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Original Article

Genetic Diversity Analysis of ten Indigenous Grey Cattle Breeds (*Bos indicus*) from different Agroclimatic regions of India using Microsatellite Markers

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Abstract

The aim of the present study was to assess the genetic variation and establish the relationship amongst the ten Indian grey cattle breeds using 21 bovine-specific microsatellite markers. A total of 460 unrelated DNA samples from Hariana, Kankrej, Mewati, Nagori, Tharparkar, Ghumusari, Hill Cattle, Kangayam, Binjharpuri and Punganur breeds of cattle were genotyped to estimate within and between breed genetic diversity indices. The estimated mean allelic diversity was from Tharparkar (9) to Hill Cattle (12.76), with a total of 448 alleles. The observed heterozygosity (H_o) ranged from 0.682 (Hill Cattle) to 0.768 (Hariana), while the expected heterozygosity (H_e) ranged from 0.700 (Tharparkar) to 0.781 (Hill cattle). The F_{ST} estimates demonstrated that approximately 92.38% of the total genetic variation was because of the genetic differentiation within population and about 7.62% of the total genetic variation corresponded to among population. Pair-wise breed differentiation, Nei's standard and D_C genetic distance estimates revealed almost close genetic similarity between Hariana-Mewati and Nagori-Mewati respectively in comparison to other breeds. In the UPGMA-based phylogenetic tree constructed from the genetic distances, the five populations of Hariana, Kankrej, Mewati, Nagori and Tharparkar individuals with admixture, whereas, Ghumusari, Hill Cattle and Kangayam individuals showed clear distinctness and formed separate clusters whereas Binjharpuri and Punganur formed separate clusters. Similar relationship among ten breeds was realized using different approaches, i.e. analysis of Molecular variance and PCA.

Keywords: Cattle, Microsatellite markers, Genetic diversity, F_{ST}, Phylogenetic relationship, Correspondence analysis, Conservation.

Introduction

The Indian cattle breeds, also known as *zebu* cattle (*Bos indicus*) are broadly categorized into dairy, draft and dual purpose breeds depending upon their utility either in dairying or in agricultural work. The dual-purpose breeds have specific qualities like disease resistance, heat tolerance, ability to survive and reproduce under stress and low feed input. *Zebu* cattle are used in cross breeding programs as they can adapt to hot and humid climates [38, 33]. However, a number of these breeds are now being bred out because of intensive cross breeding with high milk producing exotic breeds and reduction of emphasis on draft ability due to mechanization of agriculture and transport. As a result, some of the native draft breeds are on the verge of extinction. Hence, there is an urgent need to conserve these breeds. Breed characterization is the primary step in any conservation programme. However most of such studies have been done on European cattle breeds and very little information is available concerning the genetic diversity of cattle breeds native to India.

Microsatellites in particular are useful in conservation genetics because the high degree of polymorphism makes them extremely informative and gives them very high discriminating power [31], allowing for a thorough assessment of genetic variation and structure within and among populations [46]. Microsatellites have proved to be useful polymorphic markers for the analysis of genetic diversity. Microsatellite based studies in livestock have mainly concentrated on pig, cattle and sheep and there are now more than a thousand cattle microsatellite markers to choose from [54, 52]. Awareness of the value of genetic resources in livestock has stimulated the study of the genetic diversity of native breeds. The purpose of the present study was to examine genetic diversity among ten native Indigenous grey cattle breeds from different agro-climatic regions of India through examining microsatellite DNA polymorphisms. We also estimated the genetic differentiation and the genetic relationship within and between the ten native Indian cattle breeds to serve as guide for decisions regarding conservation and management.

Materials and Methods

Collection of Blood and Extraction of DNA

Fresh blood samples (7-8 ml) were collected randomly from 40-50 genetically unrelated animals of Hariana, Kankrej, Mewati, Nagori, Tharparkar, Ghumusari, Hill Cattle, Kangayam, Binjharpuri and Punganur cattle breeds from their respective native breeding tracts. Blood samples were collected by jugular vein puncture, using *Vacutainer*® blood collection tubes treated with 0.25% ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Genomic DNA was isolated from blood samples as described by [30].

