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Genetic diversity of native Turkish cattle breeds: Mantel, AMOVA and bottleneck analysis

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ABSTRACT

This study was conducted to evaluate potential extinction risk of Turkish native cattle breeds using Mantel and AMOVA tests and Bottleneck analysis. A total of 271 DNA samples were isolated from Anatolian Black, Anatolian Grey, South Anatolian Red, Native Southern Anatolian Yellow, East Anatolian Red, and Zavot cattle. In this study, genotypes of 20 microsatellites were determined by capillary electrophoresis and fragment analysis. A total of 269 different alleles were detected. The maximum and minimum numbers of total alleles were observed in TGLA122 (n=26) and INRA005 (n=7) loci, respectively. The highest average observed and expected heterozygosity values were determined as 0.619–0.852 and 0.669–0.877, respectively. The average F_{IS} value was 0.068. Results of AMOVA and Mantel tests illustrated statistically significant differences in populations (p<0.001) and correlation (p<0.01). Bottleneck analysis revealed a normal distribution of L–shaped curve indicating that there was no recent risk of extinction for these breeds.

Keywords

AMOVA, Bottleneck, Cattle, Mantel, Microsatellite

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INTRODUCTION

Genetic characterization studies could provide valuable information needed for several works such as determination of genetic level diversity within and between populations, development of breed, and conservation strategies. Several genetic characterization studies have been conducted on cattle located in Asia (Zhou et al., 2005; Sun et al., 2008), Europe (Edwards et al., 2000; Cañón et al., 2001; Mateus et al., 2004; Martin-Burriel et al., 2007; Özşensoy et al., 2010, 2014), Africa (MacHugh et al., 1997; Freeman et al., 2004) and America (Egito et al., 2007; Novoa and Usaquén, 2010).

Approximately, one out of five cattle, goat, pig, horse and poultry breeds are currently at risk of extinction worldwide (FAO, 2007). Also, it was reported that 14 indigenous cattle breeds or types have been lost in Turkey (Ertugrul et al., 2000). In 1981, majority of cattle population were comprised of native cattle breeds (55.84%) or their crosses (33.69%) in Turkey. However, the numbers of native cattle have dramatically decreased as low as 16.30% in 2013 (TUIK, 2014). The remaining indigenous cattle breeds of Turkey including Anatolian Black (AB), Anatolian Grey (AG), South Anatolian Red (SAR), Native Southern Anatolian Yellow (SAY), East Anatolian Red (EAR) and Zavot (ZAV) have also decreasing drastically.

Anatolian Black (AB) is considered as the most widespread native breed which is reared in the central

Anatolia. Skin of AB is thick, which is covered with black hairs. These cattle are smaller in size having taller back side than the front. AG is reared in the Thrace, Marmara and North-Aegean regions of Turkey. Similar gray cattle are also found in Bulgaria, Greece, Hungary and Romania, therefore AG is known as a common breed of Balkans. Body colors of AGs range from light silver-gray to dark ash-gray. Because of having strong body and an aggressive nature, managing and handling of the cattle is often difficult. They have superior digestive system and naturally can survive and reproduce inside the forests and mountainous areas without any human intervention.

South Anatolian Red (SAR; also known as Kilis) breeds are reared at the South Anatolian part of Turkey expanding from Mersin to Sanliurfa. Body color is generally yellowish-red, but may range from yellow to brown. Comparing with the other Turkish native cattle breeds, it has the largest body size, and the cow gives highest milk yield. They are well adapted to hot climates.

Native Southern Anatolian Yellow (SAY) breeds are distributed in the South Anatolian part of Turkey between Mediterranean Sea and Taurus-Amanos Mountains. Body size of SAY is generally smaller, and mature body weight is about 150-250 kg. Body color is dark yellow to red-cinnamon.

