



Assessment of genetic diversity and admixture in two newly recognized cattle breeds of Eastern India

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ABSTRACT

The objective of this study was to quantify the genetic diversity of the 2 newly recognized cattle breeds, Ghumusari and Binjharpuri adapted in the coastal state of Odisha of India. Twenty-one microsatellite loci were used to estimate parameters of genetic diversity. Genotypes of 48 animals from each of the 2 breeds and 46 of Hariana were analyzed at 21 microsatellite markers for genetic diversity and genetic distance. There was high allelic as well as gene diversity in all the 3 breeds. Mean gene diversity ranged between 0.756 and 0.769. Genetically Ghumusari was closer to distantly located Hariana breed. Greater genetic distance was detected between Binjharpuri and Ghumusari, which are geographically closer to each other (about 250 km) and lowest genetic distance between geographically far away (about 1,800 km) Ghumusari and Hariana. Similar relationship between the 3 breeds was realized using different approaches, i.e. phylogenetic relationship, F_{ST} and PCA. The breed relationship inferences, which are based on genetic distance, provide additional tools for consideration in future conservation and breeding plans.

Key words: Conservation, Gene diversity, Genetic distance, Genetic diversity, Phylogenetic relationship

A large variety of breeds developed over centuries, which are favorably adapted to varying ecological niches and to the divergent necessities and preferences of their breeders. Of late Indian cattle genetic resources were represented by 30 acknowledged breeds and a large number of uncharacterized populations. Seven new cattle populations, 4 from Odisha (Ghumusari, Binjharpuri, Motu and Khariar) have been characterized and registered as new breeds (NBAGR 2009) bringing the total number of Indian recognized breeds to 37. Because of declining number of adapted breeds in diverse regions of the world, a systematic method for rapid genetic evaluation of such diverse cattle breeds is needed. Rare and newly recognized breeds provide opportunities for increasing genetic diversity (Notter 1999). The present study was undertaken to quantify the genetic diversity and genetic distance between the 2 newly recognized cattle breeds Ghumusari and Binjharpuri cattle from Odisha and their genetic relationship with Hariana breed.

MATERIALS AND METHODS

Samples: Blood samples were collected from 46 animals of Hariana and 48 each of Ghumusari and Binjharpuri cattle breeds in line with MoDAD suggestions. Animals for genotyping were selected to ensure that they were a

representative sample of each breed, covering wider geographical areas in which the breeds are reared.

Laboratory techniques: Genomic DNA was extracted by standard phenol-chloroform method. DNA was amplified at the following 21 microsatellite loci (suggested by FAO-MoDAD) — CSRM60, ETH10, ILSTS11, TGLA122, INRA05, INRA63, TGLA227, CSSM08, HEL05, ILSTS05, ILSTS33, INRA35, BM1824, CSSM66, ETH03, ETH225, MM12, CSSM33, HEL01, HEL09, ILSTS34 —with polymerase chain reaction (PCR) using primer sequences as suggested in the literature. PCR products were detected by capillary electrophoresis using DNA genetic analyzer.

Statistical analysis: Various diversity indices like observed number of alleles, allele frequency, observed and expected heterozygosity were estimated using programme POPGENE (Yeh *et al.* 1999). Polymorphism information content (PIC) was calculated using allele frequencies according to the formula given by Botstein *et al.* (1980). Wright's F_{ST} was estimated to quantify breed differentiation, both pair wise and among populations. Variance based method was employed to compute global F-statistics and heterozygote deficiency using FSTAT program. Possible divergence from Hardy-Weinberg expectations was determined by POPGENE (Yeh *et al.* 1999). Microsatellite allele frequency data were used to calculate Nei's genetic distance (D_A) (Nei *et al.* 1983). Pair-wise chord distances between breeds were utilized to derive radiation tree visualized using TREEVIEW version 1.6.6 (Page 1996). Bootstrap resampling ($n=1,000$) was performed to test the

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robustness of the topologies. The degree of gene flow (number of migrants per generation) was estimated using the program Genetix Version 4.05 (Belkhir *et al.* 2004). The geometric relationship between breeds was examined using principal components analysis. Pair-wise chord distance measures between individual animals were utilized to perform principal components analysis using SPSS version 10.5.

