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Multilocus Genotyping to Study Population Structure in Three Buffalo Populations of India

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ABSTRACT : Three buffalo populations viz. Bhadawari, Tarai and local buffaloes of Kerala were genotyped using 24 heterologous polymorphic microsatellite loci. A total of 140 alleles were observed with an average observed heterozygosity of 0.63. All the loci were neutral and 18 out of the 24 loci were in Hardy Weinberg Equilibrium. The F_{IS} values (estimate of inbreeding) for 16 loci in all the three populations were negative. This indicated lack of population structure in the three populations. The effective number of immigrants was 5.88 per generation between the Tarai and Bhadawari populations which was quite high suggesting substantial gene flow. The genetic distances revealed closeness between the Tarai and Bhadawari populations which was expected from geographical contiguity. The F_{ST} values were not significantly different from zero showing no population differentiation. The Correspondence Analysis based on the allelic frequency data clustered the majority of the Tarai and Bhadawari individuals as an admixture. (**Key Words :** Buffalo, Microsatellites, F-statistics, Genetic Distance, Correspondence Analysis)

INTRODUCTION

Microsatellite are short segments of DNA of a specific motif made up of 1-6 base repeats which may be repeated upto 50-60 times. Microsatellite markers have exceptional variability, are easy to score and thus are considered to be the most powerful genetic markers for population studies. It is typical for microsatellite loci to have 10 or more alleles with a heterozygosity values of 0.6 or more which can be detected even from relatively small samples (Bowcock et al., 1994; Primmer et al., 1996). Microsatellite are rapidly replacing other polymorphic markers for identifying relatives to infer various demographic parameters (Bloom et al., 1996; Goldstein et al., 1996; James and Lagoda, 1996; Fan et al., 2005). The primers are designed to amplify the genomic regions which include a well defined repeat structure responsible for the observed variations. The variation at the locus with the repeat structure allows for the development of inferential methods based on explicit models of microsatellite evolution. Microsatellites are the best markers for population genetic studies and demographic inferences.

India has 10 recognized breeds of buffaloes distributed in the various agro climatic regions of the country. Most of the buffaloes are reared for milk production and have their breeding tracts in the north and the northwestern regions of the country. In addition to the recognized breeds, there are several distinct populations of buffaloes in various parts of the country. Three populations were used for this study. Among these, the Bhadawari breed is an established breed which inhabits the borders of Uttar Pradesh and Madhya Pradesh. Tarai buffaloes are medium sized and found in the foot hills of Uttaranchal state, Bareilly and Pilibhit districts of Uttar Pradesh. The distributions of Tarai and Bhadawari buffaloes are in geographical contiguity. Another distinct population in the southernmost part of India is the Kerala buffalo locally known as Kuttanad buffalo. These buffaloes are mainly utilized as work animals in the paddy fields. They are considered to be good swimmers (Anilkumar and Raghunandanan, 2003). The animals in Kuttanad area are of short stature with an average height of 109 cm. These animals yield 1-2 kg of milk and are reared as work animals. The present study was undertaken to find out the genetic parameters of the three buffalo populations.

MATERIALS AND METHODS

Blood samples were collected from the breeding tract of three buffalo populations. Ten ml of whole blood was collected from jugular vein of each animal using

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heparinised vacutainer tubes and transported to the lab at 0-5°C.

DNA was extracted from the whole blood following the standard protocol (Sambrook et al., 1989). The DNA isolation procedure encompassed lysis of RBC's, digestion of protein using Proteinase K and precipitation of protein using phenol:chloroform:isoamylalchol. DNA was precipitated by gentle addition of 2.5 volumes ethanol and 250 µl of 3 M sodium acetate pH 5.2. The resulting DNA strands were spooled out and washed twice with ice cold 70% ethanol to remove excess salts. DNA was re-dissolved in 500-750 µl of TAE buffer pH 8.0. The concentrations of DNA were adjudged using comparison with the standard DNA marker concentrations on the agarose gel. The quality of the DNA

