Genetic variation and relationships among Turkish water buffalo populations

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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 was assessed using a set of twenty-six heterologous (bovine) microsatellite markers. Between 7 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 analyzed populations was found to be 12.57. The expected mean heterozygosity (H_E) per population was between 0.5 and 0.58. Significant departures from Hardy-Weinberg equilibrium were observed for 44 locus-population combinations. Population differentiation was analyzed by estimation of the F_{ST} index (values ranging from 0.053 to 0.123) among populations. In the PCA analysis the Merzifon population showed the highest differentiation compared to the others. Also some individuals of the Danamandira population appeared clearly separated. Instead Afyon, Coskun, Pazar and Thural populations represented one single cluster. The assignment of individuals to their source populations, performed using the Bayesian clustering approach implemented in STRUCTURE 2.2 software, has evidenced a high differentiation of Merzifon and Danamandira populations as well. The results of this study could be useful for the development of conservation strategies of the Turkish buffalo.

Keywords: Microsatellites, Anatolian water buffalo, heterozygosity, population structure

1 The number of water buffaloes in the world has decreased rapidly over the past three decades
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3 (Georgoudis *et al.* 1998) and according to FAO statistics (2003) (<http://faostat.fao.org/>) there are
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5 about 158 million buffaloes in the world. However, water buffalo (*Bubalis bubalis*) play an
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7 important role in the world rural economy (Cockrill 1981) although, compared to cattle, buffalo
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9 productivity of meat and milk is generally lower (Moioli *et al.* 2001).
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11 Also in Turkey the buffalo population has declined dramatically over the last decades. The current
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13 total population according to FAO statistics (2003) is 164.000 head. Their breeding area is mainly
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15 the central Black Sea and Marmara regions, where 40-60 % of the total buffalo population is raised
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17 (Soysal *et al.* 2005). From 1984 to 1997, there was a 65 % decrease in the breeding population due
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19 to the preference for cattle over buffalo in these regions. However, Turkey still remains the country
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21 with the largest number of river buffaloes.
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23 In Turkey only one breed, the Anatolian water buffalo, is present and classified as ‘Mediterranean’
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25 type (Soysal *et al.* 2007). This category is classified as ‘River’ type, which all buffaloes of Europe
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27 and countries of the Near East belong to. Buffaloes of the River type show similar phenotype but are
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29 variable in size (Borghese & Mazzi 2005). Anatolian water buffalo is reared for a triple aptitude: for
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31 meat, milk and as draught animals. The most prevalent system found in villages consists of farmers
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33 keeping 1-2 heads for family consumption; while farms with around 100 head are located near big
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35 cities (Soysal *et al.* 2007).
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37 In the present study, microsatellite markers were used to analyse the relationships among six
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39 Turkish water buffalo populations.
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41 One hundred and fifty-five animals were sampled in six different Turkish districts belonging to four
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43 regions that represent the most important sites of water buffalo breeding (Fig.1). Pazar and Turhal
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45 are two districts of the Tokat province located in central East Anatolia. Merzifon is the district of
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47 Samsun province located in the Black Sea region and Afyon is the province located in central
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49 Anatolia. Danamandira and Coskun are found in the Silivri district of Istanbul province. Coskun is a
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large farm which buys and breeds stock all over Turkey. The other buffaloes are local populations belonging to very small farms.

We have chosen 26 bovine heterologous microsatellites primers listed in the BOVMAP database (available at www.marc.usda.gov/) and recommended by FAO 1998 (Lenstra 2004) for diversity studies in cattle (Tab.S1). Microsatellites were analyzed 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 pair wise 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) (Tab.S1). Only ETH10 and TGLA126 markers 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). The observed (H_o) and expected heterozygosity (H_e) over all loci varied from 0.5 (Afyon) to 0.58 (Merzifon and Turhal) and 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) (Backer *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 for sure implied, but we cannot rule out Wahlund effect since 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 analyzed 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 that from another genetic study on Asian buffalo which reports 16.8% (Bucker *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 GENEALX 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 to the others. Also some individuals of the Danamandira population appeared clearly separated. Instead Afyon, Coskun, Pazar and Thural populations represented one single cluster.