RESULTS AND DISCUSSION

Genetic variability: The genetic diversity parameters evaluated for the 3 breeds are given in Table 1. A total of 368 alleles were observed in the 3 breeds from the 21 loci investigated. Mean numbers of alleles per locus (Na) differences observed between breeds were negligible, despite wide differences in the range of alleles. The allelic variability detected in the 3 breeds is relatively higher than reported in majority of Indian indigenous breeds, which ranged from 3.88 to 9.60 (Prakash *et al.* 2011) as well as international breeds (Groeneveld *et al.* 2010). However, comparable or higher allelic diversity was reported in 27 Chinese breeds (Zhang *et al.* 2007) and 10 Brazilian breeds (Egito *et al.* 2007). Lower allelic diversity reported in earlier years may be due to the use of silver staining which does not detect all the alleles, specially the alleles differing by few base pairs (Prakash *et al.* 2011). In general higher number of alleles per locus were detected in zebu breeds as compared to taurine breeds (Egito *et al.* 2007), which may be due to lesser use of AI in Indian cattle. The mean effective number of alleles in all the 3 breeds is far lower (even less than 50%) than the actual mean observed number of alleles

per locus (Table 1) indicating that alleles with frequencies away from the 'average' contribute very little to the effective number of alleles.

The mean observed (H_o) as well as expected heterozygosities (H_e) were very high (Table 1). The genetic variability computed in the 3 breeds was either higher or comparable with other Indian breeds, ranged from 0.460 to 0.789 in 24 Indian breeds (Prakash *et al.* 2011). Heterozygosity estimates, in general, were moderate to high (>0.45) in different breeds investigated worldwide (Groeneveld *et al.* 2010), but lower than estimated in the 3 breeds in this study. Higher estimates of observed and expected heterozygosities observed in the 3 breeds are indicative of low inbreeding, which is also substantiated by very low mean inbreeding coefficients (F_{IS}) across 21 loci in all the 3 breeds (mean $F_{IS}=0.044$, ranged from 0.0 to 0.20 across loci), (Table 2). High heterozygosity is indicative of lots of variability and is a desirable attribute of a population for improvement and conservation purposes. The high PIC values obtained for most of the markers further suggested their usefulness in the evaluation of the biodiversity of native Indian cattle breeds (Table 1).

The exact test for Hardy-Weinberg disequilibrium within breeds showed a significant deviation in all the 3 breeds ($P < 0.05$). These breeds presented 11, 12 and 10 loci with significant heterozygote deviations ($P < 0.05$) respectively in Hariana, Ghumusari and Binjharपुरi (Table 2). The heterozygote deviations were in both directions, some loci exhibiting heterozygote excess and others heterozygote deficit (Table 1). Significant departure from HWE is a general occurrence and was reported in several cattle breeds

Table 1. Diversity parameters in Binjharपुरi, Ghumusari and Hariana cattle

	Ghumusari					Binjharपुरi					Hariana				
	Na	Ne	H_o	H_e	PIC	Na	Ne	H_o	H_e	PIC	Na	Ne	H_o	H_e	PIC
CSRM60	21	7.732	0.792	0.871	0.862	18	7.024	0.833	0.858	0.845	12	3.661	0.652	0.727	0.695
ETH10	7	3.044	0.646	0.671	0.619	13	4.551	0.696	0.780	0.757	9	4.161	0.696	0.760	0.726
ILSTS11	7	2.540	0.458	0.606	0.556	9	3.308	0.667	0.698	0.666	8	2.366	0.457	0.577	0.538
TGLA122	13	7.642	0.896	0.869	0.856	15	8.727	0.813	0.885	0.875	14	8.798	0.870	0.886	0.875
INRA05	7	5.195	0.875	0.808	0.780	6	4.247	0.771	0.765	0.726	7	4.236	0.891	0.764	0.728
INRA63	9	2.727	0.646	0.633	0.594	5	3.431	0.750	0.709	0.660	7	3.094	0.630	0.677	0.638
TGLA227	6	1.940	0.375	0.485	0.457	8	1.520	0.292	0.342	0.324	10	3.228	1.000	0.690	0.641
CSSM08	5	1.895	0.188	0.472	0.408	8	3.421	0.744	0.708	0.680	11	4.402	0.689	0.773	0.741
HEL05	11	6.136	0.738	0.837	0.818	7	4.142	0.889	0.759	0.729	10	5.153	1.000	0.806	0.781
ILSTS05	26	5.908	0.792	0.831	0.818	14	6.564	0.889	0.848	0.831	13	6.151	0.870	0.837	0.819
ILSTS33	22	13.322	0.800	0.925	0.920	13	6.847	0.875	0.854	0.838	13	4.093	0.587	0.756	0.721
INRA35	11	6.847	0.792	0.854	0.837	16	9.289	0.733	0.892	0.883	15	6.972	0.870	0.857	0.841
BM1824	3	1.947	0.167	0.486	0.378	7	3.211	0.938	0.689	0.640	7	2.622	0.739	0.619	0.549
CSSM66	10	5.020	0.717	0.801	0.776	8	4.640	0.708	0.785	0.758	13	6.551	0.761	0.847	0.832
ETH03	8	4.655	0.833	0.785	0.755	14	3.949	0.854	0.747	0.722	10	4.015	0.761	0.751	0.714
ETH225	15	5.064	0.750	0.803	0.781	11	3.869	0.771	0.742	0.719	10	2.782	0.652	0.641	0.622
MM12	23	11.973	0.574	0.916	0.911	13	4.482	0.771	0.777	0.756	16	4.422	0.652	0.774	0.747
CSSM33	12	7.758	0.833	0.871	0.858	16	9.580	0.875	0.896	0.887	16	8.687	0.902	0.885	0.875
HEL01	14	3.678	0.771	0.728	0.691	8	1.971	0.542	0.493	0.473	17	5.286	0.732	0.811	0.798
HEL09	11	7.178	0.938	0.861	0.845	11	6.747	0.750	0.852	0.835	13	8.977	0.976	0.889	0.878
ILSTS34	15	4.315	0.771	0.768	0.749	20	9.804	0.833	0.898	0.890	17	5.511	0.732	0.819	0.804
Mean	12.19	5.548	0.683	0.756	0.727	11.43	5.301	0.762	0.761	0.738	11.81	5.008	0.768	0.769	0.741
S.D	6.298	3.079	0.216	0.143	0.163	4.214	2.490	0.141	0.136	0.140	3.281	2.021	0.145	0.089	0.101

