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Genetic diversity and population structure of four cattle breeds raised in Turkey using microsatellite markers

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Abstract: In the present study, genetic diversity and population structure of Holstein Friesian and three native cattle breeds of Turkey including Turkish Grey Steppe, Eastern Anatolian Red and Anatolian Black were assessed. Totally 120 individuals of 4 breeds were genotyped using 20 microsatellite markers and 204 different alleles, of which 31 were private alleles, were detected. The average observed and expected heterozygosity values were 0.63 and 0.74, respectively. Observed heterozygosity at the marker level ranged from 0.30 (*DRBP1*) to 0.88 (*ILSTS011*), while expected heterozygosity ranged from 0.51 (*INRABERN172*) to 0.88 (*SPS113*). Inbreeding coefficient values for Turkish Grey Steppe, Eastern Anatolian Red, Anatolian Black and Holstein Friesian were 0.216, 0.202, 0.128 and 0.069, respectively. The lowest pairwise F_{ST} value (0.030) was detected between Turkish Grey Steppe and Anatolian Black breeds, while the highest value (0.070) was detected between Turkish Grey Steppe and Holstein Friesian. Results of structure and factorial correspondence analysis revealed that Turkish native cattle breeds and Holstein Friesian were genetically different enough to separate the two breeds. Results of bottleneck analysis indicated heterozygosity deficiency in Turkish Grey Steppe ($P < 0.05$).

Keywords: bovine; genetic characterization; indigenous population

Turkey has great genetic diversity of animals due to a wide range of geographical conditions and diverse environment. In addition, Turkey is situated near the Fertile Crescent, in which cattle, sheep and goats were first domesticated (Zeder and Hesse 2000). In Turkey, there are 17 million head of cattle belonging to Holstein Friesian breed (pure and crossbreeds) and 6 distinct native cattle breeds including Turkish Grey Steppe, Eastern Anatolian Red and Anatolian Black (http://tuik.gov.tr/PreTablo.do?alt_id=1002). Turkish native cattle breeds are raised in limited regions such as Anatolian Black in Middle Anatolia, Eastern Anatolian Red in East Anatolia and Turkish Grey Steppe in the Thrace region. Although Turkish native cattle breeds have low milk and meat yields, they are resistant to diseases and temperature changes. These cattle breeds are raised by small farmers for milk and meat production. However, most of the farmers prefer breeding Holstein Friesian due to their high performance. The use of Holstein Friesian semen in artificial insemination caused a decrease in the number and purity of Turkish native cattle breeds. In fact, Turkey, which has 1.6 million head of native cattle today, had 4.2 million head of native cattle in 2000. As a result, some Turkish native cattle breeds such as Karacadag, Eleskirt and Karaisali breeds have gone extinct while the remaining breeds have lost their population sizes (Ertugrul et al. 2015). The reduction in population size and purity of breeds may cause a reduction in genetic diversity.

A reduction in genetic diversity brings about conservation efforts. It is necessary to reveal the

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genetic diversity in order to carry out conservation programs. Thanks to developing molecular techniques, it is possible to examine the genetic diversity in populations at the DNA level. Today, microsatellite markers are used extensively for determination of genetic diversity in livestock (Agung et al. 2019; Ozsensoy et al. 2019). Since microsatellites are distributed randomly throughout the genome, they are useful in genome mapping studies. In addition, microsatellites are used for estimation of phylogenetic relationships between populations (Ozkan 2005; Ozsensoy 2011), determination of bottleneck effects (Devi et al. 2017) and calculation of inbreeding coefficient (Agung et al. 2016). The present study aimed to reveal genetic diversity, population structure and phylogenetic relationships between four cattle breeds raised in Turkey by using 20 microsatellite markers recommended by Food and Agriculture Organization of the United Nations (FAO). The results of the present study may be the basis for subsequent breeding and conservation programs for these cattle breeds.

MATERIAL AND METHODS

Ethic statement and animal material. This study was approved by Akdeniz University Animal Experiments Local Ethic Committee (Protocol No. 2017.08.007). The study was conducted on a total of 120 animals representing Holstein Friesian (HF, n = 30) and 3 native cattle breeds of Turkey: Turkish Grey Steppe (TGS, n = 30), Eastern Anatolian Red (EAR, n = 30), Anatolian Black (AB, n = 30). All samples were collected from several representative herds. The blood samples of HF were collected from dairy cattle bred on the farm of Akdeniz University that has recently (2 years ago) been provided cattle from several herds kept in ten towns of Turkey. Blood samples of AB were collected from Eskisehir and Antalya, Turkey. Blood samples of EAR and TGS were collected from Erzurum and Balikesir, Turkey, respectively. The blood samples were stored at –20°C until DNA extraction steps.

Molecular markers. In this study, 20 microsatellite markers recommended by FAO (2011) for genetic characterization studies were used. More information about all used loci is given in Table 1.

DNA extraction from blood samples. In this study, DNA extraction protocol reported by Miller et al. (1988) was used to extract genomic DNA from blood samples. Agarose gel electrophoresis was applied to confirm that genomic DNA was extracted successfully.

