

## **BOTTLENECK ANALYSIS OF ANATOLIAN BLACK CATTLE (*BOS TAURUS*) USING MICROSATELLITE MARKERS**

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### **ABSTRACT**

The present study was conducted in order to reveal the genetic diversity and bottleneck in Anatolian Black Cattle (*Bos Taurus*). Animal material of the study consisted of 75 cattle raised in International Center for Livestock Research and Training. The bottleneck in the cattle breed studied was checked with 10 microsatellites markers, amplified in a multiplex polymerase chain reaction (PCR) were used according to recommendation of FAO (2011). A total of 116 alleles was observed from microsatellites studied. Overall value belongs to average number of alleles ( $N_a$ ), effective number of alleles ( $N_e$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), the polymorphic information content (PIC), average heterozygosity ( $\bar{h}$ ), and  $F_{IS}$ , known as the inbreeding coefficient, were 11.60, 5.35, 0.80, 0.78, 0.80 and 0.012, respectively. All microsatellite markers except INRA23 and ETH3 deviated from Hardy Weinberg equilibrium (HWE). Bottleneck was analyzed with Bottleneck software according to three different mutation models including the infinite allele model (IAM), two-phase mutation model (TPM) and stepwise mutation model (SMM). It can be said that there is not any ultimate risk in terms of bottleneck considering L-shaped curve showing normal distribution obtained from the analysis.

**Keywords:** *Bottleneck, microsatellite, Anatolian Black Cattle.*

### **INTRODUCTION**

Anatolian Black Cattle originates from *Bos Taurus brachyceros* and it is the most prominent breed in terms of number and spread. Middle Anatolia is its natural habitat and it is bred almost every place of this region (Alpan, 1993; FAO, 2018). It has a relatively small body and hairs are dark black. Skin is generally thick and stiff. Both males and females have horns and they are slim, crescent shaped. This breed is very resistant to harsh conditions, diseases, pests and parasites. It has an excellent digestion system and can live and reproduce in forests or mountain terrains without any support. Anatolian Black Cattle has a relatively high

adaptation capability to harsh conditions. It is usually hard to manage mainly due to its aggressive nature and tough body. It is bred for milk, meat and body power.

After several genetic characterization studies, breeds with high allelic diversity and heterozygosity have been accepted as genetic repository for genetic diversity. It has been emphasized that particularly breeds close to domestication regions have to be conserved (Giovambattista et al., 2001; Egito et al., 2007; Sharma et al., 2008; Medugorac et al., 2009; Öz ensoy et al., 2010). Extinction of Anatolian domestic animal breed is critical due to close residing to first domestication center (Bruford and Townsend, 2004). It has been reported that Anatolian Black Cattle is under the risk of extinction (Ertu rul et al., 2000). This breed is being conserved *in situ in vivo* in the International Center for Livestok Research and Training under a conservation program named “Conservation of Local Animal Genetic Resources” initiated by Turkish Ministry of Food, Agriculture and Livestock in 2004.

Microsatellites are used extensively in many species (A ao lu and Ertu rul, 2011) because they are codominant (Ramamoorthi et al., 2009), specific to loci (Condit and Hubbell, 1991), abundant and evenly distributed along genome (Iamartino et al., 2005; Ramamoorthi et al., 2009), highly mutated (Toro et al., 2009), informative (Ramamoorthi et al., 2009) and PCR based. Microsatellites is one of most preferred markers for genetic studies due to compatibility to PCR (Weber and May 1989; Liu, 1998). Heterozygosity and allele frequency distribution can be used to determine bottleneck effects in populations. Bottleneck effects cause genetic variation losses, fixation of unfavorable alleles and inbreeding depression (Hedrick, 2005; Luikart and Cornuet, 1998).

Bottleneck analysis was conducted first time using microsatellites for the Anatolian Black Cattle herd being held in the International Center for Livestok Research and Training under the conservation program.

