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Phylogenetic relationships of native Turkish cattle breeds using microsatellite markers

Yusuf ÖZŞENSOY[1], Ercan KURAR[2,*], Müge DOĞAN[3], Zafer BULUT[3], Mehmet NİZAMLIOĞLU[3], Vahdettin ALTUNOK[3], Ayşe IŞIK[4], Aysun ÇAMLIDAĞ[4]

[1]Department of Veterinary Biometrics and Genetics, Faculty of Veterinary Medicine, Cumhuriyet University, Sivas, Turkey
[2]Department of Medical Biology, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey
[3]Department of Biochemistry, Faculty of Veterinary Medicine, Selçuk University, Konya, Turkey
[4]Eastern Mediterranean Agricultural Research Institute, Ministry of Agriculture and Forestry, Adana, Turkey

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Abstract: A total of 20 microsatellite DNA markers were used for genetic characterization and determination of phylogenetic relationships of native cattle breeds of Turkey, including the Anatolian Grey (AG), Anatolian Black (AB), South Anatolian Red (SAR), East Anatolian Red (EAR), Southern Anatolian Yellow (SAY), and Zavot (ZAV). DNA samples were isolated from 271 blood samples using an organic method. Amplified polymerase chain reaction products were separated by capillary electrophoresis and genotypes were determined for 20 microsatellites. A total of 269 different alleles were determined. The lowest (7.80) and highest (10.80) mean allele numbers were observed for the ZAV and SAY populations, respectively. TGLA122 was the most polymorphic locus; however, only 7 different alleles were observed for INRA005. A total of 40 different private alleles were determined. The general F_{IS} values were between 0.034 and 0.123. Due to the close location to the domestication center, higher genetic diversities were observed. The observed genetic diversities and the results of the phylogenetic analyses were in agreement with evolutionary history and the geographical origins of Turkish native cattle breeds.

Key words: Cattle, Anatolia, genetic diversity, microsatellite, TÜRKHAYGEN-1

1. Introduction

In order to determine the origin of breed domestications, genetic characterization and archaeological studies have been carried out. The migration routes of European breeds were reported to be two different paths from North to Central Europe along the coast of the Danube and the Mediterranean coast. In addition, cattle, sheep, goats, pigs, and buffalo were first domesticated in two different regions, including Southwest Asia and East Asia (1). The oldest of these centers contains the eastern and southeastern Anatolian regions. Previous archeological and molecular findings indicate that most animal breeds originated and spread from these regions to the rest of the world, especially from Anatolia to Europe (2–6). In particular, the main routes of cattle coming into Europe were determined to stem from the eastern region (7). Because of their closeness to this domestication center and their being relatives of the first cattle domesticates, Anatolian native breeds should be given priority as a genetic resource stock (1).

Genetic diversity studies can be performed on native breeds of a country and breeds of different countries or, more broadly, on international breeds (8). The objective of this study was phylogenetic analysis of Turkish cattle breeds by utilizing microsatellites as part of a national level project titled "*In Vitro* Conservation and Preliminary Molecular Identification of Some Turkish Domestic Animal Genetic Resources-1 (TÜRKHAYGEN-1)."

2. Materials and methods

A total of 271 blood samples were collected from Anatolian Black (AB, n = 51), Anatolian Grey (AG, n = 54), East Anatolian Red (EAR, n = 45), Native Southern Anatolian Yellow (SAY, n = 51), South Anatolian Red (SAR, n = 51), and Zavot (ZAV, n = 19) cattle. Genomic DNA was isolated using a standard organic method (9).

In the study, 20 microsatellite loci (Table 1) were selected from a list (10) recommended by the Food and Agriculture Organization's Measurement of Domestic Animal Diversity (FAO–MoDAD) and the International Society of Animal Genetics (ISAG). Based on PCR product sizes and conditions, three different multiplex systems were developed. The PCR profiles and microsatellite genotyping procedures have been previously described

* Correspondence: ekurar@erbakan.edu.tr

Table 1. Microsatellite loci used in the study.

