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Original study

Microsatellite based genetic diversity among the three water buffalo (*Bubalus bubalis*) populations in Turkey

Emel Özkan Ünal[1], M. İhsan Soysal[1], Eren Yüncü[2], Nihan Dilşad Dağtaş[2] and İnci Togan[2]

[1]Department of Animal Science, Faculty of Agricultural, Namık Kemal University, Tekirdağ, Turkey, [2]Department of Biological Sciences, Middle East Technical University, Ankara, Turkey

Abstract

In this study, twenty microsatellite loci were used to define genetic diversity among 56 water buffalo samples. Their somatic tissues (the skin and cartilage tissues from ears) and DNAs were deposited in Turkish Gene Banks. Samples of healthy animals were collected from three different geographic regions: Northern Turkey, North-Western Turkey and Eastern Thrace. Three (loci CSSM57, ETH3) to ten (locus CSSM47) different alleles were identified per micro-satellite locus in a total of 103 alleles. PIC values for the micro-satellite loci analysed ranged from 0.14 (CSSM32) to 0.82 (CSSM47) with a mean of 0.4945. In all of the populations for each of the loci, the observed heterozygosities (H_O) were greater than the expected heterozygosities (H_E), indicating that populations suffered from bottleneck. H_E per population ranged between 0.5359 in the Black Sea Region and 0.5208 in the Aegean-South Marmara Region. Within the population, inbreeding estimates (F_{IS}) was positive in only four of the 20 loci analysed. Individuals of the different geographic populations did not cluster on the neighbour joining tree which was constructed on the basis of allele sharing distances. Population differentiation was further visualized by Factorial Correspondence Analysis and determined by the pairwise estimations of fixation index (F_{ST}) and Nei's standard genetic distance (D_a). The results revealed that populations have inertia (as depicted by Factorial Correspondence Analysis), they are differentiated significantly but little (by pairwise F_{ST} values) and the least genetic distance is between Black Sea Region and Thrace Region. Comparative studies indicated that the genetic diversity of water buffalo harboured in the Gene Banks of Turkey is at the lower end of the diversity spectrum. This study thus highlights the usefulness of heterologous bovine microsatellite markers to assess the genetic variability in Anatolian water buffalo breeds. Furthermore, the results can be utilized for future breeding strategies and conservation.

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Corresponding author:
Emel Ozkan Unal; email: ozemel@nku.edu.tr,
Namık Kemal University, Faculty of Agricultural, Department of Animal Science, 59030, Tekirdağ, Turkey

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Abbreviations: D_a: Nei's standard genetic distance, DNA: deoxyribonucleic acid, F_{IS}: inbreeding coefficient, F_{ST}: fixation index, H_E: expected heterozygosities, H_O: observed heterozygosities, MFAL: Turkish Ministry of Food, Agriculture and Livestock, PIC: polymorphism information content value

Introduction

The water buffalo has prime importance in the lives of farmers and thus the economy of many countries worldwide. They are not only draught animals, but also a source of meat, horns, skin and particularly the rich and precious milk that may be converted into cream, butter, yoghurt and many different kinds of cheese (Michelizzi *et al.* 2010). The number of water buffaloes in the world has increased rapidly over the past few decades and according to FAO statistics (2011) (http://faostat.fao.org/), there are about 195 million buffaloes in the world. However, in Turkey the Anatolian buffalo population has declined dramatically in the last decade. From 2001 to 2011, there was a 42 % decrease in the breeding population because of the preference for cattle over buffalo (http://faostat.fao.org). FAO statistics (2011) show that the current total buffalo population is 84 726 head in Turkey. In Turkey, only the river type water buffalo is found (Soysal *et al.* 2007). It is believed that the buffalo arrived in Turkey from India in the seventh Century during the expansion of Islam (Moioli & Borghese 2005). Currently, they are mostly bred in the Central Black Sea, Marmara (Thrace) and Western Central Anatolia regions of Turkey (Atasever & Erdem 2008, Gürcan *et al.* 2011). The genetic characterization of buffalo populations is necessary for their effective conservation and meaningful improvement (Sajid *et al.* 2007). In the MoDAD Project proposed by FAO, microsatellite markers are recommended for the analysis of genetic variation and relationships among farm animal populations (Hoffmann *et al.* 2004). In recent years, several studies using microsatellites have been published regarding domestic river buffalo in Egypt, Greece and Italy (Moioli *et al.* 2001, El-Kholy *et al.* 2007), Iran (Aminafshar *et al.* 2008), Iraq (Jaayid & Dragh 2013), India (Kumar *et al.* 2006, Tantia *et al.* 2006, Kataria *et al.* 2009, Vijh *et al.* 2008, Mishra *et al.* 2009, Padeeri, *et al.* 2009, Kathiravan *et al.* 2009), China (Zhang *et al.* 2007, Zhang *et al.* 2011), Pakistan (Babar *et al.* 2009, Saif *et al.* 2012) as well as Turkey (Gargani *et al.* 2009, Soysal *et al.* 2007).

