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Short communication

Microsatellite based genetic structuring reveals unique identity of Banni among river buffaloes of Western India

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ABSTRACT

Genetic structure of Banni buffaloes vis-à-vis four other river buffalo breeds of Western India was assessed using short tandem repeat markers. The total number of alleles varied between 115 and 127 across five buffalo breeds. The mean estimates of global *F*-statistics over all loci were 0.330 for F_{IT}, 0.145 for F_{ST} and 0.216 for F_{IS} respectively. Analysis of molecular variance revealed that 14.33% of the total genetic variation was being explained by between breed differences. The phylogenetic tree constructed using chord distance estimates revealed the distinctness of Banni and Jaffarabadi buffaloes from other river buffalo breeds of the region. Multi dimensional scaling display of pair-wise F_{ST} values revealed the close proximity of Mehsana, Surti and Murrah buffaloes while Banni and Jaffarabadi buffaloes were placed separately. The results of principal components analysis based on pair-wise chord distance estimates between individual animals were consistent with the observation based on multidimensional scaling (MDS) analysis. This genetic structure was further supported by Bayesian clustering analysis which revealed three inferred clusters with Banni and Jaffarabadi forming separate clusters each while the remaining three breeds viz. Mehsana, Surti and Murrah together formed a single cluster. The results of the present study thus revealed the genetic uniqueness of Banni buffalo among other buffalo breeds of the region.

1. Introduction

River buffaloes (*Bubalus bubalis*) are the mainstay of Indian dairy industry playing a major role, by contributing approx. 56% of total milk production. Among the 10 well defined river buffalo breeds of India (Nivsarkar et al., 2000), buffaloes especially from Northern and Western parts of the country have been traditionally evolved for high milk production. Among the three well defined breeds of Western India, Jaffarabadi is the heaviest of all the Indian breeds of buffalo with good genetic potential for high milk and fat production (Cockrill, 1974). Surti buffalo is a lightly built animal which consumes less feed and thrives well under extensive system of management (Nivsarkar et al., 2000), while Mehsana is presumed to be a breed developed by crossbreeding Murrah and Surti buffaloes (Olver, 1938). These buffaloes are well reputed for regularity in breeding and persistency in milk production (Singh, 1992). Apart from these three breeds, buffaloes in the Banni region (Kachchh district of Gujarat state) are good producers of milk with mean peak yield as high as 15.7 ± 0.1 l/day (Mishra et al., 2008) and their production potential is comparable to Murrah (Sadana et al., 2006). Banni buffaloes were evolved by "Maldharis" a traditional livestock rearing community of the region whose livelihood largely depends on these animals.

Increased interests in sustainable livestock production systems may cause preferential shift from improved breeds to adapted breeds that are more biologically fit in low input systems and harsh environment (FAO, 1999; Drucker et al., 2001). The importance of such adapted breeds increases when they have good potential for certain production traits. Banni buffaloes are important in this respect as they are adapted to

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harsh dry conditions of the Kachchh region and are primarily maintained under extensive management system. The genetic structure analysis and resolving the genetic relationship of domesticated livestock is a complex process, because of continuous gene flow among different breeds. However, short tandem repeat (microsatellite) markers have been successfully used in many livestock species to unravel the genetic differentiation among different breeds/populations (Kumar et al., 2006; MacNeil et al., 2007). In case of riverine buffaloes of Western India, the genetic relationship between Jaffarabadi, Surti and Mehsana has been reported earlier by Kumar et al. (2006) and Vijh et al. (2008). However, Banni buffalo, an important breed of Western India with good potential for milk production was not included in these studies. We earlier reported the physical and morphometric characteristics, management practices, production performance and basic diversity indices of Banni buffalo (Mishra et al., 2008). Further, microsatellite analysis of Banni and Murrah buffaloes indicated genetic differentiation, which were earlier presumed to be related due to their resemblance in morphology and production potential (Mishra et al., 2009). However, the genetic structure of Banni buffaloes vis-à-vis other buffalo breeds of the region is poorly understood. In this study, we have attempted to quantify genetic relationship of Banni with other riverine breeds of the region using heterologus bovine microsatellite markers.

2. Materials and methods

2.1. Blood sample collection and DNA extraction

Blood samples were collected from jugular vein of 230 animals from five different buffalo breeds. The number of individuals sampled included 47 from each of the Banni, Surti and Mehsana, 41 from Jaffarabadi and 48 from Murrah. Although no parentage records were available in the field, to ensure unrelatedness, animals were selected from different villages after interviewing the farmers in detail. Genomic DNA was isolated from blood samples as described by Sambrook and Russell (2001).