No.	Locus	Chromosome	Primer	Allele	Label*
1	BM1824	1	GAGCAAGGTGTTTTTCCAATC	170–218	D2
			CATTCTCCAACTGCTTCCTTG		
2	BM2113	2	GCTGCCTTCTACCAAATACCC	116–146	D4
			CTTAGACAACAGGGGTTTGG		
3	INRA023	3	GAGTAGAGCTACAAGATAAACTTC	193–235	D3
			TAACTACAGGGTGTTAGATGAACTCA		
4	ETH10	5	GTTCAGGACTGGCCCTGCTAACA	198–234	D4
			CCTCCAGCCCACTTTCTCTTCTC		
5	ILSTS006	7	TGTCTGTATTTCTGCTGTGG	277–309	D4
			ACACGGAAGCGATCTAAACG		
6	HEL9	8	CCCATTCAGTCTTCAGAGGT	141–173	D3
			CACATCCATGTTCTCACCAC		
7	ETH225	9	GATCACCTTGCCACTATTTCCT	135–165	D2
			ACATGACAGCCAGCTGCTACT		
8	CSRM60	10	AAGATGTGATCCAAGAGAGAGGCA	79–115	D4
			AGGACCAGATCGTGAAAGGCATAG		
9	HEL13	11	TAAGGACTTGAGATAAGGAG	178–200	D4
			CCATCTACCTCCATCTTAAC		
10	INRA005	12	CAATCTGCATGAAGTATAAATAT	135–149	D4
			CTTCAGGCATACCCTACACC		
11	CSSM66	14	ACACAAATCCTTTCTGCCAGCTGA	171–209	D4
			AATTTAATGCACTGAGGAGCTTGG		
12	SPS115	15	AAAGTGACACAACAGCTTCTCCAG	235–265	D4
			AACGAGTGTCCTAGTTTGGCTGTG		
13	TGLA53	16	GCTTTCAGAAATAGTTTGCATTCA	143–191	D3
			ATCTTCACATGATATTACAGCAGA		
14	ETH185	17	TGCATGGACAGAGCAGCCTGGC	214–246	D3
			GCACCCCAACGAAAGCTCCCAG		
15	TGLA227	18	CGAATTCCAAATCTGTTAATTTGCT	64–115	D4
			ACAGACAGAAACTCAATGAAAGCA		
16	ETH03	19	GAACCTGCCTCTCCTGCATTGG	90–135	D2
			ACTCTGCCTGTGGCCAAGTAGG		
17	TGLA126	20	CTAATTTAGAATGAGAGAGGCTTCT	104–131	D3
			TTGGTCTCTATTCTCTGAATATTCC		
18	TGLA122	21	CCCTCCTCCAGGTAAATCAGC	134–193	D3
			AATCACATGGCAAATAAGTACATAC		
19	BM1818	23	AGCTGGGAATATAACCAAAGG	248–278	D2
			AGTGCTTTCAAGGTCCATGC		
20	HAUT27	26	TTTTATGTTCATTTTTTGACTGG	120–158	D2
			AACTGCTGAAATCTCCATCTTA		

*WellRED dye labels: D4 = blue; D3 = green; D2 = black.

(11). Capillary electrophoresis was conducted using the FragTest-3 protocol and Beckman Coulter CEQ-8000 genetic analysis system. The FragTest program was used to determine alleles.

General population parameters including allele numbers and Wright's F-statistics were calculated using GenAlEx6 (12). The GENETIX 4.05 program (http://www.genetix.univ-montp2.fr/genetix/genetix.htm) was used for factorial correspondence analysis (FCA). Population matrices of genetic distances were calculated according to Nei (13), using the Population 1.2.32 program (http://www.bioinformatics.org/project/?group_id=84). TreeView (14) was used to draw a phylogenetic tree using the neighbor-joining (NJ) method and Nei's D_A genetic distances. For analysis of the population structure, 7 independent runs of K (K = 1–7) were conducted for the genotype dataset using an admixture model. All model runs were based on 100,000 Markov chain Monte Carlo (MCMC) iterations and on 50,000 after an initial burn-in period. Five independent runs were performed for each K-value using the Structure 2.3.4 http://pritch.bsd.uchicago.edu/structure_software/releas.

Ethical approval was issued by the Selçuk University Faculty of Veterinary Medicine Ethics Committee (19.11.2007, #2007/063).

3. Results

General population genetics analyses (Table 2) showed that 269 different alleles were determined, and the mean allele number was 13.45. The minimum (7 alleles) and maximum (26 alleles) numbers of total alleles were determined for INRA005 and TGLA122, respectively. A total of 40 private alleles were detected. The maximum numbers of private alleles (10 alleles) were observed in the SAY population.

General F_{IS} values were calculated for the SAR (0.063), AB (0.063), AG (0.123), SAY (0.061), EAR (0.034), and

Table 2. Mean and total number of alleles of six Turkish cattle breeds.