Table 1. Details of the microsatellite primers used

S. No.	Primer	Sequence	Annealing temp.	Allele size	No of alleles
1	ILSTS087	AgCAgACATgATgACTCAgC CTgCCTCTTTTCTTgAgAgC	55	115-123	3
2	ILSTS072	ATgAATgTgAAAgCCAAggg CTTCCgTAAATAATTgTggg	55	142-146	3
3	ILSTS059	AgTATggTAAggCCAAAggg CgACTTgTgTTgTTCAAAgC	60	154-176	6
4	ILSTS029	TgTTTTgATggAACACAgCC TggATTTAgACCAgggTTgg	55	156-170	5
5	ILSTS049	CAATTTTCTTgTCTCTCCCC gCTgAATCTTgTCAAACAgg	55	141-145	3
6	ILSTS052	CTgTCCTTTAAgACCAAACC TgCAACTTAggCTATTgACg	55	144-180	7
7	ILSTS005	ggAAgCAATgAAATCTATAgCC TgTTCTgTgAgTTTgTAAgC	55	188-198	4
8	ILSTS030	CTgCAgTTCTgCATATgTgg CTTAgACAACAggggTTTgg	56	155-167	5
9	ILSTS058	gCCTTACTACCATTTCCAgC CATCCTgACTTTggCTgTgg	58	122-152	8
10	ILSTS011	gCTTg CTACATggAAAgTgC CTAAAATgCAgAgCCCTACC	58	264-272	4
11	CSSM043	AAACTCTgggAACTTgAAAAACTA gTTACAAATTTAAgAgACAgAgTT	55	222-254	5
12	CSSM047	TCTCTgTCTCTATCACTATATggC CTgggCACCTgAAACTATCATCAT	55	126-164	9
13	CSSM029	TCTCCATTATgCACATgCCATgCT CgTgAgAACCgAAAgCACACATTC	60	186-192	4
14	CSSM008	CTTggTgTTACTAgCCCTggg gATATATTTgCCAgAgATTCTgCA	55	182-196	6
15	CSSM019	TTgTCAgCAACTTCTTgTATCTTT TgTTTTAAgCCACCCAATTATTTg	55	132-156	6
16	CSSM057	TgTggTgTTTAACCCTTgTAATCT gTCCTggATAAACAATTTAAAgT	60	118-128	5
17	CSSM006	AgCTTCTgACCTTTAAAgAAAATg AgCTTATAgATTTgCACAAgTgCC	55	202-220	5
18	CSSM045	TAgAggCACAAgCAAACCTAACAC TTggAAAgATgCAgTAgAACTCAT	58	100-122	6
19	CSSM033	CACTgTgAATgCATgTgTgTgAgC CCCATgATAAgAgTgCAgATgACT	58	154-180	7
20	CSRM060	AgATgTgATCCAAgAgAgAggCA ggACCAgATCgTgAAAggCATAg	60	112-140	9
21	ETH152	ACTCgTAgggCAggCTgCCTg gAgACCTCAgggTTggTgATCAg	55	194-216	6
22	HAUT027	TTTTATgTTCATTTTTTgACTgg AACTgCTgAAATCTCCATCTTA	55	139-151	4
23	BM1818	F-AgCTgggAATATAACCAAAgg AgTgCTTTCAAggTCCATgC	56	252-278	7
24	ILSTS038	GggCATTATTTTGTTTCCC CCACTTCTgggTAATTATCC	55	152-176	4

