.250	0.178

Table 4. *Observed FIS values in the six sheep breeds for MAF209, MAF65 ve OarCP34 loci*
Tablo 4. *MAF209, MAF65 ve OarCP34 lokusları için altı koyun ırkında gözlenen FIS değerleri*

Breed	F_{IS}
Bafra	-0.197
İvesi	-0.814
Karayaka	-0.679
Morkaraman	-0.607
Sakız	-0.692
Turkish Merino	-0.149

Estimated F_{ST}, which was calculated to determine the genetic differences between breeds, was found the lowest (-0.010) between Karayaka-Sakız breeds. The highest F_{ST} value was observed between Turkish Merino and İvesi breeds (0.364).

According to the Nei's standard genetic distances values (DA), as the most genetic distance value was observed between Turkish Merino and İvesi sheep breeds (0.302). The closest genetic distance (0.004) was observed between Sakız and Morkaraman sheep breeds. F_{ST} matrix calculated by binary comparison of breeds and the DA values were also given in *Table 5*.

Table 5. *In Bafra, İvesi, Karayaka, Morkaraman, Sakız ve Turkish Merino for MAF209, MAF65 ve OarCP34 loci F_{ST} (above diagonal) and D_A (below diagonal) values*
Tablo 5. *Bafra, İvesi, Karayaka, Morkaraman, Sakız ve Türk Merinosu ırklarında MAF209, MAF65 ve OarCP34 lokusları için F_{ST} (üst diyagonal) ve D_A (alt diyagonal) değerleri*

Breeds	Bafra	İvesi	Karayaka	Morkaraman	Sakız	Turkish Merino
Bafra	****	0.27293	0.22267	0.20660	0.21782	0.06858
İvesi	0.170445	****	0.01345	0.09593	0.02534	**0.36435**
Karayaka	0.196917	0.0185288	****	0.02066	**-0.00997**	0.30024
Morkaraman	0.24387	0.0477441	0.0113966	****	0.00768	0.25759
Sakız	0.230059	0.0286601	0.00540146	**0.00347113**	****	0.28574
T. Merino	0.177278	**0.302187**	0.26992	0.289516	0.296582	****

Another important finding was that neighbor joining method (NJT) generated by the tree in three main groups were observed in the six breeds for the MAF209, MAF65 and OarCP34 loci. According to the neighbor joining tree, Morkaraman breed with Sakız breed is found in the same group. İvesi, Turkish Merino and Bafra were observed in second group. However, Bafra and Turkish Merino were nearest to each other *(Fig. 1)*.

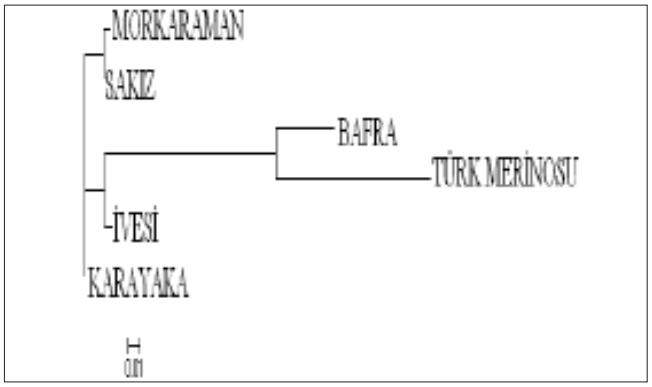

Fig 1. Formed according to the D_A values for Nei'nin Neighbor Joining Tree (NJT)

Şekil 1. Nei'nin D_A değerlerine göre oluşturulan Komşu Birleştirme Ağacı (NJT)

DISCUSSION

There are several research analysis of local sheep breeds of Turkey with the microsatellite method. For example, Soysal et al.[7], scanned 3 microsatellite loci in five breeds and Gutierrez-Gil et al.[8], scanned total of 30 microsatellite locus in five local sheep breeds which also included the populations of Karayaka and Morkaraman. Uzun et al.[9] also investigated 30 microsatellite loci for five Turkey sheep breeds. In the current study, genetic relationships within and between the sheep breeds of Turkey called Bafra, İvesi, Karayaka, Morkaraman, Sakız and Turkish Merino was determined with microsatellite method. All breeds were screened for OarCP34, MAF65, MAF209 and DYMS1 loci. Also, for the first time, Bafra and Sakız sheep breeds were analyzed using the microsatellite method in the present study.