	Populations							
Locus	SAR	AB	AG	SAY	EAR	ZAV	Mean	Total
CSSM66	13	13	12	13	13	9	12.17	14
CSRM60	10	13	11	12	7	6	9.83	15
ETH03	10	11	10	11	10	11	10.50	14
INRA023	13	10	10	11	10	9	10.50	14
HEL9	11	12	12	14	11	10	11.67	16
ILSTS006	11	11	10	9	8	5	9.00	13
SPS115	9	10	8	9	8	6	8.33	10
ETH185	12	12	12	13	10	9	11.33	17
BM1818	8	10	10	11	8	7	9.00	13
ETH225	13	11	8	9	10	10	10.17	13
ETH10	8	8	8	9	7	5	7.50	9
TGLA53	18	14	18	19	11	13	15.50	23
BM2113	10	9	9	12	8	9	9.50	13
INRA005	5	6	6	4	6	4	5.17	7
HAUT27	8	9	9	8	9	7	8.33	10
TGLA122	19	19	15	17	16	12	16.33	26
TGLA126	6	8	8	9	8	4	7.17	9
TGLA227	12	12	13	13	11	11	12.00	16
BM1824	6	7	5	5	5	4	5.33	8
HEL13	8	7	7	8	6	5	6.83	9
Mean	10.50	10.60	10.05	10.80	9.10	7.80	9.81	13.45

SAR: South Anatolian Red; SAY: Native Southern Anatolian Yellow; AB: Anatolian Black; AG: Anatolian Grey; EAR: East Anatolian Red; ZAV: Zavot.

Table 3. Population matrix of Nei's genetic distance.

Populations	SAR	AB	AG	SAY	EAR	ZAV
SAR	0.000	0.100	0.177	0.070	0.115	0.141
AB		0.000	0.151	0.073	0.082	0.143
AG			0.000	0.163	0.187	0.210
SAY				0.000	0.107	0.158
EAR					0.000	0.163
ZAV						0.000

SAR: South Anatolian Red; SAY: Native Southern Anatolian Yellow; AB: Anatolian Black; AG: Anatolian Grey; EAR: East Anatolian Red; ZAV: Zavot.

ZAV (0.035) populations. The general F_{IS} value was determined to be 0.068 for all populations. According to the assignment test results, of 271 animals, 147 (54.24%) were assigned to their own population in a 99% confidence interval.

Table 3 shows the population matrix of Nei's genetic distances. The highest genetic distance (0.210) was observed between ZAV and AG. However, the lowest genetic distance (0.070) was determined between SAR and SAY. The phylogenetic tree is given in Figure 1. Grouping possibilities for populations were found as ~70% for SAR and SAY and for ZAV and EAR; however, other populations had lower possibilities. Similar to the geographical locations of these populations, EAR to ZAV and SAY to SAR groupings were determined within the same cluster. Although the AG population was determined to be near the EAR to ZAV cluster, the AB population was determined to be in a completely different position from the other populations.

According to FCA analysis, the AB population was observed as having formed two different subgroups that were completely separate from all other populations. SAR and SAY populations were intermixed with each other, and AB was observed to be very close to these populations. ZAV and AG populations were separated from each other and from all other groups (Figure 2). Structure analysis is given in Figure 3, and the results were found to be compatible with FCA and NJ results.

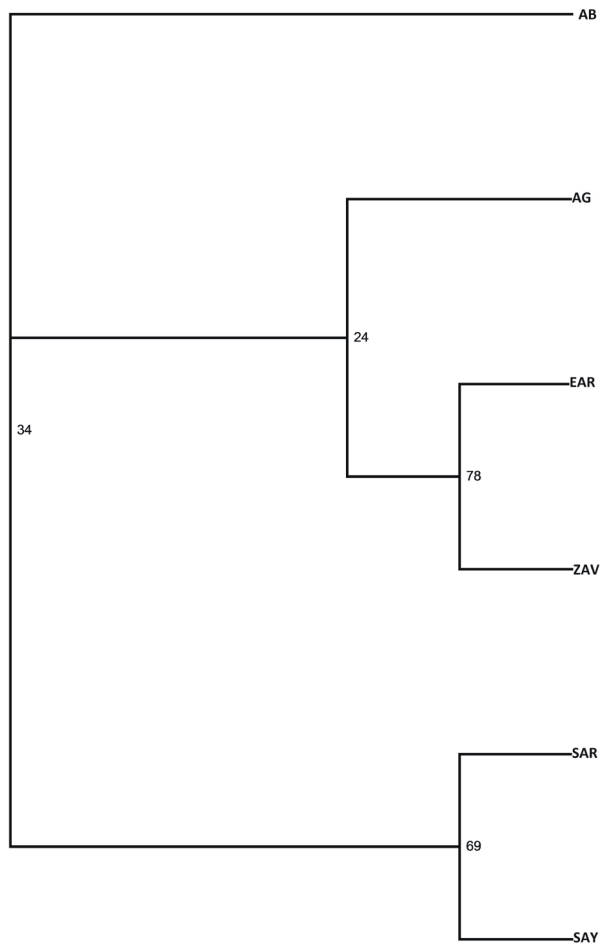

Figure 1. Neighbor-joining tree summarizing the phylogenetic relationships of Turkish cattle breeds. SAR: South Anatolian Red; SAY: Native Southern Anatolian Yellow; AB: Anatolian Black; AG: Anatolian Grey; EAR: East Anatolian Red; ZAV: Zavot.