Polymerase chain reaction (PCR) and fragment analysis. Many experiments have been carried out with a few samples in order to obtain a common PCR and PCR program for all loci. Using an Eppendorf 5331 MasterCycler Gradient Thermal Cycler (Eppendorf, Germany) PCR was carried out in a total of 15 µl volumes containing as follows: 1× PCR buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1 U Taq DNA polymerase, 10 pM of each primer and 50 ng template DNA. PCR amplifications were carried out in initial denaturation at 94°C for 10 min, followed by 31 cycles at 94°C for 40 s, at 50–65°C (Table 2) for 40 s and at 72°C for 40 s. The final extension was applied at 72°C for 10 min. In PCR steps each microsatellite marker was amplified separately. The PCR products were visualized by agarose gel electrophoresis to confirm that all loci were amplified successfully. A 96-well automatic fragment analyzer (Agilent 5200 Fragment Analyzer System; Agilent, USA) was used for fragment analysis. ProSize Version 3.0 was used to obtain allele sizes.

Statistical analysis. Convert Version 3.1.1 (Glaubitz 2004) was used to detect allele number, number of private alleles and allele size range for each locus. POPGENE Version 1.32 (Yeh et al. 1997) was used to calculate average number of alleles, effective number of alleles, observed heterozygosity, expected heterozygosity, F-statistics. Expected heterozygosity (He) was calculated from Nei's (1987) equation:

$$He = 1 - \sum_{i=1}^{k} p_i^2$$

where:

p_i = frequency of allele i

Microsatellite toolkit (Park 2001) was used to obtain polymorphism information content (PIC) values, statistical assessment of informativeness of the used markers, calculated according to Botstein et al. (1980) using the formula:

$$PIC = 1 - \sum_{i=1}^{m} p_i^2 - \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} 2p_i^2 p_j^2$$

where:

m = allele number of a microsatellite locus

p_i, p_j = frequencies of the i^{th} and j^{th} allele of a locus

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Table 1. Overview of 20 microsatellite loci in four cattle breeds

Loci	Turkish Grey Steppe						Eastern Anatolian Red						Anatolian Black						Holstein Friesian					
	Na	Ne	Ho	He	PIC	F_{IS}	Na	Ne	Ho	He	PIC	F_{IS}	Na	Ne	Ho	He	PIC	F_{IS}	Na	Ne	Ho	He	PIC	F_{IS}
BM6444	8	2.91	0.87	0.67	0.63	–0.355	10	4.51	1.00	0.79	0.75	–0.269	10	5.49	1.00	0.83	0.80	–0.207	8	5.49	1.00	0.83	0.80	–0.207
CSRM60	8	6.07	0.48	0.85	0.82	0.436	7	3.81	0.37	0.75	0.71	0.516	11	6.04	0.87	0.85	0.82	–0.022	7	4.37	0.77	0.78	0.74	0.023
CSSM66	9	6.03	0.48	0.85	0.81	0.438	7	3.96	0.21	0.75	0.72	0.731	9	5.44	0.68	0.83	0.80	0.186	10	6.40	0.56	0.86	0.83	0.358
DRBP1	5	3.70	0.57	0.74	0.68	0.239	5	2.29	0.20	0.57	0.52	0.655	5	2.34	0.30	0.58	0.51	0.489	4	1.24	0.14	0.20	0.19	0.298
ETH185	11	6.70	0.43	0.87	0.83	0.510	11	7.48	0.54	0.89	0.85	0.393	14	9.32	0.54	0.91	0.88	0.413	12	7.01	0.90	0.87	0.84	–0.028
ETH3	6	2.15	0.42	0.55	0.51	0.238	8	3.11	0.44	0.69	0.63	0.379	8	4.95	0.36	0.81	0.77	0.565	4	2.78	0.36	0.65	0.59	0.456
HAUT24	8	5.60	0.58	0.84	0.80	0.320	8	3.56	0.48	0.73	0.69	0.347	8	4.66	0.57	0.80	0.75	0.294	8	4.02	0.54	0.77	0.72	0.301
HEL1	8	5.07	0.81	0.82	0.78	0.016	7	4.42	0.75	0.79	0.74	0.049	7	4.71	0.93	0.80	0.76	–0.168	7	0.33	0.87	0.78	0.74	–0.110
ILSTS005	7	4.76	0.63	0.81	0.76	0.229	7	3.41	0.33	0.72	0.66	0.540	6	3.10	0.30	0.69	0.64	0.569	5	1.56	0.21	0.36	0.34	0.435
ILSTS006	9	5.85	0.50	0.84	0.81	0.412	5	4.15	0.38	0.77	0.72	0.513	7	4.68	0.62	0.80	0.75	0.236	8	4.00	0.59	0.76	0.71	0.227
ILSTS011	9	7.20	0.93	0.88	0.85	–0.067	9	5.66	1.00	0.84	0.80	0.198	10	8.04	1.00	0.89	0.86	–0.125	8	5.20	1.00	0.82	0.78	–0.222
ILSTS087	8	3.21	0.80	0.70	0.65	–0.145	5	3.19	0.97	0.70	0.63	–0.395	7	3.36	1.00	0.71	0.65	–0.410	8	2.50	1.00	0.61	0.52	–0.656
INRA032	13	8.06	0.96	0.89	0.86	–0.081	9	4.93	1.00	0.81	0.77	–0.238	9	5.11	0.90	0.82	0.78	–0.102	6	4.52	0.83	0.79	0.75	–0.053
INRA037	4	2.56	0.14	0.63	0.57	0.781	6	4.73	0.61	0.81	0.76	0.252	8	5.19	0.37	0.82	0.78	0.554	7	4.49	0.29	0.79	0.74	0.643
INRA063	8	4.72	0.67	0.80	0.76	0.171	5	3.20	0.80	0.70	0.65	–0.148	9	5.77	0.73	0.84	0.80	0.130	6	3.05	0.59	0.69	0.64	0.137
INRAB-ERN172	6	3.47	0.50	0.72	0.66	0.313	6	2.19	0.20	0.55	0.51	0.642	7	2.44	0.43	0.60	0.54	0.282	4	1.19	0.10	0.17	0.16	0.378
MM12	5	2.95	0.21	0.69	0.61	0.695	7	4.02	0.25	0.77	0.72	0.681	8	3.80	0.44	0.75	0.70	0.420	8	1.74	0.36	0.43	0.41	0.179
SPS113	13	8.18	0.63	0.89	0.82	0.294	10	6.23	0.90	0.85	0.82	–0.055	12	7.14	1.00	0.88	0.85	–0.146	13	9.58	0.93	0.91	0.89	–0.25
SPS115	10	5.92	0.66	0.85	0.82	0.228	7	2.20	0.37	0.56	0.52	0.344	9	3.30	0.57	0.71	0.68	0.204	8	2.96	0.59	0.67	0.63	0.132
TGLA227	4	2.99	1.00	0.68	0.60	–0.491	4	2.90	1.00	0.67	0.59	–0.513	5	2.64	1.00	0.63	0.55	–0.599	4	2.92	0.90	0.67	0.59	–0.355
All loci	7.95	4.90	0.61	0.78	0.73	0.216	7.15	4.00	0.59	0.74	0.69	0.202	8.45	4.88	0.68	0.78	0.73	0.128	7.1	3.97	0.63	0.67	0.63	0.069