Soysal et al.[7] have scanned the three microsatellite locus including MAF65 locus in five local sheep breeds of Turkey. İvesi sheep breed was scanned and as in the present study. Koban [10], was scanned 5 locus including MAF209 locus in twelve breeds which included Morkaraman, İvesi and Karayaka breeds. In another study made by Arranz et al.[11], 18 microsatellite loci were scanned in six local spanish sheep breeds including MAF65 and OarCP34 loci. Dalvit et al.[12]. Gutierrez-Espeleta et al.[13] and Arora et al.[14] also evaluated the loci. Breeds and loci that are common to the above-mentioned studies are evaluated, the number of alleles observed and heterozigosity values were lower in the present study. For example, the researches reported that they obtained typically 10 alleles for locus MAF65. However, in the current study was observed six alleles for the locus. Similarly, heterozigosity values were lower in the present study. For instance, Gutierrez-Gil et al.[8] have scanned a total of 30 microsatellite locus in five local sheep breeds totally, including Morkaraman and Karayaka breeds. They have found that He value was 0.726 for Morkaraman sheep breed and 0.720 for Karayaka sheep breed. In the current study, He values were found as 0.229 and 0.192, respectively. When we compaire the results, there is a decrease in value of He. The reason for this results may be releated to number of breed studied and number of loci screened. In addition to this, collection of blood samples from farm state may be one of the reason to have low heterozigosity due to probable inbreeding depression in the populations.

F-Statistical Values and Genetic Distance

In this study, F statistics values which are the coefficient of racial and inter-racial breeding with relatives was determined that F_{IS}: -0.3859, F_{IT}: -0.0978 and F_{ST}: 0.2079 in all breeds for MAF209, MAF65, OarCP34 and DYMS1 locus. In the current study, F_{IS} values were observed between -0.1494 and -0.8138 for all the breeds. These values were insignificant in permutation test (1000) and there was no deviation from Hardy-Weinberg equilibrium. Gutierrez-Gil et al.[8] recorded the F_{IS} value as 0.041 for Morkaraman breed, and as 0.058 for Karayaka breed which are in agreement with those of the current study and this values were found as -0.679 for Karayaka and as -0.607 for Morkaraman breed. The results were similar to each other according to the F parameters.

According to F_{ST} value calculated, genetic differences were observed at most between Turkish Merino and İvesi sheep breeds (0.364). The F_{ST} value was observed at least between Sakız and Karayaka sheep breeds (-0.010).

Original Alleles

Arranz et al.[11] reported that Merinos breed has the most genetic diversity among the six local Spain sheep breeds for 18 microsatellite loci. Similarly, in the present study, Turkish Merino was determined as breed which has the most genetic diversity. Morever, in the present study six of total nine breed-specific alleles for the all breeds, was observed in the Turkish Merino breed. But, frequencies of the specific alleles were generally smaller than 0.2. Therefore, low values of the frequency specific alleles do not allow reliably distinctive characterization of the breeds.

Unification Factorial Analysis (FCA)

When the results of factorial combination analysis are examined, it is seen that breeds are not fully separated from each other in three-dimensional plane. Although Turkish Merinos and Bafra breeds are separated from other

breeds in plane, the other breeds are observed closely. To separate individuals from each other belonging to breeds and to group according to the origin, it is necessary to evaluate more locus and samples. In the literature, limited success has been obtained in discrimination of breeds which characterizes in many studies.

In the literature, it shows that the data obtained from molecular analysis of sheep breed is associated with the number of populations, samples and locus. The greater the number of these parameters increases the ability of making the genetic discrimination of breeds. It is planned to enlarge the study by increasing the number of breeds samples and locus in the future.

Turkey, still at the beginning of molecular genetic analysis for livestock, needs knowledge obtained from similar molecular genetic analysis. It is also important in determining the priority of the breeds which must be taken under protection. In this respect, data belong to animal genetic resources of Turkey need to be increased.

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