East Anatolian Red (EAR) is reared at the East and Northeast Anatolian regions of Turkey. Body color is red. The animals are medium in size. Mature body weight can reach as high as 450 kg. ZAV is located at the north-eastern part of Turkey. Body color is generally white. It is considered that ZAV has been developed through long-term crossing of the local cattle breeds such as EAR with Simmental and Brown Swiss. These local cattle breeds are generally reared by farmers having only small number of animals. They are resistant to several diseases and parasitic infestations (Anonim, 2011).

Extinction of Anatolian native animal breed is critically important because of their close localization to the first domestication center (Bruford and Towsend, 2004). Therefore, characterization at molecular level for the determination of genetic variation might have critical importance for the development of conservation strategies of Turkish local cattle breeds. In order to protect these genetic resources, a national project has been initiated in Turkey namely- "*In vitro Conservation and Preliminary Molecular Identification of Some Turkish Domestic Animal Genetic Resources–I (TURKHAYGEN–I)*". The present study has been focused on molecular characterization of Turkish native cattle breeds based on microsatellite markers, and to evaluate the potential risks of extinction of the breeds using AMOVA, Mantel tests and Bottleneck analysis.

MATERIALS AND METHODS

Ethical approval: Ehtical approval for this study was taken from Ethics Committee of Selcuk University Faculty of Veterinary Medicine Ethics Committee (19.11.2007; No. 2007/063).

Blood samples collection and DNA extraction: A total of 271 blood samples were collected from SAR (n=51), SAY (n=51), AB (n=51), AG (n=54), EAR (n=45), and ZAV (n=19) cattle. Genomic DNA samples from the blood samples were extracted by following standard organic phenol-chloroform method (Sambrook et al., 1989).

Polymerase chain reaction (PCR): Twenty micro-satellite loci were selected (Hoffmann et al., 2004) suggested by Food and Agriculture Organization of the United Nations–Measurement of Domestic Animal Diversity (FAO–MoDAD) and International Society of Animal Genetics (ISAG) (**Table 1**). Genotyping procedures were previously described (Özşensoy et al., 2010). The primers were fluorescent-labeled. Three multiplex pool systems were done based on labeling and product size; these loci-groups included 7 (CSSM66, ETH03, HEL9, CSRM60, INRA023, SPS115, ILSTS006), 7 (INRA005, HAUT27, TGLA122, TGLA126, TGLA227, BM1824, HEL13), and 6 (BM2113, TGLA53, ETH225, ETH10, ETH185, BM1818). Each multiplex PCR was carried out in 15 µL reaction volume containing 1xMg^{++} free PCR buffer (Fermentas), 0.125 mM dNTPs (Fermentas), 1.5 mM $MgCl^{++}$, 0.375 U *Taq* polymerase (Fermentas), 2–17 pmol each primer, and ~100 ng of genomic DNA.

Touchdown PCR profile was used in two steps (Don et al., 1991). The first step was initial denaturation at 95°C for 4 min, followed by 16 cycles of denaturation at 94°C for 30 sec, annealing beginning at 60°C and ending at 52°C for 30 sec, and extension at 72°C for 30 sec. The annealing temperature was decreased 0.5°C per cycle until it reached to 52°C. At the second step, 25 cycles of 94°C for 30 sec, 52°C for 30 sec, and 72°C for 30 sec was applied. The final extension of 72°C for 10 min was applied in all reactions.

Capillary electrophoresis: The resulting PCR products were prepared for capillary electrophoresis and loaded onto a Beckman Coulter CEQ–8000 Genetic Analysis

Table 1: Microsatellite loci and oligonucleotides used in the study.

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

System. Genotypes were determined by fragment analysis using CEQ-8000 FragTest program.

Statistical analysis: Total and average allele numbers, expected (**He**) and observed (**Ho**) heterozygosities, F_{IS} values, analysis of molecular variance (AMOVA) test, Mantel test and Bottleneck analysis were conducted by using GenAlEx 6.5 (Peakall and Smouse, 2012), FSTAT (Goudet, 1995), Arlequin 3.5. (Excoffier and Lischer, 2010), and Bootleneck 1.2.02 (Piry et al., 1999) package programs, respectivley.