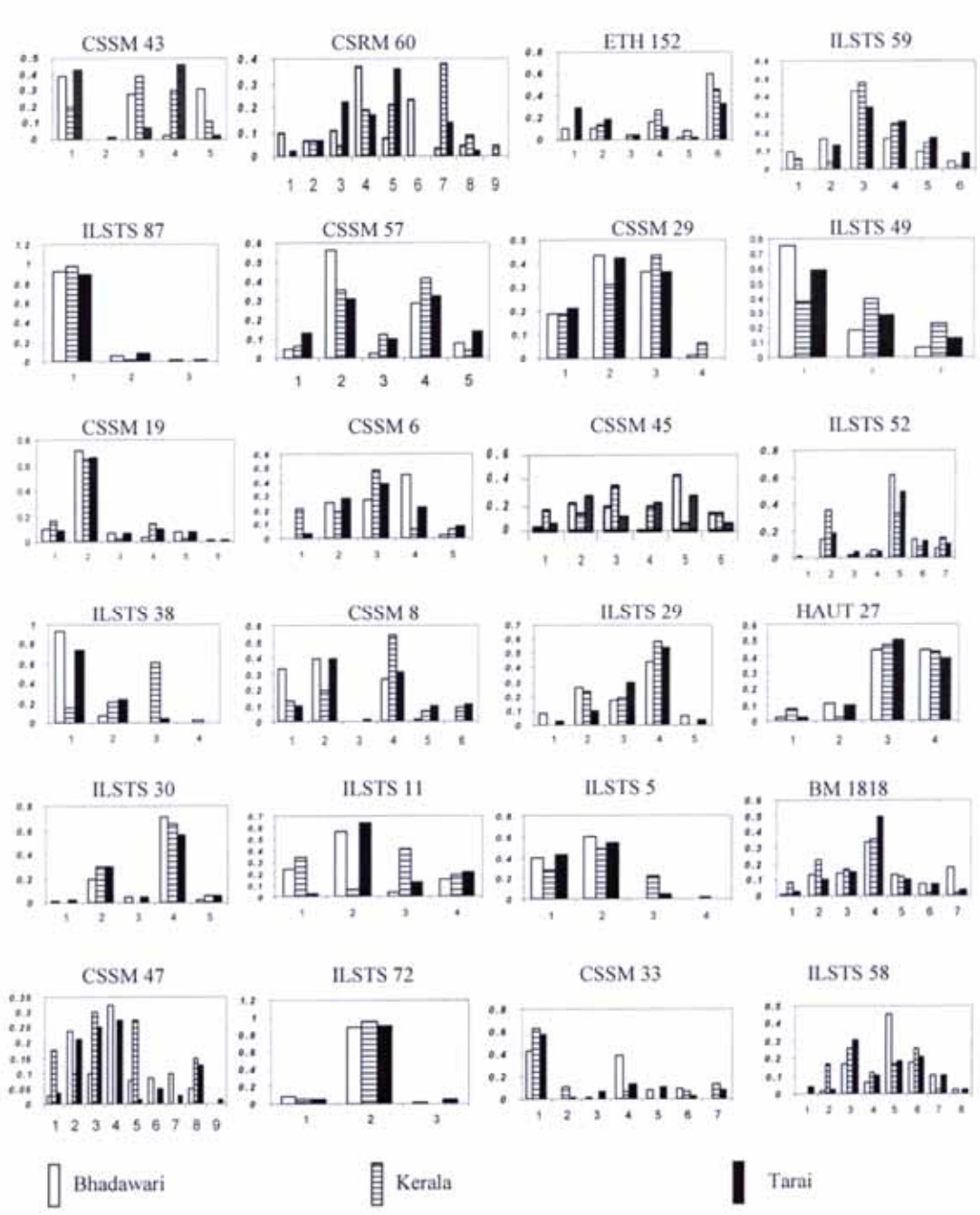

Figure 1. No of alleles and their frequencies in the three populations studied.

was checked on 0.8% agarose gel prepared in TAE buffer.

A total of 24 heterologous primers were chosen for the study. These primers were ETH152, ILSTS 5, ILSTS 11, ILSTS 29, ILSTS 30, ILSTS 38, ILSTS 49, ILSTS 52, ILSTS 58, ILSTS 59, ILSTS 72, ILSTS 87, CSSM 6, CSSM 8, CSSM 19, CSSM 29, CSSM 33, CSSM 43, CSSM 45, CSSM 47, CSSM 57, CSRM 60, HAUT 27 and BM 1818. The criterion for selection of the heterologus microsatellite loci on their polymorphism in buffaloes, PIC value and number of alleles (Navani et al., 2002).

The primer sequences, annealing temperature, $MgCl_2$ concentration, size range of PCR products along with the total number of alleles observed in the three populations are given in Table 1. The amplified PCR products from the heterologous markers were sequenced using ABI Avant 3100 to confirm the sequences of the microsatellite repeats. A total of 104 individuals were utilized for the study; 40 animals each of Bhadawari and Tarai, and 24 animals of Kerala buffalo.

The PCR conditions were standardized for all of the 24

Table 2. Heterozygosities in the three buffalo populations studied

S. No.	Locus	Bhadawari buffalo			Kerala buffalo			Tarai buffalo		
		PIC	H exp	H obs	PIC	H exp	H obs	PIC	H exp	H obs
1	CSSM 43	0.6759	0.6854	0.6250	0.7042	0.7198	0.4783	0.5991	0.6066	0.6000
2	CSRM 60	0.7842	0.7963	0.9394	0.7665	0.7828	0.9583	0.7705	0.7806	0.6667
3	ETH 152	0.5874	0.5951	0.6154	0.6866	0.7012	0.7083	0.7534	0.7630	0.7500
4	ILSTS 59	0.7384	0.7488	0.6944	0.6806	0.6950	0.5833	0.7587	0.7688	0.7368
5	ILSTS 87	0.1403	0.1421	0.1000	0.0408	0.0417	0.0417	0.1822	0.1845	0.0250
6	CSSM 57	0.5922	0.5997	0.5500	0.6797	0.6941	0.9583	0.7558	0.7656	0.8718
7	CSSM 29	0.6419	0.6500	0.5750	0.6719	0.6862	1.0000	0.6428	0.6509	0.6000
8	ILSTS 49	0.3984	0.4035	0.4250	0.6502	0.6640	1.0000	0.5566	0.5636	0.5750
9	CSSM 19	0.4713	0.4772	0.5250	0.5330	0.5443	0.5833	0.5337	0.5405	0.6000
10	CSSM 6	0.6603	0.6691	0.9211	0.6840	0.6986	0.7500	0.7118	0.7216	0.8919
11	CSSM 45	0.7097	0.7189	0.9231	0.7786	0.7972	0.8333	0.7804	0.7905	0.9744
12	ILSTS 52	0.5766	0.5841	0.6154	0.7309	0.7465	0.7083	0.6982	0.7073	0.6410
13	ILSTS 38	0.1387	0.1405	0.1500	0.5558	0.5687	0.6364	0.4131	0.4187	0.4595
14	CSSM 8	0.6666	0.6754	0.6579	0.6450	0.6587	0.7500	0.7244	0.7340	0.9474
15	ILSTS 29	0.7064	0.7156	0.8718	0.5720	0.5842	0.7500	0.6091	0.6168	0.9000
16	HAUT 27	0.6088	0.6167	0.8718	0.5875	0.6026	0.6000	0.5952	0.6032	0.6316
17	ILSTS 30	0.4491	0.4527	0.4750	0.4939	0.5044	0.6250	0.5866	0.5940	0.6000
18	ILSTS 11	0.5973	0.6051	0.8462	0.6762	0.6906	0.9583	0.5322	0.5389	0.5750
19	ILSTS 5	0.4800	0.4861	0.5500	0.6437	0.6580	0.6087	0.5296	0.5365	0.5641
20	BM 1818	0.7978	0.8082	0.5641	0.7708	0.7872	0.8750	0.6972	0.7063	0.4615
21	CSSM 47	0.8016	0.8117	0.8000	0.7713	0.7910	0.6500	0.7962	0.8063	0.8750
22	ILSTS 72	0.2041	0.2066	0.2250	0.0799	0.0816	0.0833	0.1850	0.1873	0.2000
23	CSSM 33	0.6534	0.6617	0.8250	0.5652	0.5778	0.6522	0.6275	0.6354	0.7250
24	ILSTS 58	0.7232	0.7326	0.6410	0.7925	0.8118	0.7143	0.8051	0.8158	0.5263
	Mean	0.5752	0.5827	0.6244	0.6150	0.6286	0.6878	0.6185	0.6265	0.6416

