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Genetic diversity among Indian Gir, Deoni and Kankrej cattle breeds based on microsatellite markers

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The present study was conducted to examine genetic diversity, genetic differentiation and genetic relationship among Gir, Deoni and Kankrej cattle breeds using microsatellite markers. The number of alleles observed at different loci ranged from 5 (HEL5) to 8 (CSRM60) with a total of 46 alleles across three breeds. The overall heterozygosity and polymorphic information content (PIC) values were 0.730 and 0.749, respectively. Nei's standard genetic distance was least between Gir and Kankrej and highest between Deoni and Kankrej. In the analyzed loci, an overall significant deficit of heterozygotes across these breeds was found and it could be due to inbreeding within breeds. The overall genetic differentiation (F_{ST}) among breeds was moderate, but significantly different. All loci, except INRA035, contributed significantly to the overall differentiation. The highest F_{ST} values were found in HEL5 and lowest in INRA035. The overall N_em value indicated a high rate of genetic flow between the breeds, which is in agreement with their origin of close proximity in the geographical area.

Keywords: Gene flow, genetic distance, heterozygosity, microsatellite, PIC, Zebu cattle

Introduction

India is one of the mega biodiversity centers of the world and rich in farm animal diversity with 30 phenotypically characterized breeds of zebu cattle[1]. Dairy industry requires development of very standardized cattle herds to fulfill their commercial needs that reflects on selection practices in breeding programmes. The extensive selection and multiplication of superior animals cause a significant decrease in the genetic base of the germplasm, which is the major source of the genetic variation needed for the improvement in economic traits and breeds. Hence, there is an urgent need to prevent the rapid erosion of animal genetic resources. This is true for the breeds especially in developing and underdeveloped countries, where many will get vanished without even having been adequately characterized or studied.

The native breeds are multipurpose with unique genetic characteristics[2] and are often well adapted to home tract conditions, climate, diseases and nutritional environment. Conservation of such genetic groups is crucial. In such pursuit, the first step is the evaluation of genetic resources and the selection of appropriate populations for conservation. Estimation of genetic diversity is essential to decide the priority of the population to be conserved.

The traditional evaluation methods for breeding traits, such as, yield, type and morphology, have little power to detect subtle changes in the genetic variation of populations. DNA-based molecular markers with high level of polymorphism have been successfully used to evaluate genetic variation of breeds in breeding programmes and conservation. The molecular markers are the potential tool for geneticists and breeders to evaluate existing germplasm and to manipulate it to develop character specific strains, and to provide the basis for effective genetic conservation. Among the several types of molecular markers available, microsatellites represent the most powerful tools. They are highly polymorphic[3], dispersed throughout genome at a frequency of one at every 6 kb nucleotide sequence, co-dominantly inherited and amenable to PCR

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amplification, which make them potentially very useful DNA markers to investigate the genetic properties of breeds and to identify genes that code for important traits. Microsatellites are potential markers to study genetic variation[4], parentage determination, gene flow, hybridization, genetic distance and diversity[5] of indigenous cattle breeds.

The Western part of India is endowed with excellent cattle breeds, viz,. Gir, Deoni (as milch) and Kankrej (as dual-purpose). The Gir is one of the principal Zebu or *Bos indicus* milch breeds originated from Gujarat; however, it is used for both dairy and beef production in other countries. These cows are good milkers and bullocks are well suited for heavy work. The home tract of Deoni cattle is Latur, Parbhani, Nanded and Osmanabad districts of Maharashtra and Bidar district of Karnataka. Kankrej, one of the heaviest Indian cattles, is highly suitable as drought cattle breed originated from South-eastern Rann of Kutch, Gujarat, and Rajasthan.

The present study was carried out to examine the genetic diversity, genetic differentiation and genetic relationship among Gir, Deoni and Kankrej breeds of Indian cattle through microsatellite DNA polymorphisms.

