Genetic structure and relationship of three cattle breeds from Rajasthan: Implications for breeding strategies and conservation

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ABSTRACT

Information on genetic structure and variability of livestock breeds is critical for efficient conservation plans. Here, we present molecular characterization of 3 cattle breeds from Rajasthan state of India on the basis of 21 FAO recommended microsatellite markers. All the three breeds had high diversity values. The average number of alleles per locus was 9.00 in Tharparkar, 9.76 in Nagori and 10.33 in Kankrej. Gene diversity (expected heterozygosity) was high in all breeds and varied from 0.707 in Tharparkar, 0.713 in Nagori to 0.755 in Kankrej. FIS values were very low (almost zero) in all the three breeds, with mean FIS values ranging from -0.018 in Kankrej to -0.025 in Nagori. Phylogenetic analysis by Neighbour Joining tree based on Nei's genetic distance and Cavalli-Sforza Edwards chord distances generated identical phylogenies, which are in accordance with historical and geographical data. Tharparkar and Kankrej clustered together while Nagori was distinct. AMOVA revealed very low breed differentiation among the three breeds. Only 2.19% variation was due to between breed differences and the remaining 97.81% was due to individuals within breeds. PCA and MDS analysis revealed mixed breed structure. A very high migration (average 11.26 immigrants per generation) was estimated between the three breeds. The study thus reveals very low genetic differentiation despite high genetic variability in the three investigated breeds.

Key words: Gene diversity, Phylogenetic analysis, Neighbour joining tree, breed structure, genetic differentiation

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INTRODUCTION

Livestock breeds have been created by centuries of human and natural selection. Breeds have been selected to suit a wide range of environmental conditions and human needs. Cattle hold a unique position among domestic livestock species in India in that they have fulfilled key agriculture, economic, cultural and even religious roles in historical and current societies. The selection and extensive use of a few highly productive cattle breeds through crossbreeding in recent years has caused the decline, deterioration or replacement of numerous other breeds, particularly in developing countries including India. The genetic diversity found in domestic breeds allows farmers to cultivate new characteristics in response to changes in environment, diseases or market conditions. There is growing recognition that existing indigenous breeds often possess specific gene combinations and haplotypes related to special adaptations (such as disease resistance, adaptation to harsh conditions or poor-quality feeds, etc.) not found in other breeds adapted to different environmental conditions. In India, a large number of hardy cattle breeds have been selected for many centuries to suit the widely varying environmental conditions and human and cultural needs. In recent times, the populations of many indigenous domestic breeds became has reduced drastically and few are facing extinction (Vechur, Krishna Valley) by replacement or crossbreeding with exotic breeds, substitution of draft animals by technology, or unfavourable marketing (Kohler-Rollefson 2000). This necessitates that within the conservation framework in India, there is a need to ensure that unique indigenous strains are not lost.

Over the past 15 years, about 300 of 6000 breeds of farm animals identified by the FAO have become extinct. Furthermore, 1350 breeds of domestic animals currently face extinction in the near future (Scherf 2000) but these represent a unique resource to meet present and future breeding objectives in both developed and developing countries. At the worldwide level, 17% of cattle and 14% of sheep breeds have

already been lost (Scherf 2000). Molecular characterization of animal genetic resources may contribute to a rational approach to conservation (Hanotte and Jianlin 2005) by giving a high priority to breeds that are taxonomically most distinct (Barker 2000). In order to maintain breed diversity it is necessary to know how far apart they are. DNA-based molecular markers like microsatellites are now commonly used for the estimation of genetic diversity, calculation of genetic distances and detection of admixture, genetic bottlenecks, and inbreeding (Sunnucks 2000). Especially, high degree of polymorphisms is considered to be greatly useful for assessing genetic diversity and relationships among closely related livestock breeds (FAO, 1998). As for conservation of livestock genetic resources, if there are limitations of costs or breeding places, the population may be maintained as a core collection, which possesses as much genetic variability as possible with appropriate population size. To develop the core collection, exact genetic evaluation for the population would be required by molecular markers. It would lead to a correct management of the population as a genetic resource. Consequently, the obtained results would contribute to the appropriate managements avoiding loss of genetic variability in these lines and to future improvements.

