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Original Article

Comparative evaluation of Murrah breeds with buffaloes of Indo-Gangetic Plains

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Abstract

The present study was carried out in order to investigate the population genetic parameters for the buffalo of Uttar Pradesh and its relationship/differentiation with Murrah buffalo using 25 microsatellite markers. The mean observed and expected heterozygosity was found to be 0.597 and 0.703 respectively in UP buffaloes while it was 0.631 and 0.642 respectively in Murrah buffaloes. The mean estimates of F statistics were over all loci 0.139 (FIS), 0.092 (FST) and 0.219 (FIT). The values were significantly different from zero for all the three measures and point towards the existence of population structure and moderate differentiation (9.2%) in two buffalo populations. The analysis of molecular variance revealed 9.38% of the variation attributed to among populations and 78.8% within populations. The phylogenetic and correspondence analysis revealed that individuals of the two populations formed distinct clusters. Among UP buffaloes Bhadawari breed and buffaloes of Tarai regions of Uttar Pradesh clustered into distinct groups. Bayesian analysis also revealed similar type of genetic structure. The genetic distinctness of these two buffalo populations as revealed by microsatellite analysis may have significant impact development of breeding plans and improvement of buffaloes.

Keywords Buffalo, Microsatellite markers, Genetic diversity, FST, Correspondence analysis.

Introduction

In India, buffaloes play pivotal role in livestock production through contributions in terms of milk, meat and draft power for agricultural operations. India inhabits one of the best riverine breeds of buffalo in the world. There are 13 well defined registered buffalo breeds, of which Murrah, Nili-Ravi, Mehsana and Jaffarabadi are notable ones having good potential for milk production. The Indo-Gangetic plains of India are fertile basin and have more than 26% buffaloes of India [20]. The Indo- Gangetic plains have only one defined breed of buffalo named Bhadawari and rest other buffaloes are considered as Murrah type/grade undefined populations. The genetic structure of this vast buffalo population was not known.

Microsatellite markers, also known as simple sequence repeats (SSRs) or short tandem repeats (STRs), are regions of DNA that exhibit

short repetitive sequence motifs. Because of their high degree of polymorphism, random distribution across the genotypes, microsatellite markers have been proved to be one of the most powerful tools for evaluating genetic diversity and estimating genetic distances among related populations of ruminant species [31, 9, and 11]. Microsatellite markers have been used to analyze the genetic variation in cattle, sheep, pig, goats, buffaloes, chickens, horses and human beings [5, 18, 3, 4, 29, 1 and 22].

No systematic characterization of UP buffaloes has been done in the past. It is necessary to characterize and evaluate the genetic diversity of UP buffaloes. Therefore, the present study was undertaken with the objectives of evaluating the genetic diversity within UP buffalo population and to estimate the genetic relationship/differentiation with that of Murrah breed as most of the UP buffaloes are known as Murrah type/grade [28]. In this paper, we carried out the microsatellite analysis to find out whether the UP buffaloes commonly termed as Murrah grade/Murrah type are genetically similar to Murrah or have a different population structure.

Materials and Methods

A total of 573 blood samples (525 from different districts of Uttar Pradesh and 48 from Murrah buffalo) were collected from their respective breeding tract. Blood samples collected from the buffaloes were placed into an EDTA tube for DNA isolation. Genomic DNA was extracted from 10 ml blood samples using the standard phenol chloroform method [13].

A set of 25 microsatellite loci were selected from cattle genome database (Table 1). The Polymerase chain reaction (PCR) conditions were standardized for all of the 25 primer pairs selected for the study. PCR amplification was carried out in a 15µL reaction containing 50 ng of genomic DNA, 200µM dNTP, 5 pmol each of forward (labelled) and reverse primers, 1 U of Taq DNA polymerase, and 1X reaction buffer (containing 1.5mM $MgCl_2$). Amplification was carried out by using an Eppendrof ProS instrument with initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 45 s, 55°C (annealing temperature) for 45 s, and extension at 72°C for 1 min final cycle was followed by an extension step at 72°C for 10 min. The PCR products were visualized on 2% agarose gels with 1X Tris-acetate-EDTA buffer containing 200 ng/mL of ethidium bromide. Genotyping was carried out on an ABI 3130xL Genetic Analyzer, with LIZ 500 (Applied Biosystems) as the internal lane standard (size standard). Post-PCR multiplexing was used to simultaneously genotype 4 or 5 loci, depending on the PCR product size and dye label of the primers used. Sizing and allele calling were performed by using Genemapper version 3.0 software (Applied Biosystems). The allele data thus generated were used for further statistical analyses.