primer pairs selected for the study. The variables, which required standardization, included annealing temperature, $MgCl_2$ concentration, quantity of primer, Taq polymerase and dNTP's. The PCR products were loaded on 6% denatured polyacrylamide gel with urea as the denaturing agent. The standard DNA markers were simultaneously run on the gel for sizing of the alleles. The polyacrylamide gel electrophoresis was run for a sufficiently long period for proper resolution of the alleles.

The polyacrylamide gel was fixed in acetic acid (10%) and stained with silver nitrate following a standard protocol (Bassam et al., 1991). The size of the alleles were estimated by making a standard curve taking $\log_{10}$ of the molecular weights of the standard markers on the X-axis and the mobilities of the DNA bands on the Y-axis. The sizes of the alleles were calculated from the standard curve.

Statistics

The data for the 24 microsatellite loci in the three populations were subjected to statistical analysis. The locus and population wide gene frequencies, the number of alleles, effective number of alleles, and observed and expected heterozygosities were calculated with the Popgene software (Yeh et al., 1999). The Ewens Watterson test of neutrality was also carried out using 10,000 permutations.

The Hardy-Weinberg equilibria of the loci in the three populations were tested against null hypothesis of random union of gametes. The U test with heterozygotic deficiency as the alternative hypothesis was carried out using the GENEPOP software (Raymond and Rousset, 2003). The software performed a probability test utilizing Markovs' chain (dememorization 5,000, batches 100, iterations per batch 1,000). Significance levels were calculated per locus, per population and over all loci and all populations combined. Two estimates of F_{IS} (Weir and Cockerham 1984; Robertson and Hill, 1984) were estimated.

The F statistics values F_{IS}, F_{IT} and F_{ST} were estimated using Jack-knifing over loci and the confidence interval were generated using 10,000 permutations with the GDA software (Lewis and Zaykin, 2002). The number of migrants (Nm) was estimated using $Nm = 0.25\ (1\text{-}F_{ST})/F_{ST.}$

The genotypic linkage disequilibrium was estimated between all the possible pairs. The significance of population differences was tested using the exact test of population differentiation in the GENEPOP software based on the allele frequency data.

The correspondence analysis which is a weighted principal component analysis was performed using the allele frequency data for the individuals of the three populations and the 24 loci using GENETIX software (Belkhir et al., 1998).

Two genetic distances viz. Nei's standard and Nei's D_A were calculated using the DISPAN software (Ota, 1993) and $(\delta\mu)^2$ was estimated with the MICROSAT software (Minch

Table 3. F_{IS} values for microsatellite loci in the three buffalo populations studied