Microsatellite Genotyping

A total of 21 bovine specific microsatellite markers recommended in MoDAD project of FAO [18, 19] were utilized to generate data. Only forward primers at 5' end of each pair were labeled with one of the four fluorophore i.e., FAM (Blue), VIC (Green), NED (Yellow) and PET (red). The PCR amplification was carried out in 25 µl reaction volume containing 1.5 mM $MgCl_2$, 200 µM dNTPs, 50 ng each of forward and reverse primer, 50-100 ng of genomic DNA and 0.5 Units of Taq DNA polymerase (Bangalore Genei, India). PCR products were loaded on to a 2% agarose gel, electrophoresed and visualized over UV light after ethidium bromide staining to detect the amplification. Genotyping was performed on automated DNA sequencer of Applied Biosystems (ABI 3100 Avant). The electropherograms drawn through Gene Scan were used to extract DNA fragment sizing details using Gene Mapper software (version 3.0) (Applied Biosystems).

Statistical analysis

Observed number of alleles, effective number of alleles, observed and expected heterozygosity estimates were computed after Nei [40] using POPGENE software [17]. Polymorphism information content (PIC) was calculated using allele frequencies according to the formula [12]. Departure from Hardy–Weinberg equilibrium based on exact test was performed using POPGENE. Heterozygote deficiencies at each locus and linkage disequilibrium among the loci were computed using FSTAT software [7]. These parameters of population structure are F_{IT}, F_{ST} and F_{IS}.

Results and Discussion

Genetic Variation within breeds

The observed and effective number of alleles, private alleles, heterozygosity and PIC for the ten breeds is given in Table 1-2. A total of 448 microsatellite alleles were amplified in 21 loci and 460 animals belonging to the ten breeds with mean number of 21.33 alleles at each locus (Fig. 1).

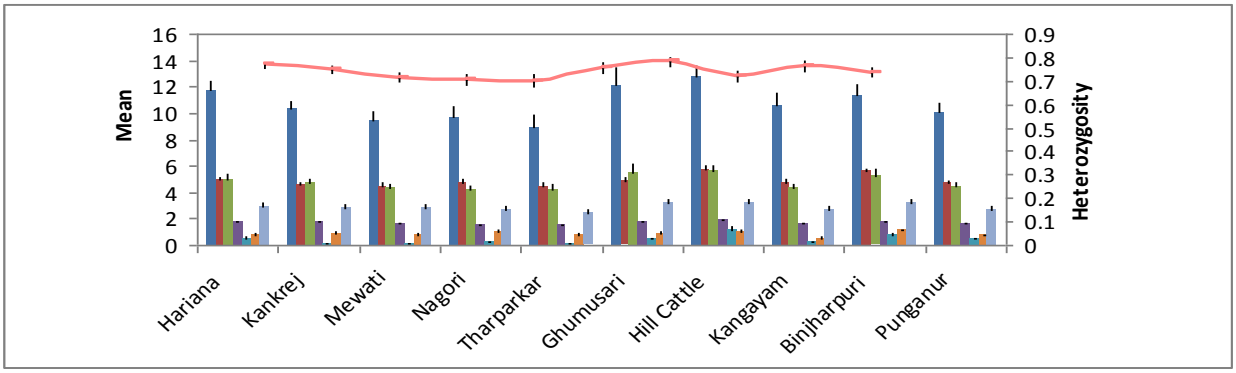

Fig. 1 The overall allelic pattern in terms of number of alleles, effective number of alleles and observed heterozygosity

The estimated mean allelic diversity was from Tharparkar (9) to Hill Cattle (12.76), with a total of 448 alleles. The mean numbers of alleles were not significantly different among the ten cattle populations. The most polymorphic marker was ILST05 with a total of 35 alleles and the least polymorphic marker war INRA05 with 11 alleles each. The Hill Cattle breed had the largest (23) private alleles, followed by Binjharpuri (16), Hariana (12), Ghumusari (10), Punganur (10), Kangayam (6), Nagori (4), Mewati (3), Kankrej (2) and Tharparkar (2). Two loci *viz*., HEL1 and HEL09 showed no private allele in any of the ten breeds (Table 1-2).

