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Y chromosome analysis of native Turkish cattle breeds by microsatellite markers

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Abstract: The aim of this study was to determine phylogenetic relationships of 6 native cattle breeds of Turkey using 7 Y chromosome-specific microsatellite DNA markers. DNA samples were isolated from Anatolian Black, Anatolian Grey, South Anatolian Red, Native Southern Anatolian Yellow, East Anatolian Red, and Zavot cattle using a standard phenol/chloroform method. PCR products were separated by capillary electrophoresis and marker genotypes were determined. A total of 41 different alleles were observed. The mean allele number was 5.86 and mean F_{IS} value was 0.427 for all populations. The INRA189 locus was monomorphic for all populations except for South Anatolian Red. UMN0307 and INRA124 loci were monomorphic in the East Anatolian Red and Anatolian Black populations, respectively. The Anatolian Black population was assigned to its own population at a maximum level (78.26%). In this study, a taurine-specific allele was identified in the INRA124 locus. Zebu- and taurine-specific alleles were observed in BM861, INRA189, and UMN0103 loci. Allele 134 in INRA124 seems to be a specific allele of taurine or zebu. The alleles of the UMN0307 and UMN0504 loci need to be investigated for zebu- or taurine-specific alleles. The resulting neighbor-joining tree and structure analysis suggested that the breeds analyzed are consistent with their modern geographical locations.

Key words: Cattle, microsatellite, Turkey, Y chromosome

1. Introduction

The Y chromosome follows paternal ancestry and is often investigated for determination of paternal origins (Li et al., 2007). The first analysis of the Y chromosome was made based on karyological features of different species and it was identified as metacentric/submetacentric and acrocentric in taurine (*Bos taurus*) and zebu (*Bos indicus*), respectively (Potter and Upton, 1979; Halnan and Watson, 1982).

The understanding of the origin, relationships, and paternal inheritance of native breeds indicated that there is large share of Y chromosome-specific markers (Edwards et al., 2000; Hellborg and Ellegren, 2004; Li et al., 2007). Y chromosome-specific markers are preferred for testing paternity, examining contamination risks of DNA samples (analysis of male component in male/female mixtures), and handling criminal cases (Jobling et al., 1997; Gill et al., 2001; Jobling, 2001). Y chromosome-specific single nucleotide polymorphisms (SNPs) and microsatellites were also used to investigate the genetic diversity and origins in dogs (Bannasch et al., 2005; Erdoğan et al., 2013), cattle (Bradley et al., 1994; Budowle et al., 2005; Cai et al., 2006; Yang et al., 2011), sheep (Niemi et al., 2013), and human populations of different regions (Cinnioglu et al., 2004; Rootsi et al., 2004). A Y chromosome haplotype reference database of Asian, European, and American countries has been made available to the scientific community (http://www.yhrd.org/; Willuweit and Roewer, 2007).

SNP markers were used to identify genetic variations in both X and Y chromosomes of taurine and zebu cattle breeds in Africa. SNP markers were reported to be useful in the determination of a mixture of zebu in African cattle breeds (Anderung et al., 2007). The Y chromosome-specific markers were also determined in cattle (Antoniou and Skidmore, 1995). The genetic diversity of the Y chromosome was determined as lower than that of autosomal chromosomes (Liu et al., 2003; Hellborg and Ellegren, 2004; Ginja et al., 2009). For prenatal sex determination in cattle, fetal sexes were successful determined at 99.9% after 55 days of pregnancy using circulating fetal DNA (da Cruz et al., 2012).

Previous studies based on archaeological and genetic data indicated that there are 2 domestication centers for cattle, sheep, and goat (Loftus et al., 1994; Luikart et al., 2001; Troy et al., 2001; Hiendleder et al., 2002; Bruford and Towsend, 2004). The Fertile Crescent region, including

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part of Anatolia, is accepted as the oldest and also the most important domestication center for these species. Molecular genetics studies also indicated that the European cattle, sheep, and goat breeds originated and spread from Anatolia (Loftus et al., 1999; Luikart et al., 2001; Troy et al., 2001; Bruford and Towsend, 2004; Cymbron et al., 2005). Bruford and Towsend (2004) suggested that conservation and genetic characterization of Anatolian native animal breeds are critically important due to them being the closest relatives of the first domesticated animals. Therefore, phylogenetic relationships of Turkish native cattle breeds were previously studied using autosomal and maternal marker systems (Özşensoy et al., 2010; Kurar et al., 2011) as part of a national project titled "In Vitro Conservation and Preliminary Molecular Identification of Some Turkish Domestic Animal Genetic Resources-I (TURKHAYGEN-I)".