Loci	Bhadawari			Kerala			Tarai		
	P-val	W and C[1]	R and H[2]	P-val	W and C	R and H	P-val	W and C	R and H
CSSM43	0.7549	0.088	0.05	0.9575	0.341	0.228	0.4892	0.011	-0.013
CSRM60	0.4248	-0.183	-0.021	0.0114*	-0.23	-0.114	0.9894	0.148	0.177
ETH152	0.2904	-0.035	-0.05	0.6133	-0.01	0.011	0.8117	0.017	0.053
ILSTS59	0.5364	0.074	-0.003	0.8869	0.164	0.085	0.7251	0.042	0.045
ILSTS87	0.9927	0.299	0.188	-	-	-	1	0.866	0.447
CSSM57	0.9536	0.084	0.141	0.001*	-0.392	-0.204	0.0359*	-0.141	-0.136
CSSM29	0.6417	0.117	0.023	0.0*	-0.472	-0.319	0.4969	0.079	-0.002
ILSTS49	0.7996	-0.054	0.08	0.0*	-0.523	-0.489	0.1062	-0.02	-0.127
CSSM19	0.4706	-0.102	-0.011	0.4562	-0.073	-0.012	0.484	-0.112	-0.008
CSSM6	0.0001*	-0.384	-0.252	0.8069	-0.075	0.077	0.0044*	-0.24	-0.163
CSSM45	0.0053*	-0.289	-0.153	0.2607	-0.049	-0.065	0.0052*	-0.236	-0.144
ILSTS52	0.3503	-0.054	-0.034	0.8139	0.052	0.07	0.881	0.095	0.087
ILSTS38	0.8175	-0.068	-0.069	0.1767	-0.122	-0.115	0.0874	-0.099	-0.118
CSSM8	0.5619	0.026	0.009	0.2278	-0.142	-0.079	0.0077*	-0.296	-0.118
ILSTS29	0.0095*	-0.222	-0.142	0.0292*	-0.292	-0.27	0.0*	-0.468	-0.193
HAUT27	0.0002*	-0.421	-0.225	0.4809	0.004	-0.015	0.3288	-0.048	-0.048
ILSTS30	0.3991	-0.045	-0.031	0.059*	-0.245	-0.192	0.468	-0.01	-0.021
ILSTS11	0.0*	-0.406	-0.232	0.0008*	-0.399	-0.285	0.4091	-0.068	-0.02
ILSTS5	0.3044	-0.133	-0.135	0.726	0.076	0.057	0.4145	-0.052	-0.037
BM1818	0.9999	0.305	0.252	0.2976	-0.114	-0.043	0.9966	0.35	0.222
CSSM47	0.5056	0.015	-0.01	0.9509	0.182	0.193	0.1373	-0.086	-0.047
ILSTS72	0.6037	-0.09	-0.051	0.9787	-0.022	-0.023	0.6792	-0.068	-0.043
CSSM33	0.0059*	-0.251	-0.133	0.2089	-0.132	-0.092	0.0474*	-0.143	-0.075
ILSTS58	0.938	0.126	0.092	0.9329	0.123	0.131	0.997	0.358	0.212

* p-value significant; [1] Weir and Cockerham (1984); [2] Robertson and Hill (1984).

Table 4. Private alleles in the three buffalo populations studied

Locus	Allele	Frequency	Found in
CSSM 43	126	0.012500	Tarai
CSRM 60	140	0.041667	Kerala buffalo
CSRM60	132	0.227273	Bhadawari
ILSTS 52	144	0.012821	Bhadawari
ILSTS 58	176	0.022727	Kerala buffalo
CSSM 8	190	0.013158	Tarai
ILSTS 5	198	0.021739	Kerala buffalo
CSSM 47	164	0.012500	Tarai
ILSTS 58	122	0.039474	Tarai

et al., 1996). The dendrograms were constructed by using the PHYLIP software (Felsenstein, 1993).

RESULTS AND DISCUSSION

In total, 140 alleles were observed from the 24 loci analysed. The number of alleles per locus ranged from 3 (ILST 49, ILST 72, ILST 87) to 9 (CSRM 60, CSSM 47) with a mean of 5.83 alleles. The number of alleles was similar to Indian water buffaloes (Arora et al., 2004), but different from Sri Lankan water buffaloes (Barker et al., 1997). The comparative allele frequencies are given in Figure 1. Nine loci out of the 24 had one allele which was predominant in all the populations. The expected heterozygosity varied between 0.1350 for ILSTS 87 and 0.834 for CSRM 60 with a mean value of 0.6337 across the three populations (Table 2). There was no linkage disequilibrium among the 24 loci utilized in this study. 18 loci in the three populations were in HW equilibrium. ILSTS 29 was the only locus which was not in HWE in all the three populations (Table 3). The population wide F_{IS} (Robertson and Hill, 1984) values were negative for 16, 15 and 17 loci in Bhadawari, Kerala and Tarai populations, respectively. This indicated lack of population structure in all the three populations. The private alleles identified in the three populations are given in Table 4.

Heterozygosity is defined as the probability that a given individual randomly sampled from a population will be heterozygous at a given locus. The statistics F_{ST} is an estimate of the variation due to differentiation among populations, which is the reduction in heterozygosity of a population due to genetic drift. The statistics F_{IS} is an estimate of variation with in populations that measures the reduction in heterozygosity in an individual due to nonrandom mating within sub populations. F_{IT} is the over all inbreeding coefficient of an individual relative to the total population. This includes the contribution due to nonrandom mating within sub populations (F_{IS}) and that due to population subdivision (F_{ST}). The mean values of F, θ (estimate of population differentiation-F_{IT}) and f (within population inbreeding estimate-F_{IS}) from Jackknifing over loci were 0.0092, 0.0648 and -0.0593, respectively (Table 5). All the three values did not deviate significantly from