RESULTS AND DISCUSSION

A total of 269 different alleles were observed in 20 microsatellites (**Table 2**). The maximum and minimum numbers of total alleles were observed in TGLA122 (26 alleles), and INRA005 (7 alleles) loci, respectivelly. The mean allele number was 13.45. The highest averages of

Table 2: Average and total number of alleles, average expected (**He**) and observed (**Ho**) heterozygosities and total F_{IS} values.

SN	Locus	Average Allele	Allele (n=269)	Average		Total F_{IS}
				(Observed) Ho	(Expected) He	
1	CSSM66	12.17	14	0.822	0.856	0.046
2	CSRM60	9.83	15	0.761	0.762	0.038
3	ETH03	10.50	14	0.762	0.804	0.055
4	INRA023	10.50	14	0.779	0.808	0.058
5	HEL9	11.67	16	0.793	0.834	0.049
6	ILSTS006	9.00	13	0.673	0.755	0.123
7	SPS115	8.33	10	0.661	0.768	0.166
8	ETH185	11.33	17	0.797	0.788	0.033
9	BM1818	9.00	13	0.767	0.771	0.038
10	ETH225	10.17	13	0.742	0.814	0.115
11	ETH10	7.50	9	0.644	0.669	0.053
12	TGLA53	15.50	23	0.801	0.877	0.093
13	BM2113	9.50	13	0.806	0.840	0.071
14	INRA005	5.17	7	0.671	0.685	0.027
15	HAUT27	8.33	10	0.619	0.734	0.166
16	TGLA122	16.33	26	0.794	0.842	0.065
17	TGLA126	7.17	9	0.750	0.759	0.051
18	TGLA227	12.00	16	0.852	0.859	0.037
19	BM1824	5.33	8	0.719	0.711	**-0.004**
20	HEL13	6.83	9	0.728	0.788	0.070
Average		**9.81**	**13.45**	**0.747**	**0.786**	**0.068**

Table 3: Analysis of Molecular Variance (AMOVA). Populations were evaluated as a single group.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Variation (%)	*p*-value
Among populations	5	103 596	0.14909 Va	1.96	
Within populations	536	3 988,665	7.44152 Vb	98.04	**0.000**
Total	**541**	**4 092,251**	**7.59061**		

Table 4: Mantel test results

Populations	Criterion	Correlation coefficient	*p*-value	Importance
Single group	D_A matrix / Geographical Distance	0.715911	0.003	***p*<0.01**
	F_{ST} values / Geographical Distance	0.990176	0.003	***p*<0.01**

observed (Ho) and expected (He) heterozygosities were ranged from 0.619 to 0.852, and 0.669 to 0.877, respectively. General F_{IS} value was 0.068 in all populations. A negative total F_{IS} value (-0.004) was determined only for BM1824 (**Table 2**).

Genetic variation within and among the breeds were determined by AMOVA. All populations were analyzed to be a single group by AMOVA (**Table 3**). A total genetic variation of 98.04% was found within populations, whereas 1.96% vatiation was recorded among the populations. Total genetic variation in the populations were found to be statistically significant (p<0.001) (**Table 3**).

A relationship between the genetic distance matrix (D_A) and F_{ST} values matrix with geographical distance for populations were analyzed by the Mantel test. The results were evaluated to be a single group of all population (**Table 4**). When all populations were evaluated as a single group, the correlation between genetic distance (D_A) and geographic distance was positive, weak (0.715911) and statistically significant (p<0.01). The correlation value (0.990176) between F_{ST}

