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Determination of Phylogenetic Relationships of Turkish Native Cattle Breeds with Other Cattle Breeds Using Mitochondrial DNA D-loop Sequence Polymorphism

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ABSTRACT : The aim of this study was to determine the specific polymorphic sites in cattle breeds and inter- and interbreed genetic variation among breeds and to develop a databank of Turkish native cattle mtDNA using sequence analysis. The entire D-loop region was analyzed based on DNA sequences in Turkish Grey, East Anatolian Red, South Anatolian Red, and Anatolian Black native breeds. In total, 68 nucleotide differences were observed at 26 different sites. The variable positions consisted of 22 transitions, two transversions, and two insertions, but no deletions. Haplotype number, haplotype diversity, nucleotide diversity, and mean number of pairwise difference values were found to be 17, 0.993, 0.00478, and 4.275, respectively. In addition, a phylogeny was developed by comparison among cattle populations for which the entire D-loop sequence was available. A high level of genetic variation was observed within and among the native cattle breeds. (**Key Words** : Genetic Resource, Genetic Diversity, Mitochondrial DNA, D-loop Sequencing, Cattle)

INTRODUCTION

The analysis of mtDNA sequence diversity has provided important insights into the origin and diversity of modern cattle populations. The main phenotypic subdivision of cattle into *Bos taurus* and *Bos indicus* has been shown to correlate with a marked sequence differentiation at the mtDNA level (Loftus et al., 1994a). It has been found using either microsatellite or mtDNA analysis that there are at least two regions of cattle breed domestication. One of these regions is the Near East, where taurine or humpless cattle (*B. taurus*) are bred, and the other is the Indus Valley of India, where Zebu or humped cattle (*B. indicus*) are bred (Loftus et al., 1999; Troy et al., 2001; Bruford et al., 2003). A subsequent study has also argued that modern mtDNA sequence distributions suggest biologically distinct origins for the indigenous *B. taurus* populations of Africa and Europe (Bradley et al., 1996).

Turkey has some original cattle breeds saved as a genetic resource. Some breed characteristics of four Turkish native breeds examined in this study are as follows. East Anatolian Red (EAR) is a native breed adapted to the harsh climate, poor quality pasture in the hills and uplands of East Anatolia which is 1,300-2,000 m above sea level. EAR cattle have been raised for their meat and milk. Common color of the EAR is light red, but color varies from light to dark red. Average mature weight ranges from 250-300 kg. The average lactation period and milk yields are 170 days and 700-800 kg with a 5-8% fat content (Soysal et al., 2004). Anatolian Black cattle are raised all over Turkey, except eastern Anotolia and the European part of Turkey. Characteristic features of the Anatolian Black cattle breed are relatively small body size, black color and short horns. Under village conditions, live weight varies between 200-300 kg and milk production between 1,500-2,000 kg with a 5-8% fat content. The conformation of the breed tends to vary. Some animals exhibit a more typical beef build and others tend toward dairy. The legs are short and thick and the neck long with a large dewlap. The coat is brown-black to dark gray. Anatolian Black cattle have relatively high adaptability for inadequate conditions and some improvement programs have been carried out. Anatolian Black is hardy, disease resistant and tolerant of poor care, meager diet and adverse climate conditions (Soysal et al., 2004). Turkish Grey originated from the Balkan region of Europe, so the other name of this breed is Plevne. They are raised in Trakya (European part of Turkey), Marmara

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Region, Western Anotolia. Turkish Grey cattle have big horns and usually a grey colour. Their body size is relatively large and they are used especially as a power source. The live weight, which depends on levels of nutrition, management and housing facilities, varies from 300-350 kg and milk production can reach up to 800-1,000 kg and sometimes 1,500-2,000 kg. Since meat fibers are thick and strong the meat quality is not good. Turkish Grey is a tri-purpose breed kept for milk and meat as well as being used as a work animal (Soysal et al., 2004). South Anatolian Red (SAR) Cattle are raised in the southern Anatolia region of Turkey. SAR is known for giving greater milk yield than other Turkish native cattle breeds. SAR has a relatively small body size and a live weight of 200-350 kg. Their height is 125-135 cm and lactation period is 275 days; the milk yield varies between 1,500-3,200 kg. For several years, SAR adapted for poor conditions and endurance against disease (Soysal et al., 2004).