Materials and Methods

Experimental material for the present study comprised of blood samples collected at random from their respective breeding tracts, i.e., Junagadh, Bhavnagar and Rajkot districts (Gujarat) for Gir; Banaskatha, Patan and Mehsana districts (Gujarat) for Kankrej; and Latur, Osmanabad and Parbhani districts (Maharashtra) for Deoni. The number of animals used for different breeds for microsatellite analysis varied as per the details given in the Table 1. The DNA was isolated from blood as per John's method[6] using standard phenol-chloroform extraction method. The evaluation of quality and purity of DNA was done by agarose gel electrophoresis, and the concentration of DNA was estimated by UV spectrophotometer.

The seven microsatellite loci, viz., ETH225, CSRM60, HEL5, INRA005, INRA035, ILSTS002 and ILSTS006, were selected from the available list of 30 microsatellites for estimation of genetic diversity in cattle[7]. PCR reaction was carried out in a final reaction volume of 25 µL in a thermal cycler (Biometra Ltd., Eppendorf). The annealing temperature for INRA005 and HEL5 was optimized at 50°C; ETH225, INRA035, CSRM60, ILSTS002 at 54°C; and ILSTS006 at 55°C. Optimization for $MgCl_2$ was carried out depending up on the demand of microsatellite. Initially, dNTP was taken at 200 µ*M*, further it was reduced to 150 µ*M*. Similarly, Taq polymerase was initially attempted with 1 U, later it was reduced to 0.5 U.

Microsatellite analysis was done in 7% polyacrylamide gel electrophoresis using Sequi-Gen GT nucleic acid sequencing cell (Bio-Rad Laboratories). Microsatellite alleles were visualized by silver staining. The allele and genotype frequencies were scored by directly counting the bands. The average heterozygosity and polymorphic information content (PIC) values of all selected marker loci were calculated using the appropriate equations[8]. The D_s (genetic distance) was estimated online[9] using the gene frequencies of microsatellite loci. A phylogenic tree was constructed by Neighbor-Joining algorithm[10] using POPGENE program version 1.31. The population differentiation was estimated by fixation indexes[11] F_{IT}, F_{ST} and F_{IS} across the populations according to the variance based method[12] using GENEPOP program (version 1.31). The gene flow (N_em) was calculated from F_{ST} values using GENEPOP computer programme[13] (where N_e is effective population size and m is the proportion of migrants).

Results

The number of alleles, size range of alleles, PIC, heterozygosity and effective number of alleles for the three breeds are given in Table 1. All the seven loci were polymorphic, and the number of alleles varied between 5 (HEL5) and 8 (CSRM60) with little difference between the cattle breeds.

In the present study, except HEL5, all other microsatellites (ETH225, CSRM60, INRA005, INRA035, ILSTS002 & ILSTS006) presented high levels of variability. The total numbers of observed alleles across all loci studied were found to be 46. In total 37 alleles were observed in Gir cattle with maximum alleles (7) contributed by locus CSRM60 and the least (3) alleles by HEL5; while 40 alleles were observed in Deoni breed with maximum 8 alleles contributed by locus CSRM60 and the least 4 alleles by HEL5 and INRA035. In case of Kankrej breed also, 40 alleles were observed with maximum alleles (8) contributed by locus CSRM60 and the least (5) contributed by HEL5, INRA035 and ILSTS002.

Table 1—Genetic characteristics of seven microsatellite loci in three Indian native cattle breeds