The aim of this study was to determine phylogenetic relationships of 6 native cattle breeds of Turkey using 7 Y chromosome-specific microsatellite DNA markers.

2. Materials and methods

A total of 146 blood samples were collected from South Anatolian Red (SAR, n = 26), Native Southern Anatolian Yellow (SAY, n = 25), Anatolian Black (AB, n = 23), Anatolian Grey (AG, n = 29), East Anatolian Red (EAR, n = 26) and Zavot (ZAV, n = 17) cattle. Genomic DNA samples were extracted using a standard organic phenol/chloroform method (Sambrook et al., 1989).

A total of 7 Y-specific microsatellite loci and 2 fluorescence-labeled M13 primers (Table 1) were selected from previous studies (Liu et al., 2003; Li et al., 2007; Ginja et al., 2009). For pigtailing, the forward primers were synthesized with tails that matched one of the fluorescence-labeled M13 primers. Each polymerase chain reaction (PCR) was carried out in a 15-µL reaction volume including 1X Mg^{++}-free PCR buffer (Fermentas), 0.125 mM dNTPs (Fermentas), 1.5 mM $MgCl^{++}$, 0.375 U of Taq polymerase (Fermentas), 2–10 pmol of each primer, and ~100 ng of genomic DNA.

A touchdown PCR profile (Don et al., 1991) was used with 2 steps. The first step was initial denaturation at 95 °C for 4 min, followed by 16 cycles of denaturation at 94 °C for 30 s, annealing beginning at 60 °C and ending at 52 °C for 30 s, and extension at 72 °C for 30 s. The annealing temperature was decreased by 0.5 °C per cycle until it reached 52 °C. In the second step, 25 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s were applied. A final extension of 72 °C for 10 min was applied in all reactions.

The resulting PCR products were prepared for capillary electrophoresis and loaded onto a Beckman Coulter CEQ 8000 Genetic Analysis System. Genotypes were determined by fragment analysis. Genotypes were analyzed after the removal of the added nucleotides (fluorescence-labeled M13 primers).

Table 1. Description of microsatellite loci used in the study.

No.	Locus	Primer	Add.	Allelic range (bp)*
1	BM861	TTGAGCCACCTGGAAAGC	m13-Cyb5	135–192
		CAAGCGGTTGGTTCAGATG		
2	DDX3Y	TGAACCACTAGGGAGGTCATC	m13-IRD700	249
		TTCCAATTTAGCTGTGGTTATCTG		
3	INRA124	GATCTTTGCAACTGGTTTG	m13-Cyb5	126–190
		CAGGACACAGGTCTGACAAT		(58–67)
4	INRA189	TACACGCATGTCCTTGTTTCGG	m13-IRD700	148–156
		CTCTGCATCTGTCCTGGACTGG		(43–44)
5	UMN0103	ACACAGAGTATTCACCTGAG	m13-IRD700	124–136
		ATTTACCTGGGTCAAAGCAC		
6	UMN0307	GATACAGCTGAGTGACTAAC	m13-IRD700	101–162
		GTGCAGACATCTGAGCTGTG		
7	UMN0504	AGGCCATCTGCATAGTGAAG	m13-Cyb5	106–144
		TGCTGGACTGCTCATCTCTG		
8	m13-IRD700	TTTCCCAGTCACGACGTTG		
9	m13-Cyb5	TAAAACGACGGCCAGTGC		

*: Liu et al., 2003; Ginja et al., 2009.