Na = number of alleles, Ne = number of effective alleles, Ho = observed heterozygosity, He = expected heterozygosity, PIC = polymorphism information content, F_{IS} = inbreeding coefficient

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Table 2. Characterization of 20 cattle microsatellite markers used

Loci	Chromosome	Primer sequence (5'-3')	Annealing temperature (°C)	Genbank Acc. No./reference	Allele size (bp)
BM6444	2	CTCTGGGTACAACACTGAGTCC TAGAGAGTTTCCCTGTCCATCC	65	G18444	118–200
CSRM60	10	AAGATGTGATCCAAGAGAGAGGCA AGGACCAGATCGTGAAAGGCATAG	55	Devi et al. (2017)	79–115
CSSM66	14	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	58	Agung et al. (2016)	171–209
DRBP1	23	ATGGTGCAGCAGCAAGGTGAGCA GGGACTCAGTCTCTCTATCTCTTTG	58	M55069	195–229
ETH185	17	TGCATGGACAGAGCAGCCTGGC GCACCCCAACGAAAGCTCCCAG	65	Z14042	214–246
ETH3	19	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	65	Z22744	103–133
HAUT24	22	CTCTCTGCCTTTGTCCCTGT AATACACTTTAGGAGAAAAATA	50	X89250	104–158
HEL1	15	CAACAGCTATTTAACAAGGA AGGCTACAGTCCATGGGATT	54	X65202	99–119
ILSTS005	10	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	51	L23481	176–194
ILSTS006	7	TGTCTGTATTTCTGCTGTGG ACACGGAAGCGATCTAAACG	55	L23482	277–309
ILSTS011	14	GCTTGCTACATGGAAAGTGC CTAAAATGCAGAGCCCTACC	58	L23485	250–300
ILSTS087	6	AGCAGACATGATGACTCAGC CTGCCTCTTTTCTTGAGAG	58	L37279	135–155
INRA032	11	AAACTGTATTCTCTAATAGCAC GCAAGACATATCTCCATTCCTTT	50	X67823	160–204
INRA037	10	GATCCTGCTTATATTTAACCAC AAAATTCCATGGAGAGAGAAAC	50	X71551	112–148
INRA063	18	ATTTGCACAAGCTAAATCTAACC AAACCACAGAAATGCTTGGAAG	50	X71507	167–189
INRABERN172	26	CCACTTCCCTGTATCCTCCT GGTGCTCCCATTGTGTAGAC	58	Nagamine et al. (2008)	234–256
MM12	9	CAAGACAGGTGTTTCAATCT ATCGACTCTGGGGATGATGT	52	Z30343	101–145
SPS113	10	CCTCCACACAGGCTTCTCTGACTT CCTAACTTGCTTGAGTTATTGCCC	58	Agung et al. (2019)	134–158
SPS115	15	AAAGTGACACAACAGCTTCTCCAG AACGAGTGTCCTAGTTTGGCTGTG	55	FJ828564	131–159
TGLA227	18	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAAACTCAATGAAAGCA	50	Devi et al. (2017)	75–105