Table 1 Microsatellite markers used for molecular genetic studies in two buffalo populations

S.No	Markers Name	Primers sequence (5'→3')	Allele size Range (bp)	Chromosome	Dye
1.	BMS2722	F:TAGAGCCCACGTGAAAAATG R: GCAGGCTTTCACAAATACACA	105-113	XY	PET
2.	BMS2785	F:ACAAACCTGTGCGCCTTG	96-118	18	VIC

		R: GGCAATCAGTCGGACACAC			
3.	CSSM08	F: CTTGGTGTTACTAGCCCTGGG	179-199	-	PET
		R: GATATATTTGCCAGAGATTCTGCA			
4.	CSSM19	F: TTGTCAGCAACTTCTTGTATCTTT	125-159	1	PET
		R: TGTTTTAAGCCACCCAATTATTTG			
5.	CSSM47	F:TCTCTGTCTCTATCACTATATGGC	109-167	8	NED
		R: CTGGGCACCTGAAACTATCATCAT			
6.	ILSTS05	F:GGAAGCAATGAAATCTATAGCC	162-198	10	VIC
		R: TGTTCTGTGAGTTTGTAAGC			
7.	ILSTS11	F: GCTTGCTACATGGAAAGTGC	244-292	14	VIC
		R: CTAAAATGCAGAGCCCTACC			
8.	ILSTS49	F: CAATTTTCTTGTCTCTCCCC	132-192	11	FAM
		R: GCTGAATCTTGTCAAACAGG			
9.	RM232	F:ATCTGTCCCTCAACTGGGTG	111-129	4	FAM
		R: GTCACAAGGGGGTCCATG			
10.	BL1071	F:AGAAGGACAGAGACCACAGGC	170-210	13	HEX
		R: TTGAGGTGAAGAGGTCCACC			
11.	BM6548	F: CGTTTTGGGAGTTTTCTTATGG	225-251	13	FAM
		R: TAGCATGCACACATACACGC			
12.	BMS2819	F: GCTCACAGGTTCTGAGGACTC	81-123	9	HEX
		R: AACTTGAAGAAGGAATGCTGAG			
13.	DIK4861	F: TTAGGTCTGTGAGCCCCATC	195-219	18	FAM
		R: GAGGGCCTTGACAAAGAGTG			
14.	DIK545	F: ATTTTCCCCAGGTTGAGGAG	114-174	5	FAM
		R: GGTTAGGATTGGGAGGGAAT			
15.	AGLA226	F: CCAGCACCATGGAAATTCTCAGTC	134-154	12	FAM
		R: CTTAACAAGCCATGCTGAATGGTCT			
16.	BM6507	F: ACTTAGCACAATGCCCTCTAGG	139-165	18	HEX
		R: ATGTTATTCCATCAGGAGGAGC			
17.	BMS1580	F: GGTACCATGTGCAAGCAGG	110-128	13	FAM
		R: CCATCCCATTAGCTGTAAGAGG			
18.	DIK2866	F: GAAACTTTTGGGCAAATGGA	179-217	10	FAM
		R: GCTATCTTCCCTCCCAGCTT			
19.	DIK4241	F: CAAGCGCTCAGAGACCAAAC	154-170	18	FAM
		R: TGCTCTAAAGCTTGAAGGGTTATT			
20.	BM875	F: ACCTATCTCATTTGGCTTCTGG	97-117	10	FAM
		R: AAAAAACCCCAAACAACAACC			
21.	BMS2658	F: TCCCTGGACTTCTTGCAGAG	117-139	28	FAM
		R: CTGGCCCCAGACACAATC			
22.	CA002	F: AGCAATGTCAAACATTGCTCC	96-114	23	HEX
		R: TCCATCTTTACAACTTTTGAATC			
23.	DIK2325	F: TCAATGCAAAAACCATCGTG	186-246	13	HEX
		R: CTTGAGGCGCTTAAAAGACA			
24.	DIK4609	F: ACAAAAATAGCCTGGGGACA	161-185	5	HEX
		R: ACCAGAGCTGGTTGTGGAAG			
25.	DIK5212	F: CCCTTCACACTCATGCACAC	160-194	5	HEX
		R: ACCAGAGCTGGTTGTGGAAG			

For 25 microsatellite loci analyzed, observed and effective numbers of alleles, observed and expected heterozygosity were calculated using POPGENE software version 1.32 [10]. The F-statsticts values FIS, FIT and FST were estimated using Jack-Knifing over loci

using FSTAT software [12]. Analysis of molecular variance was carried out using the software Arlequin version 3.5 [19].