In Turkey, in a large scale national project with the acronym TURKHAYGEN-I (http://www.turkhaygen.gov.tr), five livestock species (cattle, sheep, goat, horse, and water buffalo) have been sampled by the Turkish Ministry of Food, Agriculture and Livestock (MFAL). Replicates of somatic tissue and DNA samples of the water buffalo individuals were deposited in the two National Gene Banks. The aim of the present study, 20 microsatellite loci were used to describe the genetic diversity of Turkish water buffalo whose tissues are in the gene banks. The results of the present study will contribute to the understanding of »What the genetic diversity level is« and »How distinct the geographic populations of buffalo are in Turkey«. Furthermore, it will provide information on the genetic relatedness of the individuals, which can be employed for instance to support the natural populations in the coming years if necessary and infer strategies for the enrichment of the Gene Bank samples for buffalo in Turkey.

Material and methods

Samples, geographic populations and DNA isolation

Randomly chosen, unrelated animals were collected by the personnel of MFAL from 8 different provinces in Turkey (İstanbul-Silivri, Tekirdağ-Saray, Balıkesir, Bursa, Afyon, Tokat, Sinop and Samsun) in the year 2012 (Figure 1). These provinces were known for their relatively large buffalo populations in Turkey. Throughout the national project (TURKHAYGEN-I) candidate animals were first screened for several diseases (IBR, IBR-IPV, tuberculosis, Leptospirosis, Brucella, etc.). Then, somatic tissues (the skin and cartilage tissues from ears) and blood were taken from the healthy animals. DNA was extracted from the blood. The tissues and the DNA were deposited, in replicate, into two Gene Banks of Turkey. There were 56 tissues from healthy individuals in the Gene Banks. From those water buffalo individuals, eight to 10 mL of whole blood was collected from the jugular vein in EDTA-coated Vacutainer tubes (BD Vacutainer Systems, Plymouth, UK) and transported to the laboratory at 0 to 5 °C. Genomic DNA was extracted by using a standard phenol-chloroform extraction method (Sambrook & Russell 2001). The concentration of DNA was judged in comparison with the standard DNA marker concentration on agarose gels. The quality of DNA was checked on 0.8 % agarose gels prepared with a Tris-Boric acid-EDTA buffer. Since there was no pedigree recording for the water buffalo and thus no genetic isolation between the individuals in Turkey, they were considered in three geographic groups: the Aegean-South Marmara Region (Afyon, Balıkesir and Bursa), the Black Sea Region (Sinop, Samsun and Tokat) and the Thrace Region (Istanbul-Silivri, Tekirdağ-Saray).

Figure 1
Water buffalo sampling sites from the eight different Turkish provinces grouped in three regional populations: ASM – Aegean-South Marmara Region (Afyon, Balıkesir, Bursa), BSR – Black Sea Region (Sinop, Samsun, Tokat), THR – Thrace Region (Istanbul, Tekirdag)

Microsatellite loci and PCR-based profiling

A total of 20 heterologous microsatellite loci from the recommended list by FAO for the buffalo were chosen for the study (http://www.fao.org/docrep/014/i2413e/i2413e00.pdf) and presented in Table 1.