Genetic diversity among and within populations was analysed by AMOVA using Arlequin Version 3.5 (Excoffier et al. 2005). GENETIX Version 4.05 (Belkhir et al. 2004) was used for a factorial correspondence analysis (FCA) which locates breeds in three-dimensional space. STRUCTURE Version 2.2 (Pritchard et al. 2000) was used to determine the genetic structure of populations and to cluster all individuals to show the genetic admixture. The program was run using 100 independent runs for each K value between 2 and 5 with a burn-in period of 200 000 iterations followed by 500 000 iterations of Markov Chain Monte Carlo (MCMC) algorithm. An online program

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STRUCTURE HARVESTER (Earl and vonHoldt 2012) implementing the Evanno method (Evanno et al. 2005) was applied to detect the optimal K value. BOTTLENECK Version 1.2.02 (Piry et al. 1999) detected genetic signatures of the recent reduction in population size. Standardized differences are recommended when the number of loci ≥ 20 (Piry et al. 1999). The Two-Phase Model of Mutation (TPM) is recommended to be applied instead of the Stepwise Mutation Model (SSM) in a microsatellite analysis (Luikart and Cornuet 1998).

RESULTS AND DISCUSSION

Genetic diversity. A total of 204 different alleles, of which 31 were private alleles, were detected for 20 microsatellite loci in all populations. All the private alleles showed frequencies lower than 3%. The number of alleles per locus ranged from 5 (*TGLA227*) to 17 (*ETH185*) with a mean of 10.2, while the number of effective alleles per locus ranged from 2.39 (*DRBP1*) to 7.78 (*SPS113*) with a mean of 4.44.

Observed heterozygosity ranged from 0.30 (*DRBP1*) to 0.98 (*ILSTS011*) with a mean of 0.63, while expected heterozygosity ranged from 0.51 (*INRABERN172*) to 0.88 (*SPS113*) with a mean of 0.74. Generally, observed heterozygosity was lower than expected heterozygosity, which may be due to the presence of more homozygous individuals in the analysed samples.

The PIC value ranged from 0.47 (*INRABERN172*) to 0.84 (*SPS113*) with a mean of 0.70. All microsatellite loci except *INRABERN172* showed high polymorphism (PIC > 0.5) to reveal genetic diversity in the studied populations.

Genetic differentiation and relationship among the populations. *F*-statistics including F_{IS} (within-population inbreeding estimate), F_{IT} (total inbreeding estimate) and F_{ST} (estimate of population differentiation) are used to determine the inbreeding level in a breed or all populations and for genetic differentiation among populations. Mean values of F_{IS}, F_{IT} and F_{ST} for all loci were 0.154, 0.185 and 0.055, respectively. F_{ST} values could indicate small (0–0.05), medium (0.05–0.15), high (0.15–0.25) and very high (F_{ST} > 0.25) differentiation between breeds (Hartl and Clark 2007). In this study, a medium level of F_{ST} value was detected in all breeds and only 5.5% of genetic diversity was attributed to breed differences. The lowest pairwise F_{ST} value (0.030) was between TGS and AB breeds, while the highest pairwise F_{ST} value (0.070) was between TGS and HF breeds (Table 3). All pairwise F_{ST} values were significant ($P < 0.01$), indicating that all breeds could be considered genetically different.

Table 3. Pairwise F_{ST} values in breeds

Breeds	TGS	EAR	AB	HF
TGS	0.000			
EAR	0.040***	0.000		
AB	0.030***	0.032***	0.000	
HF	0.070***	0.049***	0.040***	0.000

TGS = Turkish Grey Steppe, EAR = Eastern Anatolian Red, AB = Anatolian Black, HF = Holstein Friesian
***$P < 0.001$

The FCA method revealed a very clear separation between HF and the Turkish native cattle breeds (Figure 1). Axis 2, which explained 32.13% of total variance, separated the HF breed from Turkish native cattle breeds. FCA results indicated that individuals from native cattle breeds (especially the TGS and AB breeds) were mixed, which indicates a closer relationship between them.

The structure analysis is presented in Figure 2. The TGS and EAR breeds as well as the AB and HF breeds clustered together at K = 2. At K = 3, HF breed was clustered separately from Turkish native cattle breeds. Using STRUCTURE HARVESTER, the most likely number of clusters was obtained at K = 4. At K = 4, all the cattle breeds were identified as a separate cluster. In addition, a distinct genetic difference in the degree of admixture was observed between TGS and the other cattle breeds. Results of FCA and structure analysis were in accordance with the origin of the studied populations. AMOVA results presented in Table 4 showed that the highest and the lowest part of variation was observed within populations (80.068%) and among populations (5.186%), respectively.