The mitochondrion has some advantages in scientific studies, since it is a source of cytoplasmic inheritance and has its own DNA passed only through the maternal line. Mitochondrial DNA has unique features, including a mutation rate considerably higher than that of nuclear DNA, multiple copies in a single cell, clonal inheritance characteristics, and no recombination (Carracedo et al., 2000; Rokas et al., 2003).

Since Anderson et al. (1982) published the complete sequence of bovine mtDNA, mtDNA D-loop sequences have been used for phylogenetic studies of cattle (Loftus et al., 1994b; Bradley et al., 1996; Mannen et al., 1998, 2003; Steinborn et al., 1998; Troy et al., 2001; Kim et al., 2003; Mirol et al., 2003; Pfeiffer et al., 2005; Lai et al., 2006).

The aims of this study were to locate a specific polymorphic site of the mtDNA D-loop in Turkish native cattle, determine genetic variation using mtDNA, and examine the phylogenetic relationships among East Anatolian Red, South Anatolian Red, Turkish Grey, and Anatolian Black cattle along with other cattle breeds from different countries. This Turkish native breeds have been conserved as gene resources in some Agricultural Research Institutes, in Anatolia next to the Near East.

MATERIALS AND METHODS

DNA extraction and PCR amplification of mtDNA D-loop

Genomic DNA was extracted from whole blood samples of four Turkish Grey (TG), four Anatolian Black (AB), four East Anatolian Red (EAR), and six South Anatolian Red (SAR) cattle using the Purgene DNA isolation kit (Gentra system, Minnesota, USA). The D-loop region was amplified by PCR using the following primers: L-strand, 5'-CTGCAGTCTCACCATCAACC-3' and H-strand, 5'-GTGCCTTGCTTTGGGTTAAG-3' (sequence from positions 15,737-16,338 and 1-394 of bovine mtDNA, accession number V00654).

PCR was performed in 50 μl volumes, each containing approximately 200-300 ng of template DNA, 10 μM of each primer, 2 μl of dNTPs (D7595: Sigma, St. Louis, MO, USA), 2.5 U of *Taq* polymerase (D1806: Sigma, St. Louis, MO, USA), 5 μl of 10×PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, and 0.01% gelatin), 3 μl of 25 mM-$MgCl_2$, and ddH_2O added to a final volume of 50 μl. The upper part of the tube was covered with 15 μl of mineral oil. Reaction profiles included a 5-min denaturation step at 95° followed by 30 cycles of denaturation for 45 s at 95°, 45 s annealing at 56°, and 45 s extension at 72°, and then a final 7-min extension step at 72°.

The D-loop PCR product sequences for each animal were analyzed by Iontek Limited Company (www.iontek.com.tr). Analysis outputs for the sequences of each individual were obtained using both the H-strand and the L-strand.

Phylogenetic analysis

Using the NCBI GenBank, we identified 75 samples from 19 foreign cattle breeds for which the entire mtDNA D-loop region sequences were available and added the data to allow comparison with the studied samples; in total, 93 samples were used for phylogenetic analyses (GenBank accession numbers: AB065119-31; AB085918-26; AB117037-38; AF022916; AF034438-42,46; AY378133-36, 38-42; AY521119-20, 27-28; AY676865-73; DQ124403-13, 16-18; EF126306-23; L27720-23, 32-33, 36-37; U92230; and reference V00654). The 93 individuals (18 Turkish native cattle and 75 from GenBank) represented 23 different cattle breeds.

In evaluation of the DNA sequence results, BIOEDIT version 7.041 software (Hall, 1999) was used to edit the sequence, and the ClustalX 1.83 software package (Thompson et al., 1997) was used to align the sequence, which was prepared for analysis by exclusion of the gaps at each end. Phylogenetic analysis was performed using MEGA version 3.1 software (Kumar et al., 2004). A neighbor-joining tree (Saitou and Nei, 1987) was constructed using the 93 sequences of the D-loop on the basis of Kimura's two-parameter method (Tamura and Nei, 1993).

