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Genetic relationships among four Turkish sheep breeds using microsatellites

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Abstract: The genetic structure of 4 Turkish breeds of sheep, Gökçeada, Kıvırcık, Karacabey Merino, and Sakız (SZ), was analyzed by genotyping with 17 microsatellite markers in 250 individuals. The microsatellites showed high levels of polymorphism, with means for total and effective number of alleles per locus of 20.71 and 7.04, respectively. The average observed and expected heterozygosity values were 0.66 and 0.87, respectively. It was found that a higher level of breed admixture existed in the 4 breeds, which was an important finding. It was also found that 7.9% of genetic variation resulted from difference between the populations, whereas 92.1% resulted from difference between individuals. Our results showed that the used microsatellite markers were adequately polymorphic and can be successfully used for investigating genetic diversity in the 4 breeds studied. The genetic variation in Turkish breeds was not significantly higher than that of European breeds, which might be a consequence of the recent sharp decrease in sheep numbers.

Key words: Genetic diversity, microsatellite, sheep, native sheep breeds

1. Introduction

Turkey's proximity to domestication centers, along with its climate, topographical pattern, and agricultural structure that is formed according to plant cover, has created an appropriate environment for sheep breeding. Therefore, there are many sheep breeds in various regions and territories (1,2). Although there are many varieties and crossbred populations of sheep breeds in Turkey, 20 sheep breeds have been officially registered by the Ministry of Food, Agriculture, and Livestock (3). Even though Gökçeada (GA), Kıvırcık (KIV), Karacabey Merino (KM), and Sakız (SZ) sheep breeds, which are raised in the western Anatolian and Marmara regions, have a low percentage of native sheep population, each breed has certain significant characteristics. SZ sheep, known for their high fertility and milk production, and GA sheep, known for their milk production and high lamb survival rate, are raised along the coastline of the Aegean region. KIV and KM sheep breeds are known for their meat quality, wool, and meat production and are raised in western Turkey. For the purpose of improving the meat quality and fertility of the sheep population in western Turkey, the SZ and KIV breeds are commonly used in crossbreeding (4).

Molecular genetic methods for identifying genetic structure and diversity in farm animals have shown a rapid development in recent years and have become widely used. Various molecular genetic methods have been developed for this purpose. Microsatellites that are specific to DNA regions are more widely used than other methods (5).

This study aims to determine the genetic structure of GA and SZ sheep breeds, which are bred on the coastline of the Aegean region, and that of the KIV and KM sheep breeds, which are commonly bred in the Aegean and Marmara regions, by means of microsatellites. The information obtained from this study is based on microsatellite markers and will clarify the genetic relationships between these sheep breeds. It will also be helpful for determining current and future breeding programs and breed management and conservation strategies.

2. Materials and methods

2.1. Animal resources and DNA isolation

The animal material of the study consisted of a total of 250 animals belonging to GA, KIV, KM, and SZ sheep populations bred in the Aegean and Marmara regions. The origins and sample sizes of these 4 breeds are presented in Table 1. A DNA isolation kit (Applied Biological Materials Inc., Canada) was used for DNA extraction from blood samples.

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Table 1. Origin and size of samples from GA, KIV, TM, and SZ sheep breeds.

Breeds	N	Location	Flock type	Flock
GA	49	SRS*	GRCF**	1
KIV	69	Uşak, Turkey	Breeder's farm	12
KM	91	SRS	Nucleus flock	1
SZ	41	SRS	GRCF**	1

*SRS: Sheep Research Station, Bandırma, Turkey. **GRCF: genetic resource conservation flock.

2.2. Polymerase chain reaction and fragment analysis

Seventeen bovine, ovine, and caprine microsatellite markers were selected according to the recommendations of the Food and Agriculture Organization of the United Nations (6). Two multiplex groups were formed with 17 microsatellites. The first and second multiplex groups consisted of 9 (BM1818, D5S2, INRA0023, INRA0132, OARAE0129, OARCP34, OARFCB193, OARFCB20, and OARFCB304) and 8 (BM1329, BM8125, CSRD0247, HSC, MAF214, McM0527, OARFCB128, and OARJMP29) microsatellites, respectively.