The Hardy-Weinberg equilibria (HWE) of the loci in the two 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 [21]. The software performed a probability test utilizing Markovs' chain (dememorization 10,000, batches 100 and iterations per batch 10,000). Significance levels were calculated per locus, per population and over all loci and all populations combined. Weir and Cockerham (1984) and Roberston and Hill (1984) estimates of FIS [8, 2] were calculated.

Correspondence analysis was performed using GENETIX v4.05 software [16] for multivariate analysis. Inter-individual distance based on Nei's DA and Allele sharing distance (DAS) were estimated using Population 1.2.30 [24] software and were utilised for the construction of Neighbor joining tree [23]. The Inter-individual genetic distances were visualised using MEGA Ver 5.10 software [17].

Bayesian analysis was used to infer the populations structure which simultaneously assigns individuals to the populations of their origin was implemented using STRUCTURE software [14]. This software generates clusters of individuals based on their multilocus genotypes. We used an admixture model with a burn-in period of 40,000 iterations and 100,000 Markov chain Monte Carlo (MCMC) replications to calculate the probable number of genetic clusters (K). The allele frequencies were taken as correlated.

Results

A total of 573 animals representing UP and Murrah buffaloes were analysed using 25 microsatellite markers. The observed and effective numbers of alleles at different loci investigated are presented in Table 2. All the loci were amplified successfully and exhibited substantial genetic diversity.

Table 2 Observed number of allele (Na) and effective number of allele (Ne) and summary of heterozygosity of two buffalo populations at 25 microsatellite loci

	UP buffalo				Murrah buffalo			
Locus	**Na**	**Ne**	**Obs_Het**	**Exp_Het**	**Na**	**Ne**	**Obs_Het**	**Exp_Het**
BMS2722	5	3.095	0.5441	0.6776	4	2.712	0.3542	0.6379
BMS2785	11	3.603	0.3424	0.7232	8	3.051	0.5208	0.6794
CSSM08	11	3.788	0.6667	0.7367	7	3.373	0.7083	0.7110
CSSM19	18	5.189	0.7332	0.8081	9	4.81	0.8333	0.8007
CSSM47	26	10.621	0.8267	0.9067	11	4.114	0.8958	0.7649
ILSTS05	11	3.763	0.6610	0.7350	4	1.936	0.5417	0.4888
ILSTS11	16	4.342	0.5143	0.7705	9	1.965	0.5625	0.4963
ILSTS49	11	1.859	0.3524	0.4627	3	1.501	0.2917	0.3375
RM232	9	2.582	0.3750	0.6134	2	1.652	0.0417	0.3991
BL1071	13	3.681	0.8805	0.7291	6	2.591	0.9375	0.6206
BM6548	14	4.626	0.7797	0.7846	10	6.847	0.9167	0.8629
BMS2819	13	3.516	0.6138	0.7163	13	3.952	0.7708	0.7548
DIK4861	11	2.340	0.4762	0.5733	4	2.436	0.2708	0.5958
DIK545	24	3.218	0.9561	0.6899	9	1.916	0.5417	0.4831

AGLA226	10	4.000	0.7124	0.7508	8	3.642	0.7708	0.7331
BM6507	13	2.703	0.4393	0.6307	6	2.860	0.6250	0.6572
BMS1580	10	3.436	0.5296	0.7097	7	2.451	0.5208	0.5982
DIK2866	17	4.248	0.7176	0.7653	7	3.480	0.6667	0.7202
DIK4241	9	5.584	0.4980	0.8218	9	4.343	0.5417	0.7779
BM875	11	3.102	0.6371	0.6783	6	2.032	0.4583	0.5134
BMS2658	8	2.309	0.2515	0.5676	4	3.090	0.8542	0.6836
CA002	10	5.954	0.9222	0.8329	6	3.958	0.9792	0.7553
DIK2325	20	5.445	0.7318	0.8172	12	5.165	0.8750	0.8149
DIK4609	11	3.423	0.5864	0.7086	7	3.945	0.8125	0.7544
DIK5212	13	1.572	0.2008	0.3642	9	1.734	0.5000	0.4281
Mean	13.000	3.920	0.5979	0.703	7.200	3.182	0.6317	0.6428
St. Dev	4.8563	1.795	0.2032	0.119	2.7988	1.280	0.2385	0.1424

The mean number of alleles in UP and Murrah buffaloes were 13.00±4.85 and 7.20±2.80 and effective number of alleles were 3.92 and 3.18 alleles respectively (Table 2). The value of the Shannon information index (I) in UP and Murrah buffaloes were 1.576 and 1.315 which is a measure of genetic diversity. Observed heterozygostity varied between 0.200 (DIK5212) to 0.9561 (DIK545) in UP buffalo and 0.0417 (RM232) to 0.9792 (CA002) in Murrah buffalo (Table 2). The mean expected heterozygostity was 0.703 and 0.642 in UP and Murrah buffaloes respectively. At all loci, the observed heterozygosity were significantly lower than the expected heterozygosity pointing towards the existence of population structure. The number of alleles (Na) and effective number of alleles (Ne) are presented graphically in Fig. 1.