Table 2. F_{IS} , HWE (p-values) and F_{ST} values in three breeds of cattle

Marker	F_{IS}			HWE (p-values)			F_{ST}
	Hariana	Ghumusari	Binjharपुरi	Hariana	Ghumusari	Binjharपुरi	
CSRM60	0.114	0.101	0.039	0.000***	0.000***	0.000***	0.016
ETH10	0.095	0.049	0.119	0.752	0.000***	0.000***	0.027
ILSTS11	0.220	0.254	0.055	0.000***	0.000***	0.000***	0.001
TGLA122	0.030	-0.020	0.093	0.453	0.203	0.000***	0.012
INRA05	-0.156	-0.073	0.002	0.188	0.041*	0.829	0.051
INRA63	0.079	-0.009	-0.048	0.006**	0.318	0.383	0.010
TGLA227	-0.440	0.236	0.158	0.070	0.144	0.993	0.175
CSSM08	0.120	0.610	-0.040	0.000***	0.000***	0.663	0.224
HEL05	-0.230	0.130	-0.153	0.222	0.052	0.101	0.08
ILSTS05	-0.027	0.058	-0.037	0.010**	1.000	0.001**	0.087
ILSTS33	0.234	0.146	-0.014	0.000***	0.041*	0.000***	0.027
INRA35	-0.004	0.083	0.189	0.001***	0.000***	0.001***	0.025
BM1824	-0.184	0.663	-0.352	0.000***	0.000***	0.219	0.07
CSSM66	0.113	0.115	0.108	0.295	0.266	0.034*	0.064
ETH03	-0.002	-0.051	-0.134	0.164	0.000***	1.000	0.075
ETH225	-0.007	0.076	-0.029	0.940	0.774	1.000	0.186
MM12	0.168	0.382	0.018	0.000***	0.000***	0.869	0.071
CSSM33	-0.007	0.054	0.034	0.162	0.044*	0.016*	0.018
HEL01	0.110	-0.048	-0.089	0.248	1.000	0.872	0.302
HEL09	-0.087	-0.079	0.130	0.000***	0.521	0.000***	0.002
ILSTS34	0.118	0.007	0.082	0.003**	0.002**	0.508	0.111
Mean	0.013	0.107	0.010				0.080

Key: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

from different parts of the world (Prakash *et al.* 2011 for Indian breeds; Groeneveld *et al.* 2010 for global breeds). In domestic species, heterozygote deficiencies can be explained by several factors such as the presence of unamplified alleles ("null" alleles), selection against heterozygotes, population subdivision (Wahlund's effects) or inbreeding. The exact test for population differentiation based on allele frequency variations showed that all breeds tested were significantly different from each other ($P < 0.0001$).

