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Microsatellite based polymorphism and genetic diversity among Indian buffalo breeds

BHEEMASHANKAR H KANNUR[1], MD. NADEEM FAIROZE[2], P S GIRISH[3], C RENUKAPRASAD[4], NAGAPPA KARABASANAVAR[5], SHAILESH BAGALE[6] and B H RUDRESH[7]

Karnataka Veterinary, Animal and Fisheries Sciences University, Hebbal, Bengaluru, Karnataka 560 024 India

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ABSTRACT

Autosomal microsatellite markers allow estimation of expected heterozygosity and allele frequencies which may reveal the effects of genetic isolation, inbreeding, introgression and subdivision within livestock breeds. In the present study, 7 buffalo breeds (Bhadawari, Jaffrabadi, Murrah, Mehsana, Nagpuri, Pandharpuri and Surti) were studied using a panel of eight microsatellite markers selected based on polymorphic information content (PIC) for buffalo (*Bubalus bubalis*) species as per the guidelines of Food and Agricultural Organization. Frequency of breed-specific alleles was found lower in all the breeds studied; 4 out of 49 alleles showed a frequency of 8% while only one allele showed >10% frequency. In the present study, highest genetic distance was observed between Murrah and Surti (0.896) indicating their isolated breeding over several generations.

Key words: Buffalo breed, Genetic diversity, Microsatellite, Polymorphism

Present pattern of diversity of animal genetic resources (AnGR) is the result of local adaptation, artificial selection, mutations and genetic drift that lead to differences in the appearance, physiology and economic traits i.e., evolution of breeds. Of the several livestock species, diverse buffalo population contributes substantially to the Indian dairy sector, meat industry and draught power (Sangwan 2012). India is the largest repository of elite buffalo genetic diversity. Autosomal microsatellite markers allow estimation of expected heterozygosity and allele frequencies which reveal effects of genetic isolation, inbreeding, population bottlenecks, introgression and subdivision within breeds (Sangwan 2012). Present study was carried out with the objective of deciphering microsatellite polymorphism and genetic diversity among major buffalo breeds of India.

Present address: [1]Veterinary Officer (drbhkannur @gmail.com), Department of A.H.V.S., Government of Karnataka.[2]Professor and Head (prof.nadeem@gmail.com), Department of Livestock Products Technology. [3]Senior Scientist (girishlpt@gmail.com), ICAR – NRCP, Rani, Guwahati. [4]Vice Chancellor (c.renukaprasad @gmail.com), KVAFSU, Bidar. [5]Assistant Professor (pub.nag@gmail.com), Department of Veterinary Public Health and Epidemiology. [7]Associate Professor (rudreshbh1906@yahoo.co.in), Department of Animal Genetics and Breeding, Veterinary College, KVAFSU, Shivamogga. [6]Veterinary Officer (shaileshsbagale@gmail.com), Department of Animal Husbandry and Veterinary Services, Belgaum.

MATERIALS AND METHODS

Sample collection: Blood samples (~10 mL) were drawn from jugular vein into EDTA-coated tubes from seven Indian buffalo breeds i.e., Bhadawari (20), Jaffaarabadi (24), Murrah (20), Mehsana (15), Nagpuri (15), Pandharpuri (45) and Surti (10) from their respective breeding tracts. Blood samples were brought to laboratory under chilled conditions (0–5°C) for DNA isolation.

DNA isolation: Genomic DNA was extracted from meat samples by phenol-chloroform method (Sambrook and Russel 2001) while commercial DNA extraction kit was used for blood samples.

Microsatellite genotyping: Panel of microsatellite markers for buffalo (*Bubalus bubalis*) species was selected based on the PIC (polymorphic information content) score as recommended by ISAG-FAO (International Society for Animal Genetics - Food and Agriculture Organization) advisory group (FAO 2011). Selected microsatellite markers were custom synthesized and 5^1 end of forward primers was labeled with either FAM (6 carboxyflouroscien) or HEX (hexachloro–6-carboxyflouroscien) florescent dyes; while reverse primers were kept unlabeled. Chromosome location, annealing temperature, Genebank accession number and allele range of the selected primers are given in Table 1.