Table 5. F-Statistics over all the populations

S. No.	Locus name	Jackknifing over loci		
		F	Theta P	F
1	CSSM 43	-0.0033	0.0607	-0.0681
2	CSRM 60	0.0074	0.0631	-0.0595
3	ETH 152	0.0068	0.0649	-0.0621
4	ILSTS 59	0.0051	0.0677	-0.0671
5	ILSTS 87	0.0041	0.0654	-0.0655
6	CSSM 57	0.0136	0.0663	-0.0564
7	CSSM 29	0.0113	0.0678	-0.0606
8	ILSTS 49	0.0128	0.0644	-0.0552
9	CSSM 19	0.0128	0.0670	-0.0581
10	CSSM 6	0.0178	0.0645	-0.0499
11	CSSM 45	0.0158	0.0638	-0.0513
12	ILSTS 52	0.0065	0.0663	-0.0640
13	ILSTS 38	-0.0035	0.0519	-0.0585
14	CSSM 8	0.0130	0.0649	-0.0555
15	ILSTS 29	0.0225	0.0666	-0.0472
16	HAUT 27	0.0173	0.0676	-0.0539
17	ILSTS 30	0.0116	0.0667	-0.0590
18	ILSTS 11	0.0120	0.0589	-0.0499
19	ILSTS 5	0.0103	0.0664	-0.0600
20	BM 1818	-0.0025	0.0675	-0.0750
21	CSSM 47	0.0057	0.0649	-0.0634
22	ILSTS 72	0.0100	0.0654	-0.0594
23	CSSM 33	0.0142	0.0646	-0.0539
24	ILSTS 58	-0.0040	0.0664	-0.0754
Over all loci		0.0092	0.0648	-0.0593
Upper boundary		0.0724	0.0994	0.00338
Lower boundary		-0.0531	0.0358	-0.12078

zero and lay between upper and lower bound calculated on the basis of 10,000 replicates. The negative value of F_{IS} indicated mating between individuals which are less closely related than the average relationship in the population. The F_{IS} values of most of the loci were negative indicating outbreeding or mating with migrants. This could be explained by the indiscriminate use of Murrah bulls in all the three populations. This view was supported by the higher values of Nm obtained in the present study. Such possibilities have also been reported by Pundir et al. (1997) and Sethi (2001) on the basis of surveys of the areas. The pairwise F_{ST} values estimated were 0.1024 (between Bhadawari and Kerala) 0.0408 (between Bhadawari and Tarai) and 0.0644 (between Kerala and Tarai) buffalo populations. These values were obtained using exact test for population differentiation. The F_{ST} values were significant between Bhadawari and Kerala, while the values were non-significant between Bhadawari and Tarai, and Kerala and Tarai populations.

The three populations were tested for genotypic differentiation using the null hypothesis that the genotypic distribution was identical across populations. The unbiased estimates of the p-value of the log likelihood (G) based exact test revealed that the null hypothesis was accepted in 17 of the 24 loci studied. There was no genotypic differentiation for the loci ILSTS 087, CSSM 029, CSSM 019, HAUT 027, ILSTS 030, BM 1818 and ILSTS 072. The overall X^2 value was infinity at df 48 and the genotypic distribution was significantly different across all the populations. The genic differentiation using the null hypothesis that the allelic distribution was identical across populations was not accepted, suggesting a significant difference in the allelic distributions among populations.

Table 6. Genetic distances among the three buffalo populations studied

Distance		Bhadawari	Kerala	Tarai
(Ds)	Bhadawari	0		
Nei's Standard	Kerala	0.2134	0	
	Tarai	0.0844	0.1512	0
Nei's D_A	Bhadawari	0		
(Nei et al., 1983)	Kerala	0.1346	0	
	Tarai	0.0633	0.0908	0
$(\delta\mu)^2$	Bhadawari	0		
(Goldstein	Kerala	5.9007	0	
et al., 1995)	Tarai	3.0795	4.7192	0

The three genetic distances, Nei's standard and D_A, and Goldstein ($\delta\mu^2$), also revealed closeness between Bhadawari and Tarai populations, while Kerala buffaloes were distant (Table 6). These were in agreement with the genetic distances reported by Arora et al. (2004) and Vijh et al. (2005). The tree of inter-individual genetic distance (Nei' D_A) using the Neighbour Joining algorithm revealed that all the 24 Kerala individuals clustered together while the Tarai and Bhadawari individuals grouped in distinct and mixed clusters (Figure 2). The genetic basis of the samples was quite wide since using Nei's D_A 20% of the individuals did not cluster in their own populations. It has been used by several authors for clustering purposes (Cho, 2005).

The Correspondence Analysis (which is a weighted Principle Component Analysis) was performed on the three populations using allele frequencies of the 24 loci to summarize the breed relationships. Figure 3 shows close relationship among individual belonging to the Bhadawari and Tarai populations. The first four factors contributed a total of 17.3% with value of 6.08, 4.43, 3.67 and 3.11% respectively. The analysis indicated that all individuals of Kerala buffaloes clustered together while two clusters one each for Bhadawari and Tarai buffaloes were found. Some of the individuals of these latter two populations were clearly overlapping with one another and thus form another group.