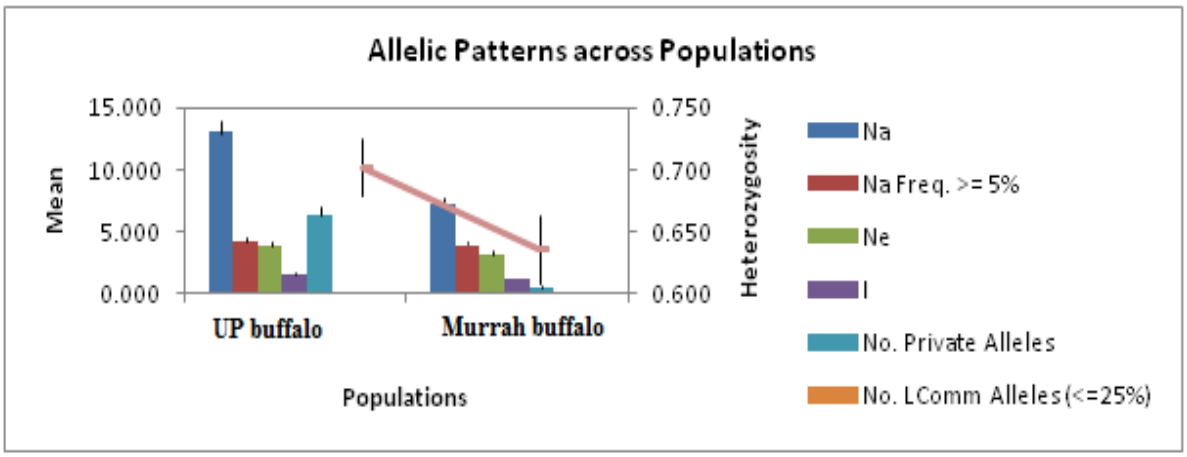


Fig. 1 Allelic patterns across populations

The hierarchical analysis of molecular variance revealed 9.38% of variance explained by between buffalo populations while 11.8% of the variation was explained by differences between individuals within each population (Table 3). In this study, 23 loci in UP buffalo

were in HW equilibrium while most of the loci in Murrah buffalo showed significant deviations from HWE (Table 4). The reasons for the deviaition from HWE in Murrah buffalo may be attributed due to the presence of null alleles, small sample size and Wahlund effect [15].

Table 3 Analysis of Molecular Variance in two buffalo populations

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Among populations	1	165.448	0.88548 Va	9.38%
Among individuals within populations	571	5525.543	1.11727 Vb	11.83%
Within individuals	573	4264.500	7.44241 Vc	78.80%
Total	1145	9955.491	9.44517	-