The primary objective of the present work was to quantify and compare levels of genetic variability within three cattle breeds from livestock biodiversity rich state of Rajasthan representing different uses and population sizes. Tharparkar is a dual type breed acclimatized to the desert area of Rajasthan. Though, Kankrej, a milch type animal, is described as a breed from Gujarat but it also has a vast breeding area in Rajasthan where Kankrej animals are managed as migratory herds. In contrast, in Gujarat, there is hardly any migration of Kankrej herds during extreme summer when there is acute shortage of water in Rajasthan. Nagori is an excellent draft type breed. The breeding tracts of the three breeds are quite distinct and isolated, about 300 to 500 Km away from each other. An additional aim was to assess the extent and pattern of gene admixture and the dynamics of the introgression in the studied cattle populations. The impact of the geographic proximity on genetic differentiation was also considered.

MATERIALS AND METHODS

Samples: Blood samples were collected from 44 animals of Kankrej, 49 of Nagori and 50 of Tharparkar cattle breeds covering a large geographical range of the respective breeding tract 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 following standard procedure. DNA was PCR-amplified at 21 microsatellite loci (suggested by FAO-MoDAD) namely— CSRM60, ETH10, ILSTS11, TGLA122, INRA05, INRA63, TGLA227, CSSM08, HEL05, ILSTS05, ILSTS33, INRA35, BM1824, CSSM66, ETH03, ETH225, MM12, CSSM33, HEL01, HEL09, ILSTS34 —using primer sequences as suggested in the literature. These markers are dispersed across the genome, covering 15 of the autosomes. Amplification of the microsatellite loci was done by multiplex PCR by combining a maximum of five markers per multiplex. PCR products were detected by capillary electrophoresis using an ABI Prism 310 DNA Genetic Analyzer (Applied Biosystems). The size of alleles was determined by using GeneScan-500 ROX Size Standard, which detects different alleles through comparing sizes with standard DNA sizes.

Statistical analysis: Various diversity indices like observed number of alleles, allele frequency, observed and expected heterozygosity, population differentiation (FST) (Weir and Cockerham, 1984), global F-statistics and heterozygote deficiency were calculated using Microsatellite Analyzer version 4.05 (Dieringer and Schlotterer, 2003). Possible divergence from Hardy-Weinberg expectations was determined running the GENEPOP version 3.1 (Raymond and Rousset, 1995). Analysis of molecular variance was performed using ARLEQUIN version 3.0 (Excoffier et al. 2005). Pair-wise chord distances between breeds were utilized to derive radiation tree visualized using TREEVIEW version 1.6.6. Bootstrap re-sampling (n=10,000) was performed to test the robustness of the topologies. The geometric relationship between the 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

Molecular Markers: A total number of 277 alleles were detected at the 21 loci across the three breeds investigated. Measures of genetic variability for each marker in the three breeds are presented in Table 1. The total number of alleles (TNA) per locus ranged between 4 for BM1824 and 21 for CSSM33. Allelic richness per locus was high, with an overall average of 9.697. Genetic diversity, along with the degree of differentiation, was evaluated using microsatellite markers within and between the three cattle breeds from Rajasthan state of India. The diversity parameters of the three breeds are presented in Table 1.

Within Breed Variability: Kankrej cattle had higher total number of alleles (217; range 6-18) across the 21-microsatellite markers evaluated. The corresponding allelic count in Nagori cattle was 205 (4-21) and in Tharparkar cattle 189 (5-21) (Table 1). The mean allelic diversity (number of alleles per locus) varied from 9.00 in Tharparkar to 9.76 in Nagori and 10.33 in Kankrej. As a general observation, the gene frequencies and the evenness of allele frequencies varied greatly between the three populations and the loci vary greatly in number of alleles, presumably reflecting differences in the mutation rate across loci. The number of alleles per locus in the three investigated breeds is, in general, higher than those previously reported for a majority of the acknowledged indigenous breeds and hitherto uncharacterized populations from India, which varied from 3.88 to 9.60 using microsatellite markers (Prakash et al. 2010) as well as majority of exotic cattle breeds studied worldwide (Groeneveld et al. 2010).