Polymerase chain reaction (PCR) amplifications were carried out in 25-µL total volumes, each containing 0.10 µM of each primer (with the forward primer labeled as D2, D3, or D4), 0.20 mM dNTPs (Applied Biological Materials Inc., Canada), 2.0 mM $MgCl_2$, 1X PCR buffer, 1 U of Taq DNA polymerase (Applied Biological Materials Inc.), and ~50 ng of DNA. Specific genomic regions were amplified by using different touchdown PCR protocols for each multiplex group (Table 2).

Fluorescently labeled PCR fragments were separated by capillary electrophoresis in the GenomeLab GeXP Genetic Analysis System (Beckman Coulter, Inc., USA).

Table 2. Thermal cycling conditions used for touchdown PCR.

Loci	Multiplex group	First denaturation	Denaturation	Annealing	Extension	Cycles	Final extension
BM1818	1	95 °C (5 min)	95 °C (40 s)	63–54 °C (40 s)	72 °C (60 s)	40	72 °C (10 min)
D5S2							
INRA0023							
INRA0132							
OarAE0129							
OarCP34							
OarFCB193							
OarFCB20							
OarFCB304							
BM1329	2	95 °C (5 min)	95 °C (40 s)	60–50 °C (40 s)	72 °C (60 s)	34	72 °C (10 min)
BM8125							
CSRD0247							
HSC							
MAF214							
McM0527							
OarFCB128							
OarJMP29							

2.3. Statistical analysis

Total number of alleles per locus (TNA), mean number of alleles (MNA), effective number of alleles (NE), polymorphic information content (PIC), observed heterozygosity (Ho), expected heterozygosity (He), average heterozygosity ($\hat{H}$), and Hardy–Weinberg equilibrium were calculated using GenAlEx (7) and Arlequin 3.0 (8). For the breed genetic distance dendrogram that was drawn with MEGA 4 software (9) according to Nei's minimum genetic distance matrix (10), the bootstrap resampling methodology (1000 replicates) was performed to test the robustness of the dendrogram topology. Wright's F-statistics (F_{IT}, F_{IS}, F_{ST}) (11) were calculated with POPGENE (12). Nei's gene diversity (H_T), diversity between breeds (D_{ST}), and coefficient of gene differentiation (G_{ST}) values were calculated with FSTAT 2.9.3 (13). The genetic structure of the populations was investigated using STRUCTURE (14). Analysis was performed with a burn of 20,000 in length, followed by 100,000 Markov chain Monte Carlo iterations for each from K = 2–4, with 20 replicate runs for each K, using independent allele frequencies and an admixture model. Evanno's method (15) was used to identify the appropriate number of clusters using ΔK, based on the rate of change in the log probability of the data.

3. Results

A total of 352 alleles from 17 microsatellite loci were observed. The number of alleles ranged from 15 (OARCP34) to 31 (MAF214), and the mean number of alleles per locus was 20.71. The mean number of alleles per locus was 12.29. The effective number of alleles varied from 3.32 (BM1329) to 11.89 (OARJMP29). PIC values were found to be between 0.68 and 0.91 (Table 3). The $\hat{H}$ value for all loci studied was 0.77. The highest observed Ho value was observed in the MAF214 locus (0.83), and the

Table 3. Allelic range, total number of alleles per locus (TNA), mean number of alleles (MNA), effective number of alleles (NE), polymorphic information content (PIC), Wright's F-statistics (F_{IT}, F_{IS}, F_{ST}), observed heterozygosity (Ho), expected heterozygosity (He), average heterozygosity ($\hat{H}$), diversity between breeds (D_{ST}), coefficient of gene differentiation (G_{ST}), and Nei's gene diversity (H_T) for each locus and all loci in KM, KIV, GA, and SZ breeds.