Table 4 FIS values for microsatellite loci in two buffalo populations studied

	UP buffalo			Murrah buffalo		
Locus	P-val	W&C[1]	R&H[2]	P-val	W&C[1]	R&H[2]
BMS2722	0.0000*	0.1973	0.3271	0.0000*	0.4474	0.6153
BMS2785	0.0000*	0.5268	0.2809	0.0044*	0.2353	0.1747
CSSM08	0.0017*	0.0952	0.0622	0.5790	0.0037	-0.0115
CSSM19	0.0000*	0.0927	0.1904	0.0163*	-0.0413	0.1131
CSSM47	0.0000*	0.0884	0.0822	0.9926	-0.1733	-0.0576
ILSTS05	0.0049*	0.1008	0.0636	0.8133	-0.1094	-0.0363
ILSTS11	0.0000*	0.3327	0.1389	0.9641	-0.1351	-0.0353
ILSTS49	0.0000*	0.2386	0.1222	0.2482	0.1370	0.0771
RM232	0.0000*	0.3889	0.2619	0.0000*	0.8966	0.9147
BL1071	0.0011*	-0.2080	0.0591	0.2519	-0.5189	0.0192
BM6548	0.0007*	0.0062	0.0612	0.6661	-0.0630	-0.022
BMS2819	0.0000*	0.1433	0.1204	0.6080	-0.0214	-0.0099
DIK4861	0.0002*	0.1695	0.0735	0.0019*	0.5481	0.3090
DIK545	0.9237	-0.3863	-0.0096	0.9738	-0.1226	-0.0339
AGLA226	0.0000*	0.0512	0.1184	0.7762	-0.0520	-0.0257
BM6507	0.0000*	0.3037	0.1881	0.7244	0.0495	-0.0282
BMS1580	0.0000*	0.2539	0.1035	0.0312*	0.1306	0.1001
DIK2866	0.0115*	0.0624	0.0356	0.0647	0.0705	0.0666
DIK4241	0.0000*	0.3942	0.3249	0.0012*	0.3059	0.1955
BM875	0.0296*	0.0608	0.0306	0.1536	0.1082	0.0526
BMS2658	0.0015*	0.5572	0.0958	0.9744	-0.2529	-0.1385
CA002	0.5697	-0.1074	-0.0032	1.0000	-0.3006	-0.1604
DIK2325	0.0000*	0.1046	0.0914	0.0148*	-0.0746	0.0974
DIK4609	0.0000*	0.1725	0.1670	0.1667	-0.0779	0.0523
DIK5212	0.0001*	0.4490	0.1114	1.0000	-0.1701	-0.0315
Total	-	0.150	-	-	0.017	-

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

The results of global analysis for genetic differentiation among UP and Murrah buffaloes are presented in Table 5. The mean estimates of F-statistics over all the loci were 0.1393 for FIS (Within population inbreeding estimate), 0.0926 for FST (estimate for population differentiation) and 0.219 for FIT (total inbreeding estimate). An overall heterozygosity deficiency of 13.9% and a mean FST of 9.2% were observed between the two buffalo populations. The mean estimate of gene flow (Nm) was found to be 4.53 indicating large amount of gene flow between the populations.

Table 5 Global F-Statistics and gene flow (Nm) for each of 25 microsatellite loci analyzed across two buffalo populations

Locus	FIS	FST	FIT	Nm
BMS2722	0.2172	0.2575	0.4187	1.3710
BMS2785	0.5034	0.2636	0.6343	1.3212
CSSM08	0.0878	-0.0031	0.085	157.2643
CSSM19	0.0816	0.0193	0.0994	19.1407
CSSM47	0.0699	0.1135	0.1755	3.4615
ILSTS05	0.0892	0.0695	0.1525	5.2650
ILSTS11	0.3071	0.2558	0.4843	1.2001
ILSTS49	0.2325	0.0043	0.2358	38.9291
RM232	0.4180	0.0561	0.4506	6.2662
BL1071	-0.2302	0.2412	0.0665	1.4471
BM6548	-0.0001	0.0104	0.0103	31.9266
BMS2819	0.1288	0.1544	0.2634	2.6870
DIK4861	0.2023	0.1554	0.3262	2.6473
DIK545	-0.3705	0.1387	-0.1804	2.6377
AGLA226	0.0428	-0.0037	0.0392	222.2132
BM6507	0.2814	0.1135	0.363	3.7368
BMS1580	0.2452	0.0452	0.2793	8.5277
DIK2866	0.0635	0.0000	0.0635	79.8715
DIK4241	0.3869	0.0310	0.4059	12.816
BM875	0.0641	0.0203	0.0831	16.6683
BMS2658	0.4762	0.1021	0.5297	4.4053
CA002	-0.1222	0.0138	-0.1067	25.1798
DIK2325	0.0893	0.0017	0.0908	62.7879
DIK4609	0.1501	0.0025	0.1522	56.2579
DIK5212	0.3885	-0.0040	0.3861	136.1823
All	0.1393	0.0926	0.2190	4.5349

The correspondence analysis was carried out using allele frequencies at 25 loci to infer the relationship among individuals (Fig. 2) of the two populations. The analysis indicated that all individuals of Murrah buffaloes clustered together while two clusters one each for individuals of Bhadawari and Tarai region buffaloes were found and there was large admixture of Western and Central UP buffaloes.

In this study, Bhadawari region buffaloes clustered near to Murrah. This can be attributed to use of Murrah semen under various buffalo improvement programs in the Bhadawari tract.