The number of migrants per generation based on F_{ST} values were lowest (2.19) between Kerala and Bhadawari. The values were highest (5.88) between the Bhadawari and Tarai populations. The estimated Nm value could be explained on the basis of contiguity of the breeding tract between these two populations. The large number of migrants detected in the Tarai and Bhadawari populations

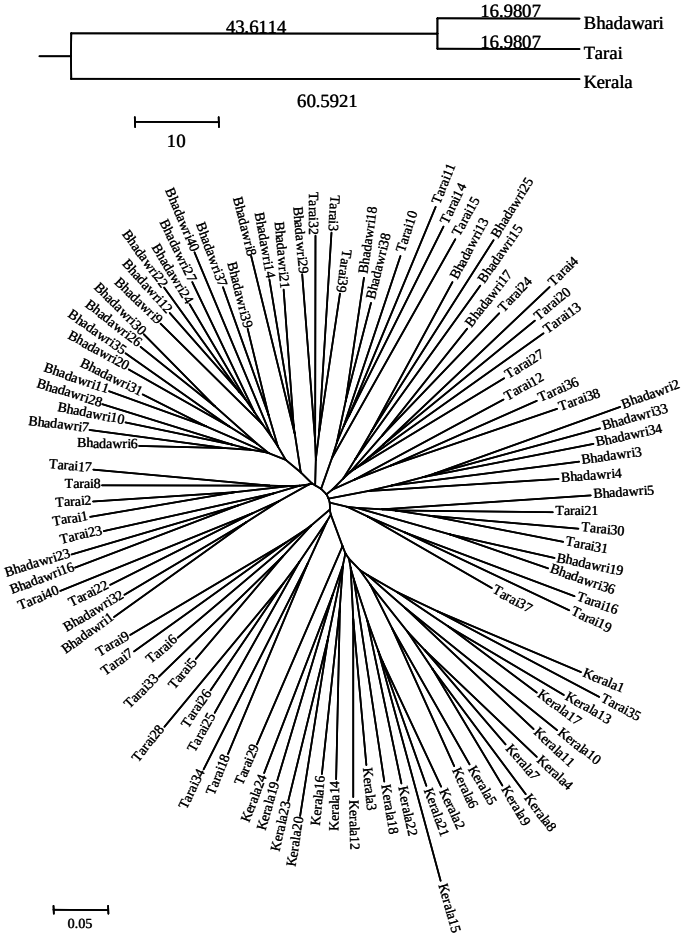

Figure 2. (a) Dendrgram obtained by using UPGMA $(\delta\mu)^2$, (b) radiation tree based on Nei's D_A.

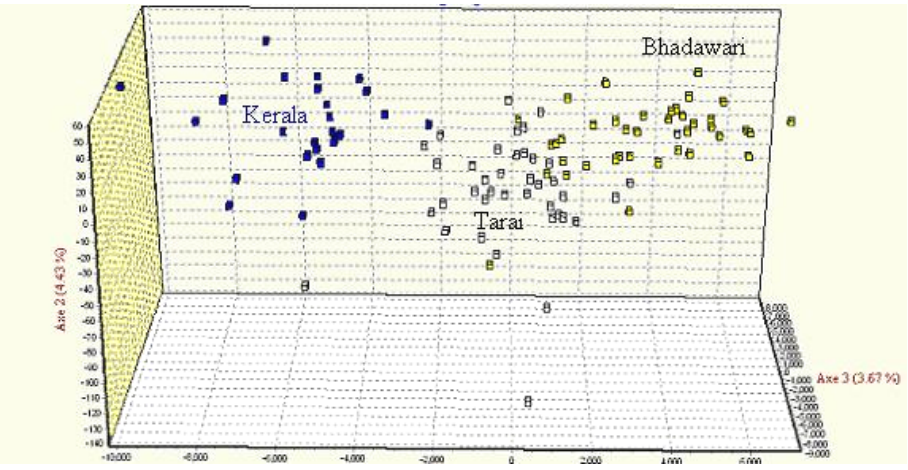

Figure 3. Clustering of individuals based on correspondence analysis.

might be due to larger exchange of individuals between these two populations or use of Murrah buffaloes in both populations. The number of migrants, estimated by using the private alleles as implemented in GENEPOP (Barton and Slatkin, 1983) was 1.191. This could be because of few private alleles among the three populations studied.

The study indicated that there is an abundance of within population genetic variation in the three buffalo populations. This is further supported by the fact that the genetic distance among the three populations were quite low. The homogeneity among the breeds may be due to immigration as there is excess of heterozygosity in all the three

populations. The Nm values obtained from F_{ST} also supported this. However, the explanation to such a situation is that since only three populations were taken for the study it is very possible that the migrants came from populations other than these three. It is well known that to improve milk production there has been indiscriminate use of Murrah bulls/ semen in the breeding tract of the three populations under investigation.

REFERENCES

Anilkumar, K. and K. V. Raghunandanan. 2003. The dwarf cattle and buffalo of kerala. College of Veterinary and Animal Sciences, KAU, Mannuthy, Thrissur.