Polymerase chain reaction: PCR reactions were standardized for 8 sets of microsatellite markers. Amplified PCR products were analyzed using horizontal submarine gel electrophoresis. Post-PCR multiplexing was performed simultaneously for genotype multiple loci depending on the

Table 1. Characteristics of microsatellite markers

Name	Chromosome[1]	Annealing temperature (°C)	Gene bank accession number	Allele range (bp)[2]
CSSM033	17 (17)	65	U03805	154-175
CSSM047	3q (8)	55	U03821	127-162
ETH003	3p (19)	65	Z22744	96-192
CSSM061	Unknown	60	-	100-126
DRB3	2p (23)	50-55	-	142-198
CSSM019	1q (1)	55	U03794	131-161
CSSM041	21 (22)	55	U03816	129-147
HMH1R	21 (22)	60	D10197	169-187

[1]Cattle chromosome assignments in parentheses. [2]No multiplex developed.

Table 2. Allele patterns across the microsatellite loci

Locus/ marker	Product size (bp)	No. of alleles	Mean number of alleles (N_a)	Effective number of alleles (N_e)	Most frequent alleles
CSSM033	141-162	8	3.714	2.334	160
CSSM047	123-163	21	8.571	5.173	128
ETH003	127-151	10	3.143	2.330	136
CSSM061	86-147	18	8.000	4.921	111
DRB3	150-195	24	8.571	4.623	192
CSSM019	126-174	23	8.571	4.484	131
CSSM041	120-143	16	6.714	3.432	139
HMH1R	129-180	7	2.714	1.786	163
Overall	86-195	127	—	—	—
Mean	—	15.875	6.249	3.635	—

PCR product size and primers were labeled with dye. Fluorescently end-labeled microsatellite PCR product-multiplex mix was then run on capillary based genetic analyzer against the internal lane (size) standard. Raw data were collected using 310 Data collection Software Version 3.1.0.

Statistical analysis: Allele frequency, mean and effective allele number, heterozygosity (Nei 1987), PIC, genetic distances, F-statistics and Hardy-Weinberg equilibrium were calculated using Microsatellite Analyzer GenAlEx 6.5–Genetic Analysis in Excel software.

RESULTS AND DISCUSSION

Genetic variation: Buffaloes (149) belonging to 7 breeds were genotyped using 8 microsatellite markers according to FAO recommendations to establish diversity among the buffalo breeds. About 127 alleles were observed with an average of 15.8 alleles per locus which was lower compared to 151 or 388 alleles observed across 30 or 22 loci by Vijh *et al.* (2008). Surti and Pandharpuri breeds showed 87 and 34 alleles per locus; these numbers were comparatively less against 158 and 129 alleles as reported by Vijh *et al.* (2008) for these breeds, respectively. Such variation in the allele number could be attributed to larger sample size, genotyping technique and extent of polymorphism at different loci.

Overall mean for N_a and N_e alleles observed in the buffalo was 6.250 and 3.636, respectively (Table 2). The mean number of alleles (N_a) ranged from 4.25 to 10.87; with lowest number in Surti (4.25) and highest in Pandharpuri (10.87); however, Vijh *et al.* (2008) observed N_a of 5.86 and 9.41 in Pandharpuri and Nagpuri breeds, respectively. Further, Kumar *et al.* (2006) also observed N_a of 6.15 in Toda and 7.19 in Murrah and Mehsana breeds. The highest N_a observed in Pandharpuri breed in the present study was even greater than the observations made by Vijh *et al.* (2008) and Kumar *et al.* (2006) for the Pandharpuri breed. The mean number of effective alleles (N_e) was least in Mehsana (2.62) and highest in Pandharpuri (10.87); while, Vijh *et al.* (2008) observed N_e of 3.30 in Bhadawari and 4.88 in Nagpuri breed of buffalo.

