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***A STUDY ON GENETIC RELATIONSHIP OF NATIVE  
AND EXOTIC CATTLE BREEDS IN TURKEY BY  
USING MICROSATELLITE MARKERS***

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## ABSTRACT:

Four native Turkish cattle breeds and three exotic (western breed) breeds were investigated with seven microsatellite loci in order to characterise their genetic relationship.

It was determined that observed average number of alleles ranged between 7.571-11.286 and expected heterozygosity values ranged between 0.7345-0.8114. Furthermore, totally 11 private alleles with low frequencies were observed in the native breeds.

A cline in the frequencies of alleles of some microsatellite loci was observed from eastern to western Turkey. The proposed explanations were 1) Turkey is geographically close to the cattle domestication center and cline is parallel the migration from the center to the Europe, 2) There is admixture of zebu individuals from the east. Also, it was determined that there was genetic contribution from exotic breeds to native breeds in Turkey.

Results of several analyses (structure, breed integrity values, factorial correspondence analysis) showed that, although the exotic breeds could be, the native breeds with distinct phenotypes could not be resolved genetically with respect to 7 microsatellite loci

# NATIVE CATTLE BREEDS OF TÜRKİYE





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# TURKISH GREY



# EASTERN ANATOLIAN RED



# NATIVE BLACK CATTLE



# SOUTHERN YELLOW&SOUTHERN RED



## ***INTRODUCTION***

**In Turkey, cattle population decreased from 18 million to 11 million within 30 years.**

**Therefore, Ministry of Agriculture in Turkey carried out different cattle improvement projects to increase the number of cattle and the productivity of the native cattle breeds.**

**These projects mainly included cross breeding of native animals, which have low productivity, with those of modern exotic breeds such as Jersey, Holstein, Brown Swiss and Simmental.**

**Today, native cattle breeds are potentially endangered because of cross breeding. There are mainly three sources of genotypes for Turkish cattle breeds named as; native cattle breeds, imported cattle breeds (exotic cattle breeds) and cross breeds (hybrids of imports and native breeds).**

Turkish native cattle breeds are especially adapted to difficult local environmental conditions; such as rough climatic conditions; insufficient food availability, high resistant for disease and long distance travelling towards summer pasture located over 1000-2000 m above the sea level.

Therefore, native breeds should be conserved and the conservation of native cattle breeds has become an important part of the economic and environmental promotion to prevent a decline of job opportunities and depopulation because of environmental deterioration.

Cattle population has decreased in the last 30 years and there is not any conservation strategy for Turkish native breeds. In order to improve conservation strategies for Turkish native cattle breeds; firstly their genetic diversity and genetic distinctness should be determined (Bruford, et al; 2003).

In this study, the genetic variation within and among native and exotic cattle breeds in Turkey was analyzed by using 7 microsatellite loci (TGLA122, TGLA227, ETH10, ETH225, HEL5, ILSTS005, ILSTS006). The admixture, genetic diversity and genetic structure of the Turkish native and exotic cattle breeds were also examined.

## Breeds and Molecular Analyses

Blood samples were obtained from a total of 315 individuals, including seven different native (Turkish Grey Steppe, Anatolian Black, East Anatolian Red and South Anatolian Red) and exotic (western) cattle breed (Jersey, Brown Swiss and Holstein) in Turkey.

DNAs were extracted from 10 ml blood using standard phenol – chloroform procedure (Sambrook, et. al.; 1989). All the DNA's were extracted at Middle East Technical University, Department of Biology.

Seven microsatellite loci used in this study are ETH10, ETH225, TGLA122, TGLA227, HEL5, ILSTS005 and ILSTS006. These microsatellite loci are all present in the international selected set of markers recommended by FAO and ISAG for cattle diversity studies.

For all microsatellites, allele sizes were determined with a Perkin Elmer ABI Prism 310 Genetic Analyzer, using the Genescan software (Perkin Elmer). PCR analyses and size detection was

## Data Analysis

For each breed and for each locus, the number of alleles ( $n_A$ ), the observed heterozygosity ( $H_O$ ) and unbiased expected heterozygosity were calculated using the Genetix 4.02 program (Belkhir et al., 1996-2006).