2.2. Microsatellite genotyping

A total of 24 bovine specific microsatellite markers were chosen for the present study. All these markers were selected based on their reported level of polymorphism in terms of heterozygosity and allele numbers (Kathiravan et al., 2009). The PCR amplification was carried out in 25 µl reaction volume containing 1.5 mM $MgCl_2$, 200 µM dNTPs, 50 ng of each of forward and reverse primer, ~100 ng of genomic DNA and 0.5 U of Taq DNA polymerase (Bangalore Genei, India). PCR was carried out in PTC — 200 Thermal cycler (MJ Research, Inc, MA, USA) using cycling conditions: 2 min at 94 °C followed by 30 cycles of 1 min at 94 °C, 1 min at precise annealing temperature, 1 min at 72 °C and for 10 min at 72 °C. The PCR products were resolved on 6% denaturing polyacrylamide gels (Sequi GT system, Bio-Rad, USA) and sized using a 10 bp ladder (Invitrogen, Life Technologies, CA, USA). In order to compare the allele size across different breeds, comparative analysis of samples containing different alleles was performed for each of the investigated loci. Gels were stained by silver staining (Bassam et al., 1991) and genotypes scored manually.

2.3. Statistical analysis

Various diversity indices like mean observed and effective number of alleles per locus, mean observed and expected heterozygosity were computed using POPGENE software package (Yeh et al., 1999). The global *F*-statistics (Weir and Cockerham, 1984), heterozygosity deficiency and test for linkage disequilibrium were performed using FSTAT version 2.9.3.2 (Goudet, 2002). Gene flow, defined as the number of reproductively successful migrants among populations was calculated from Nei's coefficient of gene differentiation as described in Slatkin and Barton (1989). Analysis of molecular variance was performed using ARLEQUIN version 3.0 (Excoffier et al., 2005). The relationship among different breeds of buffaloes was analyzed using two different approaches. Firstly, genetic divergence between the breeds was estimated according to Cavalli-Sforza and Edwards (1967) using MICROSATELLITE ANALYZER version 3.15 (Dieringer and Schlotterer, 2003). Pair-wise chord distances among breeds were utilized to derive dendrogram and radiation tree respectively using PHYLIP version 3.5 (Felsenstein, 1993) and the tree was visualized using TREEVIEW version 1.6.6 (Page 1996). Bootstrap resampling ($n = 10{,}000$) was performed to test the robustness of the topologies.

Secondly, the geometric relationship between different buffalo breeds was examined using two ordination techniques viz. multidimensional scaling and principal components analysis. Pair-wise F_{ST} values between all possible breed pairs were displayed by multidimensional scaling (MDS) using SPSS version 10.5. Pair-wise chord distance measures between individual animals were utilized to perform principal components analysis using SPSS version 10.5. Breed differentiation was further investigated using Bayesian clustering approach as implemented in STRUCTURE program (Pritchard et al., 2000). Individual animals were assigned to different clusters based on their multilocus genotypes. Admixture model was used with a burn in period of 1,000,000 iterations and 100,000 Markov Chain Monte Carlo (MCMC) repetitions to calculate the probable number of genetic clusters.

3. Results and discussion

In the present study, allelic and genotypic data of 230 animals at 24 different microsatellite loci were investigated for a comprehensive genetic analysis of Banni buffaloes vis-à-vis other four buffalo breeds of the region. The total observed number of alleles per breed varied between 115 (Surti) to 127 (Banni) with an overall mean of 122 alleles per breed. The mean observed number of alleles per locus per breed varied between 4.79 (Surti) to 5.29 (Banni) while the mean effective number of alleles per locus per breed varied between 2.64 (Jaffarabadi) and 3.08 (Banni). The mean observed and expected heterozygosity per locus varied between 0.441 (Banni) to 0.541 (Surti) and 0.572 (Banni) to 0.659 (Mehsana) respectively. Allelic diversity and heterozygosity in different buffalo breeds investigated in the present study were lower than that reported by Kumar et al. (2006), although using a different set of microsatellite markers. The pair-wise F_{ST} estimates among different buffalo breeds are presented in Table 1, and the estimates of global *F*-statistics are presented in Table 2. The pair-wise F_{ST} values ranged between 0.101 (Surti-Mehsana) to

Table 1
F_{ST} estimates and Cavalli-Sforza and Edwards chord distances between each pair of five Indian buffalo breeds.