Locus	Breed	No. of animals	No. of alleles	Size range (bp)	PIC	*H*	n_e
ETH225	Gir	52	5	142-160	0.599	0.644	2.76
	Deoni	43	6	142-160	0.651	0.699	3.24
	Kankrej	46	6	142-162	0.482	0.511	2.02
	Across population	141	7	142-162	0.602	0.633	2.70
CSRM60	Gir	46	7	96-120	0.722	0.764	4.11
	Deoni	40	8	94-120	0.717	0.755	3.94
	Kankrej	43	8	94-120	0.673	0.721	3.48
	Across population	129	8	94-120	0.836	0.856	4.10
HEL5	Gir	47	3	153-165	0.258	0.292	1.41
	Deoni	22	4	151-165	0.582	0.656	2.84
	Kankrej	50	5	151-167	0.491	0.563	2.50
	Across population	119	5	151-167	0.514	0.572	5.00
INRA005	Gir	52	6	138-150	0.761	0.801	4.83
	Deoni	24	5	138-148	0.602	0.649	2.79
	Kankrej	49	7	138-150	0.759	0.799	4.76
	Across population	125	7	138-150	0.797	0.825	5.60
INRA035	Gir	52	5	104-112	0.687	0.743	3.77
	Deoni	31	4	104-112	0.664	0.725	3.53
	Kankrej	42	5	104-114	0.681	0.735	3.65
	Across population	125	6	104-114	0.684	0.735	3.70
ILSTS002	Gir	39	5	132-140	0.686	0.738	3.71
	Deoni	47	6	124-140	0.491	0.543	2.15
	Kankrej	38	5	124-140	0.702	0.749	3.85
	Across population	124	6	124-140	0.665	0.705	3.40
ILSTS006	Gir	30	6	290-300	0.774	0.729	4.28
	Deoni	45	7	288-300	0.766	0.719	4.12
	Kankrej	47	6	290-300	0.681	0.617	3.06
	Across population	122	7	288-300	0.749	0.785	4.60

PIC: Polymorphism Information Content
H: Heterozygosity
n_e: Effective Number of Alleles

The mean observed alleles and effective alleles were found to be 6.57±0.97 and 4.16±0.16, respectively across the breeds and loci studied. In Gir cattle, the mean observed and effective number of alleles were found to be 5.29±1.25 and 3.55±1.13, respectively across all loci; whereas, the mean observed and effective number of alleles in Deoni cattle breed were 5.71±1.5 and 3.24±0.693, respectively across all loci. On the other hand, in case of Kankrej cattle, mean observed and effective number of alleles were found to be 6.0±1.5 and 3.30±0.94, respectively across all loci studied.

The average heterozygosity was highest (0.856) for locus CSRM-60 and least (0.572) for HEL5 locus. In Gir, the average heterozygosity was observed to be 0.679±0.09 across all the loci; the observed and expected heterozygosity were highest (0.769 and 0.800, respectively) for INRA005 locus and least (0.127 and 0.291, respectively) for HEL5 locus. In Deoni cattle, average heterozygosity was observed to be 0.674±0.09 across all the loci; the observed heterozygosity (0.444) and expected heterozygosity (0.766) were highest for ILSTS006 locus and least (0.489 and 0.542, respectively) for ILSTS002 locus. In Kankrej, average heterozygosity was observed to be 0.674±0.09 across all the loci; the observed heterozygosity (0.816) and expected heterozygosity (0.798) were highest for INRA005 locus and least (0.500 and 0.511, respectively) for ETH225 locus.

The PIC values across the loci were found to be highest (0.836) for locus CSRM60 and least (0.514) for HEL5 locus. For Gir cattle, the highest PIC value (0.761) was observed at INRA005 locus and least (0.258) at HEL5 locus. For Deoni cattle, the highest PIC value (0.719) was observed at ILSTS006 locus and least (0.491) at ILSTS002 locus. For Kankrej cattle, the highest PIC value (0.759) was observed at INRA005 locus and least (0.482) at ETH225 locus.

Hardy-Weinberg equilibrium for genotype distributions in all systems was not always maintained ($p<0.05$). Other than HEL5, all other microsatellites exhibited high levels of variability. The results for Nei's standard genetic distance indicated that Deoni breed was distinct from other two breeds. Genetic distance was least (0.203) between Gir and Kankrej and highest between Deoni and Kankrej (0.444).