## MATERIAL AND METHODS

The study was carried out on 75 Anatolian Black Cattle breed is raised in International Center for Livestock Research and Training Institute genetic conservation flock. Blood samples were collected from *vena jugularis* into containing K3EDTA tubes and stored at -20°C until DNA extraction. Genomic DNA was extracted from 200 µL whole blood using QIAamp 96 DNA QIAcube HT Kit (Qiagen, Hilden, Germany) by the standard protocol of QIAcube HT extraction robot. Amount and quality of DNA were checked using Titertek® micro amount spectrometer. In the present study, a panel of ten microsatellite markers, recommended by FAO (2011), was used to reveal intra-breed genetic diversity and bottleneck test. Microsatellite loci were amplified by a commercial multiplex kit, “StockMarks® Bovine Genotyping (Thermo Fischer Scientific) in a programmable thermocycler (ABI 9700). Fluorophore labelled amplicons were loaded to ABI Prism 3130 capiller electrophoresis device for fragment analysis with ROX® Gene Scan 500 internal size standard. Results were evaluated using GeneScan® software.

### Statistical Analysis

The numbers of alleles ( $N_a$ ), effective alleles ( $N_e$ ), observed heterozygosity ( $H_o$ ), expected ( $H_e$ ) heterozygosity, mean heterozygosity ( $\bar{h}$ ) and Hardy-Weinberg equilibrium were calculated using the GenAlEx genetic analysis program (Peakall and Smouse, 2006; Peakall and Smouse, 2012). Inbreeding coefficient ( $F_{IS}$ ) values were obtained using POPGENE statistical software (Yeh et al., 1997) The Cervus 3.0.3 (Marshall et al., 1998; Kalinowski et al., 2007) program was used to calculate polymorphic information content (PIC) and null allele frequency ( $F(\text{Null})$ ).

Bottleneck events were tested with Sign, Standardized differences and Wilcoxon sign-rank tests under the different mutation models such as Infinite Allele Model (IAM), Stepwise Mutation Model (SMM), and Two Phase Model of Mutation (TPM) model in Bottleneck software version 1.2.02 (1 000 simulation) (Cornuet and Luikart, 1996; Luikart and Cornuet, 1998; Piry et al., 1999).

### RESULTS AND DISCUSSION

A total 116 alleles were observed from 10 microsatellites used in this study. Computed genetic diversity statistics was given in Table 1.

Table 1. Genetic polymorphism statistics across 10 microsatellite loci

Locus	N	$N_a$	$N_e$	$H_o$	$H_e$	PIC	$\bar{h}$	$F_{IS}^*$	HWE	$F(\text{Null})$
BM2113	75	14	8.44	0.80	0.88	0.87	0.88	0.099	***	0.0474
TGLA53	75	11	4.09	0.75	0.76	0.72	0.76	0.019	***	0.0117
ETH10	75	10	4.32	0.69	0.77	0.73	0.77	0.104	*	0.0524
SPS115	74	14	4.56	0.81	0.78	0.76	0.78	-0.032	***	-0.016
TGLA126	75	11	4.15	0.75	0.76	0.73	0.76	0.023	***	0.011
TGLA122	75	17	5.84	0.84	0.83	0.81	0.83	-0.007	**	-0.008
INRA23	74	10	6.52	0.88	0.85	0.83	0.85	-0.031	ns	-0.0196
ETH3	75	10	4.74	0.88	0.79	0.76	0.79	-0.109	ns	-0.0619
ETH225	75	9	3.98	0.75	0.75	0.71	0.75	0.009	***	-0.0082
BM1824	75	10	6.90	0.83	0.86	0.84	0.86	0.040	**	0.0179
<b>Overall</b>		<b>11.60</b>	<b>5.35</b>	<b>0.80</b>	<b>0.80</b>	<b>0.78</b>	<b>0.80</b>	<b>0.012</b>		