Total and average allele numbers, expected (He) and observed (Ho) heterozygosities, deviation from Hardy–Weinberg equilibrium (HWE), F_{IS} values, structure, and neighbor-joining (NJ) analyses were done using GenAlEx6 (Peakall and Smouse, 2006), FSTAT (Goudet, 1995), Populations 1.2.32 (http://www.bioinformatics.org/project/?group_id=84), TreeWiev (Page, 1996), and Structure 2.3.4 (http://kinglab.eeb.lsa.umich.edu/EEID/eeid/evolution/Popgen_EEID_2012/Manuals/STRUCTURE_Manual.pdf) package programs. For determining the best number of clusters, the ΔK criterion in structure analysis was calculated according to Evanno et al. (2005). In this study, 6 independent runs of K (K = 1–6) were done for the whole dataset using an admixture model. All model runs were based on 100,000 Markov chain Monte Carlo iterations and 50,000 after an initial burn-in period. Five independent runs were performed for each K value.

3. Results

A total of 41 different alleles were observed in 6 populations for 7 microsatellite markers. The minimum and maximum numbers of total alleles varied from 3 (INRA124 and UMN0504) to 8 (DDX3Y), respectively. The mean allele number was 5.86 (Table 2). Allele frequencies are presented in Table 3. The highest average observed (Ho) and expected (He) heterozygosities ranged from 0.054 to 0.809 and from 0.061 to 0.704, respectively. The mean F_{IS} value was 0.427 for all populations. The F_{IS} value was significant in all populations (Table 4). The INRA189 locus was monomorphic for all populations except for SAR. The UMN0307 and INRA124 loci were monomorphic in the EAR and AB populations, respectively. HWE was found in all populations (Table 5).

A pairwise population matrix of Nei genetic distances is presented in Table 6. The highest genetic distance was found between ZAV and SAR (0.388). The SAY population was closest to the AG (0.105) and EAR (0.103) populations.

The mean polymorphism rate for all populations was 83.33%. The assignment test results illustrated that 47% of the populations were assigned to their own populations. The AB population was assigned to its own population at the maximum level (78.26%). SAR (53.85%), SAY (40%), AG (13.79%), EAR (46.15%), and ZAV (64.71%) were also assigned to their own populations.

The NJ tree illustrated that there was an admixture between EAR and SAY, AB and ZAV, and SAR and AG populations and these populations were closely localized (Figure 1). Findings of the structure analysis and assignment tests were similar. The AB and AG populations appear to have the least mixture (Figure 2).

4. Discussion

In this study, a total of 41 different alleles were observed, and the mean allele number was 5.86. A total of 12 alleles were observed in a previous study (Edwards et al., 2000) using the same 4 loci. The number of alleles ranged from 2 to 8 in this study, but numbers of alleles were lower (2 or 3 alleles) in other studies used the same loci (Hanotte et al., 1997; Liu et al., 2003; Li et al., 2007; Ginja et al., 2009; Pérez-Pardal et al., 2011). Genetic characterization studies using autosomal microsatellites and mtDNA reported that Turkish native cattle breeds had rich genetic diversity (Özşensoy et al., 2010; Kurar et al., 2011). Similarly, the Y chromosome-specific markers in Turkish cattle breeds showed high genetic diversity.

Seven loci used in the present study were found to be polymorphic, as reported in other studies (Budowle et al., 2005; Cai et al., 2006). The UMN0504 locus was found to be monomorphic in a previous study (Ginja et al., 2009) in which the same loci were also used. In this study, however, the UMN0504 locus was polymorphic in all populations and INRA189 was found to be monomorphic in all populations except for SAR.

African zebu and taurine can be easily distinguished by Y chromosome loci (Bradley et al., 1998). It was previously

Table 2. Observed number of alleles (Na) of 7 microsatellite DNA in 6 cattle populations.

	Populations							
Locus	SAR	AB	AG	SAY	EAR	ZAV	Mean	Total
BM861	5	5	5	5	7	5	5.33	7
DDX3Y	5	4	5	5	5	6	5.00	8
INRA124	2	1	3	2	2	2	2.00	3
INRA189	6	1	1	1	1	1	1.83	6
UMN0103	4	4	5	6	4	3	4.33	7
UMN0307	3	5	6	3	1	3	3.50	7
UMN0504	3	3	3	3	3	3	3.00	3
Mean	4.00	3.29	4.00	3.57	3.43	3.29	3.57	5.86

Table 3. Allele frequencies of 6 Turkish cattle populations.