Table 1
Microsatellite loci used in the present study to characterize water buffalo individuals and populations

Loci	Genebank access number	Chromosome*	Size Range, bp**	Annealing temperature, °C	Fluorescent dye
CSSM33	U03805	17 (17)	152-169	58	D_4
ILSTS033	L37213	13 (12)	142-156	55	D_2
CSSM43	U03824	1p (27)	222-258	54	D_4
CSRM60	AF232758	11 (10)	109-129	57	D_2
CSSM47	U03821	3q (8)	127-165	58	D_2
CSSM32	U03811	1q (1)	210-224	56	D_2
BRN	X59767	11 (10)	232-238	59	D_3
CSSM41	U03816	21 (22)	133-146	53	D_4
CSSM38	U03817	11 (10)	161-183	58	D_4
BMC1013	G18560	3p (19)	225-259	55	D_3
CSSM19	U03794	1q (1)	129-147	52	D_3
ILSTS005	L23481	11 (10)	177-185	59	D_3
CSSM46	U03834	11 (10)	150- 160	53	D_2
CSSM57	U03840	9 (7)	185-189	55	D_4
CSSM29	U03807	9 (7)	242-254	58	D_4
ETH003	Z22744	3p (19)	104-110	57	D_4
CSSM36	U03827	1p (27)	164-174	55	D_4
CSSM22	U03806	4q (5)	205-213	54	D_2
ILSTS030	L37212	2q (2)	152-164	54	D_3
ETH121	Z14037	2q (2)	183-201	52	D_2

*cattle chromosome assignments in parentheses, **allele size range in the present study

The criterion for selection of the heterologous microsatellite loci was based on the polymorphism information content value (PIC) and the number of exhibited alleles (Navani *et al.* 2002) of the loci. The 5′ ends of the forward primers were labeled with D2, D3 or D4 dyes (Beckman Coulter, Inc., Pasadena, CA, USA). The PCR conditions were standardized for all of the 20 primer pairs selected for the study. Polymerase chain reaction amplification was carried out in a 20 µl reaction containing 50 ng of genomic DNA, 200 mM dNTP, 20 pmol of each forward (labeled) and reverse primers, 1 U of Taq DNA polymerase and 1X reaction buffer (containing 1.5 mM $MgCl_2$). Amplification was carried out using a BioRAD instrument with initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 52 to 59 °C (as a primer specific manner) for 20 to 45 s, and extension at 72 °C for 40 to 60 s. The final cycle was followed by an extension step at 72 °C for 15 min. The PCR products were visualized on 2 % agarose gels with 1×TBE buffer containing 100 ng/mL of ethidium bromide. Amplified PCR products were analyzed using Beckman Coulter CEQ8000 capillary automated DNA sequencer (Beckman Coulter, Inc., Pasadena, CA, USA), and the CEQ 8000 Genetic Analysis System (Beckman Coulter, Inc., Pasadena, CA, USA) was used to obtain allele designations.

Statistical analyses

Allele frequencies, the number of alleles, observed heterozygosity and unbiased estimates of expected heterozygosities (H_E: Nei 1987), Nei *et al.* (1983) D_A distances and significant tests (1 000 permutations) for the D_A were calculated using GENETIX v4.05.2 (Belkhir *et al.* 1996-2004). Heterozygosity deficiency within populations for each loci and all loci was estimated by F_{IS} and pairwise genetic differentiation of the populations was estimated by F_{ST} (Weir & Cockerham 1984), using FSTAT v2.9.3.2 (Goudet 2002). In order to evaluate the significance of the F_{IS} values, permutation tests were carried out by permuting the alleles within samples over all loci in each breed using the GENEPOP software (Raymond & Rousset 1995, Rousset 2008). Similarly, to test the significance of pairwise genetic differentiation (F_{ST}), permutation tests (1 000 permutations) implemented in the package GENETIX v4.05.2 were used. The Bonferroni correction (Weir 1996) was employed to determine the levels of probability for multiple tests. To visualize the genetic relationship between all of the individuals, a neighbour joining tree was built on the basis of allele sharing distances by employing Populations v1.2.31 software (Langella 1999), Factorial Correspondence Analysis using Genetix software was carried out also.