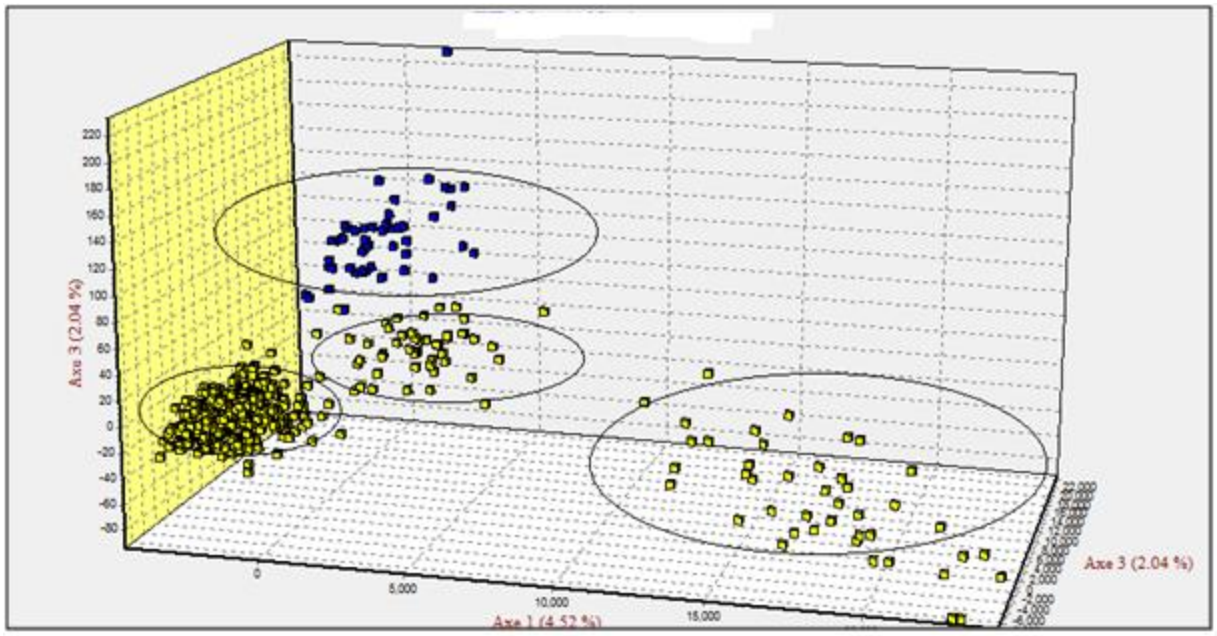
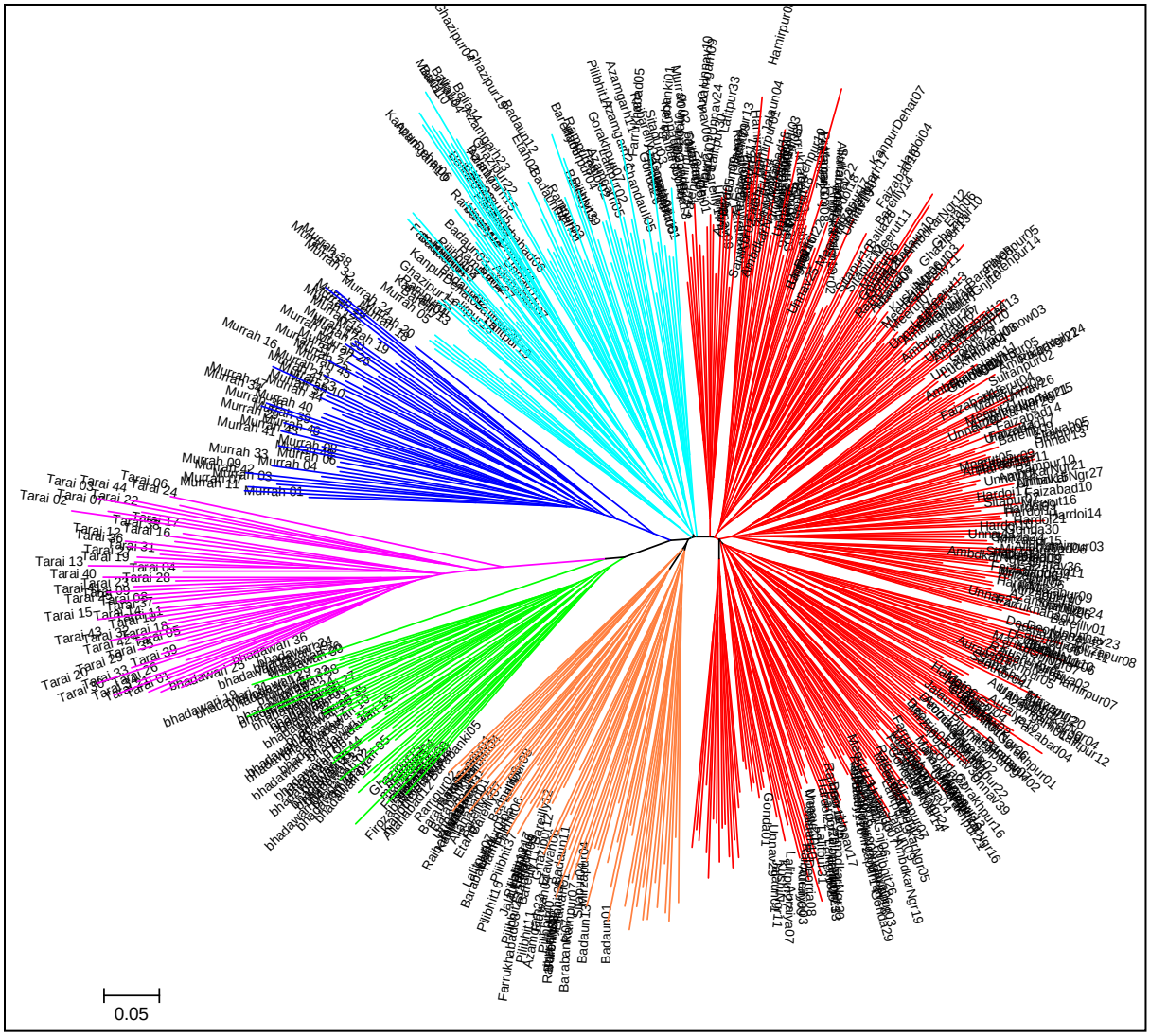