$N_a$ : Number of alleles,  $N_e$ : Effective number of alleles, **PIC**: Polymorphic information content,  **$H_o$** : Observed heterozygosity,  **$H_e$** : Expected heterozygosity,  $\bar{h}$ : average heterozygosity, **HWE**: Significance level of Hardy-Weinberg Equilibrium,  **$F(\text{Null})$** : Null allele frequency, \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ , ns: non-significant

The highest number of alleles and effective number of alleles were obtained from TGLA122 (17) and BM2113 (8.44), respectively. It was seen that the microsatellites used in the present study were highly informative for defining the genetic diversity in the population studied, given that PIC values varied between 0.71 and 0.87. Overall mean of observed heterozygosity value was equal the expected heterozygosity value. According to the diversity parameters we obtained,

high genetic variation was detected in Anatolian Black cattle breed. These parameters were higher than some of cattle breeds raised in different location (Özkan et al., 2009; Mateus et al., 2004; Karthickeyan et al., 2008). This situation may be accepted as evidence of the accumulation of high genetic diversity in domestication centres. The average of  $F_{IS}$  value, also known as inbreeding coefficient and described as Wright' F statistics, was 0.012. All microsatellite loci except INRA23, and ETH3 deviated from the Hardy-Weinberg equilibrium ( $P < 0.05$ ). Null alleles that is defined as a non-amplifiable allele due to mutations in the PCR binding site, causing only a single allele to peek like a homozygote, thus causing erroneous reading. It has been reported by Dakin and Avise (2004) that the null allele frequency value should be below 20% in order for molecular genetic studies to be performed without errors. When the null allele frequencies obtained are examined, it is seen that the null allele frequency values of 10 microsatellites to be studied are below 0.20. Taking this value into consideration, it has been demonstrated that working locus can be used safely in genetic diversity and bottleneck study in the population studied. It is necessary to understand the processes that cause decreasing genetic diversity such as genetic bottleneck, genetic drift and inbreeding especially in small populations. For this reason, genetic bottleneck analysis was performed to investigate whether there was a bottleneck in Native Black cattle population conserved as a genetic resource. Since the mutation pattern of evolution and microsatellites are not clearly known, the data set obtained was tested with 3 different mutation models, Infinite Allele Model (IAM), Stepwise Mutation Model (SMM), and Two-Phase Model of Mutation (TPM) model reported by Cornuet and Luikart, 1996; Luikart and Cornuet, 1998 and Piry et al., 1999. Sign, Standardized differences and Wilcoxon sign rank tests were used to predict excess of heterozygosity (Table 2).

Table 2. Test for null hypothesis under three microsatellite evolution models for bottleneck analysis

Models	Sign Test	Standardized	Wilcoxon rank test (one tail for H excess)
IAM (The infinite allele model)	Hee	6.03	0.00098
	Hed	1	
	He	9	
	P	0.04798	
TPM (Two-phase mutation model)	Hee	5.99	0.61523
	Hed	5	
	He	5	
	P	0.36838	
SMM (The stepwise mutation model)	Hee	5.82	0.99658
	Hed	8	
	He	2	
	P	0.01662	

**Hee:** Expected number of loci with heterozygosity excess, **Hed:** heterozygosity deficiency, **He:** heterozygosity excess

It was reported that two-phase mutation model (TPM) is the best alternative for bottleneck analysis with microsatellites comparing to other models namely infinite allele model (IAM) and stepwise mutation model (SMM) due to inconsistent results and also reported that TPM was the most useful model with regard to revealing heterozygosity (Piry et al., 1999; Di Rienzo et al., 1994; Luikart et al., 1998). On the other hand, Wilcoxon test with high statistical power was reported to be useful even for bottleneck analysis with limited loci (<20) with high reliability (Piry et al., 1999). Considering the both TPM and Wilcoxon test results it can be said that there is no bottleneck for Anatolian Black Cattle population.