Table 4. AMOVA analysis of four cattle breeds

Source of variation	Sum of squares	Variance components	Variation (%)
Among breeds	91.844	0.406	5.186
Among individuals within breeds	909.245	1.156	14.746
Within individuals	714.000	6.276	80.068
Total	1715.089	7.838	

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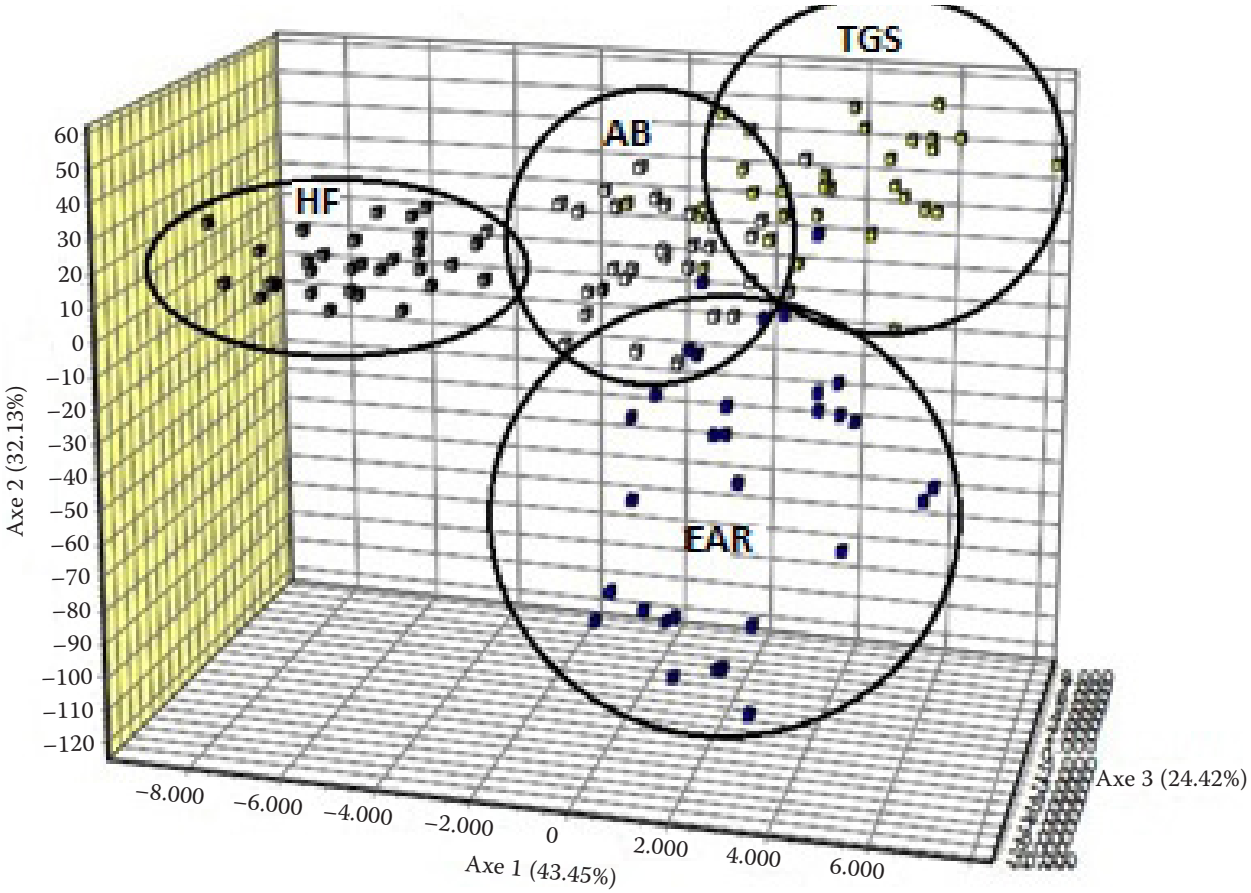

Figure 1. Plot of multivariate factorial correspondence analysis
HF = Holstein Friesian, TGS = Turkish Grey Steppe, EAR = Eastern Anatolian Red, AB = Anatolian Black

Bottleneck effects cause a rapid decrease in effective population size, resulting in a reduction in heterozygosity (Nagamine et al. 2008; Devi et al. 2017). Bottleneck analysis was applied to reveal the reduction in effective population size. The probability values obtained based on TPM model and standardized difference test are presented in Table 5. The result of bottleneck analysis revealed a reduction in heterozygosity in TGS breed ($P < 0.05$). The EAR, AB and HF breeds were in mutation-drift equilibrium ($P > 0.05$).