The explanation for detection of lower number of alleles per locus in earlier studies may be the use of denaturing polyacrylamide sequencing gels and silver staining, which probably does not locate all the alleles, especially the alleles differing by few nucleotides. This is appropriately reflected in the findings of Sodhi et al. (2006) and Sodhi et al. (2008), who used PAGE-silver staining as well as automated DNA sequencer for genetic characterization of Tharparkar breed using the same set of microsatellite markers and animals. Using PAGE-silver staining, only 6.2 alleles per locus were identified (Sodhi et al. 2006) in contrast to 9.0 alleles using automated DNA sequencer (Sodhi et al. 2008). Comparable allelic diversity in Tharparkar and Rathi (Sodhi et al 2008) and Kenkatha and Gaolao (Chaudhari et al. 2009) breeds of Indian cattle was achieved using automated DNA sequencer. Higher allelic diversity reported in 9 European breeds (Na=10.60; Lubieniecka et al. 2001), 27 Chinese breeds (Na=9.73; Zhang et. al. 2007) and 10 Brazilian breeds (Na=13.18; Egito et. al. 2007) was also achieved using automated DNA sequencer. By and large higher number of alleles per locus has been encountered in zebuine breeds as compared to taurine breeds (Egito et. al. 2007). Higher allelic diversity in Indian cattle might be accredited to lack of any appreciable designed selection pressure due to negligible utilization of AI under filed conditions and thus implies the existence of larger effective population sizes of the explored Indian cattle breeds.

Heterozygosity is of decisive relevance in evaluating genetic variation in natural populations. It can clarify a great deal about the structure and even history of a population. The mean observed heterozygosity (Ho) estimated across the 21-microsatellite markers in the 3 breeds was very high ranging from 0.721 in Tharparkar to 0.788 in Nagori (Table 1). Correspondingly, the expected heterozygosity or gene diversity in the investigated breeds was high, varying from 0.707 in Tharparkar to 0.819 in Nagori. The observed as well as expected heterozygosity estimates in the three breeds are in general higher than those assessed for Indian (Prakash et al. 2010) as well as exotic cattle breeds evaluated earlier (Groeneveld et al. 2010). In the reported 28 observed heterozygosity values for different Indian cattle breeds, 17.9% were lower than 0.50, 32.1% varied between 0.50 to 0.60, 35.7% between 0.60 and 0.70 and only 14.3% were above 0.70. The observed and expected heterozygosities in all the breeds are more or less comparable suggesting that mating is almost random within each breed. Higher estimates of observed and expected heterozygosities observed in the 3 breeds are indicative of low inbreeding, which is also supported by close to zero mean inbreeding coefficients (FIS) across the 21 loci in the investigated breeds (ranged from –0.025 in Nagori to 0.018 in Kankrej) Table 2.

Between Breed Variability and Genetic Differentiation: Mean estimates of F-statistics from Jackknifing over loci were: FIT (total inbreeding estimate) =0.009, FIS (within-population inbreeding estimate) = -0.0136 and FST (population differentiation) = 0.0218 (Table 3).

These values were not significantly different from zero ($p > 0.05$). The non-significant values of FIS 0.009), F IT (0.0136) and FST (0.0218) suggested random mating in the three populations. Genetic differentiation between the three populations was low (FST = 0.0218). This implied that only 2.18% of the total variation

Table 1. Genetic diversity parameters of three breeds of cattle from Rajasthan

Locus	Kankrej				Nagori				Tharparkar			
	Na	Ho	He	PIC	Na	Ho	He	PIC	Na	Ho	He	PIC
CSRM60	10	0.705	0.700	0.650	9	0.469	0.486	0.445	7	0.640	0.631	0.5896
ETH10	10	0.818	0.784	0.742	7	0.694	0.745	0.693	6	0.800	0.729	0.6732
ILSTS11	8	0.500	0.568	0.523	6	0.408	0.485	0.443	6	0.300	0.375	0.3454
TGLA122	13	0.791	0.882	0.858	12	0.936	0.850	0.824	10	0.800	0.820	0.7887
INRA05	8	0.864	0.829	0.795	7	0.776	0.794	0.756	6	0.740	0.716	0.6685
INRA63	8	0.523	0.577	0.538	7	0.531	0.589	0.534	5	0.660	0.675	0.6029
TGLA227	7	0.955	0.627	0.560	8	1.000	0.608	0.526	8	0.920	0.601	0.5275
CSSM08	8	0.841	0.819	0.784	7	0.714	0.724	0.672	6	0.640	0.770	0.7243
HEL05	8	0.907	0.771	0.731	9	1.000	0.736	0.698	8	0.959	0.794	0.761
ILSTS05	12	0.932	0.825	0.795	9	0.796	0.801	0.762	11	0.840	0.796	0.759
ILSTS33	8	0.659	0.682	0.618	8	0.612	0.594	0.538	7	0.680	0.705	0.6662
INRA35	11	0.659	0.839	0.811	9	0.735	0.820	0.786	8	0.820	0.802	0.7646
BM1824	6	0.659	0.636	0.559	4	0.551	0.521	0.440	5	0.420	0.491	0.4486
CSSM66	11	0.841	0.797	0.759	12	0.796	0.814	0.783	10	0.780	0.798	0.7606
ETH03	8	0.750	0.645	0.577	6	0.571	0.646	0.573	7	0.680	0.671	0.6147
ETH225	9	0.636	0.649	0.618	10	0.653	0.600	0.561	7	0.360	0.368	0.35
MM12	13	0.750	0.777	0.739	11	0.714	0.698	0.644	9	0.680	0.708	0.653
CSSM33	15	0.841	0.876	0.854	21	0.898	0.892	0.874	21	0.960	0.901	0.8832
HEL01	18	0.674	0.898	0.880	18	0.682	0.873	0.855	20	0.766	0.907	0.8891
HEL09	13	0.837	0.896	0.8744	12	0.787	0.890	0.869	11	0.894	0.877	0.8536
ILSTS34	13	0.805	0.790	0.752	13	0.867	0.809	0.776	11	0.809	0.711	0.6707
Mean	10.3	0.759	0.755	0.740	9.76	0.788	0.819	0.709	9.0	0.721	0.707	0.7117