	Banni	Surti	Jaffarabadi	Mehsana	Murrah
Banni	0	0.138	0.155	0.167	0.189
Surti	0.368	0	0.115	0.101	0.128
Jaffarabadi	0.379	0.368	0	0.180	0.185
Mehsana	0.365	0.342	0.435	0	0.107
Murrah	0.372	0.358	0.409	0.310	0

F_{ST} — above diagonal values; Cavalli-Sforza and Edwards chord distances — below diagonal values.

0.189 (Banni-Murrah). The global *F*-statistics revealed the mean F_{ST} overall loci as 0.145, while the global heterozygosity deficit was found to be 0.216. Estimation of genetic sub-division showed that the average proportion of genetic differentiation among breeds as 14.3% ($P<0.01$). This is higher than that of reported for eight Indian buffalo breeds by Kumar et al. (2006), while it is comparable to that reported for two North Indian buffalo breeds viz. Tarai and Bhadawari (Arora et al., 2004). The gene flow between different pairs of buffalo populations varied between 2.02 (Banni-Murrah) and 3.96 (Surti-Mehsana) while the global gene flow across different breeds overall loci was found to be 1.674. The estimates of gene flow between Banni and other buffalo breeds were found to be lowest among all the pair-wise estimates for different buffaloes of the region. Similarly the estimates for Jaffarabadi were also found to be less except with that of Surti. These estimates of gene flow give indications regarding the geographic and reproductive isolation of Banni and Jaffarabadi buffaloes. However, these indirect estimates of gene flow need to be looked cautiously as they have many underlying assumptions which may not be realistic in actual populations (Whitlock and Mccauley, 1999).

Table 2
Global *F*-statistics and gene flow (Nm) for each of 24 microsatellite loci analyzed across five breeds of buffaloes.

Locus	F_{IT} (F)	F_{ST} (θ)	F_{IS} (f)	Nm
CSRM 060	0.252	0.116	0.153	2.086
ILSTS 026	0.161	0.057	0.11	4.189
HEL 013	0.233	0.104	0.144	2.431
ILSTS 030	0.321	0.154	0.197	1.546
ILSTS 033	0.454	0.162	0.348	1.406
ILSTS 017	0.382	0.206	0.222	1.111
ILSTS 019	0.318	0.379	−0.098	0.394
ILSTS 045	0.745	0.408	0.57	0.450
ILSTS 034	0.29	0.299	−0.012	0.717
ILSTS 058	0.396	0.113	0.32	2.174
ILSTS 056	0.434	0.078	0.386	2.925
ILSTS 068	0.293	0.062	0.247	3.910
CSSM 066	0.243	0.086	0.171	2.861
ILSTS 036	0.257	0.11	0.165	2.280
ILSTS 095	0.711	0.189	0.643	1.281
ILSTS 029	0.587	0.421	0.287	0.438
ILSTS 028	0.243	0.063	0.192	3.775
ILSTS 025	0.238	0.096	0.157	2.510
ILSTS 052	0.109	0.068	0.043	3.575
ILSTS 031	−0.02	0.006	−0.026	16.055
ILSTS 073	0.309	0.118	0.216	2.205
BM 1818	0.408	0.161	0.294	1.613
ILSTS 061	0.099	0.023	0.078	8.399
ILSTS 008	0.334	0.032	0.312	6.221
Overall	0.330	0.145	0.216	1.674

Cavalli-Sforza and Edwards (1967) was utilized to construct the phylogenetic trees of five buffalo breeds and pair-wise distance values are presented in Table 1. The smallest chord distance was found to be between Murrah and Mehsana (0.310), while the highest distance was found between Jaffarabadi and Mehsana (0.435). The dendrogram constructed based on pair-wise chord distance measures following UPGMA revealed the clustering of Mehsana and Murrah at a single node (Fig. 1). Surti joined the cluster later followed by Jaffarabadi and Banni. This is understandable, as Mehsana is presumed to be developed by crossbreeding Murrah and Surti animals and its characteristics are intermediate of these two buffalo breeds (Olver, 1938). This is also supported by the higher gene flow estimates (3.08 to 3.96) observed between these three breeds.