RESULTS

Analysis of the mtDNA D-loop sequences of the four EAR, four TG, four AB, and six SAR cattle identified 26 polymorphic sites representing 2.9% of the total D-loop sequence analyzed. Genetic variation sites at 910 bp of

Table 1. Polymorphic regions detected in D-loop regions of 18 Turkish native cattle

Breeds	Base order numbers of mtDNA sequences																										
	15953	15966	16055	16057	16073	16085	16112	16119	16133	16135	16138	16140	16143	16185	16247	16248	16255	16260	16300	16302	8	163	169	203	215	216-223
Reference[a]	C	G	T	G	A	T	T	T	T	T	T	C	A	G	C	C	T	C	A	G	G	A	A	T	+	+
TG 1	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	-	-
TG 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	G	.	-	C
TG 3[b]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	C
TG 4	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	-	C
EAR1	.	.	.	C	.	C	.	.	.	.	.	.	.	A	.	.	C	T	.	.	.	.	G	.	-	C
EAR 2	G	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	C	.	.	.	.	.	G	.	-	C
EAR 3	.	A	.	C	.	.	.	.	.	.	.	.	.	A	.	.	C	.	.	.	.	.	G	.	-	C
EAR 4[b]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	C
SAR 1	.	.	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	G	.	-	-
SAR 2	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	-	C
SAR 3	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	-	C
SAR 4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	G	.	-	C
SAR 5	.	.	.	C	.	.	.	.	.	.	.	.	.	A	.	T	.	.	.	A	.	.	G	.	T	C
SAR 6	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	T	.	.	.	A	.	.	G	.	T	C
AB 1	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	-	C
AB 2	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
AB 3	.	.	.	C	.	.	.	.	.	C	.	.	.	A	.	.	C	.	.	.	.	.	G	.	-	-
AB 4	.	.	.	.	G	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	C	-	C

[a] Reference associated with Gen-Bank accession number V00654 (Anderson et al., 1982). [b] Having the same sequence and haplotype.
Dot (.) = Shows the same sequence as the reference.

surveyed native cattle breeds are presented in Table 1, and a genetic variation summary is presented in Table 2. While one member each of the TG and EAR breeds shared a common haplotype, other individuals represented unique haplotypes (Table 1). We identified 26 polymorphic regions with a total of 68 nucleotide differences: at variable positions, 22 transitions, two transversions, two insertions, and no deletions were found (Table 2). The insertions were T at 215 bp and C at 221 bp.

In the TG breed, we identified six polymorphic sites with a total of nine mutations: six transitions and three insertions; in the EAR breed, there were 10 polymorphic sites with a total of 19 mutations: 12 transitions, three transversions, and four insertions; in the SAR breed, for 13

Table 2. Observed mutations, region numbers, and their distributions among the breeds

Mutation \ Breeds	TG		EAR		SAR		AB		General	
	N	S	N	S	N	S	N	S	N	S
T→C[a]	2	2	4	2	5	4	5	5	16	9
C→T[a]	-	-	2	2	2	1	1	1	5	4
A→G[a]	3	2	3	1	8	3	4	2	18	5
G→A[a]	1	1	3	2	3	2	1	1	8	4
G→C[b]	-	-	2	1	1	1	1	1	4	1
C→G[b]	-	-	1	1	-	-	-	-	1	1
T[c]	-	-	-	-	2	1	-	-	2	1
C[c]	3	1	4	1	5	1	2	1	14	1
Total	9	6	19	10	26	13	14	11	68	26

N = Observed mutation number. S = Number of polymorphic sites.
[a] Transition. [b] Transversion. [c] Insertion.