Table 5. Bottleneck analysis of four cattle breeds

Breeds	Mutation model	Probability
TGS	TPM	0.041*
EAR	TPM	0.350
AB	TPM	0.152
HF	TPM	0.128

TGS = Turkish Grey Steppe, EAR = Eastern Anatolian Red, AB = Anatolian Black, HF = Holstein Friesian, TPM = Two-Phase Model of Mutation
*$P < 0.05$

Genetic characterization studies are the first step of conservation programs (Devi et al. 2017). Especially the genetic structure of native cattle breeds, which contribute to the world animal genetic resources, should be estimated using strong molecular markers. Microsatellite markers are commonly used to estimate both genetic diversity and phylogenetic relationship of indigenous cattle breeds of the world. Although there are numerous published studies focusing on genetic characterization of indigenous cattle breeds using microsatellite markers (Suh et al. 2014; Agung et al. 2016; El-Sayed et al. 2016; Hussain et al. 2016; Devi et al. 2017), there are fewer studies focusing on native cattle breeds of Turkey (Ozkan 2005; Ozsensoy 2011; Ozsensoy et al. 2019).

Genetic diversity parameters including the number of alleles, number of effective alleles, observed heterozygosity and expected heterozygosity values of Turkish native cattle breeds were higher than

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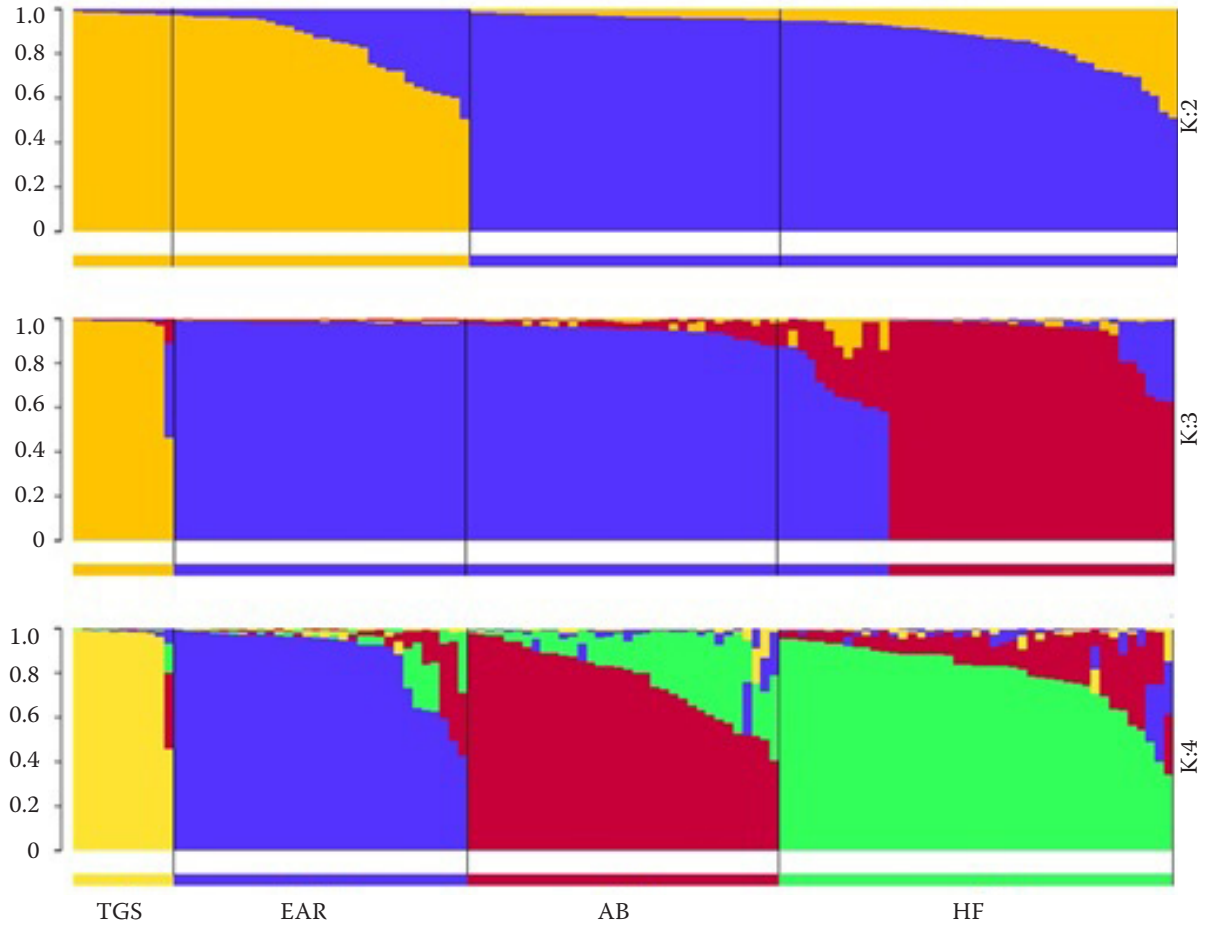

Figure 2. Population structure of four cattle breeds obtained by STRUCTURE analysis