Loci	Allelic range (bp)	TNA	MNA/ locus	NE	PIC	F_{IS}*	F_{IT}*	F_{ST}*	Ho	He	$\hat{H}$	D_{ST}	G_{ST}	H_T
OarFCB304	146–192	23	14.00	5.17	0.79	0.355	0.437	0.127	0.46	0.81	0.73	0.100	0.118	0.841
OarFCB193	96–136	20	12.25	5.20	0.79	-0.068	-0.032	0.034	0.81	0.81	0.77	0.022	0.027	0.791
INRA0023	187–251	20	12.50	8.57	0.87	0.548	0.629	0.180	0.36	0.88	0.74	0.151	0.167	0.899
OarFCB20	83–127	20	11.50	6.71	0.84	-0.045	0.088	0.127	0.73	0.85	0.73	0.101	0.120	0.870
OarAE0129	127–169	16	8.25	3.56	0.68	0.408	0.437	0.048	0.42	0.72	0.67	0.027	0.038	0.708
BM1818	240–282	20	11.75	5.72	0.81	0.105	0.178	0.082	0.73	0.83	0.76	0.061	0.074	0.827
INRA0132	146–182	19	13.75	11.14	0.90	0.171	0.213	0.051	0.73	0.91	0.86	0.039	0.043	0.911
OarCP34	108–138	15	10.00	6.86	0.84	0.034	0.100	0.068	0.75	0.85	0.80	0.052	0.061	0.855
D5S2	164–214	16	8.50	4.25	0.73	0.295	0.369	0.105	0.50	0.76	0.69	0.074	0.096	0.770
CSRD0247	211–269	23	13.50	8.94	0.88	0.032	0.098	0.069	0.79	0.89	0.82	0.054	0.061	0.885
MCM0527	157–267	18	13.25	8.73	0.88	0.162	0.231	0.083	0.64	0.89	0.80	0.066	0.075	0.873
BM8125	104–146	19	13.00	7.65	0.86	0.076	0.145	0.075	0.72	0.87	0.81	0.059	0.068	0.874
HSC	261–301	21	13.00	7.02	0.84	0.133	0.179	0.053	0.68	0.86	0.82	0.036	0.041	0.868
BM1329	115–189	22	9.25	3.32	0.68	-0.055	0.108	0.155	0.63	0.70	0.62	0.106	0.143	0.736
OARFCB128	96–140	21	12.75	8.92	0.88	0.149	0.211	0.072	0.68	0.89	0.82	0.057	0.064	0.878
OARJMP29	94–178	28	17.00	11.89	0.91	0.143	0.210	0.079	0.68	0.92	0.84	0.065	0.071	0.913
MAF214	179–265	31	14.75	6.06	0.82	-0.066	0.015	0.076	0.83	0.83	0.76	0.057	0.070	0.822
Mean		20.71	12.29	7.04	0.82	0.137	0.212	0.087	0.66	0.87	0.77	0.066	0.079	0.842

*: Wright's statistics according to Weir and Cockerham (11).

lowest value was observed in the INRA0023 locus (0.36). The lowest and highest He values were 0.70 (BM1329) and 0.92 (OARJMP29), respectively. The lowest and highest F_{IS} values, which indicate the loss of heterozygosity and are important parameters in describing population characteristics, were found to be –0.068 (OARFCB193) and 0.548 (INRA0023), respectively.

The highest F_{ST}, which was calculated to determine the genetic differences between breeds, was observed in INRA0023 (0.180). The F_{IT} value, which represents general heterozygosity loss, was observed in the range from –0.032 (OARFCB193) to 0.629 (INRA0023). The mean value of Nei gene diversity (H_T), between-breed diversity value (D_{ST}), and coefficient of gene diversity (G_{ST}) were determined as 0.842, 0.066, and 0.079, respectively (Table 3).

The results of the breed-based evaluation are summarized in Table 4. The highest value in terms of mean allele number was seen in the KM breed (15.18). F_{IS} values changed between 0.1100 (SZ) and 0.2490 (KM).

Nine loci in the GA breed, 14 in the KIV breed, and the loci studied in KM and SZ breeds did not fit Hardy–Weinberg equilibrium. A total of 115 private alleles were observed in the GA, KIV, KM, and SZ breeds. However, only 7 had a frequency higher than 5%. The highest number of alleles among breeds was found in the KM breed.

When the dendrogram (Figure 1) was examined, 2 different groups emerged with KM–KIV and GA–SZ breeds belonging to the same groups.

The results of STRUCTURE analysis containing different numbers of clustering are given in Figure 2. The results obtained from structure analysis are similar to the dendrogram given in Figure 1. For the purpose of presenting the suitable cluster number (K) in structure analysis, the results are given in Table 5. The ΔK value, which was taken from 4 studied breeds, shows that the most suitable group number was 3 (K = 3).