Table 3. Intra-breed parameters of the analysis results

Breeds	N	S	h	Hd	k	π_n
TG	4	6	4	1.00	2.685	0.00298
EAR	4	10	4	1.00	4.872	0.00540
SAR	6	13	6	1.00	3.961	0.00439
AB	4	11	4	1.00	5.046	0.00560
GENERAL	18	26	17[a]	0.990	4.275	0.00478
GENERAL[b]	93	99	76	0.992	17.361	0.01966

S = The number of polymorphic sites. h = Haplotype number.
Hd = Haplotype diversity. k = Mean number of pairwise differences.
π_n = Nucleotide diversity.
[a] TG and EAR breeds have one common haplotype.
[b] Values including 93 individuals.

polymorphic sites there were 26 mutations: 18 transitions, one transversion, and seven insertions; and in the AB breed, in 11 polymorphic sites there were 14 total mutations: 11 transitions, one transversion, and two insertions (Table 2).

In the sequences from the 18 native Turkish cattle, the number of haplotypes was 17, haplotype variety was 0.990, nucleotide variability was 0.00478, and the mean number of pairwise differences was 4.275. Intra-breed variation values ranged between 2.685 and 5.046, and the lowest variation value occurred in the TG population while the highest was in the AB population (Table 3).

Addition of the GenBank data associated with the various cattle breeds for which the entire D-loop sequence data were available allowed evaluation of the relative position of Turkish native cattle breeds. The analysis involving sequences from 93 individuals and 23 cattle breeds showed that the number of polymorphic regions belonging to all populations was 99 and the number of haplotypes was 76; haplotype variety was 0.992, nucleotide variability was 0.01966, and the mean number of pairwise differences was 17.361.

Within populations, the dendrogram of the distances obtained according to the Kimura two-parameter method indicated that Turkish native cattle breeds exhibit a high variation and fall into the same group as the European