Table 1 Observed and effective number of alleles in Hariana, Kankrej, Mewati, Nagori and Tharparkar cattle breeds

	Hariana		**Kankrej**		**Mewati**		**Nagori**		**Tharparkar**	
	Na	**Ne**	**Na**	**Ne**	**Na**	**Ne**	**Na**	**Ne**	**Na**	**Ne**
CSRM60	12	3.661	10	3.248	8	2.271	9	1.928	7	2.661
ETH10	9	4.161	10	4.445	8	3.901	7	3.811	6	3.595
ILSTS11	8	2.366	8	2.279	4	2.143	6	1.923	6	1.589
TGLA122	14	8.798	13	7.769	10	7.071	12	6.276	10	5.302
INRA05	7	4.236	8	5.547	7	5.329	7	4.667	6	3.429
INRA63	7	3.094	8	2.326	7	2.163	7	2.396	5	3.012
TGLA227	10	3.228	7	2.629	9	2.326	8	2.512	8	2.468
CSSM08	11	4.402	8	5.247	7	3.569	7	3.531	6	4.209
HEL05	10	5.153	8	4.198	8	4.772	9	3.683	8	4.662
ILSTS05	13	6.151	12	5.415	9	5.446	9	4.816	11	4.717
ILSTS33	13	4.093	8	3.066	9	3.284	8	2.429	7	3.311
INRA35	15	6.972	11	5.867	11	5.391	9	5.306	8	4.859
BM1824	7	2.622	6	2.696	5	2.535	4	2.065	5	1.944
CSSM66	13	6.551	11	4.716	10	4.068	12	5.152	10	4.753
ETH03	10	4.015	8	2.758	7	2.640	6	2.776	7	2.978
ETH225	10	2.782	9	2.792	8	2.108	10	2.460	7	1.573
MM12	16	4.422	13	4.321	13	4.050	11	3.234	9	3.342
CSSM33	16	8.687	15	7.461	19	10.476	21	8.529	21	9.294
HEL01	17	5.286	18	8.932	15	3.639	18	7.306	20	9.710
HEL09	13	8.977	13	8.742	11	7.670	12	8.383	11	7.552
ILSTS34	17	5.511	13	4.549	15	5.641	13	5.012	11	3.373
Mean	11.810	5.008	10.333	4.714	9.524	4.309	9.762	4.200	9.000	4.206
SE	0.716	0.441	0.660	0.454	0.777	0.471	0.876	0.449	0.934	0.489

Na-Number of alleles, Ne-Effective number of alleles

Table 2 Observed and effective number of alleles in Ghumusari, Hill Cattle, Kangayam, Binjharpuri and Punganur cattle breeds

	Ghumusari		Hill Cattle		Kangayam		Binjharpuri		Punganur	
	Na	**Ne**	**Na**	**Ne**	**Na**	**Ne**	**Na**	**Ne**	**Na**	**Ne**
CSRM60	21	7.732	21	7.706	16	4.418	18	7.024	13	5.344
ETH10	7	3.044	10	4.820	10	5.120	13	4.551	5	3.051
ILSTS11	7	2.540	6	2.566	4	2.212	9	3.308	7	2.308
TGLA122	13	7.642	18	8.597	14	5.505	15	8.727	12	6.464
INRA05	7	5.195	8	6.050	8	5.731	6	4.247	5	3.384
INRA63	9	2.727	14	4.907	4	1.518	5	3.431	6	3.580
TGLA227	6	1.940	14	3.182	8	2.309	8	1.520	6	2.236
CSSM08	5	1.895	6	2.411	5	3.308	8	3.421	8	2.490
HEL05	11	6.136	11	6.242	11	6.024	7	4.142	12	4.699
ILSTS05	26	5.908	16	4.166	18	3.291	14	6.564	15	6.261
ILSTS33	22	13.322	20	10.990	18	7.556	13	6.847	15	5.355
INRA35	11	6.847	14	8.662	9	4.452	16	9.289	13	6.964
BM1824	3	1.947	7	2.515	4	1.819	7	3.211	9	3.933
CSSM66	10	5.020	13	4.664	10	4.902	8	4.640	11	4.670
ETH03	8	4.655	8	3.689	9	5.893	14	3.949	10	2.979
ETH225	15	5.064	10	2.952	13	2.876	11	3.869	10	2.802
MM12	23	11.973	20	9.640	19	7.622	13	4.482	12	5.301
CSSM33	12	7.758	13	9.089	14	5.870	16	9.580	14	8.125
HEL01	14	3.678	12	3.986	10	3.287	8	1.971	7	1.899
HEL09	11	7.178	11	6.463	10	4.046	11	6.747	9	5.226
ILSTS34	15	4.315	16	4.902	10	3.586	20	9.804	14	7.714
Mean	12.190	5.548	12.76	5.629	10.67	4.350	11.43	5.301	10.14	4.514
SE	1.375	0.672	0.998	0.566	1.002	0.384	0.919	0.544	0.723	0.407

Na-Number of alleles, Ne-Effective number of alleles

The allelic diversity detected in the ten investigated breeds is generally higher than those previously reported for 22 acknowledged indigenous breeds and 5 hitherto uncharacterized populations from India, which varied from 3.88 to 9.60 using microsatellite markers [5] as well as international breeds [13, 34, 37, 28, 27, 11, 1, 53, 32, 25, 24, 36].