4. Discussion

The total allele numbers, mean allele number per locus, effective number of alleles, and mean heterozygosity (0.87) observed in 17 microsatellite loci were higher than those reported in the literature (16–24). These findings indicate that the used microsatellite markers can be reliably used to measure genetic diversity for these breeds. The high level of heterozygosity observed can be explained by Turkey's geographic closeness to sheep domestication centers. In accordance with these findings, all loci used in this study were highly informative, as the PIC values indicated.

The mean values of F_{ST}, F_{IS}, and F_{IT} were higher than the values reported by Cemal et al. (18), Hoda and Marsan (20), and Santos-Silva et al. (22) and lower than the values reported by Agaviezor et al. (25). The mean F_{ST} value determined for all the loci can be accepted as a sign of the weakness of genetic diversity among the breeds. F_{IS} values showed that there was heterozygosity loss in 13 microsatellite markers, excluding OarFCB20, BM1329,

Table 4. Mean number of alleles (MNA), mean observed (Ho), and expected (He) heterozygosity, private alleles, within-breed heterozygote deficiency (F_{IS}), and number of loci not in Hardy–Weinberg equilibrium ($P < 0.05$) for each breed across 17 loci.

Breeds	N	MNA/ locus	Mean heterozygosity		F_{IS}	HWE	NPA		
			Ho (SE)	He (SE)			>5%*	<%5**	Total
GA	49	8.77	0.64 (0.325)	0.73 (0.160)	0.1130	9.00	2	7	9
KIV	69	12.82	0.74 (0.112)	0.81 (0.071)	0.1170	14.00	1	27	28
KM	91	15.18	0.57 (0.221)	0.78 (0.107)	0.2490	17.00	3	51	54
SZ	41	12.41	0.66 (0.205)	0.75 (0.098)	0.1100	17.00	1	23	24

NPA: Number of private alleles; *: frequency higher than >5%; **: frequency lower than <5%.

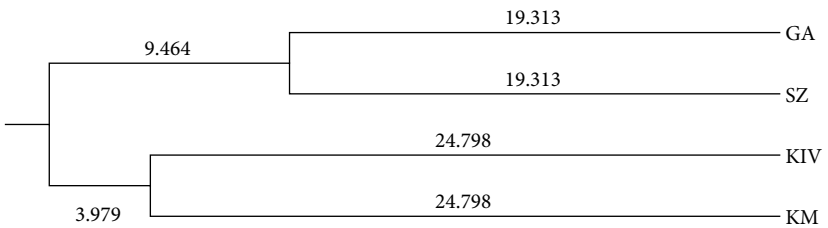

Figure 1. Dendrogram based on Nei's minimum genetic distances among 4 breeds (bootstrap resampling methodology (1000 replicates)).

