Genetic Characterization of Indigenous Anatolian Water Buffalo Breed Using Microsatellite DNA Markers

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One indigenous water buffalo population to Anatolia were characterised with 11 cattle autosomal microsatellite loci. A set of 4 cattle microsatellite loci was found to be polymorphic in the Anatolian buffalo genome. Genotyping of these polymorphic microsatellite loci revealed alleles ranging from 3 to 9. The observed heterozygosity ranged from 0.550 to 0.775 and the expected heterozygosity ranged from 0.494 to 0.815. The F_{IS} value changed from –0.101 to 0.205. This result shown that, Anatolian water buffalo population samples seemed to be in Hardy- Weinberg expectation.

Keywords: water buffalo, DNA , microsatellite DNA polimrphism

Anadolu Mandalarının Mikrosatellit DNA İşaretleyicileri Kullanılarak Genetik Tanımlanması

Anadolu manda populasyonunun karekterize edilmesi için 11 sığır mikrosatellit lokusu kullanılmıştır. Çalışmada bu lokusların dört tanesi Anadolu mandalarında polimorfik bulunmuştur. Gözlenen heterozigotluk aralığı 0,550 ile 0,775 arasında bulunmuştur. Beklenen heterozigotluk aralığı ise 0,494 ile 0,815 arasında hesaplanmıştır.. F_{is} değeri ise -0,101 ile 0,205 arasında olmuştur. Bu sonuçlar incelenen Anadolu Mandaları populasyonunun Hardy–Weinberg kuramına uyum gösterdiğini belirtmektedir.

Anahtar kelimeler: anadolu mandası, DNA , mikrosatellit DNA polimorfizmi

Introduction

The number of water buffaloes in the world has decreased rapidly over the past three decades *(Georgoudis et al., 1998)*. Most of world buffaloes live in Asia, Egypt, Southern and south-eastern Europe. Also buffaloes have played an important role in the rural economy of developing Asian country from ancient times.

According to FAO (2000) data, there are about 166 million domesticated buffaloes raised in the five world continents. However, there are about 158 million buffaloes left in the world *(FAO statistics, 2003)*. Roughly 97 percent of them or 153 million heads are water buffaloes essentially found in the Asian region.

Also, in Turkey the buffaloes population have declined dramatically over the last decades. The total population according to FAO (2003) statistics is 164.000 heads. Their breeding areas are especially middle of Black sea region in Turkey. These animals are mainly used for milk and meat production in these areas. The creamy part of milk fat of water buffaloes milk is popular accompanies to famous Turkish desert. Water buffaloes milk is preferably take place at least in some percentage in Turkish sausage making industry.

It is estimated that, 4-5 % of total milk and meat production comes from buffaloes sources. 3.65 % percent of red meat production sources from buffaloes genotypes. Total 40-60 % of buffaloes population are raised in Middle of Black Sea region. The largest number of buffalo population existed in Black sea region. Eastern Anatolian buffalo population has second biggest number of population. Third biggest number of population in the Marmara region existed in Istanbul and surroundings this city. Feeding is based on grazing, straw and concentrates. Their purpose of raising is firstly milk and secondly meat production.

Table 1 shown that several characteristics about Anatolian water buffalo raised in Turkey. This study was estimated to examine the within population genetic diversity using microsatellite markers.

Materials and Methods

The numbers of animals sampled from the Anatolian water buffalo were 40 individuals. Blood samples of unrelated animals were collected in slaughterhouse in Silivri of Marmara region. Bloods were collected in 10 ml tubes containing K_2EDTA and stored at –20 ^{0}C until the DNA was extracted by the standard Phenol – Chloroform technique *(Sambrook, J. et all, 1989)*. The microsatellite loci used in the study and their characteristics are given in Table 2.

The PCR analyses were carried out using an Applied Biosystems GeneAmp® PCR System 2700 thermal cycler. The reaction mixture was composed of genomic DNA (100 ng), 200µm dNTPs, 2.0 mM $MgCl_2$, 1X PCR buffer, 5 pmol forward and reversed primers and Taq DNA polymerase (0.5 u/sample) in a total volume of 20 µl. All samples were amplified in a reaction volume of 20 µl containing 11.7 µl of (dH_2O) distilled water.