1 Data was then analysed using the Bayesian clustering approach implemented in the STRUCTURE
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3 program (Pritchard *et al.* 2000). A number of clusters (K) ranging from 2 to 7 was tested using the
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5 admixture model, considering allele frequencies correlated among populations. The likelihood and
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7 its variance in bootstrap replicates were plotted versus K for choosing the optimal K value leading
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9 to the most reliable results (Pariset *et al.* 2006). The likelihood showed a maximum from K=3 to
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11 K=5, and likelihood variance among runs showed a sharp rise for K> 5. Therefore, we may assume
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13 that at least 3 genetically distinct groups are present. The analysis showed a high differentiation of
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15 Merzifon and Danamandira populations (Fig.S1), thus confirming the PCA clustering results.
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17 Coskun individuals were not assigned to a unique group but distributed with most of the other
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19 buffalo populations. This could reflect the heterogeneity of the breeding stock which is probably
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21 due to farmers buying animals from other regions (Soysal pers. communication). Danamandira
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23 appears as a well separated cluster. In this population, belonging to Istanbul province, husbandry is
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25 very popular and most of the farmers rear few heads for family consumption.
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29 Most of the Merzifon individuals belong to a distinct cluster, separated from Thural and Pazar
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31 populations despite their geographic proximity. In the Merzifon district the buffalo population size
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33 is shrinking and the breeding territory is now confined to a small area. This could have caused a
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35 reduction of breeding animals which led to an increase of inbreeding as previously shown by F_{IS} .
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37 Evidence of a recent genetic bottleneck was tested using BOTTLENECK software (Piry *et al.* 1999).
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39 The test did not show any significant effect of recent population bottlenecks.
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41 These preliminary results could be useful for the conservation strategies of the Turkish buffalo,
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43 which represents an important resource for small villages far from the big cities. In particular the
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45 Merzifon population deserves special attention to prevent further increase of population inbreeding.
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47 Since the populations studied belong to the same breed and show genetic distinctiveness, it is worth
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49 trying to crossbreed individuals belonging to different populations in order to increase the genetic
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51 variability and reduce inbreeding.
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References

Baker J.S.F., Moore S.S., Hetzel D.J.S., Evans D., Tan S.G. & Byrne K. (1997a) Genetic diversity of Asian water buffalo (*Bubalus bubalis*): microsatellite variation and a comparison with protein-coding loci. *Animal Genetics* **28**, 103– 115.

Borghese A. & Mazzi M. (2005) Buffalo population and strategies in the world. In: *Buffalo Production and research* (ed.by A.Borghese), pp.1-39.FAO, Rome

Cockrill W.R (1981) The water buffalo: a review. *British Veterinary Journal* **137**, 8-16

Georgoudis A.G., Papanastatis V.P. & Boyazoglu J.G. (1998) Use of water buffalo for the environmental conservation of waterland. Review. In:*8th World Conference on Animal Production*, Seoul, Korea, 341-350.

Goudet J. (2002) FSTAT: A program to estimate and test gene diversities and fixation indices. v. 2.9.3.2. Available at: <http://www.unil.ch/izea/software/fstat.html>

1 Kumar S., Gupta J., Kumar N., Dikshit K., Navani N., Jain P. & Nagarajan M. (2006). Genetic
2 variation and relationships among eight Indian riverine buffalo breeds. *Molecular Ecology* **15**,593–
3
4 600.
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11 Lenstra J.A. (2004) Molecular differentiation of European Cattle breeds. In: *Proceedings of the 29th*
12
13 *International Conference on Animal Genetics*, Tokyo, Japan.
14
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17
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19
20 Liu K. & Muse S.V. (2005) PowerMarker: Integrated analysis environment for genetic marker data.
21
22 *Bioinformatics* **21**(9), 2128-2129.
23
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26
27
28
29 Moioli B., Georgoudis A., Napolitano F., Catillo G., Lucioli S., Ligda Ch. & Boyazoglu J. (2001)
30
31 Genetic diversity between Italian and Greek buffalo populations. *Animal Genetic Resources*
32
33 *Information* **29**, 31-40.
34
35
36

37
38
39 Nei M.(1987) *Molecular evolutionary genetics*. Columbia University Press, New York.
40
41
42
43

44
45
46 Pariset L., Cappuccio I., Ajmone Marsan P., Dunner S., Luikart G., England P.R., Obexer-Ruff G.,
47
48 Peter C., Marletta D., Pilla F. & Valentini A. (2006) Assessment of population structure by single
49
50 nucleotide polymorphisms (SNPs) in goat breeds. *Journal of Chromatography B* **833**, 117-120.
51
52
53

54
55 Peakall R. & Smouse P.E. (2006) GENEALEX 6: genetic analysis in Excel. Population genetic
56
57 software for teaching and research. *Molecular Ecology Notes* **6**, 288-295.
58
59
60

Piry S., Luikart G. & Cornuet J.M. (1999) BOTTLENECK 1.2.02: A program for detecting recent effective population size reductions from allele frequency data. Available at: <http://www.ensam.inra.fr/> URLB.