The PCR product (bp) of alleles across 8 microsatellite loci varied from 86 (CSSM061) to 195 (DRB3). The overall mean number of alleles (N_a) amplified was 6.249 and it varied from 2.714 at locus HMH1R to 8.571 at loci CSSM047, DRB3 and CSSM019 (Table 3).

Allele frequency and breed specific/ private alleles: Allele frequency was lowest in Pandharpuri (1.11%) and highest in Mehsana (86.67%). These observations were in corroboration with Ellahi *et al.* (2009) for alleles at loci INRA005, ILSTS029 and ILSTS033 for Azakheli breed buffalo of Pakistan. The allele frequency distribution in the present study was observed to be discrete, which was in agreement with other investigators (Kumar *et al.* 2006, Vijh *et al.* 2008).

Present study showed 49 alleles (Table 3) out of 127 amplified alleles to be specific to breed. Breed specific alleles were found in all the eight loci in at-least one of the breeds studied. Murrah and Pandharpuri showed highest number of specific alleles across all the loci. Out of 8 loci studied, 3 showed specific alleles in Bhadawari, 2 in Jaffarabadi, 7 in Murrah, 5 in Nagpuri, 13 in Pandharpuri and 1 in Surti; whereas no breed specific allele was found in Mehsana. Loci CSS047 and DRB3 recorded highest number of specific alleles (4) in Pandharpuri breed. However, the frequency of these breed specific alleles was low and only 4 out of 49 had a frequency of 8%, while only one allele had the frequency of more than 10%.

The most predominant private allele was detected in Mehsana and Jaffarabadi for marker CSSM047 and DRB3 with a frequency of 33 and 17%, respectively (Table 3). Tantia *et al.* (2006) also reported more than 10% private alleles in Bhadawari breed for marker CSRM60 (22%).

Polymorphic information content (PIC): PIC values in Bhadawari breed for markers CSSM047, CSSM033 and CSSM19 were 0.57, 0.51 and 0.67, respectively, and the mean PIC value was 0.60. Whereas, Tantia *et al.* (2006) reported comparatively higher PIC value for the same breed and for same markers (0.80, 0.65 and 0.47, respectively) with a lower mean PIC value of 0.57. Mean PIC value observed across the loci and breeds in the present study

Table 3. Population / breed specific alleles

Locus	Bhadawari		Jaffarabadi		Murrah		Mehsana		Nagpuri		Pandharpuri		Surti	
	bp	Fr (%)	bp	Fr (%)	bp	Fr (%)	bp	Fr (%)	bp	Fr (%)	bp	Fr (%)	bp	Fr (%)
CSSM033	124	5									141			
											150	1		
											125	1		
CSSM047					135	3	136	33	160	10	133	1		
					152	8					150	8		
											158	1		
ETH003					144	3			14		127	1		
									3	3	129	1		
									15	3	137	1		
									1					
									11					
CSSM061					112	5			6	3	103	1		
									12	7	118	2	86	10
									5					
DRB3	150	5	181	8	194	8			17	3	165	2		
	153	5	189	17					8		183	1		
											188	8		
											193	1		
CSSM019	141	5	132	8	147	5					145	1		
	150	3									166	6		
CSSM041					127	3					132	9		
					136	3					138	4		
HMH1R	137	3			129	5					162	2		
	140	3			180	5								
Total	07		03		10		01		06		21		01	

Table 4. Mean number of alleles (N_a), expected (H_e) and observed heterozygosity (H_o) estimates in buffalo populations/breeds

Population or breed	No. of animals	No. of loci	Mean no of alleles per locus (N_a)		Expected heterozygosity (H_e)		Observed heterozygosity (H_o)	
			Estimate	SE±	Estimate	SE±	Estimate	SE±
Bhadawari	20	8	6.250	1.146	0.682	0.049	0.675	0.106
Jaffarabadi	12	8	5.375	0.981	0.703	0.053	0.448	0.144
Murrah	20	8	6.500	0.945	0.677	0.060	0.606	0.111
Mehsana	15	8	4.375	0.778	0.568	0.068	0.458	0.129
Nagpuri	15	8	6.125	1.125	0.715	0.056	0.442	0.113
Pandharpuri	45	8	10.875	1.641	0.765	0.056	0.569	0.116
Surti	10	8	4.250	0.648	0.600	0.068	0.350	0.098
Overall	19.5	8	6.250	0.475	0.673	0.023	0.507	0.044

was 6.15. All 8 loci experimented in present study were found to be moderate to highly polymorphic.