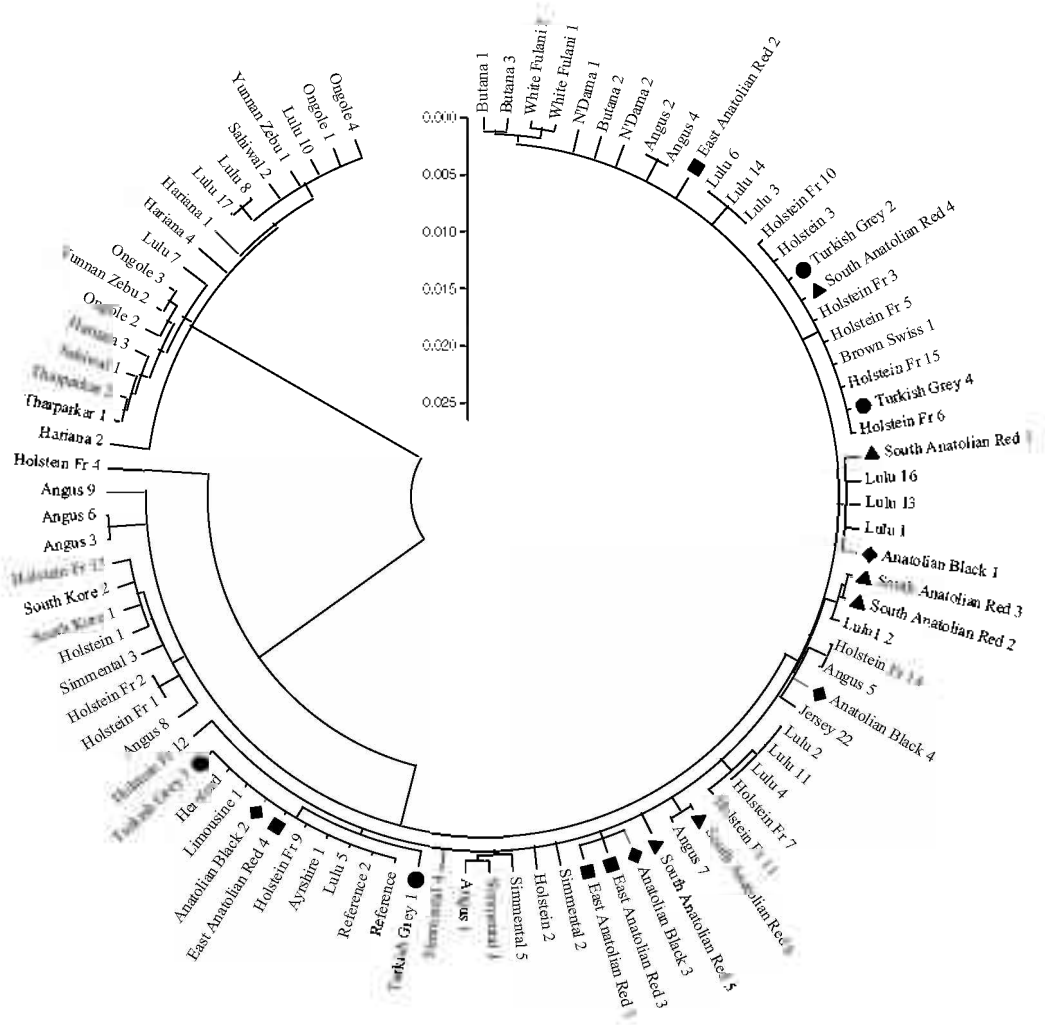

Figure 1. Individual phylogenetic tree of entire D-loop sequence analysis values.

breeds while also exhibiting a close relationship with the African breeds (Figure 1).

Interpopulation genetic distances are presented in Table 4. Although the distance values between breeds in the study ranged from 0.0055 to 0.0039, the maximum distance value was between the SAR and EAR breeds, and the minimum was between the SAR and TG populations. Figure 2 shows the dendrogram based on these values.

As Figure 2 shows, cattle belonging to the *B. taurus* and *B. indicus* breeds clustered into two main groups. While all the Turkish native breeds fell into the *B. taurus* group, TG was found to be closer to the European breeds, and SAR, AB, and EAR were positioned between the subgroup of the African breeds, such as N'Dama, Butana, and White Fulani, and the European breeds.

DISCUSSION

This study, the first to examine the entire D-loop sequence polymorphism of mtDNA in Turkish native cattle, shows that they have haplotypes in common with European and African cattle breeds and close relationships with these breeds. In this study, in which DNA sequence analysis of the D-loop region allowed analysis of differences at the nucleotide level, 68 nucleotide differences were identified in 26 different sites at 910 bp, but the total number of haplotypes was 17.

In a study carried out using mtDNA D-loop sequence variations, Mannen et al. (1998) found that one-half of the

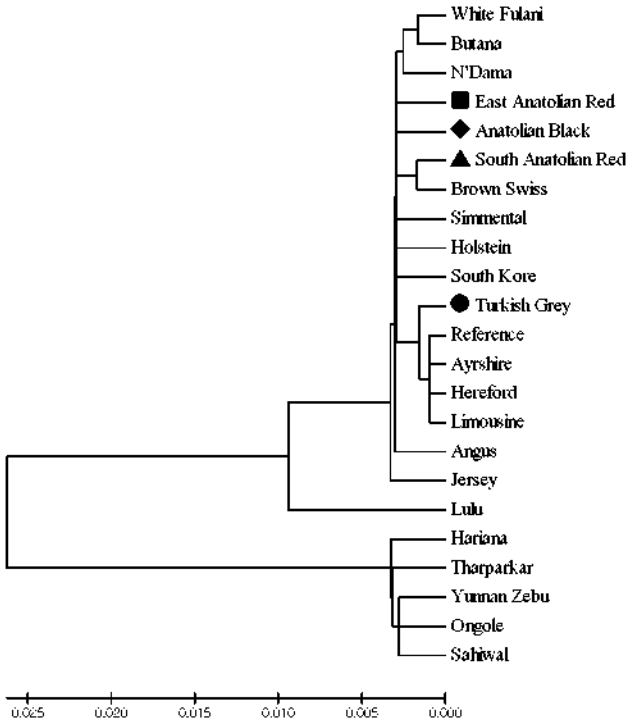

Figure 2. Phylogenetic relationships among cattle breeds according to the entire D-loop sequence analysis results.