Comparable or higher allelic diversity has been reported in 9 European breeds [29], 27 Chinese breeds [21] and 10 Brazilian breeds [2], 3 breeds of Italy [4]. This may be due to the use of denaturing

polyacrylamide sequencing gels and silver staining in earlier studies, which possibly does not discover all the alleles, especially the alleles differing by few nucleotides [5].

Elevated allelic diversity in Indian cattle might also be attributed to lack of any appreciable selection pressure due to negligible use of AI under field conditions and thus signifies the existence of larger effective population size of the explored Indian cattle breeds. In general higher number of alleles per locus has been detected in zebu breeds compared to taurine breeds [2].

The observed heterozygosity (H_o) ranged from 0.682 (Hill Cattle) to 0.768 (Hariana), while the expected heterozygosity (H_e) ranged from 0.700 (Tharparkar) to 0.781 (Hill cattle) (Table 3-4).

Table 3 Heterozygosity of Hariana, Kankrej, Mewati, Nagori and Tharparkar cattle breeds

	Hariana		**Kankrej**		**Mewati**		**Nagori**		**Tharparkar**	
	Ho	**He**	**Ho**	**He**	**Ho**	**He**	**Ho**	**He**	**Ho**	**He**
CSRM60	0.652	0.727	0.705	0.692	0.628	0.560	0.469	0.481	0.640	0.624
ETH10	0.696	0.760	0.818	0.775	0.605	0.744	0.694	0.738	0.800	0.722
ILSTS11	0.457	0.577	0.500	0.561	0.465	0.533	0.408	0.480	0.300	0.371
TGLA122	0.870	0.886	0.791	0.871	0.884	0.859	0.936	0.841	0.800	0.811
INRA05	0.891	0.764	0.864	0.820	0.721	0.812	0.776	0.786	0.740	0.708
INRA63	0.630	0.677	0.523	0.570	0.581	0.538	0.531	0.583	0.660	0.668
TGLA227	1.000	0.690	0.955	0.620	0.907	0.570	1.000	0.602	0.920	0.595
CSSM08	0.689	0.773	0.841	0.809	0.884	0.720	0.714	0.717	0.640	0.762
HEL05	1.000	0.806	0.907	0.762	0.977	0.790	1.000	0.728	0.959	0.786
ILSTS05	0.870	0.837	0.932	0.815	0.977	0.816	0.796	0.792	0.840	0.788
ILSTS33	0.587	0.756	0.659	0.674	0.674	0.696	0.612	0.588	0.680	0.698
INRA35	0.870	0.857	0.659	0.830	0.674	0.814	0.735	0.812	0.820	0.794
BM1824	0.739	0.619	0.659	0.629	0.651	0.605	0.551	0.516	0.420	0.486
CSSM66	0.761	0.847	0.841	0.788	0.791	0.754	0.796	0.806	0.780	0.790
ETH03	0.761	0.751	0.750	0.637	0.581	0.621	0.571	0.640	0.680	0.664
ETH225	0.652	0.641	0.636	0.642	0.581	0.526	0.653	0.594	0.360	0.364
MM12	0.652	0.774	0.750	0.769	0.837	0.753	0.714	0.691	0.680	0.701
CSSM33	0.902	0.885	0.841	0.866	0.977	0.905	0.898	0.883	0.960	0.892
HEL01	0.732	0.811	0.674	0.888	0.488	0.725	0.682	0.863	0.766	0.897
HEL09	0.976	0.889	0.837	0.886	0.881	0.870	0.787	0.881	0.894	0.868
ILSTS34	0.732	0.819	0.805	0.780	0.780	0.823	0.867	0.800	0.809	0.703
Mean	0.768	0.769	0.759	0.747	0.740	0.716	0.723	0.706	0.721	0.700
SE	0.032	0.019	0.027	0.023	0.036	0.027	0.036	0.029	0.040	0.032

Ho-Observed Heterozygosity, He-Expected Heterozygosity

Table 4 Heterozygosity of Ghumusari, Hill Cattle, Kangayam, Binjharpuri and Punganur cattle breeds