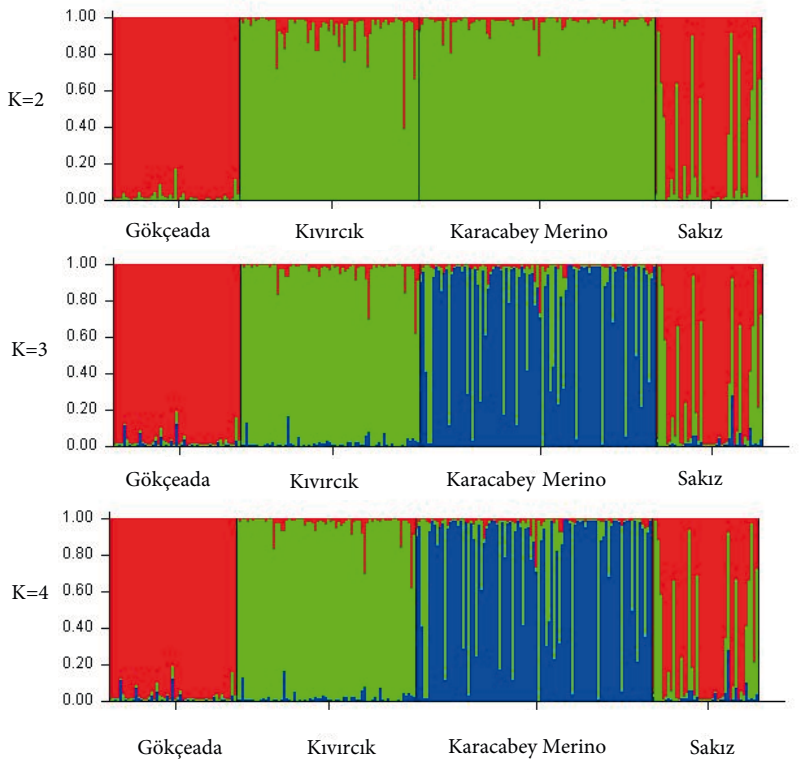

Figure 2. Estimation of the population structure with different K values (assuming K = 2 and 4).

Table 5. Estimated posterior probabilities [Ln Pr(X|K)] for different numbers of inferred clusters (K) and ΔK statistic.

K	[Ln Pr(X|K)]	ΔK
1	–16997.83	
2	–16076.04	1.751901
3	–15314.71	10.48248
4	–14802.39	

and MAF214 loci. The G_{ST} value indicates that 7.9% of total genetic variation resulted from the differences between the populations, whereas 92.1% can be explained by the difference between individuals.

Mean G_{ST} value observed in this study was higher than the values reported by Agaviezor et al. (25) and relatively lower than the values reported by Arora and Bhatia (17). The general mean D_{ST} value was found higher than the value reported by Hoda and Marsan (20). The D_{ST} value obtained from this study can be considered as an indicator of low genetic diversity between breeds. In this study, the obtained MNA value in terms of the breeds was found to be higher than in the literature (20,26,27). MNA was an indicator of within-breed diversity. The results show that obtained MNA values for the breeds were higher than in some other studies (19,27,28). Observed (Ho) and expected (He) heterozygosity values according to breeds were found to be higher than the values reported by Kusza et al. (29). The mean F_{IS} value observed in this study was lower than the values reported by Kusza et al. (29) and Yilmaz et al. (30), and higher than the values reported by Hoda and Marsan (20) and Budak Yıldıran and Çakır (31).

It was found that 17 loci in both the SZ and KM breeds, 14 loci in the KIV, and 9 loci in the GA breed did not fit Hardy–Weinberg equilibrium. This might be a result of the selection programs that have been carried out for several years for the KM and KIV breeds, and of the genetic conservation implemented for the GA and SZ breeds. The determined 7 private alleles can be said to have the property of being able to determine the breeds. Although the GA and SZ breeds were in the same group in this study, Acar (32) reported that these breeds were to be localized in different groups. The differences between the literature and the present study are expected due to the use of different samplings and a different number of microsatellites.

The contribution of the KIV breed to KM formation was clearly demonstrated with STRUCTURE analysis. However, it was interesting that two different native

breeds, GA and SZ, cannot be differentiated and show high genetic similarity, and this should be investigated further. It was understood from the dendrogram and the STRUCTURE analysis that the KM and KIV breeds were in the same group. KM was developed by a crossing of German Mutton Merino and KIV breeds (33). Considering this information, this is an expected finding.

The results of the structure analysis show a high level of breed admixture. ΔK values peaked at K = 3, indicating strong support for 3 groups. An examination of Figure 2 shows that GA and SZ breeds are in the same group and KM and KIV breeds are separated into 2 groups. The high genetic similarity between GA and SZ breeds raised on the coastline of the Aegean region has resulted in a suitable group number of 3 in STRUCTURE analysis.

Research on molecular genetics identification of Turkish sheep breeds has increased, particularly in recent years. However, there is limited literature on genetic diversity studies based on microsatellites. Therefore, this study will make a significant contribution to the literature. Our results showed that within-breed diversity was higher than between-breed diversity. This situation can be seen as an opportunity in terms of breeding programs and genetic conservation programs for these breeds. Our findings revealed that the microsatellite markers used in this study can be successfully used in genetic diversity studies.

In conclusion, the results obtained from this study showed that the used microsatellite markers can be used reliably to measure genetic variation for 4 native Turkish breeds. In addition, the results of this study, which was carried out on the KIV, SZ, GA, and KM sheep breeds, will contribute to the literature and serve as a basis for future studies.

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