Additionally, the average values of  $n_A$ ,  $H_O$ ,  $H_e$  based on seven loci were also computed for each breed. Wright's F statistics were calculated by using software program Genetix 4.0 (Belkhir et al., 1996-2000). FIS values of Wright's F statistics were estimated and their significance of deviation from the Hardy–Weinberg equilibrium was tested by permutation test.

FST values were used to determine the differentiation of the breeds. Factorial correspondence analysis was also performed by using the Genetix software program.

Additionally, Zebu admixture proportions were determined by direct counting from native and exotic cattle microsatellite data (Wright, 1969)

The Structure program (Pritchard et al., 2000) was adopted to identify the K (unknown) clusters of the genetic origin among individuals and to estimate the individual admixture proportions.

The program also allows detection of pure representatives of the formed clusters as well as potential admixed individuals between the K different groups.

It applies a Bayesian Markov Chain Monte Carlo (MCMC) approach that uses model-based clustering to partition individuals into clusters. First, the population structure was assessed using the entire sample set (n=315), and assuming that the sampled cattle belong to an unknown number of K genetically distinct clusters.

Posterior probability values for K were estimated assigning priors from two to ten at 200 000 MCMC repetitions and 200 000 burn-in period using no prior information.

To estimate K, the analysis was carried out 5 times for each potential value of K. The K value was chosen as 7, which showed the highest lnL value in this analyses.

Finally, "breed integrity" value was calculated by comparing the proportion of shared alleles (psa) between randomly chosen individuals across breeds and psa between individuals of the same breed. Breed integrity value was calculated by P. Wiener (Wiener et al., 2004).

## RESULTS and DISCUSSION

A total of 102 alleles were observed based on seven microsatellites in seven cattle breeds and 315 individuals. All of the loci were polymorphic and the number of alleles varied between 7 (ILSTS005) and 25 (TGLA122).

Average number of alleles in native cattle breeds was higher than the average number of alleles in exotic breeds. In this study, the most polymorphic microsatellites were TGLA122 with 25 alleles, while the least polymorphic was ILSTS005 with 7 alleles.

The level of polymorphism detected in seven microsatellites was highest compared to that of in the literature (Martin-Burriel et al, 1999, Schmid et al, 1999; Maudet et al, 2002; Radko et al, 2004).

The mean number of alleles detected was highest in the native breed (East Anatolian Red, 10.143; South Anatolian Red, 11.286), and lower in breeds from exotic cattle breeds, i.e. Jersey (7.571), Brown Swiss (8.143).

**As a result of the analysis of 7 microsatellite loci, totally 11 private alleles was observed.**

However, these alleles could not be used for breed determination since they have very low frequencies (< 5%) in small sample sized populations. In a study of six native Spanish breeds, Martin Burriel et al (1999), also found private alleles with low frequencies (<5%).

**Table.1. number of Alleles according to the breeds and loci**

	Grey cattle	Easter n Red	Native Black	Southe rn	Jersey	Brown Swiss	Black and White	Total	Mean/loci
TGLA122	16	16	13	17	10	9	11	92	13.143
ETH225	10	11	10	10	6	6	10	63	9
ETH10	9	8	7	9	6	6	8	53	7.571
HEL5	10	10	13	11	9	14	8	75	10.714
ILSTS005	6	4	5	6	2	3	2	28	4
TGLA227	13	14	14	15	12	10	9	87	12.428
ILSTS006	8	8	10	11	8	9	7	61	8.714
<b>Total</b>	<b>72</b>	<b>71</b>	<b>72</b>	<b>79</b>	<b>53</b>	<b>57</b>	<b>55</b>	<b>459</b>	
<b>mean. / breed</b>	<b>10.286</b>	<b>10.143</b>	<b>10.286</b>	<b>11.286</b>	<b>7.571</b>	<b>8.143</b>	<b>7.857</b>		