Arora, R., B. D. Lakhchaura, R. B. Prasad, M. S. Tantia and R. K. Vijh. 2004. Genetic diversity analysis of two buffalo populations of northern India using microsatellite markers. J. Anim. Breed. Genet. 121:111-118.

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

Barton, N. H. and M. Slatkin. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a sub divided population. Heredity 56:409-415.

Bassam, B. J., G. Coetano-Anolles and P. M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels, Anal. Biochem. 196:80-83.

Belkhir, K., P. Borsa, J. Goudet, L. Chikhi and F. Bonhomme. 1998. Genetix, logicial sous windows TM pour la genetique des populations. Montpellier. France. www.univ-montp2.fr/ ~genetix/genetix.htm

Bloom, M. S., M. Parsons, Y. Lacaille and S. Lotz. 1996. Use of microsatellite loci to classify individuals by relatedness. Mol. Ecol. 3:393-401.

Bowcock, A. M., A. Ruiz-Lenares, J. Tomfohrde, E. Minch and J. R. Kidd. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. Nature, 368:455-457.

Cho, G. J. 2005. Microsatellite polymorphism and genetic relationship in dog breeds in Korea. Asian-Aust. J. Anim. Sci. 18(8):1071-1074.

Felsestien, J. 1993. PHYLIP: A software package http://evolution. gs.washington.edu/phylip.html

Fan, B., Y. Z. Chen, C. Moran, S. H. Zhao, B. Liu, M. Yu, M. J. Zhu, T. A. Xiong and K. Li. 2005. Individual-breed assignment analysis in swine populations by using microsatellite markers. Asian-Aust. J. Anim. Sci. 18(11):1529-1534.

Goldstein, D. B., A. R. Linares, L. L. Cavalli-Sforza and H. W. Feldman. 1995. An Evaluation of genetic distance for use with microsatellite loci. Genet. 139:463-471.

Goldstein, D. B., L. A. Zhivotovsky, K. Nayar, A. RuizLinares, L. L. Cavalli-Sforza and H. W. Feldman. 1996. Statistical properties of the variation at linked microsatellite loci-implications for the history of human Y chromosome. Mol. Biol. Evol. 13:1213-1218.

Jame, P. and P. J. L. Lagoda. 1996. Microsatellite from molecules to population and back. Trends Ecol. Evol. 11:424-430.

Lewis, P. O. and D. Zaykin. 2002. GDA software available from http://lewis.eeb.uconn.edu/lewishome

Minch, E., A. Ruiz-Linares, D. Goldstein, M. Feldman and L. L. Cavalli-Sforza. 1996. Microsat 1.4d: A computer programme for calculating various statistics on microsatellite allele data. http://Lotka.stanford.edu/microsat/microsat.html

Navani, N., P. K. Jain, S. Gupta, B. S. Sisodia and S. Kumar. 2002. A set of cattle microsatellite DNA markers for genome analysis of riverine buffalo (*Bubalus bubalis*). Anim. Genet. 30:149-154.

Nei, M. 1972. Genetic distance between populations. Am. Naturalist 106:283-92.

Nei, M., F. Tajima and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol. 19:153-170.

Ota, T. 1993. DISPAN: Genetic distance and Phylogenetic analysis software. http://mep.bio.psu.edu/readme.html

Primmer, C. R., H. Ellegren, N. Sanio and A. P. Moller. 1996. Directional evolution in germline microsatellite mutations. Nat. Genet. 13:391-393.

Pundir, R. K., R. V. Singh, P. K. Vij, R. K. Vijh and A. E. Nivsarkar. 1997. Characterization of Bhadawari buffaloes. NBAGR Research Bulletin No. 7, NBAGR, Karnal, India.

Raymond, M. and F. Rousset. 2003. GENEPOP: A web software http://wbiomed.curtin.edu.au/genepop/

Robertson, A. and W. G. Hill. 1984. Deviation from Hardy Weinberg proportions; sampling variances and use in estimation of inbreeding coefficients. Genet. 107:713-718.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual 2nd Ed, Cold spring Harbour, Cold spring Laboratory Press, NY.

Sethi, R. K. 2001. Buffalo breeding in India. 2nd edn, Dairy Year Book, All India Dairy Business Directory, Sadana Publishers and Distributors, India, pp. 264-70.

Weir, B. S. and C. C. Cockerham. 1984. Estimating F statistics for the analysis of population structure. Evol. 38:1358-1370.

Vijh, R. K., B. Mishra, R. Arora, P. Chaudhary, U. Sharma and M. S. Tantia. 2005. Comparative evaluation of three buffalo populations using microsatellite markers. Ind. J. Anim. Sci. (in press).

Yeh, F. C., T. Boyle, Y. Rongcai, Z. Ye and J. M. Xian. 1999. POPGENE version 3.1 (http://www.ualberta.ca/-fyeh/fyeh).