Genetic variation and relationships among Turkish water buffalo populations

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Summary

The genetic variation and relationships among six Turkish water buffalo populations, typical of different regions, were assessed using a set of 26 heterologous (bovine) microsatellite markers. Between seven and 17 different alleles were identified per microsatellite in a total of 254 alleles. The average number of alleles across all loci in all the analysed populations was found to be 12.57. The expected mean heterozygosity (H_E) per population ranged between 0.5 and 0.58. Significant departures from Hardy–Weinberg equilibrium were observed for 44 locus–population combinations. Population differentiation was analysed by estimation of the F_{ST} index (values ranging from 0.053 to 0.123) among populations. A principal component analysis of variation revealed the Merzifon population to show the highest differentiation compared with the others. In addition, some individuals of the Danamandira population appeared clearly separated, while the Afyon, Coskun, Pazar and Thural populations represented a single cluster. The assignment of individuals to their source populations, performed using the Bayesian clustering approach implemented in the STRUCTURE 2.2 software, supports a high differentiation of Merzifon and Danamandira populations. The results of this study are useful for the development of conservation strategies for the Turkish buffalo.

Keywords Anatolian water buffalo, heterozygosity, microsatellites, population structure.

The number of water buffaloes in the world has decreased rapidly over the past three decades (Georgoudis *et al.* 1998) and according to FAO statistics (2003) (http://faostat.fao.org/), there are about 158 million buffaloes in the world. However, water buffalo (*Bubalus bubalis*) play an important role in the world rural economy (Cockrill 1981) although, compared with cattle, the productivity of buffalo meat and milk production is generally lower (Moioli *et al.* 2001). The Turkish buffalo population has declined dramatically over the last decades. The current total population according to FAO statistics (2003) is 164.000 heads. Their breeding area is mainly the central Black Sea and Marmara regions, where 40–60% of the total buffalo population is raised (Soysal *et al.* 2005). From 1984 to 1997, there was a 65% decrease in the breeding population because of the preference for cattle over buffalo in these regions. However, Turkey still remains the country with the largest number of river buffaloes.

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In Turkey, only one breed, the Anatolian water buffalo, is present and it is classified as 'Mediterranean' type (Soysal *et al.* 2007). This category is classified as 'River' type, which all buffaloes of Europe and countries of the Near East belong to. Buffaloes of the River type show similar phenotypes but are variable in size (Borghese & Mazzi 2005). The Anatolian water buffalo is reared for a triple purpose: for meat, milk and draught. The most prevalent system found in villages consists of farmers keeping 1–2 heads for family consumption, while farms with around 100 heads are located near big cities (Soysal *et al.* 2007).

In the present study, microsatellite markers were used to analyse the relationships among six Turkish water buffalo populations. One hundred and fifty-five animals were sampled in six different Turkish districts belonging to four regions that represent the most important sites of water buffalo breeding (Fig. 1). Pazar and Turhal are two districts of the Tokat province located in central East Anatolia. Merzifon is the district of Samsun province located in the Black Sea region and Afyon is the province located in central Anatolia. Danamandira and Coskun are found in the Silivri district of Istanbul province. Coskun is a large farm, which buys and breeds stock all over Turkey. The other buffaloes are local populations belonging to very small farms. We chose 26 bovine heterologous mi-
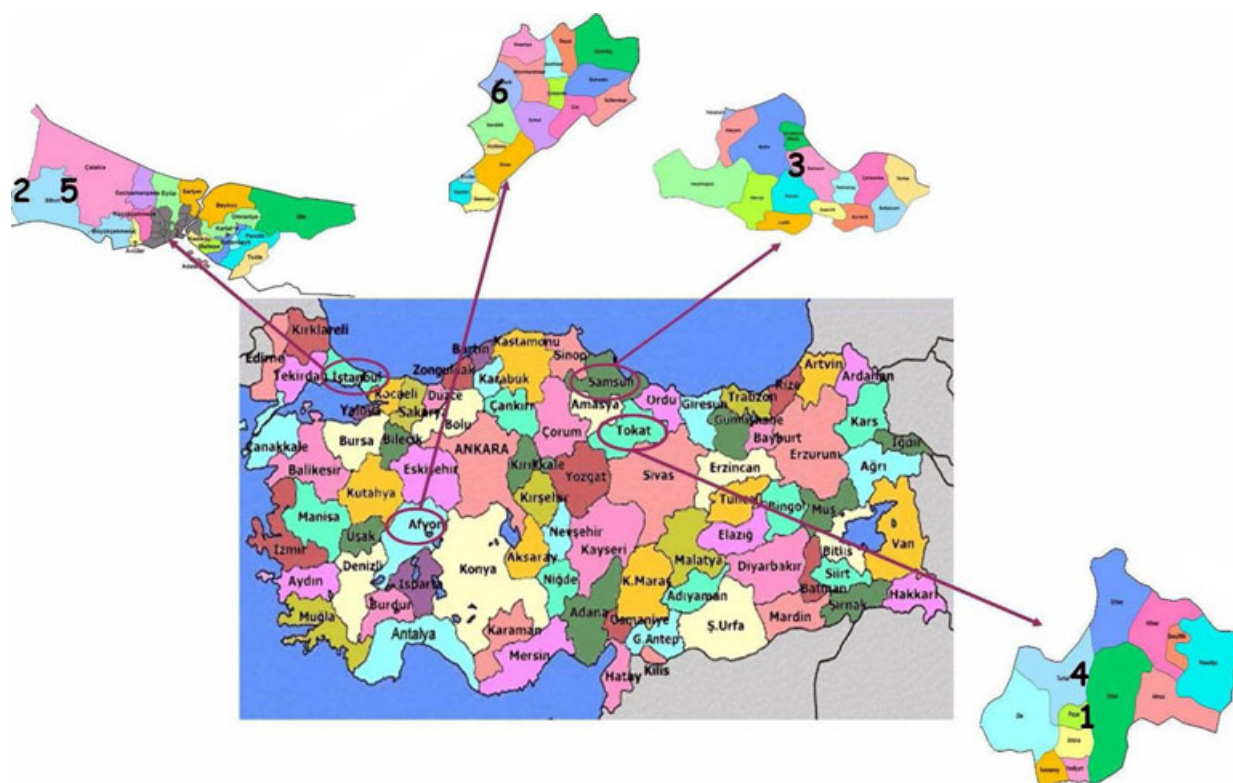
Figure 1 Sampling sites of the six Turkish buffalo populations. Numbers correspond to the following sampling sites: 1, Pazar; 2, Danamandira; 3, Merzifon; 4, Turhal; 5, Coskun; 6, Afyon.