Results and discussion

In this study, the number of alleles (n_a), expected (H_E) and observed (H_O) heterozygosity, F_{IS} and PIC values for each locus and population as well as the average values for the loci and populations are given in Table 2. Totally 103 alleles were detected in the whole sample and the number of alleles varied from 3 (loci CSSM57, ETH003) to 10 (locus CSSM47) with the mean number of alleles being 5.15 per locus (Table 2). The mean number of alleles observed in populations differed slightly: the minimum 3.70 was observed in THR and the maximum 4.35 was in BSR. The polymorphic information content (PIC) was calculated for each marker and ranged from 0.14 (locus CSSM32) to 0.820 (locus CSSM47), which has the highest number of alleles per locus in the present study. The average PIC in our populations was 0.4945. Nine microsatellites (ILSTS033, CSSM43, CSRM60, CSSM47, BMC1013, CSSM29, CSSM36, ILSTS030 and ETH121), having a PIC value higher than the threshold of 0.5 (Botstein *et al.* 1980, Seyedabadi *et al.* 2006), seemed to be highly informative and can be used in quantifying the genetic diversity and also in paternity studies in Turkey.

Importantly, for every loci and for each geographic population, H_O was more than H_E indicating the bottleneck (Cornuet & Luikart 1996) that was experienced by the buffalo populations in Turkey. The dramatic decline in the total population size of buffalo was reported by FAO (42 % between 2001 and 2011) (http://faostat.fao.org).

The decrease might have been more dramatic (76 % between 1991 and 2009) as reported by the Turkish Agricultural Engineers site (http://www.zmo.org.tr/resimler/ekler/cb16f6f3938e162_ek.pdf). Statistically significant differences (measured by F_{IS}) between the H_E and H_O were seen only for one locus (ETH003) in Aegean-South Marmara Region, two loci (CSSM32, BRN) in Black Sea Region and one locus (CSSM41) in Thrace Region. Since those loci exhibiting significant differences were different in different populations and there were not many (4/60), one can argue that there was no systematic error in genotyping on the basis of

Table 2
Genetic variability measures in water buffalo across 20 microsatellite markers