The population studied was found to be bottlenecked by the Wilcoxon test according to the infinite allele model (IAM). But it should not be forgotten that the most suitable model for microsatellites in the Wilcoxon test is the TPM model.

Mode-shift graph was drawn by means of allele frequency classes obtained from the study with ten microsatellites to reveal potential bottlenecks (Figure 1).

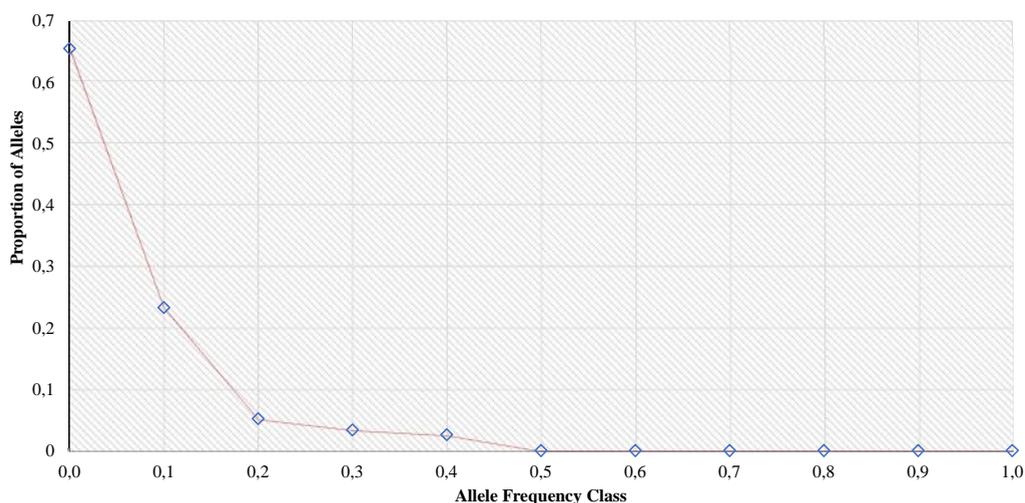


Figure 1. Mode-shift graph for bottleneck in the Anatolian Black cattle breed

As it can be deduced from the figure, L-shaped graph is consistent with the normal frequency distribution intervals. This L-shaped distribution indicates that there isn't any significant genetic bottleneck for the population in question lately (last 40-80 generations).

For Anatolian Black Cattle there are few studies conducted using microsatellites regarding genetic bottlenecks. In an earlier bottleneck study by Özensoy and Kurar (2014) it was shown that Turkish native cattle breeds' allele frequencies represents L-shaped distribution and Anatolian Black Cattle was not in a potential risk of extinction recently.

Kramarenko et al. reported in 2018 Ukrainian Red Steepe (RS) cattle had no bottleneck in near past. In another similar study by TPM assumption it was shown Kherigarh cattle hadn't have bottleneck either (Pandey et al. 2006). Ganapathi et al in 2012 reported Indian cattle breeds had no bottleneck supporting Pandey's results after a study with IAM, SMM and TPM assumptions and furthermore, IAM and TPM assumptions revealed genetic richness in 25 loci.

In contrary of abovementioned studies, in 2004, Sazaaki et al reported bottleneck in two sublines of Japanese Black Cattle.

### CONCLUSION

Consequently, the present study results indicated that despite dramatic decline in Anatolian Black cattle population, genetic diversity was significantly high in the gene pool. Our findings revealed that the microsatellite markers used in this study that can be successfully used in genetic diversity and bottleneck studies for this breed. On the other hand, obtained results will help to interpret the genetic structure of indigenous Anatolian Black cattle and will be of benefit to the efforts for conservation of this breed. The strong inference that the Anatolian Black cattle has not undergone major bottlenecks is also important for cattle breeders and other conservation programs. However, due to decreasing population numbers, conservation programs for these breeds are still necessary.

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