TGS = Turkish Grey Steppe, EAR = Eastern Anatolian Red, AB = Anatolian Black, HF = Holstein Friesian

those reported in indigenous cattle breeds of India (Devi et al. 2017), Pakistan (Hussain et al. 2016) and Egypt (El-Sayed et al. 2016). This may be attributed to the location of Turkey near the cattle domestication centre. However, these parameters were lower than in the previous genetic diversity studies on native cattle breeds of Turkey (Ozkan 2005; Ozsensoy et al. 2019). Ozşensoy et al. (2019) reported the total number of alleles as 10.05, 9.10, 10.60 in TGS, EAR and AB breeds, respectively. Ozkan (2005) reported the total number of alleles as 10.29, 10.14, 10.29 in TGS, EAR and AB breeds, respectively. This lower genetic diversity may result from many factors including the use of different microsatellite loci and different number of individuals.

In this study, the inbreeding coefficient (F_{IS}) in TGS, EAR, AB and HF was 0.216, 0.202, 0.128 and 0.055, respectively, with a mean of 0.154. It is not surprising that the inbreeding coefficient in native cattle breeds of Turkey was noticeably higher than in HF breed, because the samples of HF breed were provided from ten places of Turkey. A previous study conducted by Ozsensoy et al. (2019) reported lower F_{IS} value in TGS (0.12), EAR (0.03) and AB (0.06) breeds. It may be attributed to sampling strategy as well as to the use of different microsatellite markers.

The PIC value is the power of used microsatellite marker to reveal genetic diversity. The lowest and the highest PIC value was 0.547 (*INRABERN172*) and 0.84 (*SPS113*), respectively, with a mean of 0.70. A similar mean of PIC value (0.74) was reported in Simmental Cross cattle breed (Agung et al. 2016) while lower values were reported in Punganur cattle (0.62; Devi et al. 2017), Siwa cattle (0.45; El-Sayed et al. 2016) and Farafra cattle (0.64; El-Sayed et al. 2016).

The structure analysis showed that all breeds were distinctly different. This finding was supported by FCA in which HF breed clustered differently from Turkish native cattle breeds. Similarly, Agung et al. (2019) reported that Simmental Purebred, Simmental Cross and Holstein Friesian cattle breeds

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distinctly differed from Indonesian native cattle breeds. In addition, Korean native cattle breeds were found to be different from Holstein and Charolais (Suh et al. 2014).

AMOVA results showing that a large part of genetic variation accounted for within individuals were similar to Pakistani (Hussain et al. 2016) cattle breeds. In addition, a similar result was reported in Yellow cattle in Taiwan indicating a 4.35% proportion of genetic variation attributed to population differentiation among the studied populations (Tu et al. 2014).

The bottleneck analysis revealed a reduction in heterozygosity in TGS. It may be due to a reduction in the effective population size of TGS breed. Indeed, TGS breed has the lowest population size among native cattle breeds of Turkey. Villalobos Cortes et al. (2010) evaluated the bottleneck analysis in Guaymi (GY) and Guabala (GU) cattle populations using the Infinite Allele Mutation (IAM), TPM and SSM models with sign test, standardized difference test and Wilcoxon test. They obtained significant values in all the tests and all populations using the IAM model and no significant value using the SMM model with Wilcoxon test. They reported significant values in GY population when using the TPM model with Wilcoxon test.

CONCLUSION

The present study aimed to assess genetic diversity and population structure of three Turkish native cattle breeds and Holstein Friesian breed using 20 microsatellite markers. Medium heterozygosity was detected in four cattle breeds with medium genetic differentiation (5.5%) between them. Nevertheless, Turkish native cattle breeds had a higher level of inbreeding ranging from 0.128 (AB) to 0.216 (TGS), which indicated lower effective population sizes. In order to decrease the level of inbreeding and to increase the effective population size in native cattle breeds, comprehensive conservation programs are needed. Moreover, molecular applications such as microsatellite markers could be used to reduce the chance of inbreeding and to conserve the genetic diversity in populations. Therefore, we recommend further studies to gain more details about Turkish native cattle breeds.

REFERENCES

Agung P.P., Saputra F., Septian W.K., Lusiana, Zein M.S.A., Sulandari S., Anwar S., Wulandari A.S., Said S., Tappa B. (2016): Study of genetic diversity among Simmental cross cattle in West Sumatra based on microsatellite markers. Asian-Australasian Journal of Animal Sciences, 29, 176–183.

Agung P.P., Saputra F., Zein M.S.A., Wulandari A.S., Putra W.P.B., Said S., Jakaria J. (2019): Genetic diversity of Indonesian cattle breeds on microsatellite markers. Asian-Australasian Journal of Animal Sciences, 32, 467–476.

Belkhir K., Borsa P., Chikhi L., Raufaste N., Bonhomme F. (2004): GENETIX 4.05, Windows software for population genetics. University of Montpellier II, Montpellier, France. (in French)

Botstein D., White L.R., Skolnick M., Davis R.W. (1980): Construction of genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics, 32, 314–331.