31 Japanese Black cattle sampled were intermingled with European breeds, and the authors suggested that ancestors of Northeast Asian, European, and African cattle were interrelated. Steinborn et al. (1998) identified 43 nucleotide

Table 4. Genetic distances of the sequence analysis results (lower diagonal) and standard errors (upper diagonal)

	Ref	B.Irk	EAR	SAR	AB	Hols	Jers	Here	Sim	Ang	B.Sw	Lim	Ayr	W.F	But	Lulu	NDa	S.Ko	Y.Z.	Ong	Har	Sah	Thar
Reference		0.0008	0.0015	0.0014	0.0012	0.0009	0.0022	0.0019	0.0010	0.0017	0.0000	0.0013	0.0016	0.0024	0.0024	0.0025	0.0026	0.0024	0.0074	0.0074	0.0072	0.0075	0.0073
TG	0.0017		0.0014	0.0011	0.0010	0.0020	0.0021	0.0021	0.0011	0.0018	0.0008	0.0012	0.0013	0.0023	0.0023	0.0023	0.0024	0.0024	0.0074	0.0073	0.0072	0.0075	0.0073
EAR	0.0043	0.0051		0.0015	0.0013	0.0015	0.0023	0.0024	0.0015	0.0022	0.0015	0.0016	0.0017	0.0020	0.0021	0.0026	0.0026	0.0026	0.0075	0.0074	0.0073	0.0076	0.0074
SAR	0.0034	0.0039	0.0055		0.0012	0.0011	0.0020	0.0024	0.0013	0.0021	0.0014	0.0013	0.0013	0.0023	0.0023	0.0024	0.0021	0.0024	0.0074	0.0074	0.0073	0.0075	0.0073
AB	0.0034	0.0043	0.0054	0.0048		0.0012	0.0019	0.0023	0.0013	0.0020	0.0012	0.0014	0.0014	0.0021	0.0022	0.0021	0.0024	0.0024	0.0074	0.0073	0.0071	0.0075	0.0073
Holstein	0.0029	0.0040	0.0063	0.0049	0.0053		0.0020	0.0021	0.0012	0.0018	0.0009	0.0013	0.0013	0.0023	0.0023	0.0023	0.0020	0.0025	0.0074	0.0073	0.0072	0.0075	0.0073
Jersey	0.0045	0.0051	0.0071	0.0057	0.0057	0.0060		0.0030	0.0021	0.0027	0.0022	0.0022	0.0020	0.0028	0.0027	0.0028	0.0030	0.0026	0.0074	0.0073	0.0072	0.0075	0.0073
Hereford	0.0034	0.0051	0.0077	0.0068	0.0068	0.0063	0.0080		0.0021	0.0026	0.0019	0.0023	0.0025	0.0032	0.0031	0.0032	0.0032	0.0031	0.0077	0.0077	0.0075	0.0078	0.0077
Simmental	0.0034	0.0046	0.0065	0.0057	0.0058	0.0054	0.0067	0.0068		0.0019	0.0010	0.0014	0.0015	0.0023	0.0023	0.0023	0.0023	0.0025	0.0073	0.0072	0.0071	0.0074	0.0072
Angus	0.0023	0.0040	0.0066	0.0057	0.0057	0.0052	0.0068	0.0057	0.0057		0.0017	0.0021	0.0022	0.0030	0.0029	0.0030	0.0030	0.0029	0.0076	0.0075	0.0074	0.0077	0.0075
Brown Swiss	0.0000	0.0017	0.0043	0.0034	0.0034	0.0029	0.0045	0.0034	0.0034	0.0023		0.0013	0.0016	0.0024	0.0024	0.0025	0.0026	0.0024	0.0074	0.0074	0.0072	0.0075	0.0073
Limousine	0.0039	0.0048	0.0068	0.0055	0.0059	0.0056	0.0068	0.0073	0.0061	0.0062	0.0039		0.0015	0.0024	0.0024	0.0024	0.0021	0.0026	0.0074	0.0074	0.0072	0.0075	0.0073
Ayrshire	0.0023	0.0028	0.0048	0.0034	0.0040	0.0040	0.0045	0.0057	0.0047	0.0045	0.0023	0.0046		0.0024	0.0024	0.0024	0.0024	0.0026	0.0077	0.0076	0.0075	0.0078	0.0076
White Fulani	0.0051	0.0057	0.0057	0.0061	0.0063	0.0068	0.0074	0.0086	0.0070	0.0074	0.0051	0.0074	0.0051		0.0012	0.0015	0.0033	0.0029	0.0077	0.0076	0.0074	0.0077	0.0075
Butana	0.0065	0.0070	0.0073	0.0076	0.0074	0.0081	0.0080	0.0099	0.0081	0.0088	0.0065	0.0088	0.0065	0.0032		0.0018	0.0033	0.0029	0.0075	0.0076	0.0074	0.0077	0.0075
Lulu	0.0068	0.0074	0.0077	0.0080	0.0077	0.0083	0.0091	0.0103	0.0084	0.0091	0.0068	0.0089	0.0068	0.0040	0.0061		0.0033	0.0028	0.0075	0.0074	0.0072	0.0075	0.0073
N'Dama	0.0068	0.0074	0.0094	0.0072	0.0086	0.0069	0.0091	0.0103	0.0082	0.0091	0.0068	0.0078	0.0068	0.0097	0.0111	0.0109		0.0033	0.0082	0.0081	0.0080	0.0082	0.0081
South Kore	0.0167	0.0174	0.0194	0.0177	0.0185	0.0181	0.0184	0.0203	0.0186	0.0191	0.0167	0.0189	0.0176	0.0193	0.0205	0.0202	0.0218		0.0055	0.0054	0.0053	0.0056	0.0054
Yunnan Zebu	0.0505	0.0512	0.0540	0.0520	0.0527	0.0512	0.0505	0.0543	0.0512	0.0530	0.0505	0.0523	0.0531	0.0537	0.0526	0.0537	0.0581	0.0396		0.0019	0.0018	0.0022	0.0021
Ongole	0.0512	0.0518	0.0543	0.0524	0.0524	0.0519	0.0511	0.0550	0.0516	0.0536	0.0512	0.0529	0.0537	0.0537	0.0537	0.0531	0.0581	0.0390	0.0051		0.0017	0.0017	0.0017
Hariana	0.0499	0.0506	0.0531	0.0514	0.0521	0.0507	0.0499	0.0537	0.0507	0.0524	0.0499	0.0517	0.0524	0.0524	0.0522	0.0521	0.0572	0.0386	0.0057	0.0057		0.0020	0.0019
Sahiwal	0.0537	0.0543	0.0568	0.0549	0.0559	0.0544	0.0536	0.0575	0.0541	0.0561	0.0537	0.0554	0.0562	0.0549	0.0550	0.0546	0.0603	0.0413	0.0074	0.0060	0.0079		0.0018
Tharparkar	0.0512	0.0518	0.0543	0.0524	0.0534	0.0519	0.0511	0.0550	0.0520	0.0536	0.0512	0.0529	0.0537	0.0524	0.0524	0.0524	0.0581	0.0393	0.0063	0.0051	0.0066	0.0060	