Further, multidimensional scaling (MDS) and principal components analysis (PCA) were utilized to predict the genetic structure among different buffalo breeds. The main applications of both these techniques are to reduce the number of variables and to detect structure in the relationship between variables. The multidimensional scaling display of pair-wise F_{ST} values showed the close proximity of Mehsana, Surti and Murrah while Banni and Jaffarabadi were found to form distinct clusters each separately (Fig. 2). The stress value was found to be 0.00053. Principal component analysis based on pair-wise chord distance estimates demonstrated that the first three principal components together explained 42.01% of the total variance, which was lower than that reported by Baumung et al. (2006) although it is comparable to the reports of de Oliviera et al. (2007). The lower proportion of variance explained by the first three principal components could be attributed to comparatively more number of principal components (22) having eigen values greater than one. The three dimensional scatter gram obtained after principal components analysis showed the intermingling of Mehsana animals between Murrah and Surti, while Banni and Jaffarabadi individuals clustered separately (Fig. 3). The distinctness of Jaffarabadi buffaloes obtained in the present study is consistent with the results of Kumar et al. (2006). Distinctness of Banni buffalo indicated a separate lineage which could have evolved around the Banni grasslands of Kachchh region.

To test the validity of the clustering obtained by phylogenetic and ordination analysis, AMOVA (analysis of molecular

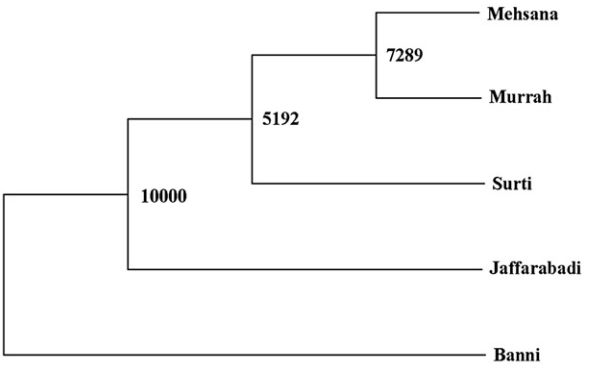

Fig. 1. Dendrogram showing genetic relationships among five Indian buffalo breeds based on Cavalli-Sforza Chord distance. The numbers at the nodes are bootstrap values from 10,000 replications (BN — Banni; MU – Murrah; MH — Mehsana; SR — Surti and JF — Jaffarabadi).

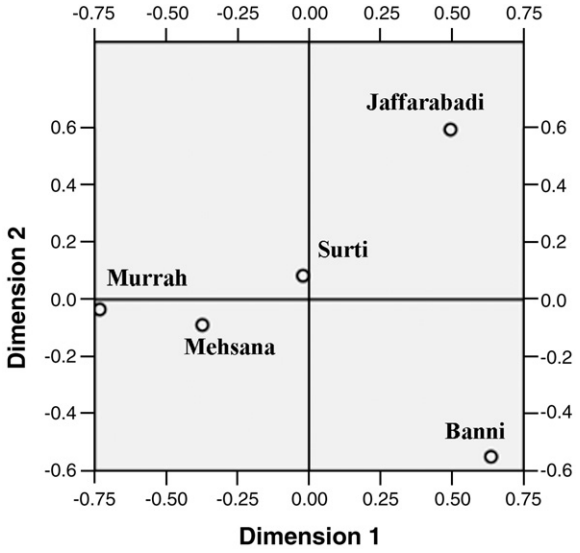

Fig. 2. Multidimensional scaling plot of pair-wise F_{ST} values between different buffalo breeds (stress value — 0.00053).

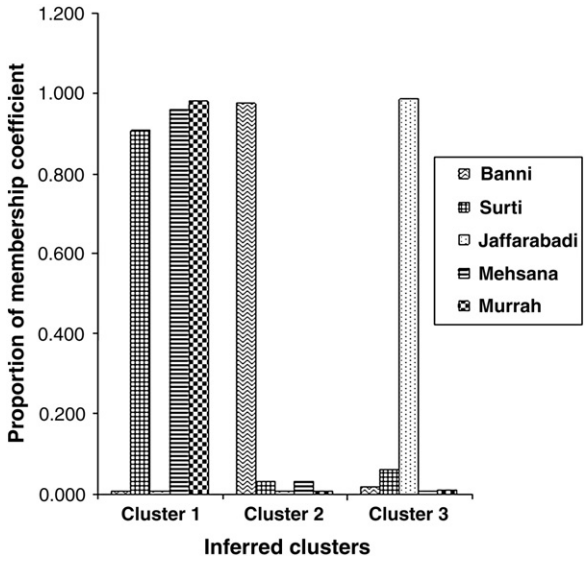

Fig. 4. Proportion of membership of each of the five buffalo breeds in three inferred clusters based on STRUCTURE program.