	Ghumusari		Hill Cattle		Kangayam		Binjharpuri		Punganur	
	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He
CSRM60	0.792	0.871	0.729	0.870	0.833	0.774	0.833	0.858	0.833	0.813
ETH10	0.646	0.671	0.792	0.793	0.979	0.805	0.696	0.780	0.686	0.672
ILSTS11	0.458	0.606	0.438	0.610	0.438	0.548	0.667	0.698	0.361	0.567
TGLA122	0.896	0.869	0.875	0.884	0.958	0.818	0.813	0.885	0.806	0.845
INRA05	0.875	0.808	0.970	0.835	0.813	0.826	0.771	0.765	0.639	0.704
INRA63	0.646	0.633	0.571	0.796	0.375	0.341	0.750	0.709	0.861	0.721
TGLA227	0.375	0.485	0.583	0.686	0.667	0.567	0.292	0.342	0.528	0.553
CSSM08	0.188	0.472	0.271	0.585	0.271	0.698	0.744	0.708	0.633	0.598
HEL05	0.738	0.837	0.761	0.840	0.854	0.834	0.889	0.759	0.742	0.787
ILSTS05	0.792	0.831	0.625	0.760	0.792	0.696	0.889	0.848	0.639	0.840
ILSTS33	0.800	0.925	0.766	0.909	0.667	0.868	0.875	0.854	0.528	0.813
INRA35	0.792	0.854	0.833	0.885	0.750	0.775	0.733	0.892	0.765	0.856
BM1824	0.167	0.486	0.458	0.602	0.319	0.450	0.938	0.689	0.889	0.746
CSSM66	0.717	0.801	0.542	0.786	0.646	0.796	0.708	0.785	0.833	0.786
ETH03	0.833	0.785	0.646	0.729	0.708	0.830	0.854	0.747	0.583	0.664
ETH225	0.750	0.803	0.479	0.661	0.542	0.652	0.771	0.742	0.556	0.643
MM12	0.574	0.916	0.609	0.896	0.591	0.869	0.771	0.777	0.639	0.811
CSSM33	0.833	0.871	0.938	0.890	0.938	0.830	0.875	0.896	0.917	0.877
HEL01	0.771	0.728	0.771	0.749	0.729	0.696	0.542	0.493	0.500	0.473
HEL09	0.938	0.861	0.917	0.845	0.792	0.753	0.750	0.852	0.889	0.809
ILSTS34	0.771	0.768	0.750	0.796	0.750	0.721	0.833	0.898	0.944	0.870
Mean	0.683	0.756	0.682	0.781	0.686	0.721	0.762	0.761	0.703	0.736
SE	0.047	0.031	0.040	0.022	0.044	0.031	0.031	0.030	0.035	0.026

Ho-Observed Heterozygosity, He-Expected Heterozygosity

These values were not significantly different among the ten cattle populations. The genetic variability computed in the ten breeds was either higher or comparable with other Indian breeds and ranged from 0.460 to 0.789 in 21 Indian breeds [5]. Heterozygosity estimates, in general, were moderate to high (>0.45) in different breeds investigated worldwide (13, 28, 27, 1, 35, 11, 53, 22, 2, 25, 41, 24, 51, 9], but lower than estimated in the 10 breeds in this study.

Higher estimates of observed and expected heterozygosity observed in the these breeds are indicative of low inbreeding, which is also substantiated by very low mean inbreeding coefficients (F_{IS}) across 21 loci in all the ten breeds (mean F_{IS}=0.033, ranged from -0.259 (TGLA227) to 0.198 (ILSTS11) across loci. The overall value obtained is not statistically different from zero. The results of Weir and Cockerham's F-

Statistics for each locus across all the populations are given in Table 5. High heterozygosity is indicative of high variability and is a desirable attribute for a population for improvement and conservation.

The high PIC values obtained for most of the markers suggest their usefulness in the evaluation of the biodiversity of native Indian cattle breeds.