Breed relationships: Allele frequencies were used to generate the genetic distances- Cavalli-Sforza distance (D_S)

Table 3. Pair wise genetic distances between breeds

Breeds	Hariana	Ghumusari	Binjharपुरi
Hariana	0.000	0.392	0.399
Ghumusari	0.213	0.000	0.424
Binjharपुरi	0.219	0.255	0.000

*Cavalli-Sforza and Edwards (D_S) above diagonal and Nei's (D_A) below diagonal.

Table 4. Gene flow between 3 Indian cattle breeds

Breeds	Hariana	Ghumusari	Binjharपुरi
Hariana	0.000	3.22	3.08
Ghumusari	0.072	0.000	2.51
Binjharपुरi	0.075	0.091	0.000

* N_m above diagonal and F_{ST} below diagonal.

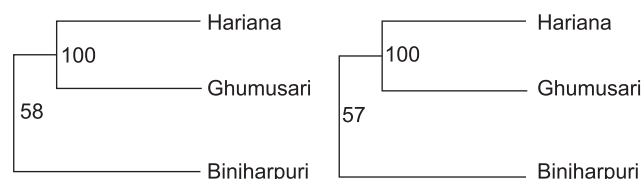


Fig. 1. UPGMA dendrogram based on Nei's genetic distance (left) and Cavalli-Sforza Edwards chord distance (right) (1000 bootstraps).

and Nei's distance (D_A) for each pair of breeds. Pair wise genetic distances calculated by the 2 methods are shown in Table 3, and F_{ST} and N_m values are presented in Table 4. Estimates of Nei's pair-wise genetic distances and Cavalli-Sforza's genetic distances were all significantly different from zero indicating independent genetic entities of the 3 breeds. Interestingly, the phylogenetic relationships among the 3 populations (Fig. 1) were not closely linearly correlated with their geographic distribution. The 2 newly recognized breeds from Odisha, Ghumusari and Binjharपुरi, had higher genetic distance than each of the breed had with distantly located Hariana breed. Ghumusari and Binjharपुरi were expected to be genetically closer due to their geographical proximity (located just about 200 km apart), but each of Ghumusari and Binjharपुरi were genetically closer to Hariana (located far away, about 1,800 km) than amongst themselves.

Principal components analysis: PCA was performed including all populations and loci using allele frequencies to summarize breed relationships (Fig. 2). The first 2