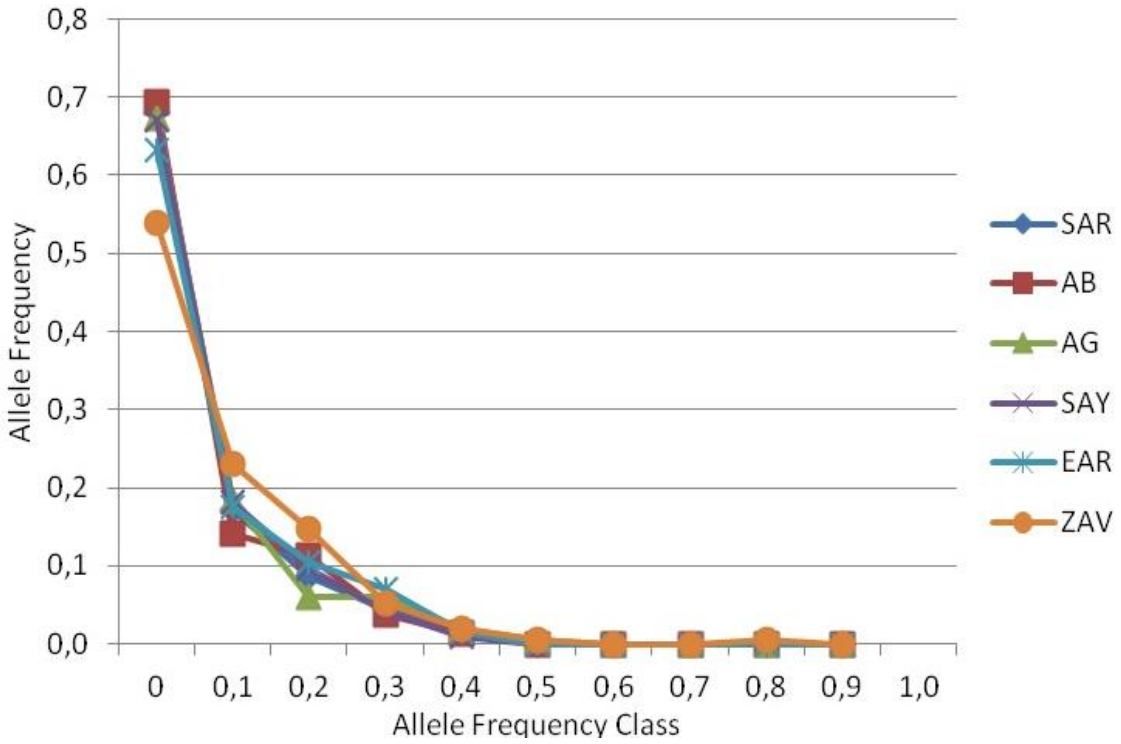

Figure 1. Bottleneck analysis. A normal distribution of L-shaped curve was observed for all cattle populations, indicating that the populations did not experience any recent potential risk of extinction..

and geographic distance was found as expected *i.e.*, positive and statistically significant (p<0.01).

According to the results obtained from the Bottleneck analysis, SAR (0.123), AB (0.057), AG (0.392), SAY (0.139) and ZAV (0.077) populations had been experienced no recent risk of extinction (p>0.05). Although EAR (0.004) populations was at the p<0.05 level, all populations revealed a normal L–shaped distribution (**Figure 1**), and thereby no recent bottleneck was determined.

Comparing with the European cattle breeds, higher allele numbers and genetic diversity were observed in this study (MacHugh et al., 1997; Martin-Burriel et al., 1999; Schmid et al., 1999; Maudet et al., 2002; Beja-Pereira et al., 2003; Mateus et al., 2004; Altınalan, 2005; Radko et al., 2005). These findings were in agreement with the previous reports indicating that the cattle breeds located in Anatolia and Middle-East regions had higher genetic diversity as compared to European, African and Indian cattle breeds (MacHugh et al., 1997; Loftus et al., 1999; Troy et al., 2001). This was explained as the result of proximity to the domestication center (Loftus et al., 1999; Özkan, 2005).