differences in the D-loop regions of 32 unrelated Austrian cattle and found that 33 of these differences were transitions, five were transversions, one was a deletion, and four were inversions in 221 consecutive cytosine series. Kim et al. (2003) identified 22 different substitution mutations in 17 sites, only one transversion, and five inversions/deletions at the 1,064-bp D-loop sequence between 15,738 bp and 463 bp in four Cheju Black, four Cheju Yellow, four Korean Yellow, and two American Brahman cattle. This group identified the phylogenetic relationships of Northeast Asian cattle to various other cattle breeds, including *B. taurus*, *B. indicus*, and *Bison bison*, which were assessed using mtDNA D-loop sequences. In addition, they constructed a neighbor-joining tree using sequences determined for four Cheju Black, four Cheju Yellow, four Korean Yellow cattle (*B. taurus*), and two American Brahman cattle (*B. indicus*), and also published sequences for 31 Japanese Black cattle, 45 European breed cattle, six African zebus, two African taurines, and six Indian zebus, using five American bisons as an outgroup. Their neighbor-joining tree showed that American bison and Indian zebu are clearly separate from other cattle breeds, but the African cattle clustered together. The results indicated that cattle in Northeast Asia, Europe, and Africa are closely related to each other, implying a recent divergence, but are separate from Indian zebu. Mannen et al. (2003) analyzed complete mtDNA sequences of eight Japanese Black cattle, identifying 13 substitutions in the D-loop region and 14 in the gene-coding regions. They reported a mean sequence displacement between the haplotypes of 0.47% in the D-loop and 0.032% in the gene coding regions. Mirol et al. (2003) assessed 489 bp in the D-loop region of 36 samples from five Creole cattle populations reared in Argentina and Bolivia and identified 23 haplotypes occurring at 35 polymorphic sites: 30 transitions, four transversions, and 1-2 insertions. They also identified a mean nucleotide variability and mean number of pairwise differences of 0.058 and 2.61, respectively. Lai et al. (2006) studied the D-loop region sequences from a total of 207 China cattle, 136 *B. taurus* cattle, and 71 *B. indicus* cattle and detected 77 substitutions and 81 haplotypes in the *B. taurus* population and 53 substitutions and 50 haplotypes in the *B. indicus* population; 76 individuals from different countries were added to this latter population.