crosatellite primers listed in the bovmap database (available at http://www.marc.usda.gov/) and recommended by FAO 1998 (Lenstra 2004) for diversity studies in cattle (Table S1). Microsatellites were analysed using a CEQ 8800 sequencer (Beckman Coulter).

Allele frequency, number of alleles, observed heterozygosity and unbiased estimates of expected heterozygosity were calculated for each population using POWERMARKER (Liu & Muse 2005). Tests for deviation from Hardy–Weinberg equilibrium (HWE) at each locus for each population were performed using GENEPOP version 3.4 (Raymond & Rousset 1995). Heterozygosity deficiency within populations (F_{IS}) and pairwise F_{ST} (Wier & Cockerham 1984) were estimated using FSTAT version 2.9.3.2 (Goudet 2002).

Among the 26 microsatellites tested, 21 loci amplified successfully, while five (*ETH3*, *ETH185*, *HEL5*, *HEL1* and *INRA023*) did not amplify. A total of 254 alleles were identified, giving a mean number of 12.57 alleles per locus. The Polymorphic Information Content (PIC) was calculated for each marker and ranged from 0.24 (*ETH10*) to 0.86 (*BMS1747*) (Table S1). Only *ETH10* and *TGLA126* showed a PIC value below 0.50. The average PIC in our samples was 0.70.

The average number of alleles, the observed and expected heterozygosity, and the F_{IS} values were calculated for each population as shown in Table 1. The average number of alleles ranged from 5.14 (Danamandira) to 9.20 (Merzifon).

Table 1 Population indices.

Population	n	H_O	H_E	A	F_{IS}
Pazar	32	0.55	0.62	5.76	0.10
Danamandira	18	0.53	0.62	5.14	0.13
Merzifon	34	0.58	0.81	9.20	0.29
Thural	20	0.58	0.64	5.42	0.08
Coskun	34	0.55	0.65	6.38	0.16
Afyon	19	0.50	0.70	7.0	0.18

n, sample size; H_O, observed heterozygosity; H_E, unbiased expected heterozygosity; A, mean number of alleles; F_{IS}, inbreeding coefficient.

The observed (H_O) and expected heterozygosity (H_E) overall loci varied from 0.5 (Afyon) to 0.58 (Merzifon and Turhal) and from 0.62 (Pazar) to 0.81 (Merzifon) respectively. The mean observed heterozygosity was 0.55, a value similar to those reported for Asian swamp buffalo (0.50) (Baker *et al.* 1997a), Chinese buffalo (0.53) (Zhang *et al.* 2007), Greek (0.59) and Italian buffalo (0.57) (Moioli *et al.* 2001), but lower than those of Indian river buffalo (0.71–0.78) (Kumar *et al.* 2006) and African buffalo (Van Hooft *et al.* 2000).

In 44 locus–population combinations, we found a deviation from H-W due to a heterozygosity deficit. Considering various populations, the Merzifon had the maximum number of loci (13) that deviated from HWE.

Our estimate of inbreeding (F_{IS}) ranged from 0.08 (Thural) to 0.30 (Merzifon), with an average of 0.19, and it was statistically significant, as shown in Table 1. The inbreeding detected in the Merzifon population is likely to be a manifestation of diminished male breeding stock. Inbreeding and occurrence of population substructure (Wahlund effect) are among factors that could be the reasons for heterozygote deficiency in this population (Nei 1987). We hypothesize that inbreeding is the most likely cause, but we cannot rule out Wahlund effect because artificial insemination is not carried out at all and the use of locally kept parents may be a cause of geographic substructuring.