Heterozygosity: In the present study, the population revealed high degree of polymorphism and genetic variation with an overall expected heterozygosity (H_e) of 0.673 (Table 4), which was in accordance with 0.67 of Vijh *et al.* (2008); however, Kumar *et al.* (2006) reported higher estimates of overall expected heterozygosity of 0.75 which may be due to higher prevalence of 2 or more alleles.

The mean expected heterozygosity estimates among breeds ranged from 0.568 to 0.765. Tantia *et al.* (2006) studied population structure in Bhadawari, Kerala buffalo and Tarai, observed the mean expected heterozygosity of 0.58 to 0.62; whereas, Kumar *et al.* (2006) who studied genetic variation and relationships among Indian riverine buffalo breeds (as in the present study) and the breed Toda, reported mean expected heterozygosity which ranged from 0.71 to 0.78. Vijh *et al.* (2008) studied seven Indian riverine buffalo breeds along with Tarai, Nili-Ravi and Toda breeds, and reported a mean expected heterozygosity ranging from 0.63 to 0.73.

The highest and lowest expected heterozygosity (H_e) estimates observed were in Pandharpuri (0.76) and Mehsana (0.56), respectively; whereas, Vijh *et al.* (2008) reported

0.65 and 0.66 estimates for Pandharpuri and Mehsana, respectively; however, Kumar *et al.* (2006) reported 0.75 in Pandharpuri and 0.76 in Mehsana.

The overall observed heterozygosity was 0.50 in the present study (Table 4), whereas higher estimates (0.60 and 0.68) were reported by Vijh *et al.* (2008) and Kumar *et al.* (2006) respectively. These higher estimates obtained may be the result of the existence of large number of alleles. The mean observed heterozygosity (Ho) estimates among breeds ranged from 0.35 to 0.67, which was lower than other published studies. Tantia *et al.* (2006) observed heterozygosity in the range of 0.62 to 0.68 in Bhadawari; whereas, Kumar *et al.* (2006) reported a range of 0.63 to 0.71; and Vijh *et al.* (2008) reported a range of 0.53 to 0.70.

The highest and lowest observed heterozygosity (H_o) estimates observed were in Bhadawari (0.67) and Surti (0.35), respectively; whereas, Vijh *et al.* (2008) reported 0.57 and 0.60 estimates in Bhadawari and Surti, respectively; and Kumar *et al.* (2006) reported 0.71 in Bhadawari and 0.69 in Surti.

The expected heterozygosity (H_e) was more than the observed heterozygosity (H_o) in the present study which may be a consequence of the presence of null alleles, the small sample size, or the Wahlund effect. The Wahlund effect refers to the reduction of heterozygosity in a population caused by the subpopulation structure. The highest observed heterozygosity and expected heterozygosity obtained in present study were 0.67 in Bhadawari and 0.76 in Pandharpuri, respectively.

The expected heterozygosity levels revealed that CSSM061 locus had highest heterozygosity of 0.816, while lowest heterozygosity of 0.442 was recorded at HMH1R. The locus HMH1R exhibited consistently lowest expected heterozygosity in Bhadawari (0.515), Jaffarabadi (0.518), Murrah (0.388), Mehsana (0.497), Nagpuri (0.515), Pandharpuri (0.395) and Surti (0.268). Hence this locus could be specifically utilized for detection of low heterozygous populations.