		Populations					
Locus	Allele	SAR	AB	SAY	AG	EAR	ZAV
BM861	136	0.000	0.000	0.000	0.000	0.026	0.000
	156	0.000	0.136	0.000	0.238	0.053	0.077
	158	0.250	0.523	0.500	0.190	0.342	0.462
	160	0.273	0.091	0.075	0.095	0.079	0.077
	166	0.045	0.000	0.200	0.000	0.053	0.000
	168	0.273	0.182	0.125	0.429	0.395	0.231
	170	0.159	0.068	0.100	0.048	0.053	0.154
DDX3Y	143	0.000	0.000	0.000	0.000	0.000	0.071
	145	0.075	0.000	0.235	0.200	0.206	0.214
	147	0.050	0.028	0.029	0.033	0.147	0.000
	149	0.250	0.417	0.206	0.167	0.088	0.071
	151	0.125	0.111	0.059	0.000	0.088	0.143
	243	0.000	0.000	0.000	0.000	0.000	0.214
	247	0.000	0.000	0.000	0.033	0.000	0.000
	249	0.500	0.444	0.471	0.567	0.471	0.286
INRA124	132	0.289	0.000	0.500	0.368	0.889	0.182
	134	0.711	1.000	0.500	0.579	0.111	0.818
	142	0.000	0.000	0.000	0.053	0.000	0.000
INRA189	46	0.789	1.000	1.000	1.000	1.000	1.000
	88	0.026	0.000	0.000	0.000	0.000	0.000
	100	0.079	0.000	0.000	0.000	0.000	0.000
	106	0.053	0.000	0.000	0.000	0.000	0.000
	108	0.026	0.000	0.000	0.000	0.000	0.000
	112	0.026	0.000	0.000	0.000	0.000	0.000
UMN0103	118	0.000	0.000	0.056	0.000	0.000	0.000
	124	0.000	0.000	0.056	0.000	0.000	0.000
	126	0.167	0.048	0.056	0.036	0.000	0.071
	128	0.000	0.048	0.000	0.286	0.045	0.000
	130	0.048	0.000	0.222	0.107	0.068	0.000
	132	0.738	0.857	0.556	0.464	0.705	0.071
	134	0.048	0.048	0.056	0.107	0.182	0.857
UMN0307	140	0.000	0.000	0.000	0.019	0.000	0.000
	142	0.000	0.174	0.000	0.019	0.000	0.250
	144	0.000	0.022	0.000	0.019	0.000	0.042
	146	0.000	0.000	0.000	0.019	0.000	0.000
	150	0.205	0.717	0.886	0.577	1.000	0.708
	152	0.659	0.043	0.091	0.346	0.000	0.000
	156	0.136	0.043	0.023	0.000	0.000	0.000
UMN0504	106	0.342	0.341	0.119	0.188	0.208	0.235
	144	0.211	0.568	0.214	0.417	0.521	0.471
	146	0.447	0.091	0.667	0.396	0.271	0.294

Table 4. Observed heterozygosity (Ho), expected heterozygosity (He), and F_{IS} values of different loci in 6 cattle populations.

		Locus							
Population		BM861	DDX3Y	INRA124	INRA189	UMN0103	UMN0307	UMN0504	F_{IS}
SAR	Ho	0.227	0.800	0.053	0.421	0.048	0.045	0.684	0.416***
	He	0.761	0.664	0.411	0.366	0.423	0.505	0.639	
AB	Ho	0.500	0.778	0.000	0.000	0.000	0.217	0.682	0.166*
	He	0.662	0.616	0.000	0.000	0.259	0.451	0.553	
AG	Ho	0.286	0.800	0.158	0.000	0.143	0.154	0.500	0.471***
	He	0.712	0.609	0.526	0.000	0.679	0.546	0.635	
SAY	Ho	0.250	0.941	0.000	0.000	0.111	0.045	0.238	0.523***
	He	0.679	0.676	0.500	0.000	0.630	0.206	0.495	
EAR	Ho	0.105	0.824	0.111	0.000	0.045	0.000	0.417	0.461***
	He	0.712	0.699	0.198	0.000	0.464	0.000	0.612	
ZAV	Ho	0.000	0.714	0.000	0.000	0.000	0.417	0.471	0.521***
	He	0.698	0.796	0.298	0.000	0.255	0.434	0.637	
Mean	Ho	0.228	0.809	0.054	0.070	0.058	0.146	0.499	0.427
	He	0.704	0.677	0.322	0.061	0.451	0.357	0.595	

*: $P < 0.05$, ***: $P < 0.001$.