Table 5 Weir & Cockerham (1984) estimation of F_{IT} (Cap F), F_{ST} (theta) and F_{IS} (small F)

Locus	Capf (F_{IT})	Theta (F_{ST})	Smallf (F_{IS})
CSRM60	0.111	0.079	0.035
ETH10	0.059	0.044	0.016
ILSTS11	0.222	0.03	0.198
TGLA122	0.033	0.031	0.002
INRA05	0.03	0.046	-0.017
INRA63	0.068	0.038	0.031
TGLA227	-0.064	0.154	-0.259
CSSM08	0.281	0.135	0.168
HEL05	-0.056	0.047	-0.108
ILSTS05	0.086	0.094	-0.008
ILSTS33	0.161	0.043	0.124
INRA35	0.134	0.042	0.096
BM1824	0.137	0.114	0.026
CSSM66	0.138	0.061	0.082
ETH03	0.078	0.056	0.024
ETH225	0.208	0.161	0.056
MM12	0.2	0.056	0.152
CSSM33	-0.003	0.018	-0.021
HEL01	0.265	0.185	0.098
HEL09	0.017	0.021	-0.004
ILSTS34	0.125	0.121	0.004
Mean	0.105	0.075	0.033

Nm Gene flow estimated from F_{ST}=0.25(1- F_{ST})/ $F_{ST.}$

In Hariana population, 21 loci were studied out of which 11 were deviated from Hardy-Weinberg Equilibrium using χ2 test, similarly other populations shows different number of loci which were deviated Hardy-Weinberg Equilibrium such as 8 in Kankrej, 6 in Mewati, 16 in Nagori, 8 in Tharparkar, 12 in Ghumusari, 13 in Hill Cattle, 15 in Kangayam, 10 in Binjharpuri and 11 in Punganur Population. Significant departure from HWE is a general occurrence and has been reported in several cattle breeds from different parts of the world [28, 10, 11, 1, 49, 50, 42, 25, 3, 45, 9, 15, 48, 16, 47, 6]. 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

The genetic relationship between the ten populations was determined using Nei's standard genetic distance (D_S) as well as Cavalli-Sforza and Edwards, D_C shown in Table 6. The largest Nei's standard genetic distance, Ds was estimated between Punganur and Kangayam cattle, 0.654, while the least distance was between Hariana-Kankrej and Hariana-Mewati cattle, 0.046, which were in similar range that was reported in Spanish cattle [26]. The D_S distance value observed in this study was also comparable to other Indian as well as taurine cattle breeds [40, 42, 44, 45, 11, 43, 20, 41 and 15]. Similar relationship was obtained in Cavalli-Sforza and Edwards, D_C method as shown in Table 6.

Table 6 Nei's standard genetic distance, D_S (1972) below diagonal and Cavalli-Sforza and Edwards, D_C (1967) above diagonal

Breeds	Hariana	Kankrej	Mewati	Nagori	Tharparkar	Ghumusari	Hill Cattle	Kangayam	Binjharpuri	Punganur
Hariana	0.000	0.231	0.234	0.255	0.278	0.392	0.405	0.430	0.399	0.427
Kankrej	0.046	0.000	0.228	0.247	0.268	0.387	0.396	0.424	0.401	0.443
Mewati	0.046	0.050	0.000	0.214	0.258	0.387	0.403	0.423	0.410	0.450
Nagori	0.070	0.071	0.052	0.000	0.244	0.421	0.438	0.450	0.432	0.467
Tharparkar	0.109	0.106	0.111	0.080	0.000	0.435	0.444	0.444	0.435	0.472
Ghumusari	0.341	0.333	0.351	0.383	0.446	0.000	0.263	0.352	0.424	0.459
Hill Cattle	0.330	0.315	0.331	0.378	0.401	0.066	0.000	0.376	0.439	0.487
Kangayam	0.374	0.367	0.367	0.400	0.423	0.234	0.243	0.000	0.482	0.508
Binjharpuri	0.361	0.366	0.416	0.425	0.461	0.435	0.468	0.593	0.000	0.319
Punganur	0.422	0.425	0.482	0.483	0.507	0.549	0.566	0.654	0.114	0.000

Distance based phylogenetic analysis describe the evolutionary relationship between the ten cattle breeds in the form of graphical representation (Fig. 2-3). The un-rooted UPGMA dendrogram constructed from the Nei's standard genetic distance, Ds reveals that the populations of Hariana-Mewati breeds showed closest relationship with a high bootstrap value of 36%. Hariana- Mewati was

joined by Kankrej, Nagori and Tharparkar with 36%, 61% and 82% bootstrap values, respectively. These five breeds further joined with another node comprising of Ghumusari, Hill Cattle and Kangayam, with a bootstrap support of 86%. The node with Ghumusari and Hill Cattle was also supported by reasonably high bootstrap value of 100% and joined by Kangayam with 86% bootstrap values. These eight breeds further joined with another node comprising of Binjharpuri and Punganur breeds with a bootstrap of 58%. In this node Binjharpuri and Punganur showed close relationship with a bootstrap value of 100% on 1,000 replications (Fig. 2). Similar profile of interbreed relationships were obtained with Cavalli-sforza and Edwards D_C genetic distance (Fig. 3).