Pritchard J.K., Stephens M. & Donnelly P. (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945– 959.

Raymond M. & Rousset F. (1995) GENEPOP (version 3.4): population genetics software for exact tests and ecumenicism . *Journal of Heredity* **86**, 248– 249.

Soysal M.I., Tuna Y.T., Gurcan E.K., Ozan E., Kok S., Castellano N., Cobanoglu O. & Barone C.M.A. (2007) Anatolian water buffaloes husbandry in Turkey: preliminary results on somatic characterization. In: *Italian Journal of Animal Science*. 8th World Buffalo Congress. Caserta.

Soysal M.İ., Tuna Y.T. & Gürcan E.K. (2005) An Investigation on the Water Buffalo Breeding in Danamandıra Village of Silivri District of İstanbul Province of Turkey. *Journal of Ttekirdag Agricultural Faculty* **2**, 73-78

Van Hooft W.F., Groen A.F. & Prins H.H.T. (2000) Microsatellite analysis of genetic diversity in African buffalo (*Syncerus caffer*) populations throughout Africa. *Molecular Ecology* **9**, 2017–2025.

Wier B.S. & Cockerham C.C. (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370

Zhang Y., Sun D., Yu Y. & Zhang Y. (2007) Genetic diversity and differentiation of Chinese domestic buffalo based on 30 microsatellite markers. *Animal Genetics* **38:6**, 569–575

Table 1. Population indices: *n*, sample size; *Ho*, observed heterozygosity; *HE*, unbiased expected heterozygosity; *A*, mean number of alleles; *F_{IS}*, inbreeding coefficient

Population	n	<i>Ho</i>	<i>HE</i>	<i>A</i>	<i>F_{IS}</i>
Pazar	32	0,55	0,62	5,76	0,1
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

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

	Pazar	Danamandira	Merzifon	Thural	Coskun	Afyon
Pazar	0					
Danamandira	0,0608	0				
Merzifon	0,1127	0,1237	0			
Thural	0,0249	0,0681	0,1	0		
Coskun	0,0206	0,0472	0,0984	0,023	0	
Afyon	0,0384	0,053	0,0736	0,0364	0,005	0

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

451x285mm (96 x 96 DPI)

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Table S1. Summary of microsatellite markers

Locus	BTA	Allele size range	Ann.temp.	PIC
BM1824*	1	170-202	57	0,666
ETH10*	5	120-226	63	0,242
ETH152*	5	196-218	63	0,721
INRA005*	12	140-158	53	0,618
SPS115*	15	248-268	59	0,688
TGLA126*	20	102-128	53	0,328
TGLA227*	18	77-109	56	0,691
TGLA53*	16	132-176	55	0,564
BMS1226†	13	130-170	58	0,829
CA004†	15	116-164	55	0,845
BM1818*	23	191-283	56	0,744
BMS1747†	14	85-101	57	0,861
BMS1352†	13	89-105	61	0,732
ILSTS058†	17	126-152	58	0,826
HEL13*	11	172-192	52	0,838
BMS462†	16	111-141	59	0,737
BL1036†	14	172-198	55	0,848
BM1316†	12	96-128	54	0,656
CSSM047†	8	128-166	55	0,822
BMS4016†	1	125-151	58	0,737
TGLA159†	4	213-261	57	0,741

BTA, cattle chromosome; † Microsatellite selected from BOVMAP database; * Microsatellite recommended by
FAO; PIC (polymorphic information content) values for each marker

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For Peer Review

Plot of PCA analysis on 156 Turkish water buffalo. Axis1=42.80%, Axis2=13.78%
135x101mm (96 x 96 DPI)

Summary plot of estimates of Q (estimated membership coefficients for each individual in each cluster) for K=3 in the 6 buffalo populations. Each individual is represented by single vertical line broken into K colored segments, with lengths proportional to each of the K inferred clusters. The numbers 1-6 correspond to the populations

151x101mm (96 x 96 DPI)