The PCR reactions were carried out in 0.2 ml PCR plates with the following PCR conditions: 1 cycle of initial denaturation for 5 minutes at 94 ^{0}C, 30 cycle of 45 seconds at 94 ^{0}C, 45 seconds at annealing temperature, 1 minute at 72 ^{0}C and 1 cycle of final extension for 10 minutes at 72^{0}C. In order to minimize the artefacts caused during the amplification leading to false size estimations, one or more positive controls were used in each PCR reaction together with a negative control. The PCR product was checked on a 2% Agarose gel together with DNA size markers standards. For all microsatellites allele size was determined on all samples with a Perkin Elmer ABI Prism 310 Genetic Analyzer using the GeneScan Software (Perkin Elmer).

Data Analysis

For the population and for each locus number of alleles (n_A), observed heterozygosity (H_0) and unbiased expected heterozygosity (H_e) were calculated using Genetix 4.0 Programs.

Also the averages of n_A, H_0, H_e based on four loci were also computed. The population F_{IS} value of Wright's F statistics based on four loci were estimated and used to test the deviation from the Hardy Weinberg equilibrium. All of the above computations were performed by using Genetix 4.0 statistical programs.

Results and Discussion

Heterologous cattle microsatellite markers have been tested on Anatolian buffalo genome. A set of 11 (TGLA227, ILSTS005, CSSM66, BM1818, ETH10, ETH225, ETH3, HAUT24, HEL5, TGLA122, TGLA126) cattle microsatellite loci was analysed in Anatolian buffalo samples. Four cattle microsatellite loci were found to be polymorphic in the Anatolian buffalo genome. Allele frequencies for each of four microsatellite loci in each of the individuals are reported. The number of alleles Per locus varied from 3 (ILSTS005) to 9 (BM1818). The mean number of alleles Per locus is about 6.75. Allele numbers distribution at the four analysed loci is given in Table 3. The observed heterozygosity ranged from 0.550 to 0.775, and the expected heterozygosity ranged from 0.494 to 0.815.

Arora et al (2003), was studied physical and microsatellite characterization of Tarai Buffalo of India. The Tarai buffalo is river with 50 chromosomes, which is similar to Anatolian water buffalo population which is called as subgroup of Mediterrenean water buffaloes.

Arora et al (2003), had studyed on heterologous cattle microsatellite loci and were used them for molecular genetic characterization of Tarai genome. A set of 22 cattle microsatellite loci was found to be polymorphic in the Tarai genome. Genotyping of these polymorphic microsatellite loci revealed alleles ranging from two to seven.

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Table 1. Several Characteristics About Anatolian Water Buffalo Raised in Turkey

	Maximum	**Minimum**	**Sources**
Lactation Yield (kg)	1070.5±279.9	709.6±23.0	Şekerden et al (2000b) Uslu, N.T. (1970b)
Lactation Length (day)	269.2±70.0	222.0±44.2	Şekerden et al (2000a) Şekerden et al (2000b)
Fat (%)	8.1±0.205	6.6±0.68	Kök, S., (1996) Şekerden et al (2000a)
Adulty Body Weight	518.6±17.2	411.0±9.07	İlarslan et al (1983) Uslu N.T, (1970a)
Calving Interval	434.3±57.1	365.2±17.5	Şekerden et al (2000a) İlarslan et al (1983)
Ageat first Insemination(day)	679.7±210.9		Şekerden et al (2000a)
Age at first calving (day)	1313.2±234.8	964.1±3.94	Şekerden et al (2000b) İlarslan et al (1983)
Birth Weight (Male)	34.3±1.20	26.7±0.52	Alaçam et al. (1992) Uslu N.T; (1970b)
Birth Weight (Female)	31.6±0.90	22.1±0.48	Alaçam et al. (1992) Uslu N.T., (1970b)
Servis Periyodu	112.45	70.8	İlarslan et al (1983) Şekerden et al (2000b)
Gestation Lenght (day)	326.5±5.8 (artificial ınsemination)	317.0±51.5 (natural insemination)	İzgi and Asker, (1989) İzgi and Asker, (1989)
Daily Live Weight Gaining (gr) (0-3 Month)	(Male)	(Female)	Şekerden et al. (2000c)
Male	0.483		
Female		0.456	
Daily Live Weight Gaining (gr) (3-6 Month)	(Male)	(Female)	Şekerden et al. (2000c)
Male	0.305		
Female		0.294	
Daily Live Weight Gaining (gr) (6-9 Month)	(Female)	(Male)	Şekerden et al. (2000c)
Male		0.314	
Female	0.357		
Daily Live Weight Gaining (gr) (9-12 Month)	(Male)	(Female)	Şekerden et al. (2000c)
Male	0.504		
Female		0.360	
Fat Content of Milk	8.1	6.1	Kök, S. (1996) (Soysal and Kök, 1997)
Total Solid Matter of Milk	17.7 (3. Lactation)	15.3(1.Lactation)	Şekerden et al.(2000b)
Ash % of Milk	0.830	0.743	Şekerden et al.(2000a) Şekerden et al.(2000b)
Water of Milk	82.3		Kök, S.; (1996)
Protein % of Milk	4.6	4.2	Şekerden et al. (2000a) (Soysal and Kök, 1997)(Kök, S., 1996)
Caseine % of Milk	3.4 (3. Lactation)	3.0 (1. Lactation)	Şekerden et al.(2000b)