N_a= Number of alleles; H_o = observed heterozygosity; H_e = Expected heterozygosity;
PIC = polymorphism information content

corresponded to between breed differences and about 97.82% of the total genetic variation corresponded to within-breed differences. This appears logical in view of the fact that the three breeds dwell in close proximity to each other and there is appreciable migration between the three breeds irrespective of their respective breeding tracts, as there is no breed society or breeding policy to check such indiscriminate breeding.

In order to further investigate the inter-breed relationship among the three breeds, different approaches were employed. Neighbour Joining tree derived from inter-individual Nei's genetic distances between the three investigated breeds exhibited wide-ranging admixture of animals of the two breeds (Fig. 1). This was further corroborated through Factorial Component Analysis (FCA, Fig. 2), which has the advantage of concurrent manifestation of genetic

Table 2. Global F-statistics among three cattle breeds at different microsatellite loci

Locus	FST	FIT	FIS	P-value
CSRM600.0286	0.0303	0.0018	0.0020	
ETH10	0.0439	0.0211	-0.0239	0.0001
ILSTS110.0089	0.1640	0.1565	0.1050	
TGLA122	0.0220	0.0288	0.0070	0.0001
INRA05 0.0216	0.0052	-0.0168	0.0017	
INRA63 0.0275	0.0939	0.0684	0.0059	
TGLA227	0.0016	-0.5744	-0.5769	0.1964
CSSM08 0.0163	0.0706	0.0552	0.0167	
HEL05	0.0051	-0.2460	-0.2524	0.0796
ILSTS050.0051	-0.0532	-0.0587	0.1266	
ILSTS330.0374	0.0515	0.0146	0.0009	
INRA35 0.0340	0.1273	0.0965	0.0001	
BM1824	0.0410	0.0541	0.0137	0.0015
CSSM66 0.0482	0.0469	-0.0014	0.0001	
ETH03	0.0144	-0.0007	-0.0153	0.0367
ETH225 0.0500	0.0292	-0.0220	0.0001	
MM12	0.0053	0.0227	0.0175	0.1456
CSSM33 0.0148	0.0016	-0.0134	0.0008	
HEL01	0.0102	0.2161	0.2080	0.0131
HEL09	-0.0017	0.0531	0.0547	0.6359
ILSTS340.0328	-0.0416	-0.0770	0.0001	
Mean	0.0218	0.0085	-0.0136	0.0001

differences that are contributed by each allele. The first three components accounted for only 36.22% of the total genetic variation. The lesser proportion of variance explained by the first three principal components could be ascribed to comparatively more number of principal components having Eigen values more than one. Diagrammatic arrangement of FCA plotted animals of the three breeds in an overlapped state and thus reflected insignificant breed-specific clustering (Figure 3).

To further authenticate the clustering obtained by NJ tree and FCA, AMOVA (analysis of molecular variance) was performed (Table 4). When no grouping was assumed, 97.82% of the total variation was found to be within breeds and a mere 2.18% was found to be among breeds. Present day subdivision of Kankrej, Tharparkar and Nagori cattle into distinct breeds is, thus, very low and much smaller than that reported for Sahiwal, Hariana and Deoni cattle (FST = 0:113; Mukesh et al. 2004); Gir, Deoni and Kaknrej cattle (FST = 0:086; Kale et. al. 2010); Ongole and Deoni cattle (FST = = 0:117; Metta et al. 2004); Deoni and Red Kandhari cattle (FST = 0:11 Sodhi et al. 2005) and Tharparkar and Rathi (FST = 0.065; Sodhi et. al. 2008) breeds of Indian cattle; 20 Northern European breeds (FST = 0:107; Kantanen et al, 2000); 7 European breeds (FST = 0:112; MacHugh et al. 1998); 18 local cattle breeds from Spain, Portugal, and France (FST = 0:07; Canon et