*Table 2 Observed ( $H_o$ ) and Expected ( $H_e$ ) Heterozygosity value in the breeds investigated*

Breed	$H_o$	$H_e$
Grey cattle	0.6823	0.7748
Eastern Red	0.6653	0.7799
Native black	0.7347	0.8114
Southern Red	0.6770	0.7837
Jersey	0.7613	0.7345
Brown Swiss	0.7351	0.7428
Black and White	0.7535	0.7435

**Table 3** *The distribution of specific alleles observed according to The Loci Investigated*

<i>Loci</i>	<i>Allele length (bp)</i>	<i>Frequency</i>	<i>Name of breed</i>
<i>TGLA122</i>	156 bp	0.0729	Brown Swiss
	160 bp	0.0435	Grey Cattle
	168 bp	0.0294	Jersey
<i>ETH225</i>	166 bp	0.0109	Grey cattle
<i>HEL5</i>	135 bp	0.0119	Native Black
	137 bp	0.0119	Native Black
	173 bp	0.0098	Jersey
<i>ILSTS005</i>	190 bp	0.0109	Grey cattle
<i>TGLA227</i>	73 bp	0.0109	Southern Red
	103 bp	0.0119	Jersey
	105 bp	0.0217	Black&white

**Table .4. The distribution of  $F_{IS}$  values of population investigated.**

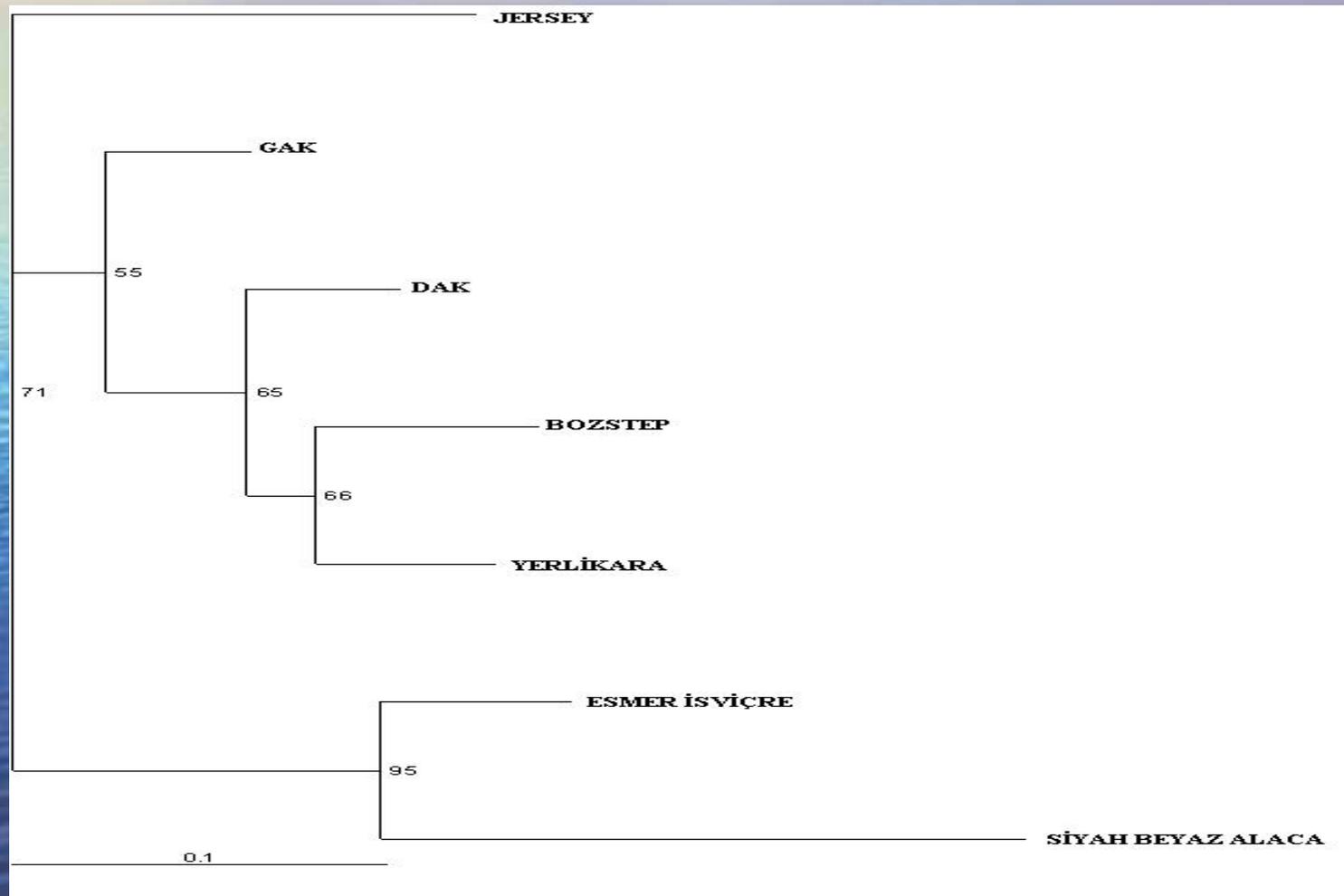
<b>Name of population</b>	<b><math>F_{IS}</math> values</b>	<b>significance level</b>
<b>Grey cattle</b>	<b>0.11930</b>	<b>ns</b>
<b>Eastern anatolian Red</b>	<b>0.14883</b>	<b>ns</b>
<b>Native Black</b>	<b>0.09563</b>	<b>ns</b>
<b>Southern Red</b>	<b>0.13743</b>	<b>ns</b>
<b>Jersey</b>	<b>-0.03683</b>	<b>ns</b>
<b>Brown Sviss</b>	<b>0.01048</b>	<b>ns</b>
<b>Black&amp; White</b>	<b>-0.01350</b>	<b>ns</b>