Observed heterozygosity of changed from 0.1316 to 0.9231. Mean observed heterozygosity of 0.60 in the Tarai buffalo population. Expected heterozygosity of changed from 0.1246 to 0.8149. BM1818, CSSM66 and ILSTS005 microsatellite loci was found polymorphic in the Tarai buffalo population and also Anatolian water buffalo

population. Anatolian water buffalo population heretozygosity was found to similar in Tarai buffalo population. Moioli et al (2001) was studied genetic diversity between Greek, Italian and Egyptian buffalo populations with using 13 polymorphic microsatellite loci. The number of alleles Per locus varied from two (ILSTS005) to 19 (ETH03). Only for two loci (CSSM33 and ILSTS005), all detected alleles were found in all three country populations (Italian, Greek and Egyptian). ILSTS005 loci was shown 3 alleles in Anatolian water buffalo population. Observed average heterozygosity was 0.135, 0.151 and 0.158 in the Italian Greek and Egyptian populations, respectively. It was lower, although not significantly different from the expected heterozygosity (0.173, 0.176 and 0.190 respectively for the Italian, Greek and Egyptian). But Anatolian water buffalo population observed and expected heterozygosity was found very high.

The Anatolian water buffalo population F_{IS} value changed from –0.101 to 0.205. This result shown that, Anatolian water buffalo population samples seemed to be in Hardy Weinberg expectation. As a conclusion, it can be said that the present study revealed the presence of high degree of genetic diversity within the water buffalo populations of Turkey.

Table 2. The table shows the name of the microsatellite loci used in the study, their primer sequences, Polymorphism information contents (PIC), annealing temperature, the chromosome number the belong to, and the references articles.

Locus Name	**Primer Sequence**	**PIC**	**Annealing Temp. (^{0}C)**	**Chromosome Number**	**Reference**
TGLA227	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAAACTCAATGAAAGCA		55	18	Steigleder et al, (2004)
ILSTS005	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	0.42	55	10	Arora et al, (2003)
CSSM66	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	0.49	58	14	Arora et al, (2003)
BM1818	AGCTGGGAATATAACCAAAGG AGTGCTTTCAAGGTCCATGC	0.40	58	23	Arora et al, (2003)

Table 3. Characteristics of Bovine Microsatellite Markers Tested on Anatolian Water Buffalo Population.

LOCUS	**Number of alleles (n_A)**	**Observed Heterozygosity (H_O)**	**Expected Heterozygosity (He)**	**$H_{n.b.}$**	**F_{IS}**
TGLA227	7	0.600	0.743	0.753	0.205
ILSTS005	3	0.550	0.494	0.500	-0.101
CSSM66	8	0.775	0.707	0.716	-0.084
BM1818	9	0.750	0.815	0.825	0.092
Mean	6.75	0.668	0.689	0.698	0.043

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