4. Discussion

The observed average allele number was similar to other studies in which Turkish native cattle breeds were used (2,6,15,16). A total of 102 (16) and 1582 (15) different alleles were observed in previous studies using the same 4 cattle breeds, including SAR, EAR, AB, and AG. The higher allele numbers found in Altınalan's study (15) can be explained by the number (26) of microsatellite loci used and the genotyping method. Relatively lower allele

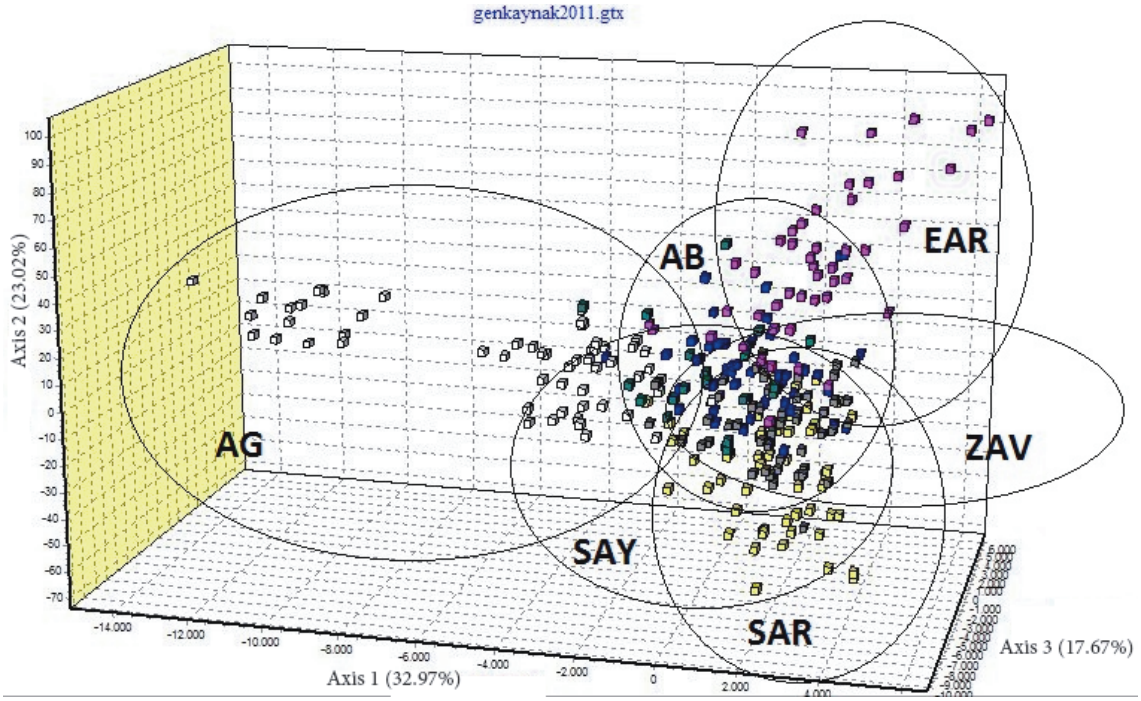

Figure 2. Factorial correspondence analysis (FCA) of Turkish native cattle breeds. SAR: South Anatolian Red; SAY: Native Southern Anatolian Yellow; AB: Anatolian Black; AG: Anatolian Grey; EAR: East Anatolian Red; ZAV: Zavot.