Fig. 2 Correspondence analysis of two buffalo populations with independent colour code; Yellow denotes- UP buffalo and Blue denotes- Murrah buffalo.

The phylogenetic analysis based on Allele sharing (DAS) distance and Nei's DA genetic distance also revealed distinct clustering of individuals of UP and Murrah buffaloes while buffaloes of Tarai and Bhadawari regions of Uttar Pradesh clustered as one group. The phylogenetic tree based on allele sharing distance has been depicted in Fig. 3.

Fig. 3 Inter-individual radiation tree based on allele sharing genetic distance (DAS) using NJ method



Further, to confirm the genetic distinctiveness of these two populations, Bayesian cluster analysis was performed with 5 different runs from K = 2 to K = 6 to assign the individuals to different clusters using STRUCTURE software. The program uses the Markov Chain Monte Carlo method to estimate the natural logarithm of the probability that a given genotype X is part of a given population K

(ln Pr (X|K)). The analysis was carried out to identify the most likely number of clusters present in the dataset.

It revealed that all individuals of Murrah buffaloes clustered themselves separately at K=6 while individuals of UP buffalo sub-structured into 5 clusters. In the present dataset, the buffaloes of Tarai hill and bhadawari region (border areas of UP and Madhya Pradesh (MP)) are different from the rest of the UP buffaloes. There was admixture between the Western and Central buffaloes. This shows continuity between the two populations. Buffaloes of Mau, Ballia and Ghazipur districts of Eastern UP showed distinctive cluster (Fig. 4).