**Table.5. Calculated  $F_{ST}$  value between the breeds investigated**

	<b>Grey Cattle</b>	<b>Eastern Red</b>	<b>Native Black</b>	<b>Southern Red</b>	<b>Jersey</b>	<b>Brown swiss</b>	<b>Black and White</b>
<b>Grey Cattle</b>	-	0.01776***	0.01553***	0.03442***	0.05654***	0.06545***	0.09318***
<b>Eastern Red</b>		-	0.01355**	0.01040*	0.05715***	0.06247***	0.07894***
<b>Native Black</b>			-	0.02860***	0.05207***	0.05582***	0.07875***
<b>Southern Red</b>				-	0.04450***	0.04723***	0.07356***
<b>Jersey</b>					-	0.06939***	0.09816***
<b>Brown Swiss</b>						-	0.05670***
<b>Black&amp;white</b>							-

\*\*\*( $P < 0.001$ ); \*\*( $P < 0.01$ ); \* ( $P < 0.05$ )

**Figure. 6.. Phylogenetic relationship drawn by neighbor joining diagram using Ds genetic distance for all breeds investigated(Nei Standart genetic Distance-1972)**



**Tablo.7..  $D_s$  Genetic distance of breeds investigated (Nei's Standart Genetic Distance-1972)**

	Grey Cattle	Eastern Red	Native Black	Southern Red	Jersey	Brown Swiss	Black and White
Grey cattle		0.115	0.107	0.178	0.235	0.283	0.425
Eastern Red			0.109	0.089	0.244	0.278	0.358
Native Black				0.171	0.232	0.260	0.383
Soputhern n Red					0.189	0.208	0.330
Jersey						0.265	0.395
Brown Swiss							0.221
Black and white							

**A decreasing gradient in the frequencies of alleles of several microsatellite loci (ETH225, ETH10, TGLA227, HEL5, and ILSTS005) was observed from eastern to western Turkey.**

It is assumed that the reasons for high observed number of alleles in native breeds and the decrease in the frequencies of several alleles from eastern to western Turkey were that Turkey is close to the domestication center of cattle (as a consequence of its geographical location) and that there is admixture with zebu and individuals of the breeds in eastern Turkey.