Table 5. Hardy-Weinberg Equilibrium statistics for each of the 7 microsatellite loci of 6 cattle populations.

	Populations					
	SAR	AB	AG	SAY	EAR	ZAV
Locus	P	P	P	P	P	P
BM861	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***
DDX3Y	0.008 **	0.000 ***	0.457 ns	0.120 ns	0.064 ns	0.360 ns
INRA124	0.000 ***	Mono-morphic	0.005 **	0.000 ***	0.063 ns	0.001 ***
INRA189	1.000 ns	Mono-morphic	Mono-morphic	Mono-morphic	Mono-morphic	Mono-morphic
UMN0103	0.000 ***	0.000 ***	0.001 ***	0.000 ***	0.000 ***	0.000 ***
UMN0307	0.000 ***	0.000 ***	0.033 *	0.000 ***	Mono-morphic	0.921 ns
UMN0504	0.021 *	0.013 *	0.089 ns	0.001 **	0.000 ***	0.002 **

ns: not significant, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

reported that INRA124, BM861 and INRA189 had taurine- and zebu-specific alleles (Edwards et al., 2000; Li et al., 2007). Two alleles (130, 132) were identified in the INRA124 locus. It was reported that 132 and 130 alleles of INRA124 were breed-specific alleles of taurine and zebu cattle, respectively (Hanotte et al., 1997; Edwards et al., 2000; Li et al., 2007; Ginja et al., 2009). In this study, 132 and 134 alleles of the INRA124 locus and a taurine-

Table 6. Pairwise population matrix of Nei genetic distances between 6 cattle populations

Populations	SAR	AB	SAY	AG	EAR	ZAV
SAR	0.000	0.184	0.202	0.124	0.331	0.388
AB		0.000	0.180	0.146	0.266	0.222
SAY			0.000	0.105	0.103	0.238
AG				0.000	0.138	0.218
EAR					0.000	0.309
ZAV						0.000

specific allele (132) were identified in all populations except for AB. To the best of our knowledge, this is the first time that allele 134 was observed in this study. Therefore, allele 134 is thought to be taurine- or zebu-specific allele due to the high level of genetic diversity (Özşensoy et al., 2010) and the close localization of Turkish cattle breeds to the domestication center (Loftus et al., 1994; Loftus et al., 1999; Troy et al., 2001; Cymbron et al., 2005). This finding was also supported by Bruford et al. (2003), indicating that European, North and West African, and Middle Eastern cattle breeds originated from taurine cattle while East