The observed heterozygosity was >0.50 in Bhadawari, Murrah and Pandharpuri; whereas it was <0.50 in case Jaffarabadi, Mehsana, Nagpuri and Surti, but across the loci it ranged from 0.025 to 0.971. The locus CSM033 was completely homozygous in Bhadawari, Jaffarabadi, Mehsana, Nagpuri and Surti while, CSSM041 in Jaffarabadi and ETH 03 in the all breeds except Surti were 100% heterozygous.

Fixation indices (F-values): Fixation indices give an idea about the population structure in terms of inbreeding coefficient and population differentiation. The F-statistics reveal fractional reduction in heterozygosity related to a random mating population with the same allele frequency. F_{IS} measures "the mean H_o expressed as fraction of mean H_e and deviation from 1" for all breeds in each locus; and is indicator of inbreeding within a breed. The F_{IS} estimates among the seven breeds, estimated based on the eight microsatellite loci in the present study varied from –0.03 in Bhadawari to 0.34 in Nagpuri (Table 5). The variation in F_{IS} estimates observed in the present study was possibly on account of different markers used. High F_{IS} estimates were observed in Nagpuri (0.34), Surti (0.33) and Jaffarabadi (0.31). These observed high values of F_{IS} may be a result of inbreeding. Overall, the genetic variation level was high in all three geographical African cape buffalo clusters. Inbreeding coefficients (F_{IS}) were low to moderate but significant for all clusters (Nathalie *et al*. 2014) The mean negative value of F_{IS} observed in Bhadawari breed was indicative of mating between individuals who were less closely related than the average relationship in the population. A similar negative F_{IS} was reported in Bhadawari by Tantia *et al.* (2006).

The F_{ST} estimates between the populations and F_{IT} coefficient of overall population (Table 5) observed in the present study were 0.152 ± 0.029 and 0.340 ± 0.138, respectively. About 15% differentiation between the sub-population (F_{ST}) observed in the buffalo breeds in the present study was within the range of moderate genetic

Table 5. Estimation of fixation indices at different loci

Locus	Fixation indices		
	F_{IS}	F_{IT}	F_{ST}
CSSM033	0.954	0.966	0.267
CSSM047	0.283	0.348	0.090
ETH003	-0.777	-0.305	0.265
CSSM061	0.364	0.411	0.073
DRB3	0.302	0.421	0.169
CSSM019	0.403	0.458	0.091
CSSM041	-0.199	-0.101	0.082
HMH1R	0.419	0.521	0.177
Overall	0.219±0.180	0.340±0.138	0.152±0.029

Table 6. Pair wise F_{ST} estimates between populations/breeds

Breed	Bhadawari	Jaffarabadi	Murrah	Mehsana	Nagpuri	Pandharpuri	Surti
Bhadawari	0.000	-	-	-	-	-	-
Jaffrabadi	0.116	0.000	-	-	-	-	-
Murrah	0.045	0.126	0.000	-	-	-	-
Mehsana	0.163	0.051	0.168	0.000	-	-	-
Nagpuri	0.107	0.029	0.119	0.070	0.000	-	-
Pandharpuri	0.036	0.066	0.042	0.109	0.066	0.000	-
Surti	0.157	0.058	0.170	0.065	0.051	0.123	0.000

Table 7. Nei's genetic distance matrix between population breeds

Breed	Bhadawari	Jaffrabadi	Murrah	Mehsana	Nagpuri	Pandharpuri	Surti
Bhadawari	0.000	-	-	-	-	-	-
Jaffrabadi	0.698	0.000	-	-	-	-	-
Murrah	0.156	0.763	0.000	-	-	-	-
Mehsana	0.871	0.111	0.851	0.000	-	-	-
Nagpuri	0.632	0.071	0.716	0.207	0.000	-	-
Pandharpuri	0.153	0.354	0.193	0.489	0.363	0.000	-
Surti	0.811	0.122	0.896	0.143	0.112	0.574	0.000

differentiation on a scale defined by earlier works. The F_{ST} values indicated absence of pure breeding efforts towards a particular breed type, while the F_{IT} values indicated tendency to accumulate towards one of these eight buffalo breeds.