Mirol et al. (2003) stated that an A→G mutation occurring at 169 bp characterized the breeds originating in the Near East; in many European and Asian breeds, G substitution was identified. These authors also found that an A insertion at 16,200 bp was found only in Indian-originated breeds; a T→C transition at 16,255 bp represented the African breeds, and this C is a discriminating feature that can distinguish European and African breeds. Among these identified mutations, the transition at 169 bp was seen dominantly in all the breeds in the present study, but the transition at 16,255 bp was found only in the EAR and AB populations.

The phylogenetic tree shows the *B. taurus* and *B. indicus* cattle breeds divided obviously into two large main groups. This finding has support from several studies carried out with either microsatellite or mtDNA (Loftus et al., 1994a; MacHugh et al., 1997). Native EAR and AB populations in this study occupied a position between breeds originated from Europe and Africa; the native TG and SAR populations fell closer to the European breeds analyzed. In considering distance values relative to European breeds, native breeds are ordered as AB, EAR, SAR, and TG, respectively. This finding may arise because of the AB and EAR populations having a larger expansion area in Anatolia, and thus far there have been no effective selection and crossbreeding studies in TG and SAR populations.

In light of these findings, the Turkish native cattle breeds studied here should have priority in preservation as genetic reserves because of their origin characteristics. Changing consumer demand and haphazard application of crossbreeding techniques can result in the rapid loss of the original genetic reserves. Genetic reserves should be conserved because native breeds can convert areas unsuitable for agriculture to fields for meat and milk production; they provide the basic genetic materials for new types while sustaining genetic diversity; and they can be used to increase resistance in hybrids that may exhibit susceptibilities (Soysal et al., 2001). With the preservation of native breeds with high adaptation potential, hybrid superiority can be achieved without loss of genetic diversity.

In this study, a large number of polymorphic regions were detected on mtDNA of the native cattle breeds via DNA sequence analysis of the D-loop region. The main reason for our finding of a large number of polymorphic regions on the mtDNA of the native cattle breeds may be the proximity of Turkey to the Near East, one of the two domestication centers. In addition, examined native breeds have not yet been affected by conventional breeding methods, such as selection and crossbreeding. Maternally inherited mtDNA sequence polymorphisms were used in the current analysis to infer phylogenetic relationships, but more accurate findings might have been obtained by using more markers, such as the Y-chromosome or microsatellite DNA, and by combining assessment of nuclear DNA polymorphisms and morphologic characteristics with the mtDNA techniques.

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