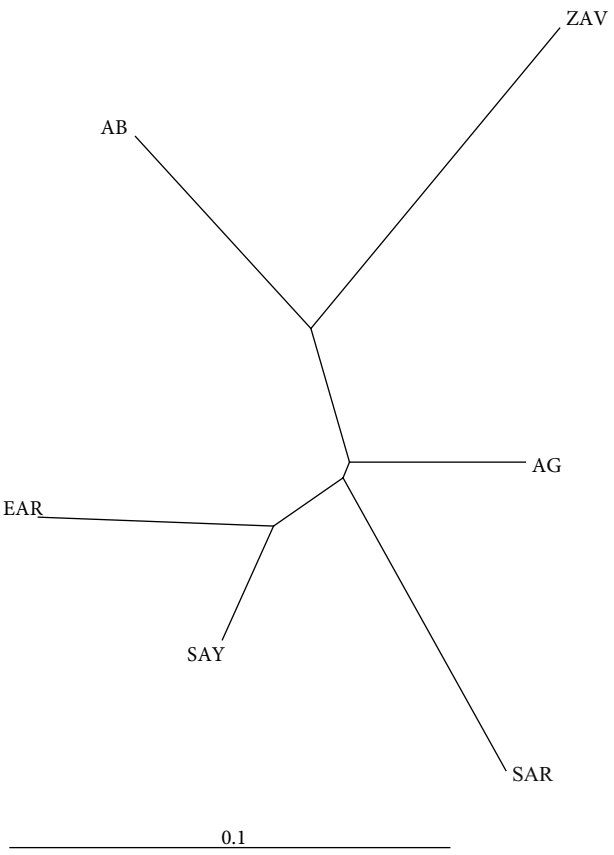

Figure 1. Neighbor-joining tree summarizing phylogenetic relationships among 6 Turkish cattle breeds.

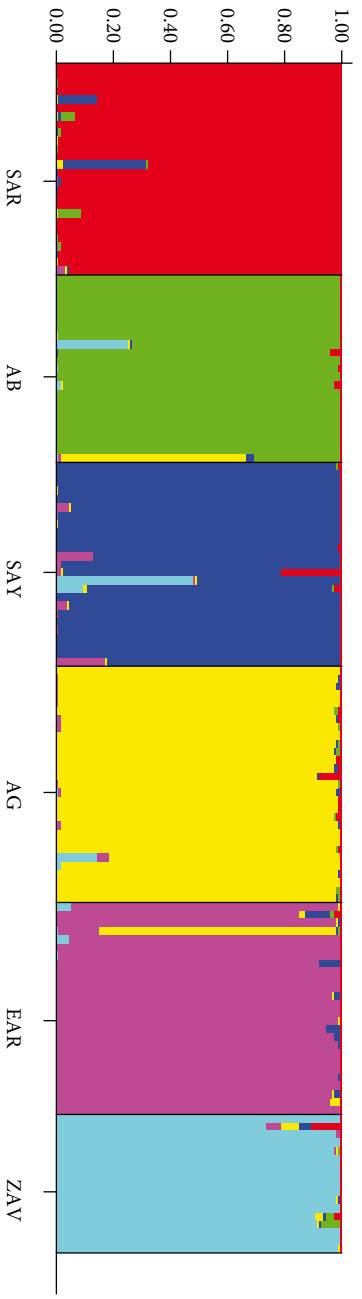

Figure 2. Bayesian assignment proportions for K = 6 clusters determined in structure analysis.

Eurasian (Indian) and East Africa cattle breeds originated from Zebu cattle. Li et al. (2007) reported that the BM861 locus contained zebu-specific (156) and taurine-specific (158) alleles. In this study, allele 156 was observed in AB, AG, EAR, and ZAV, but allele 158 was determined in all populations. The INRA189 locus contained zebu-specific (88) and taurine-specific (98 and 106) alleles (Li et al., 2007; Ginja et al., 2009). The 88 and 106 alleles were observed in only 1 animal of the SAR population. In the UMN0103 locus, zebu-specific alleles (136 and 125) were observed in the southern group and taurine-specific alleles (155 and 140) were observed in the northern group (Cai et al., 2006). In this study, the 126 allele of the UMN0103 locus was observed in all populations except for EAR. Some of the taurine alleles and the zebu allele (124), which were determined in another study (Pérez-Pardal et al., 2011), were observed in all populations and in SAR in this study, respectively. There is need for further investigation to define whether alleles of UMN0307 (150) and UMN0504 (106, 144, and 146) are zebu- or taurine-specific alleles.

Structure analysis at K = 6 separated the populations into discrete clusters. With only a few exceptions, most of the populations were clearly unified in their own clusters. Similar to the results of other markers systems (Özşensoy et al., 2010; Kurar et al., 2011), the resulting NJ tree and structure suggested that the breeds analyzed are consistent with their modern geographical locations.

As discussed above, Turkish native cattle breeds have critical importance due to their close proximity to the domestication center. Findings of this study also support the autosomal and maternal marker systems. Separation of zebu and taurine breeds focused on the genotyping data generated from both microsatellites and SNPs (Hanotte et al., 1997; Edwards et al., 2000; Li et al., 2007). On account of this, using the recommended Y chromosome SNPs seems to be an appropriate tool for investigation of zebu- and taurine-specific alleles and separation of breeds in Turkish native cattle breeds.

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