The smallest pair-wise F_{ST} value, which was 0.029, was obtained between Nagpuri and Jaffarabadi (Table 6). Low F_{ST} values were also obtained between Pandharpuri and Bhadawari (0.036) and between Pandharpuri and Murrah. The largest F_{ST} value was obtained between Murrah and Mehsana (0.168) followed by Murrah and Bhadawari buffaloes (0.163). Vijh *et al.* (2008) in their genetic diversity study on twelve Indian water buffalo breeds reported that smallest F_{ST} value (0.0213), which was obtained between Mehsana and Surti buffaloes. Low F_{ST} values were also obtained between Marathwada and Pandharpuri buffaloes (0.0468) and between Toda and Kalasthi buffaloes (0.0553). The largest F_{ST} was obtained between Jaffarabai and Pandharpuri buffaloes (0.1797), followed by Jaffarabadi and Bhadawari buffaloes (0.1744). Kumar *et al.* (2006) reported smallest F_{ST} value (0.0075), which was obtained between Bhadawari and Murrah buffaloes and the largest F_{ST} was obtained between Toda and Pandharpuri buffaloes (0.0604).

Genetic distances: In the present study, least genetic distance was observed between Jaffarabadi and Nagpuri (0.159). The results were in corroboration with Shukla *et al.* (2006) who reported least genetic difference between these breeds (0.05) as shown in Table 7.

Highest genetic distance was observed between Murrah and Surti (0.896) in the present study; whereas, Vijh *et al.* (2008) reported the highest distance between Jaffarabadi and Pandharpuri (0.598). Kumar *et al.* (2006) reported highest genetic distance between Toda and Pandharpuri and Shukla *et al.* (2006) reported highest between Mehsana and Bhadawari. Highest genetic distance observed between Murrah and Surti (0.896) in the present study indicated their isolated breeding over several generations.

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REFERENCES

Ellahi M B, Tanveer H, Asif N, Rahila J and Maryam J. 2009. Genetic characterization of Azakheli buffalo breed of Pakistan using microsatellites DNA markers. *Pakistan Journal of Zoology* **9**: 361–66.

FAO. 2011. *Molecular Genetic Characterization of Animal Genetic Resources*. FAO Animal Production and Health Guidelines. No. 9. Rome.

Kumar S, Gupta J, Kumar N, Dikshit K, Navani N, Jain P and Nagarajan M. 2006. Genetic variation and relationships among eight Indian riverine buffalo breeds. *Molecular Ecology* **15**: 593–600.

Nathalie Smitz, Daniel Cornélis, Philippe Chardonnet, Alexandre Caron, Michel de Garine Wichatitsky, Ferran Jori, Alice Mouton, Alice Latinne, Lise-Marie Pigneur, Mario Melletti, Kimberly L Kanapeckas, Jonathan Marescaux, Carlos Lopes Pereira and Johan Michaux.2014. Genetic structure of fragmented southern populations of African Cape buffalo (Syncerus caffer caffer). *BMC Evolutionary Biology* **14**: 203.

Nei M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.

OIE. 2006. General definitions. Terrestrial animal health code. Article 1.1.1.1. World Organization for Animal Health.

Sambrook J and Russel D W. 2001. *Molecular Cloning, A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sangwan M L. 2012. Analysis of genetic diversity of Indian buffalo breeds by DNA markers. *Journal of Buffalo Science* **1**: 91–101.

Shukla S, Yadav B R and Bhattacharya T K. 2006. Characterization of Indian markers. *Asian Australatian Journal of Animal Sciences* **19**: 1556–60.

Tantia M S, Vijh R K, Mishra B, Bharanikumar S T and Arora R. 2006. Multilocus genotyping to study population structure in three buffalo populations of India. *Asian Australatian Journal of Animal Sciences* **19**(8): 1071–78.

Vijh R K, Tantia M S, Mishra B and Bharanikumar S T. 2008. Genetic relationship and diversity analysis of Indian water buffalo *(Bubalus bubalis). Journal of Animal Science* **86**: 1495–1502.