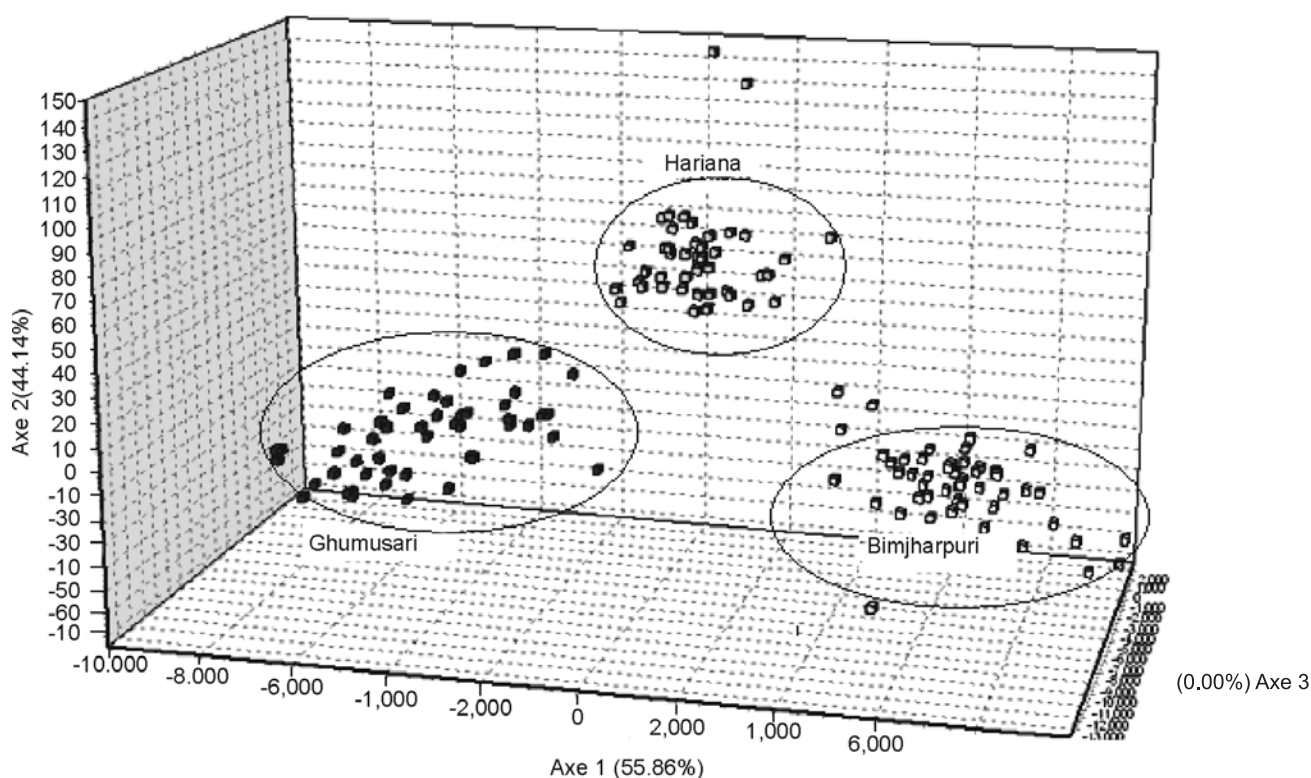


Fig 2. Principal component analysis based on pair-wise inter individual chord distance (First 2 PCs explain 100% of variance; PC1 = 55.86%; PC2=44.14%).

dimensions of the PCA accounted for the entire variance (100%). The analyses indicated distinct grouping of all the 3 breeds (Ghumusari, Binjharpuri and Hariana). Consistent with the results obtained from PCA, the UPGMA phylograms computed from D_A and D_S distances showed 2 groups (Fig. 1). Identical phylogenetic relationships were obtained for the 2 distance measures computed. Hariana and Ghumusari breeds joined with 100% of the bootstrap values. Binjharpuri joined the tree with 57% bootstrap values. These groupings are interesting as the geographically farthest breeds Hariana and Ghumusari are closer on the phylogram than the geographically closer breeds Ghumusari and Binjharpuri indicating Hariana inheritance.

Population differentiation: To validate the clustering obtained by phylogenetic and PCA analysis (Fig. 3), population differentiation was evaluated by F_{ST} (Table 4). Based on F_{ST} derived from genetic distances, moderate level of breed differentiations was observed. Lowest differentiation was obtained between Hariana-Ghumusari (7.2%), followed by Hariana-Binjharipuri (7.5%) and maximum differentiation between the 2 new breeds from Odisha, Ghumusari- Binjharpuri (9.1%). The results are thus consistent with all the methodologies employed, i.e., genetic distances, PCA, phylogenetic relationship and F_{ST} . Closer relationship of either of the 2 breeds from Odisha with distantly located Hariana breed is supported by the historical account. Hariana breed has been used in Odisha as an improver breed for improving the draughtability of Odisha grey cattle including Ghumusari and Binjharpuri. On the

other hand there was lesser intermixing of Ghumusari and Binjharpuri cattle of Odisha due to their distinct locations. This is truly reflected by higher gene flow between Hariana and Ghumusari ($N_m=3.22$), Hariana and Binjharpuri ($N_m=3.08$) as compared to lower gene flow between the 2 local breeds of Odisha, Ghumusari and Binjharpuri ($N_m = 2.51$) (Table 4).

The differentiation of the 2 newly recognized breeds from Odisha along with the distantly located Hariana breed is, thus, high and higher than that reported for 20 Northern European breeds ($F_{ST} = 0.107$; Kantanen *et al.* 2000); 18 local cattle breeds from Spain, Portugal and France ($F_{ST} = 0.07$; Canon *et al.* 2001); 8 Southwest European beef cattle breeds ($F_{ST} = 0.068$; Jordana *et al.* 2003); 3 Indian breeds ($F_{ST} = 0.117$ and $D_0: 113$; Metta *et al.* 2004 and Mukesh *et al.* 2004); and 10 Brazilian breeds ($F_{ST} = 0.098$; Egito *et al.* 2007). Very low differentiation between Kenkatha and Gaolao breeds of Indian cattle was recently detected ($F_{ST} = 0.0219$; Chaudhari *et al.* 2009).

This study, thus, revealed high genetic variability in the 2 newly recognized cattle breeds from Odisha, reflected as allelic as well as gene diversity. Comparison using genetic distances, PCA and F_{ST} with distantly located Hariana breed showed closer genetic relationship of Ghumusari as well Binjharpuri with Hariana and comparatively distant relationship between the 2 breeds from Odisha. High breed differentiation (14.0%) among breeds was revealed by AMOVA. This genetic diversity analysis of newly recognized cattle breeds and their comparison with Hariana will help in conservation prioritization and in making plans

that reconcile their genetic improvement with maintenance of genetic variation.

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