The other important aspects of the study were to measure the deviations of genotype frequencies using three parameters F_{IS}, F_{IT}, and F_{ST} and gene flow (N_em) among the breeds. The global deficit of heterozygotes (F_{IT}) across breeds amounted to 20.38% ($p<0.001$). An overall significant deficit of heterozygotes (F_{IT}) across breeds of 20.38% ($p<0.001$) occurred in the analyzed loci because of inbreeding within populations. Four loci contributed significantly to the heterozygote deficit (F_{IS}) within breeds, while all the other loci, except ETH225, affected the global heterozygosity deficit. Among all the breeds, the overall genetic differentiation (F_{ST}) was moderate (8.56%) but significantly different from zero. All the loci except INRA035 contributed significantly ($p<0.001$) to the overall differentiation. The highest F_{ST} values were found for HEL5 (0.232) and lowest for INRA035 (0.004). The overall N_em value indicated a high rate of genetic flow between the breeds (2.67).

Discussion

In the present study, the genetic polymorphism in the three cattle breeds, Gir, Deoni and Kankrej, were analyzed using seven microsatellite markers, which were known to be polymorphic in taurine cattle[14]. All the seven microsatellite loci were polymorphic, and the number of alleles varied between 5 (HEL5) and 8 (CSRM60), with little difference between the cattle breeds. The Food and Agriculture Organization (FAO) suggests that five different alleles per locus are required for estimation of genetic differences between breeds. A total of seven alleles were observed in locus ILSTS006, which is in agreement with the result reported in zebu population[15,16]. The 7 alleles were reported for HEL5 in French cattle[17]; however, Indian Tharparkar and Rathi cattle exhibited 7 and 10 alleles[18]. The genetic diversity in the breeds was expressed in terms of average heterozygosity. In the present study, average heterozygosity across all the loci was 0.730, indicating high genetic variation. The zebu cattle displayed a relatively high heterozygosity compared with European cattle breeds[19].

The PIC is a parameter indicative of the degree of informativeness of a marker. In contrast to Deoni, ILSTS002 locus was found to be highly informative locus with 0.772 PIC value in Spanish cattle[20]. Following the criteria, in the present study, microsatellite locus HEL5 appeared to be only moderately informative (0.51), whereas the other mirosatellite loci studied were highly informative (0.60-0.84). According to the selection standard[21], microsatellite loci ought to have at least 4 alleles to be considered useful for the evaluation of genetic diversity. Based on these observations, it can be stated that the seven microsatellite loci used in the present study can be considered useful for the evaluation of genetic diversity in cattle breeds.

The three cattle breeds screened were not found to be in accordance with Hardy-Weinberg equilibrium proportions for several microsatellite frequencies. The Nei's standard genetic distance matrix was generated from calculated allele frequencies that were in turn used to build a dendrogram following UPGMA clustering (Fig. 1). The clusters obtained on phylogenetic tree agreed with the geographic origin of the breeds. The high gene flow between the breeds is in agreement with their origin of close proximity in the geographical area.

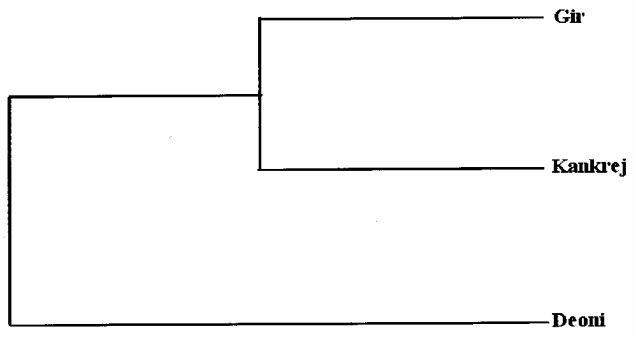

Fig. 1—The phylogenetic relationship among Indian Gir, Deoni and Kankrej breed based on seven microsatellite loci (Nei's genetic distance with bootstrap 100)

The present study is an useful attempts to understand genetic diversity of native Indian cattle breeds using microsatellite DNA markers. Further investigations including more native Indian cattle breeds would be useful to clarify their recent origin and relationships between them. Estimation of genetic diversity is important to explore heterosis in future complementary crosses within Zebu breeds and also for crosses between Zebu and European breeds.

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