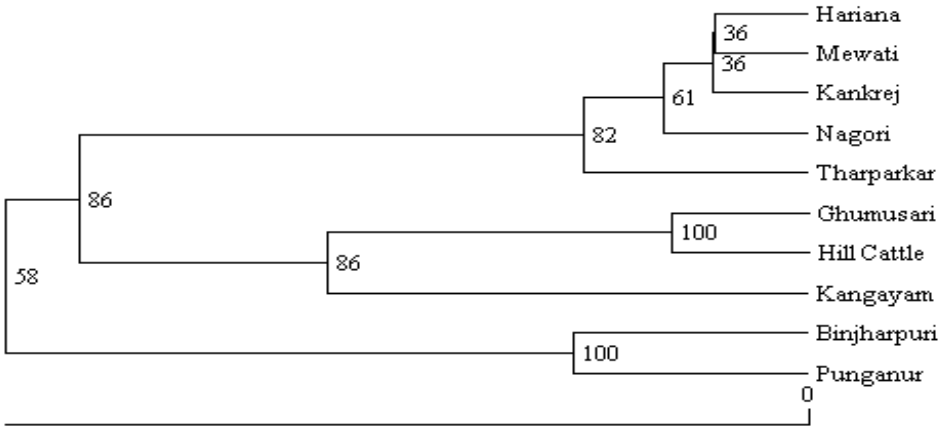

Fig. 2 Nei's standard genetic distance, Ds (1972), distance based UPGMA phylogram of ten Indian cattle breeds

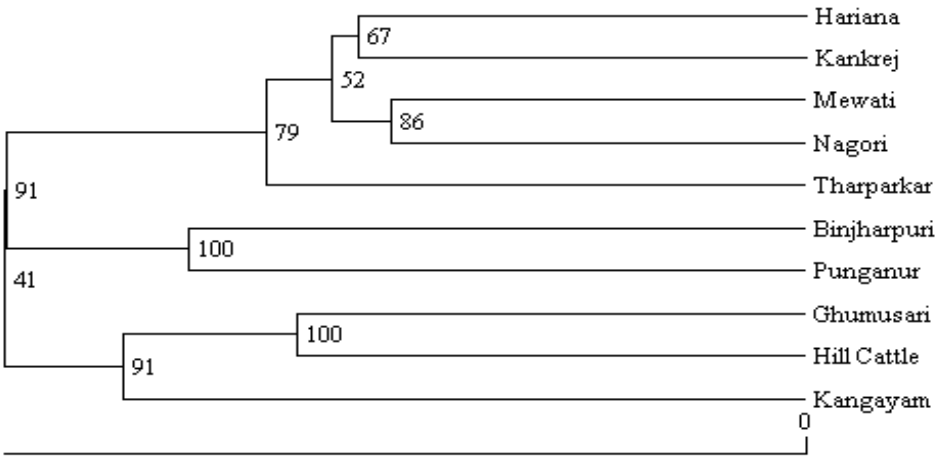

Fig. 3 Cavalli-Sforza and Edwards, Dc (1967), distance based UPGMA phylogram of ten Indian cattle breeds

Fig 4 represents the Principal component analysis based on pair-wise chord distance estimates demonstrated that the first three principal components together explained 69.35% (PC1 = 28.83, PC2 =

25.93 and PC3 = 14.59%) of the total variance. The plot reveals a similar pattern as genetic relationship from phylogenetic analysis. The five populations of Hariana, Kankrej, Mewati, Nagori and Tharparkar individuals with admixture, whereas, Ghumusari, Hill Cattle and Kangayam individuals showed clear distinctness and formed separate clusters whereas Binjharpuri and Punganur formed separate clusters.