Population differentiation was analysed by estimation of F_{ST} index (values ranging from 0.005 Coskun-Afyon to 0.123 Merzifon-Danamandira) between populations (Table 2). Thus, the average proportion of genetic differentiation among breeds was 6.2%. This value is lower than the 16.8% found in another genetic study on Asian buffalo (Baker *et al.* 1997a), but higher than those of Indian buffalo (3.4%) (Kumar *et al.* 2006) and Chinese buffalo (2.8%) (Zhang *et al.* 2007).

Furthermore, the genetic structure and the population relationships were investigated using two approaches. First, a Principal Coordinates Analysis (PCA) on pair-wise genetic distances among all 156 animals was performed using the

Table 2 Pairwise F_{ST} values of microsatellite for six Turkish water buffalo populations.

	Pazar	Danam.*	Merzifon	Thural	Coskun	Afyon
Pazar	0					
Danam.	0.060	0				
Merzifon	0.112	0.123	0			
Thural	0.024	0.068	0.1	0		
Coskun	0.020	0.047	0.098	0.023	0	
Afyon	0.038	0.053	0.073	0.036	0.005	0

*Danam. = Danamandira.

GENEALEX software (Peakall & Smouse 2006). The first two components explained 56.58% of the total variation (axis 1 = 42.80, axis 2 = 13.78). In the PCA plot (Fig. 2), the Merzifon population showed the highest differentiation compared with the others. Some individuals of the Danamandira population also appeared clearly separated. In contrast, Afyon, Coskun, Pazar and Thural populations represented one single cluster.

Data were then analysed using the Bayesian clustering approach implemented in the STRUCTURE program (Pritchard *et al.* 2000). A number of clusters (K) ranging from 2 to 7 was tested using the admixture model, considering allele frequencies correlated among populations. The likelihood and its variance in bootstrap replicates were plotted vs. K to enable choosing the optimal K value leading to the most reliable results (Pariset *et al.* 2006). The likelihood showed a maximum from $K = 3$ to $K = 5$, and likelihood variance among runs showed a sharp rise for $K > 5$. Therefore, we may assume that at least three genetically distinct groups are present. The analysis showed a high differentiation of Merzifon and Danamandira populations (Fig. S1), thus confirming the PCA clustering results. Coskun individuals were not assigned to a unique group but were distributed with most of the other buffalo populations. This could reflect the heterogeneity of the breeding stock, which is probably because of farmers buying animals from other regions (Soysal personal communication). Danamandira appears as a well-separated cluster. In this population, belonging to Istanbul province, husbandry is very popular and most of the farmers rear few heads for family consumption.

Most of the Merzifon individuals belong to a distinct cluster, separated from Thural and Pazar populations despite their geographic proximity. In the Merzifon district, the buffalo population size is shrinking and the breeding territory is now confined to a small area. This could have caused a reduction in breeding animals, which led to an increase of inbreeding as previously shown by F_{IS}. Evidence of a recent genetic bottleneck was tested using the BOTTLE-
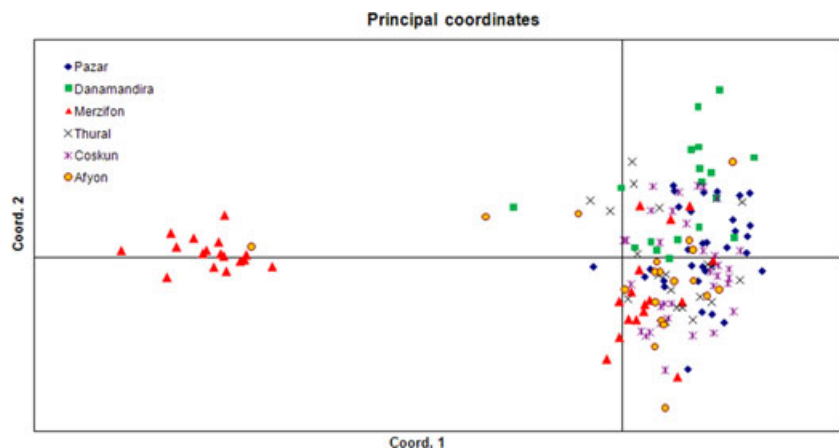

Figure 2 Plot of PCA analysis on 156 Turkish water buffalo. Axis1 = 42.80%, Axis2 = 13.78%.

NECK software (Piry *et al.* 1999). The test did not show any significant effect of recent population bottlenecks.

These preliminary results could be useful for developing conservation strategies for the Turkish buffalo, which represents an important resource for small villages that are far away from the big cities. In particular, the Merzifon population deserves special attention to prevent further increases in population inbreeding. As the populations studied belong to the same breed and show genetic distinctiveness, it is worth trying to crossbreed individuals belonging to different populations to increase the genetic variability and reduce inbreeding.

Acknowledgements

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 Summary plot of estimates of Q (estimated membership coefficients for each individual in each cluster) for K = 3 in the six buffalo populations.

Table S1 Summary of microsatellite markers used in this study.

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