variance) was performed. When no grouping was assumed, 85.21% of the total variation was found to be within breeds while the remaining 14.79% was found to be among different breeds ($P<0.01$). When the buffalo breeds were grouped according to geographical location and gross morphology (Cockrill, 1974), among group variation was not significant statistically ($P>0.05$) with the values of 1.24% and close to zero respectively. However, when the breeds were grouped based on the clusters obtained from MDS display and UPGMA tree (Group I: Murrah, Mehsana and Surti; Group II: Jaffarabadi; Group III: Banni), among group variation was found to be significantly higher with the value of 5.07% ($P<0.05$). Further, Bayesian clustering analysis was performed to assign individuals to different clusters using STRUCTURE program. Several independent runs were performed for each K with $K=2$ to $K=7$ in order to identify the appropriate K and to verify the consistency of the estimates across runs (Evanno et al., 2005). Three inferred clusters were obtained from five breeds (Fig. 4). Suti, Mehsana and Murrah formed the first cluster with 90.7%,

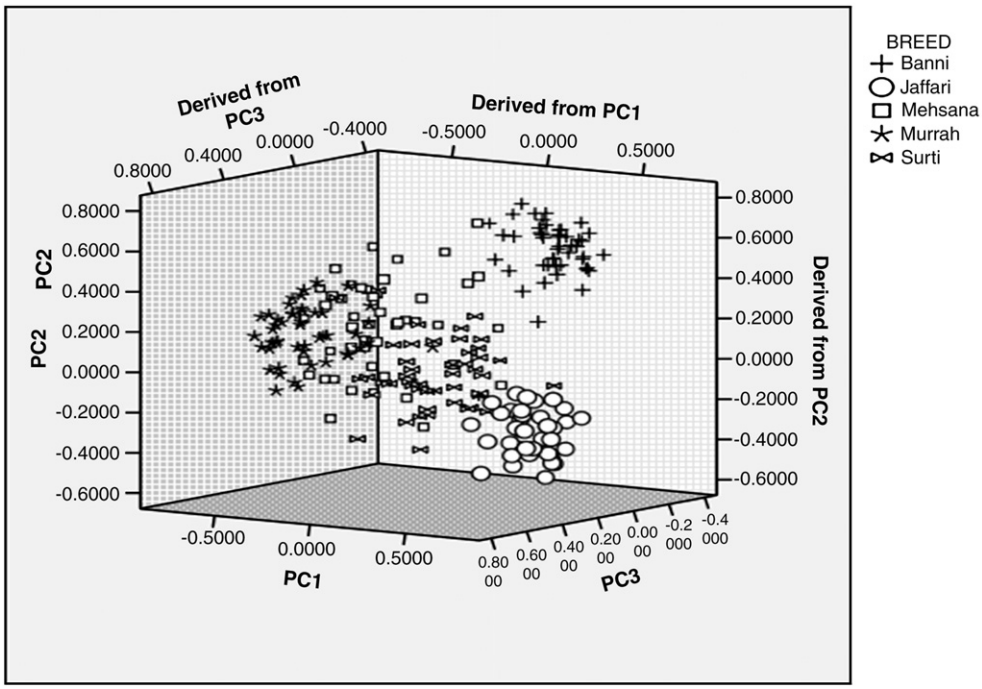

Fig. 3. Scattergram showing relative position of individual animals of five buffalo breeds as defined by three largest principal component scores; First three PCs contribute to 42.01% variance.

96% and 98% of the sampled individuals from the respective populations contributing to this cluster. The second cluster has predominant contribution from Banni buffaloes while only 3% each of Surti and Mehsana breeds contributed to this cluster. The third cluster showed majority contribution from Jaffarabadi buffaloes, while 6% of Surti buffaloes also contributed to this cluster. Thus, the Bayesian analysis with no *a priori* information confirmed the genetic uniqueness of Banni buffalo among the river buffalo breeds of the region.

4. Conclusion

Banni buffaloes, with a population of 0.52 million, have assumed significance in recent years owing to their high production potential in a unique grassland ecosystem (Singh, 2009). The present study revealed the genetic uniqueness of these buffaloes vis-à-vis other buffalo breeds available in the region which will be the basis for establishment of further conservation and selection strategies in Banni buffalo.

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