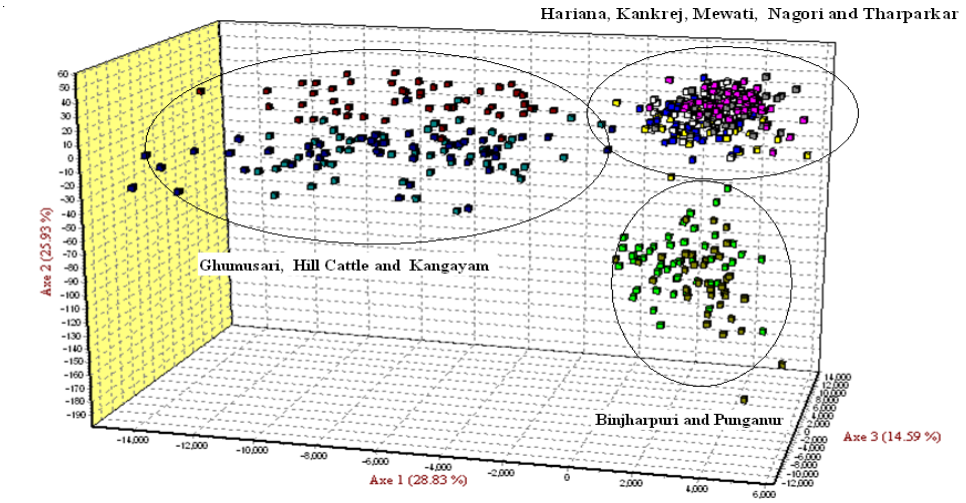

Fig. 4 Correspondence Analysis of ten Cattle Populations taking individuals as a unit

Population Differentiation

To validate the clustering obtained by phylogenetic and PCA analysis, population differentiation was evaluated by F_{ST} and analysis of molecular variance. The analysis of Molecular variance (AMOVA) revealed significant differentiation among the populations. Among population degree of freedom was 9 and percentage of variation contributed by these populations was 7.62%, the rest being attributable to variation within population (Fig. 5).

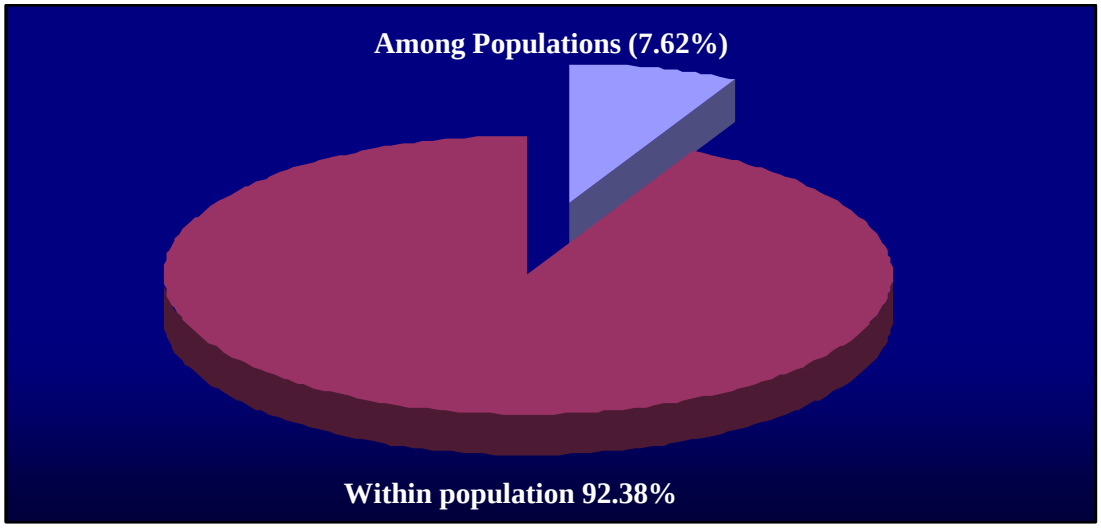

Fig. 5 Analysis of Molecular Variance (AMOVA)

This is evident by low F_{ST} values and three distinctive clusters in Correspondence Analysis.

The Nm values present an indirect measure of the gene flow and gave values of 77.15 migrants between Hariana and Kankrej animals and least values of 1.53 between Punganur and Kangayam cattle. This large value suggests almost no differentiation amongst the two breeds/population since both have contiguous breeding tract and migration of Kankrej cattle to Haryana during winter time. There is also large geneflow among populations leading to lower assignment accuracy. Nm in the analysis represents the effective number of migrants per generation.

Conclusion

This study, thus, presents a comprehensive analysis of the 10 grey cattle breeds from different agro-climatic regions of India covering a vast expanse of India. The present study contributes to the knowledge of existing genetic diversity, population structure and relationships across ten important native cattle from different agroclimatic regions of India. The results generated in the presented study can be utilized for future conservation programs and developing breeding programs for increase in production with use of improved and recognised breeds of Indigenous cattle *(Bos indicus)* and their sustainable utilisation.

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