Loci	n_a	PIC	ASM (n=21)		BSR (n=21)		THR (n=14)		ASM-F_{IS}	BSR-F_{IS}	THR-F_{IS}
			H_O	H_E	H_O	H_E	H_O	H_E			
CSSM33	5	0.37	0.3740	0.3651	0.2915	0.2846	0.5370	0.5179	0.241	0.020	0.071
ILSTS033	5	0.63	0.6109	0.5964	0.7329	0.7154	0.5741	0.5536	−0.094	0.225	0.261
CSSM43	8	0.56	0.4611	0.4501	0.6794	0.6633	0.6958	0.6709	0.072	0.091	−0.135
CSRM60	6	0.62	0.6330	0.6179	0.6655	0.6497	0.7540	0.7270	0.252	0.071	0.345
CSSM47	10	0.82	0.8780	0.8571	0.7538	0.7358	0.8571	0.8265	0.135	−0.011	0.172
CSSM32	4	0.14	0.1800	0.1757	0.0476	0.0465	0.2619	0.2526	−0.060	0.000***	−0.095
BRN	4	0.47	0.4843	0.4728	0.5912	0.5771	0.4735	0.4566	−0.185	0.523***	−0.058
CSSM41	4	0.34	0.3333	0.3254	0.4704	0.4592	0.2540	0.2449	0.146	0.091	1.00***
CSSM38	4	0.45	0.5714	0.5578	0.4495	0.4388	0.5185	0.5000	0.170	0.156	0.179
BMC1013	8	0.73	0.7782	0.7596	0.7747	0.7562	0.7593	0.7321	0.084	0.017	−0.135
CSSM19	5	0.39	0.3984	0.3889	0.4727	0.4615	0.3730	0.3597	0.045	0.198	0.044
ILSTS005	4	0.42	0.5285	0.5159	0.5218	0.5088	0.4762	0.4592	−0.270	0.141	0.409
CSSM46	4	0.38	0.5203	0.5079	0.4286	0.4184	0.2646	0.2551	0.087	0.114	−0.083
CSSM57	3	0.40	0.4379	0.4274	0.5540	0.5408	0.5159	0.4974	0.243	−0.032	−0.258
CSSM29	5	0.52	0.4866	0.4751	0.5424	0.5295	0.6640	0.6403	−0.078	0.214	0.144
ETH003	3	0.40	0.5017	0.4898	0.3717	0.3628	0.5794	0.5587	0.626***	0.494	0.142
CSSM36	4	0.58	0.6411	0.6259	0.6492	0.6338	0.6111	0.5893	0.035	−0.103	−0.054
CSSM22	4	0.42	0.5285	0.5159	0.5679	0.5544	0.3492	0.3367	0.193	0.079	0.600
ILSTS030	6	0.58	0.6074	0.5930	0.6725	0.6565	0.6455	0.6224	0.141	0.225	0.345
ETH121	7	0.67	0.7154	0.6984	0.7445	0.7268	0.6878	0.6633	−0.135	0.042	−0.040
Mean	5.15	0.4945	0.5335	0.5208	0.5491	0.5359	0.5426	0.5232			
All									0.0825^{ns}	0.125***	0.122^{ns}
T			85		88		74				
A			4.25		4.35		3.70				

ASM: Aegean-South Marmara Region, BSR: Black Sea Region, THR: Thrace Region, n_a: observed number of alleles, H_E: expected heterozygosity, H_O: observed heterozygosity, F_{IS}: inbreeding coefficient, n: sample size, T: total alleles, A: mean no. of alleles, ***$P<0.001$, ns: not significant

the loci employed and inbreeding is not yet very high. Perhaps the only significant $+F_{IS}$ in Black Sea Region is due to the significances observed in two of its loci. In terms of the mean expected heterozygosity of the populations, the range was 0.5208 (Aegean-South Marmara Region) to 0.5359 (Black Sea Region).

The genetic relatedness of the individuals was depicted by a neighbor joining tree where the allele sharing distances among 56 buffalo on 20 microsatellites were used (Figure 2). Generally, consecutive numbers belonging to a geographic region represent those individuals which were close in their collection sites. Most of the time, there are high similarities between the pairs of individuals coming from the same geographic regions, such as AS8 and AS9. However, in many instances, geographically distant individuals, even from different geographic regions, exhibited high genetic resemblances (e.g. TH12 ASM10, BS20). There seems to be no consistent genetic differentiation between the samples of the three geographically different populations, on the basis of the loci and samples used in the study (Figure 2).