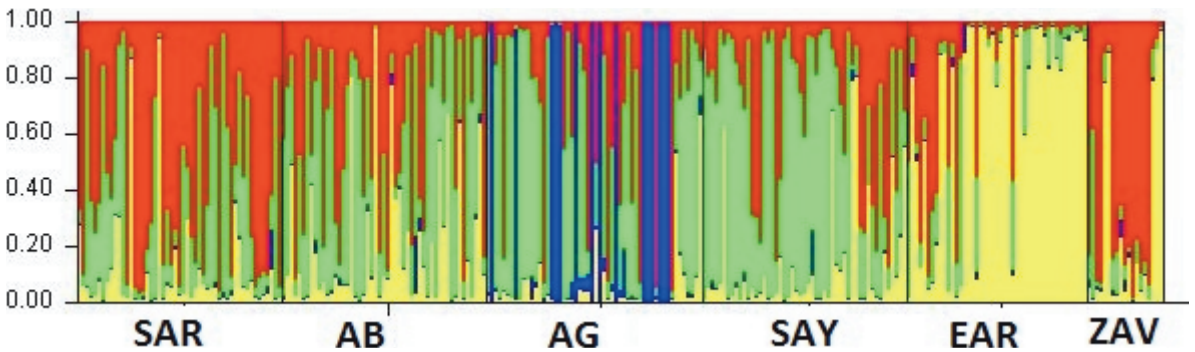

Figure 3. Structure test (K = 4) of SAR, AB, AG, SAY, EAR, and ZAV populations.

numbers were reported by Özkan (16), and these findings might be due to a limited number (7) of microsatellite loci. In this study, total (269) and average allele numbers (13.45) were found to be higher than in Europe (17–24), the United States (25), Asia (26,27), Africa, and India (28,29) and in consortium (2,18,30) studies.

The observed highest allele number in the TGLA122 locus has also been reported in previous studies (16,17,19,24,26,27,31,32). In addition, the lowest allele number in the INRA005 locus was also determined in other research (21,26,29,31,32).

Breeds with lower genetic diversities were found to have lower heterozygosities (23,26,27). The expected and observed heterozygosity values of Turkish native cattle breeds were previously reported elsewhere (11) and were found to be higher than values found in previous studies (2,17–22,27–30). Compared with several other previous studies (21,24), higher F_{IS} values were also observed in this study.

Loftus et al. (2) used and reported the NJ method for the SAR, AB, EAR, and AG breeds. In this study, (2) the EAR and AB breeds were found to be focused toward each other, whereas SAR and AG were separated in a different radiation. According to another study conducted on the native cattle breeds of Turkey (16), AG and AB were found to be close to each other, while the SAR population was especially separated from AB and also other populations. However, the SAY and AB

populations were expected to be close to each other in terms of their geographical localization. Özkan (16) reported that SAR–AB–AG were localized as separate locations, and EAR was reported to be mixed with these populations. These findings are similar to the NJ results of this study. In another study (15), AB and SAR populations were clustered into a single group, whereas AG and EAR populations were separated and found in another group. The findings related to the NJ results in this study were compatible with respective regions where these populations are reared.

FCA indicated that Turkish native cattle breeds were generally located close to each other with no differentiation. It is obvious that animals in the AG group were separated into 2 different subgroups. It was not surprising that the group close to the other breeds was sampled from the genetic conservation herd and the other subgroup was from village herds. A typical steppe cattle, AG is believed to have been brought from Thrace. AG is also a common cattle breed of the Balkans because similar animals are also raised in Bulgaria, Greece, and Romania (33). Bayesian assignment proportions for K = 4 clusters were determined in the structure analysis. In general, the cattle were unified in their own clusters.

Populations were observed to be separated from each other from the east towards the west of Turkey, which is similar to a study using Y-chromosome specific markers (34). Similarly, relations with other Asian (31) and European (17,20,23,35) cattle breeds were found to be compatible with the geographical and historical developments of these breeds. SNP analysis from an international consortium study (36) also included the 5 cattle breeds used in this study. These cattle breeds were reported to be intermixed with several different cattle breeds and cannot be considered as part of the Taurine cattle population. The ZAV population specifically was reported to be close to the Holstein breed, and it has been stated that Anatolian cattle breeds were not close to the same domestication area. The lower sample number (8 samples only) may be the main reason for this conclusion. We believe that the sampling strategy in the present study, which includes genetically unrelated animals from different herds, reflects the natural genetic structure of these cattle breeds.

In conclusion, according to results from the phylogenetic tree and from the FCA graphics and structure test, EAR to ZAV and AB to AG populations were determined to have different genetic structures. However, the SAY population was found to be a distinct breed. Also, SAY had a similar genetic structure to and was intermixed with SAR in geographically close regions.

Genetic diversities of the wild ancestors of breeds located in the first domestication area were previously reported to be higher (8). In this study, the average number of alleles was found to be decreasing from east to west, and higher diversity levels were observed compared to cattle breeds of other countries. These results can be explained by the fact that Anatolia is close to the initial domestication center. The findings of genetic diversities and phylogenetic analyses indicate that the Turkish native cattle breeds analyzed here are consistent with their modern geographical locations.

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