Table 3. Pair-wise Nei's genetic distance (upper triangle) and Cavalli-Sforza and Edward's chord distance (lower triangle) among three cattle breeds

	Kankrej	Nagori	Tharparkar
Kankrej 0.0000	0.0823	0.0944	
Nagori	0.2472	0.0000	0.0780
Tharparkar	0.2683	0.2436	0.0000

Table 4. Pair-wise FST among three cattle breeds

	Kankrej	Nagori	Tharparkar
Kankrej 0.0000			
Nagori	0.0156	0.0000	
Tharparkar	0.0281	0.0216	0.0000

Table 5. Analysis of molecular variance

Source of variation	Df	Variance Components	% of Variation
Among populations	2	0.14999 Va	2.19
Within Populations	283	6.70534 Vb	97.81
Total	285	6.85533	

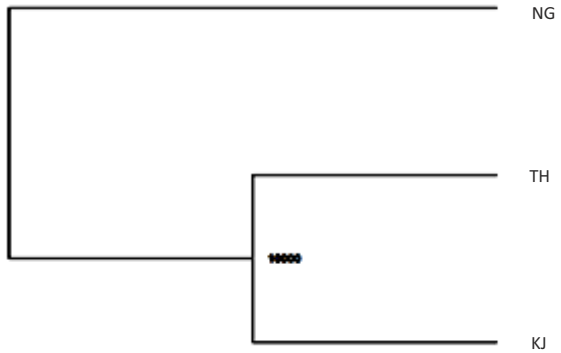

Fig.1 : Neighbour Joining tree based on Nei's Genetic Distance (Figure at nodes indicate bootstrap value)

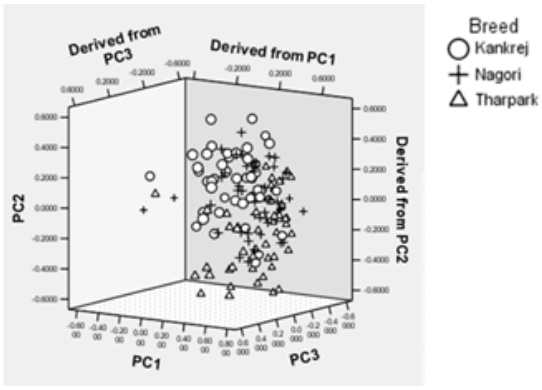

Fig.2 : Scattergram showing relative position of different individuals of three cattle breeds (First 3 PCs explain 36.22% of total variance)

Fig. 3 : Radial tree based on inter-individual Nei's genetic distance following NJ algorithm

al. 2001); 7 French breeds with 23 loci (FST = 0:08; Maudet et al. 2002); eight Southwest European beef cattle breeds (FST = 0:068; Jordana et al. 2003); 5 Korean native breeds (FST = 0:11; Chung et al. 2006) and 10 Brazilian breeds (FST = 0:098; Egito et al. 2007). However, similar lower differentiation between Kenkatha and Gaolao breeds of Indian cattle has been lately described (FST = 0.0219; Chaudhari et al. 2009). Very little differentiation has also been reported between Camelus dromedarius and L. pacos, and between the regional populations of C. dromedarius (southern Africa and the Sudan) ($P < 0.01$) (Nolte et al. 2005). Very low genetic differentiation between the three cattle breeds is exciting in view of the fact that each breed exhibited very high genetic diversity. Poor genetic structuring in the three closely located breeds is, in all probability, due to very high rate of migration between the breeds which is practically operative in their breeding tracts.

This study, thus, presents a comprehensive diversity analysis of closely located Kankrej, Tharparkar and Nagori cattle breeds from Rajasthan state of India. The geographically contiguous breeds revealed very high values of genetic diversity parameters viz. number of

alleles per locus, observed and expected heterozygosity, and polymorphism information content as assessed from 21 microsatellite markers. Despite high genetic diversity values, there was very low differentiation between the three breeds as revealed by FST, AMOVA and FCA (Table 5). This genetic diversity analysis of such closely related cattle breeds will help in conservation prioritization and in making plans that reconcile their genetic improvement with maintenance of genetic variation.

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