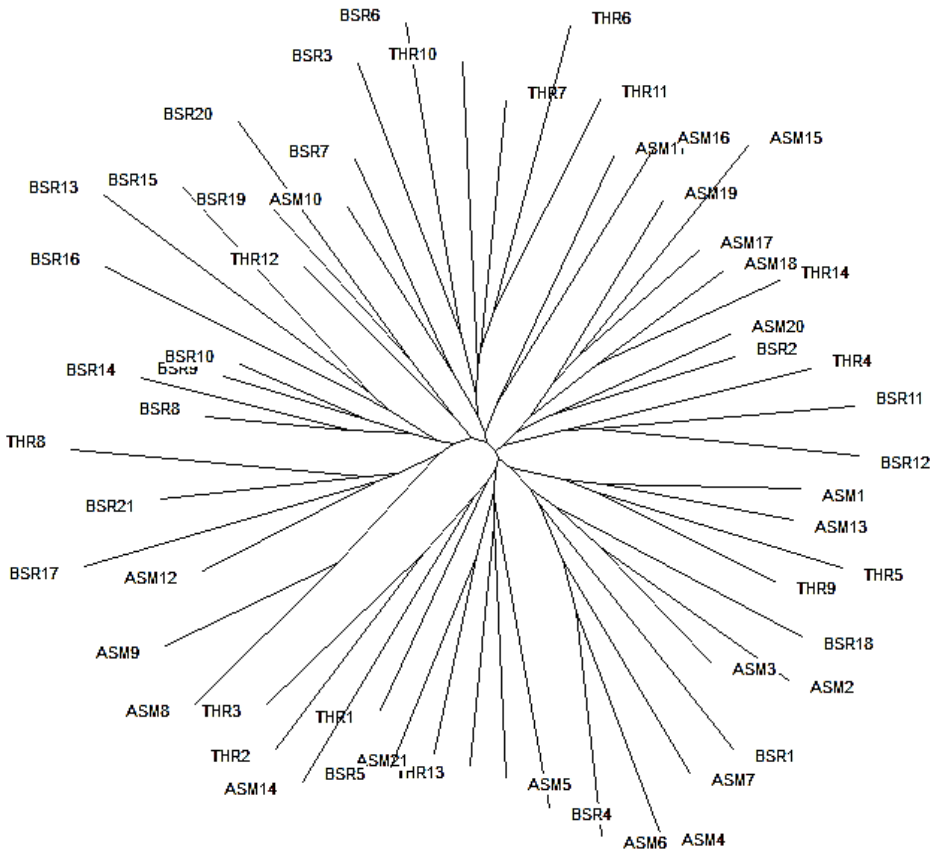

Figure 2
Neighbour joining tree where allele sharing distances among 56 buffalo on 20 microsatellites were used. ASM – Aegean-South Marmara Region (Afyon, Balıkesir, Bursa), BSR – Black Sea Region (Sinop, Samsun, Tokat), THR – Thrace Region (Istanbul, Tekirdag)

The results of Factorial Correspondence Analysis in Figure 3 were shown in two dimensions. The axes represent 53.08 % (first), and 46.92 % (second axis) of the overall variability that exists between the buffalo individuals. On the Factorial Correspondence Analysis, individuals from the similar geography were indicated by the contours labeled as Black Sea Region (BSR-blue), Aegean-South Marmara Region (ASM-yellow) and Thrace Region (THR-white). Figure 3 depicted that although three populations representing three different geographic regions have some inertia, they are not very different from each other and there is also an overlap between the individuals of the populations.