Devi K.S., Gupta B.R., Vani S. (2017): Genetic diversity and bottleneck studies in Punganur cattle through microsatellite markers. International Journal of Science, 6, 303–307.

Earl D.A., vonHoldt B.M. (2012): STRUCTURE HARVESTER: A web-site and program for visualizing structure output and implementing the Evanno method. Conservation Genetics Resources, 4, 359–361.

El-Sayed M.A., Al-Sudy A., Teleb F. (2016): Assessment of genetic diversity among two Egyptian cattle populations (Bos taurus) based on autosomal microsatellite markers. Egyptian Journal of Animal Production, 53, 65–73.

Ertugrul M., Akin A.O., Yildirim M., Dellal G., Togan I., Pabuccuoglu S., Koyuncu M., Oner Y., Yilmaz O., Koncagul S., Pehlivan E., Elmaci C., Dag B., Ozder M. (2015): Conservation and Sustainable Use of Farm Animal Genetic Resources in Turkey. In: Conf. Proc. Turkey Agricultural Engineering VIII, Ankara, Turkey, 212–236. (in Turkish)

Evanno G., Regnaut S., Goudet J. (2005): Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology, 14, 2611–2620.

Excoffier L., Laval G., Schneider S. (2005): Arlequin version 3.01: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics, 1, 47–50.

FAO (2011): Molecular Genetic Characterization of Animal Genetic Resources. FAO Animal Production and Health Guidelines, No. 9. Available at http://www.fao.org/3/i2413e/i2413e00.pdf (accessed July 9, 2019).

Glaubitz J.C. (2004): A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Molecular Ecology Resources, 4, 309–310.

Hartl D.L., Clark A.G. (2007): Principles of Population Genetics. Sinauer Associates, Sunderland, USA.

https://doi.org/10.17221/62/2019-CJAS

Hussain T., Babar M.E., Peters S.O., Wajid A., Ali A., Azam A., Ahmad Z., Wasim M., Ali A., Kizilkaya K., De Donato M., Imumorin I.G. (2016): Microsatellite markers based genetic evaluation of Pakistani cattle breeds. Pakistan Journal of Zoology, 48, 1633–1641.

Luikart G., Cornuet J.M. (1998): Empiricial evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology, 12, 228–237.

Miller S., Dykes D., Plesky H.A. (1988): Simple salting out procedure for extracting DNA from human cells. Nucleic Acids Research, 16, 1215.

Nagamine Y., Nirasawa K., Takahashi H., Sasaki O., Ishii K., Minezawa M., Oda S., Visscher P.M., Furukawa T. (2008): Estimation of the time of divergence between Japanese Mishima Island cattle and other cattle populations using microsatellite DNA markers. Journal of Heredity, 99, 202–207.

Nei M. (1987): Molecular Evolutionary Genetics. Columbia University Press, New York, USA.

Ozkan E. (2005): An investigation on genetic structure of native and cultural cattle breeds in Turkey by using microsatellite markers. PhD Thesis, University of Trakya, Edirne, Turkey. (in Turkish)

Ozsensoy Y. (2011): Genetic characterization of some Turkish cattle breeds. PhD Thesis, University of Selcuk, Konya, Turkey. (in Turkish)

Ozsensoy Y., Kurar E., Dogan M., Bulut Z., Nizamioglu M., Altunok V., Isik A., Camlidag A. (2019): Phylogenetic relationships of native Turkish cattle breeds using microsatellite markers. Turkish Journal of Veterinary and Animal Sciences, 43, 23–29.

Park S.D.E. (2001): Trypanotolerance in West African cattle and the population genetic effects of selection. PhD Thesis, Dublin University, Dublin, Ireland.

Piry S., Luikart G., Cornuet J.M. (1999): Bottleneck: A computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity, 90, 502–503.

Pritchard J.K., Matthew S., Donnelly P. (2000): Inference of population structure using multilocus genotype data. Genetics, 155, 945–995.

Suh S., Kim Y., Cho C., Byun M., Choi S., Ko Y., Lee C.W., Jung K., Bae K.H., Kim J. (2014): Assessment of genetic diversity, relationships and structure among Korean native cattle breeds using microsatellite markers. Asian-Australasian Journal of Animal Sciences., 27, 1548–1553.

Tu P., Lin D., Li G., Huang J., Wang D., Wang P. (2014): Characterization of the genetic diversity and population structure for the yellow cattle in Taiwan based on microsatellite markers. Animal Biotechnology, 25, 234–249.

Villalobos Cortes A.I., Martinez A.M., Escobar C., Vega-Pla J.L., Delgado J.V. (2010): Study of genetic diversity of the Guaymi and Guabala bovine populations by means of microsatellites. Livestock Science, 131, 45–51.

Yeh F.C., Yang R.C., Boyle T.B.J., Ye Z.H., Mao J.X. (1997): POPGENE, the User Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Canada.

Zeder M.A., Hesse B. (2000): The initial domestication of goats (Capra hircus) in the Zagros mountains 10,000 years ago. Science, 287, 2254–2257.

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