In population genetics studies, AMOVA allowed to determine the differentiation among populations (evolutionary origin). Through this analysis, levels of genetic diversities were tested among the groups, among populations, within groups, and within populations (Excoffier et al., 1992). When all populations were assessed into a single group, 98% of the genetic variation found as significant (p<0.001) that was observed within populations. Wiener et al. (2004) determined total genetic variations as 87% (within population), and 13% (among populations), respectivelly using 8 British cattle breeds (Aberdeen Angus, Ayrshire, Dexter, Friesian, Guernsey, Hereford, Highland and Jersey). AMOVA analysis of Portugal native cattle breeds (Alentejana, Arouquesa, Barrosa, Brava de Lide, Garvonesa, Minhota, Mertolenga, Marones and Mirandesa) showed that 91.04 and 8.96% of the total genetic variations were present within and among populations, respectivelly (Mateus et al., 2004). In other studies, total genetic variations were obtained as 87% among populations (Wiener et al., 2004), and 94.56% in within populations (Casellas et al., 2004). A consortium study including Europe, Asia and Near East regions demonstrated that 90% of genetic variation was present within the populations by AMOVA (Li et al., 2007).

Mantel test could reveal the correlation between two different matrixes (Mantel, 1967; Rousset and Raymond, 1997). A midpoint was determined on map for each population based on the geographical localization of sampling areas. The distances between each population were measured in 1/1,000 mile scale based on the map distances. A relationship between the genetic distance matrix (D_A) and F_{ST} values matrix with geographical distance for populations were analyzed by the Mantel test. Significance testing was performed using permutation tests (Mantel, 1967; Rousset and Raymond, 1997). As previously suggested (Rohlf, 1998), the relationships were determined as very good ($r \geq 0.9$), good ($0.8 \leq r < 0.9$), poor ($0.7 \leq r < 0.8$) or very poor ($r < 0.7$) using the correlation coefficient.

A positive (r=0.990176) significant ($p<0.01$) correlation was observed between F_{ST} values and geographical distances. However, the correlation between the genetic distance (D_A) and geographical distance values was poor (r=0.715911). Similar to the study of Özkan (2005), a significant correlation value (r=0.76: $p<0.02$) between the geographical distance and standard genetic distance ($D_{S)}$ was determined. In another study in France, an important correlation (r=0.70; $p=0.012$) was recorded between geographic and genetic distance (Maudet et al., 2002). However, Chikhi et al. (2004) reported an insignificant correlation between geographical and genetic distances (r=0.036, insignificant).

Bottleneck analysis was done based on the hypothesis that Ho were greater than He in populations, and calculation of the possible recent reduction in population size (Cornuet and Luikart, 1996; Luikart and Cornuet, 1998; Piry et al., 1999). Different bottleneck tests (Sign, Standardized differences and Wilcoxon sign–rank tests) were used for determining the number of loci with a significant excess of heterozygosities in populations. Wilcoxon test was reported to be the most suitable for Bottleneck test, where *p*-value was calculated by 1,000 simulation under Infinite Allele Model (IAM), Stepwise Mutation Model (SMM), and Two Phase Model of Mutation (TPM) model (Cornuet and Luikart, 1996; Luikart and Cornuet, 1998; Piry et al., 1999).

In Bottleneck analysis of Turkish native cattle breeds, TPM model with 1,000 permutations was used, and the Bayesian approach significance was determined using the Wilcoxon test. According to the results, SAR, AB, AG, SAY, and ZAV populations were revealed a normal L–shaped distribution (**Figure 1**) indicating that these populations did not experience any recent potential risk of extinction. The extinction probabilities of AB (0.057) and ZAV (0.077) populations were calculated to be very low. A previous study conducted by Özkan (2005) on SAR, AB, EAR, and AG showed that all probabilities were >0.41 ($p>0.05$), and we found similar findings in this study except EAR population. Ganapathi et al. (2012) used 3 different models (IAM, SMM and TPM) for analyzing genotypic data of Indian cattle breeds, and a genetic richness was observed in 25 loci when IAM and TPM models were used. Also, a probability of $p<0.01$ and graphical representation suggested that there was no recent bottleneck, which was in support of the findings of Pandey et al. (2006).

CONCLUSIONS

Population genetic analyses indicated that native Turkish cattle breeds have high level of heterozygosity. Also, it was determined that these breeds had not experienced any recent risk of extinction. However, there is still need of protection programs for these cattle breeds due to their decreasing population sizes.

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