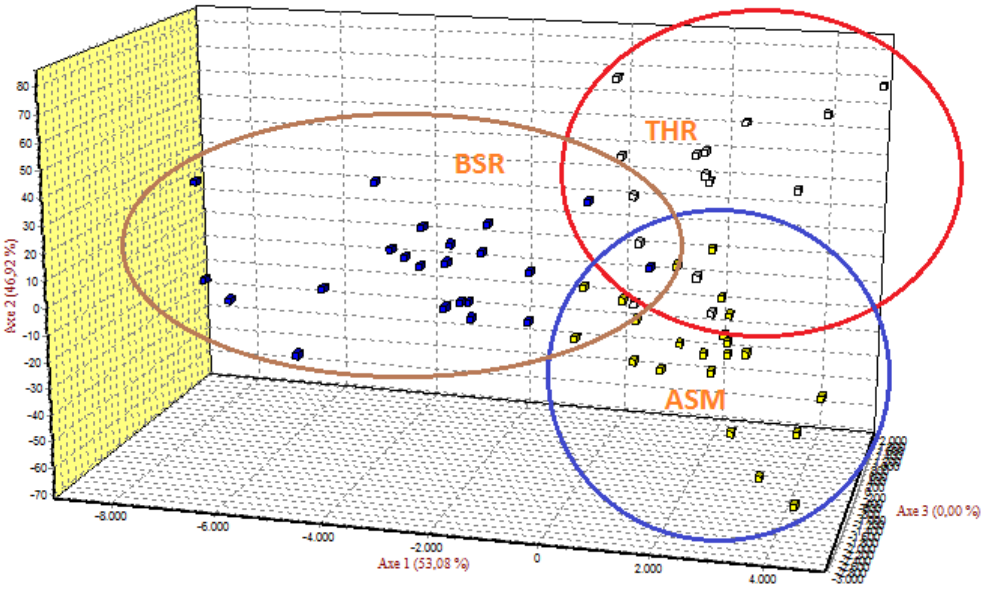

Figure 3
Factorial Correspondence Analysis results showing the relationship between all of the individuals analysed in the study

Population differentiations were compared on the basis of F_{ST} and Nei's D_A in Table 3. It can be seen that buffalos of Thrace and Black Sea Regions are the most similar ones because the minimum genetic distance (0.02194) was observed between this pair. However, since all pairwise F_{ST} values are significantly ($P<0.05$) different from each other, it can be concluded that all geographical populations are significantly different from each other. Because F_{ST} values are less than 0.05, pairwise differentiations can be considered as minor (Hartl 1980) as had been visualized by the Factorial Correspondence Analysis in Figure 3.

For the water buffalo, the genetic diversity (in terms of the mean number of alleles, expected heterozygosity and pairwise differentiation between the populations) observed within Turkey was compared with the data available from the Mediterranean or neighbouring countries (Italy, Egypt, Greece [Moioli *et al.* 2001, El-Kholy *et al.* 2007, Elbeltagy *et al.* 2008], Iraq [Jaayid & Dragh 2013] and Iran [Aminafshar *et al.* 2008]) as well as that from the domestication centers (India and China [Yue *et al.* 2013]) of water buffalo (Kumar *et al.* 2006, Tantia *et al.* 2006,

Kataria *et al.* 2009, Padeeri *et al.* 2009, Vijh *et al.* 2008, Kathiravan *et al.* 2009, Mishra *et al.* 2009, Zhang *et al.* 2007, Zhang *et al.* 2011). First of all, in these different studies, both the loci and the number of loci involved (Table 4) were different from each other. Thus, differences in the results may partly be attributable to the differences in the loci employed. In the two previous studies covering water buffalo from Turkey (Gargani *et al.* 2009, Soysal *et al.* 2007) and in the present study, samples were collected from nearly the same geographic regions. However, in the present study, except for one (CSSM047), the loci used were different from those of the previous studies. Within the afore-mentioned limitation, it is noted that genetic diversity (measured by MNA and H_E) seemed to decrease among the water buffalo of Turkey, even in the last decade.

Table 3
Pairwise F_{ST} values (belows diagonal) and Nei's D_A genetic distances (aboves diagonal) based on 20 microsatellite loci for water buffalo populations from 3 different regions of Turkey

	Aegean-South Marmara Region	Black Sea Region	Thrace Region
Aegean-South Marmara Region	–	0.02708***	0.03453***
Black Sea Region	0.033***	–	0.02194*
Thrace Region	0.042***	0.027***	–

*** $P<0.001$; * $P<0.05$

Table 4
Published data for genetic diversity in different buffalo (*Bubalus bubalis*) populations