Approximate ratios of zebu contribution to native and exotic breeds in Turkey were calculated by using the frequencies of alleles that were thought to have contribution from zebu alleles (Loftus et al, 1999).

Ratios of zebu contribution ranged from 12.58% (East Anatolian Red) to 8.11% (Turkish Grey Steppe) in native breeds and ranged from 0.34% (Jersey) to 6.2% (Holstein) in exotic breeds. Additionally; in the present study it was determined that there is allele contribution from exotic cattle breeds to native cattle breeds in Turkey

Approximate ratios of contribution of alleles from Jersey to native breeds ranged from 18.84% (Anatolian Black) to 30.5% (Turkish Grey Steppe).

Ratios of contribution of alleles from Holstein to native breeds ranged from 7.52% (South Anatolian Red) to 15.63% (Turkish Grey Steppe) and ratios of contribution of alleles from Brown Swiss to native breeds ranged from 6.54% (East Anatolian Red) to 24.82% (Turkish Grey Steppe).

Based on expected heterozygosity, Turkish native and exotic cattle breeds showed a relatively high degree of genetic diversity of 0.8114 and 0.7435, respectively. Single locus  $H_e$  values varied between 0.4669 (ILSTS005, Jersey) and 0.9085 (TGLA122, East Anatolian, Red). Average  $H_e$  values were very similar.

The range was between 0.7345 (Jersey) and 0.8114 (Anatolian Black). However, the lowest average  $H_e$  values were observed for exotic cattle breeds (Jersey,

In the present study, it was determined that  $F_{IS}$  values ranged from -0.0368 to 0.1488. When  $F_{IS}$  values were tested by permutation test, none of them were found to be significant.

Hence, all of the breeds were found to be in Hardy-Weinberg expectation.  $F_{ST}$  values ranged from 0.0104 to 0.0344 in native breeds and this value was same or lower when calculated for comparisons with different populations of the same breed.

Similar to these results, in different Jersey populations  $F_{ST}$  values ranged from -0.0090 to 0.0718 (Chikki et al, 2004).  $F_{ST}$  values in exotic breeds ranged from 0.0445 to 0.0982 and these values were at least three times higher than that of native breeds.

The Bayesian approach of the STRUCTURE software program has been proven to be a powerful method in studying population structure (Pritchard et al, 2000).

In this study, a high degree of concordance was observed in individual assignments by the STRUCTURE program at  $K=7$ . The results of all population and individual level admixture analyses showed that the present Turkish native cattle gene pools consisted of high number of admixed individuals.

However, Turkish exotic cattle gene pools consisted of a very limited number of admixed individuals. Each individual cattle is represented on the graph by a vertical line divided into  $K$  coloured segments corresponding to  $K$  genetic clusters.

The length of each coloured segment is proportional to the individual's membership in the cluster of corresponding colour. As a result, native cattle breeds (Grey Steppe, East Anatolian Red, Anatolian Black and South Anatolian Red) seems to be very admixed but Jersey, Holstein and Brown Swiss breeds are less admixed and considered as more purebred than the native cattle breeds

Holstein (0.757) and that with the lowest value was South Anatolian Red (0.469).

That is, proportion shared alleles within breeds were more likely to exceed proportion of shared alleles between breeds for Holstein samples than for South Anatolian Red. In a study of British cattle breeds, Wiener et al, (2004) determined breed integrity value by using 30 microsatellites.

In this study, British Jersey and Holstein cattle breeds showed a relatively high degree of breed integrity value of 0.924 and 0.751, respectively.

Results of several analysis performed in native and exotic cattle breeds in Turkey based on 7 microsatellite loci showed that native cattle breeds phenotypically belonging to a specific breed were not differentiated from each other genetically and individuals from different breeds were clustered together.

Therefore; it was observed that genetically, individuals of all of the native cattle breeds acted as

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