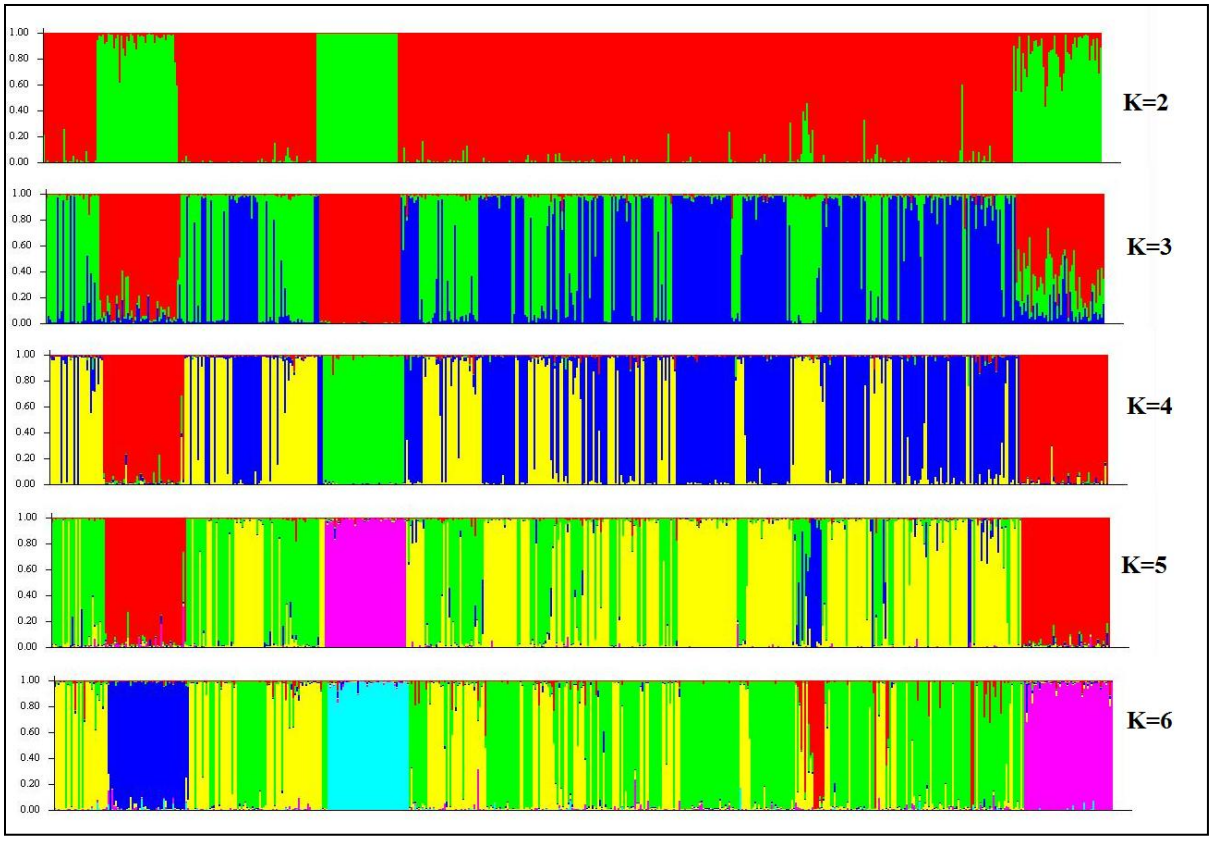


Fig. 4 Bar representation of the STRUCTURE analysis of UP and Murrah buffaloes (K=2 to K=6)

Discussion

Our study presents the first comprehensive genetic analysis using microsatellite markers and provides information on UP buffaloes usually described as Murrah type/graded Murrah buffaloes.

The mean number of alleles in these two populations was higher indicating more genetic diversity than reported in Marathwada (4.5) buffaloes [25] and Tarai (4.2) and Bhadawari (4.0) buffaloes [27]. The observed heterozygosity is lower than the expected heterozygosity pointing towards the existence of population structure. The mean observed heterozygosity for UP (0.597) and Murrah (0.631) were comparatively higher than that reported in other Indian breeds of buffaloes [25, 7] and but lower than that reported for Bhadawari and Murrah buffaloes [30].

Deviation from HWE had also been reported in Banni and Murrah buffaloes [6]. Considerable level of heterozygote deficiency was found to exist in both Banni and Murrah buffalo populations with a global deficit of 23.2%. This has resulted in the significant deviations from Hardy- Weinberg equilibrium at many of the loci. This can be attributed to selection and use of sires utilising artificial insemination.

The mean values of FIS, FST and FIT from Jackknifing over loci were 0.1393, 0.0926 and 0.2190, respectively. An overall heterozygosity deficiency of 13.9% for UP and Murrah buffaloes are comparatively lower than that reported in other Indian water buffaloes [6, 26]. Estimates of genetic differentiation (mean FST value) indicate 9.2% of the variation attributed

to between populations. This is higher than the range of values (0.75% to 6%) reported among other Indian buffalo breeds [30].

The Bayesian analysis and inter-individual genetic distance estimates based on different methods revealed existence of five population substructure of buffaloes belonging to Uttar Pradesh and confirmed the distinctness of UP and Murrah buffaloes. The Neighbor-Joining tree constructed from allele sharing distance measure among individual animals also revealed six clusters.

Conclusion

There is substantial genetic variation and polymorphism across studied microsatellite loci in the UP buffaloes. Multivariate analysis and Neighbor Joining tree constructed among individual animals revealed distinct clustering of these two buffalo populations. The UP buffaloes further had 5 distinct clusters. Bayesian analysis result revealed clear membership of UP buffalo into five clusters indicating a genetic subdivision within the UP buffalo population. The genetic distinctness of these two buffalo populations as revealed by microsatellite analysis may have significant impact on development of breeding plans for improvement of buffaloes.

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