Country	a	n	NL	MNA	H_E	F_{ST}	Reference
Turkey	6	157	26	12.57	0.62 to 0.81	0.005 to 0.123	Gargani *et al.* 2009
	1	40	11	6.75	0.494 to 0.815	nd	Soysal *et al.* 2007
	3	56	20	3 to 4	0.5208-0.5359	0.027 to 0.042	Present study
Italy, Greece, Egypt		110	9	6.1	0.222 to 0.247	0.021	Moioli *et al.* 2001
Egypt	6	92	3	12 to13	0.832 to 0.893	-0.01 to 0.067	El-Kholy *et al.* 2007
Iran	1	60	14	4.14	0.67	nd	Aminafshar *et al.* 2008
Iraq	3	96	6	11.4	0.11 to 0.80	nd	Jaayid & Dragh 2013
Mediterranean	3	104	15	4 to11	nd	0.014 to 0.083	Elbeltagy *et al.* 2008
India	1	40	449	5.3	nd	nd	Padeeri, *et al.* 2009,
	3	104	24	5.83	0.6244	0.041 to 0.064	Tantia *et al.* 2006
	12	527	22	5 to 9	0.63 to 0.73	0.021 to 0.179	Vijh *et al.* 2008
	1	48	25	5.24	0.10 to 0.84	nd	Kataria *et al.* 2008
	1	48	23	7.83	0.712	nd	Kathiravan *et al.* 2009
	2	95	24	5.75	0.572 to 0.610	0.187	Mishra *et al.* 2009
	8	383	27	6 to 7	0.71 to 0.78	0.007 to 0.060	Kumar *et al.* 2006
Pakistan	1	20	3	4 to 6	0.23 to 0.70	nd	Babar *et al.* 2009
	2	50	9	3 to 8	0.26 to 0.741	0.1304	Saif *et al.* 2012
China	20	933	30	1 to 17	0.517 to 0.609	0.00 to 0.352	Zhang *et al.* 2007
	5	168	30	1 to 13	nd	nd	Zhang *et al.* 2011

a: number of studied population; n: number of individuals; NL: number of loci, MNA: mean number of allele, H_E: expected heterozygosity, F_{ST}: pairwise F_{ST} values, nd: no data available

In Table 4, similar decline in diversity pattern is observed for Egypt (Moioli *et al.* 2001, El-Kholy *et al.* 2007). It seems that (MNA: 3-4; Mean H_E: 0.5208-0.5359) the water buffalo populations of Turkey harbor less diversity than those of centers of domestication (Kumar *et al.* 2006, Tantia *et al.* 2006, Kataria *et al.* 2009, Muraleedharan *et al.* 2009, Vijh *et al.* 2008, Kathiravan *et al.* 2009, Mishra *et al.* 2009, Zhang *et al.* 2007, Zhang *et al.* 20011), even from Mediterranean

(Moioli *et al.* 2001, El-Kholy *et al.* 2007, Elbeltagy *et al.* 2008) and neighbouring countries (Aminafshar *et al.* 2008, Jaayid & Dragh 2013) as seen in Table 4. Furthermore, it can be seen that on the basis of the samples studied in Turkey no distinct population/breed (F_{ST}: 0.027-0.042) was seen, different from what was observed in for instance India (F_{ST}: 0.179 or 0.187), China (0.352) or Pakistan (0.1304) Table 4 also shows that the differentiation between the populations is slightly more than what was observed (F_{ST}: 0.021) among the buffalo populations of Mediterranean countries (Moioli *et al.* 2001).

In Turkey, if the genetic diversity in water buffalo is decreasing, as predicted by the present study, it can be said that the cryoconservation of tissues of 56 individuals was timely. Since the usefulness of bovine microsatellite markers to assess the genetic variability in Anatolian water buffalo is confirmed in the present study, they can be utilized to minimize the loss of genetic diversity in the water buffalo populations of Turkey. Because individuals of different populations are not very distinct, the tissues of individuals kept in the gene banks might be used to support the populations (by nuclear transfers) in the future if populations further lose their existing genetic diversity. Finally, in Southeast Anatolia there are some other water buffalo populations (Atasever & Erdem 2008, Gürcan *et al.* 2011) which were not represented in the Gene Banks of Turkey and therefore were not included in the present study. Their samples must also be considered as a possible additional source of genetic diversity in the Gene Banks.

In conclusion, the results of the present study suggest that the water buffalo individuals represented by their tissues in Turkish Gene Banks confirmed that a bottleneck had been experienced in Turkey. They are genetically related to each other within and between geographic regions. The genetic diversity level captured in the gene banks for the buffalo populations in Turkey is within the range observed for the buffalo populations distributed around the Mediterranean countries but at the lower end. This information's may be useful in acquiring new samples for the Turkish Gene Banks and in supporting the buffalo populations of Turkey in the future.

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