# Minor differentiation despite high genetic diversity among closely related Hariana and Mewati cattle breeds from Northern India

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## ABSTRACT

Information on genetic organization and variability of livestock breeds is crucial for proficient conservation plans. The present investigation was undertaken to realize molecular characterization of closely related Hariana and Mewati cattle breeds from northern part of India on the basis of 21 FAO recommended microsatellite markers. Hariana breed has its native tract in the state of Haryana and Mewati inhabits adjoining areas of bordering states of Rajasthan and Uttar Pradesh. Both the breeds displayed high genetic diversity values. The average number of alleles per locus was 9.25 in Mewati and 11.81 in Hariana cattle. Gene diversity (expected heterozygosity) was very high and varied from 0.724 in Mewati to 0.777 in Hariana. $F_{IS}$ values were very low (almost zero) in both the breeds. Neighbour Joining radiation tree based on Nei's genetic distance showed thorough admixture of animals of the two breeds. Only 0.56% variation was due to between breed differences and the remaining 99.44% due to individuals within breeds. PCA and AMOVA analysis revealed virtual absence of breed structure. High migration is the most probable factor for negligible differentiation between the two breeds. The study thus reveals extraordinarily low differentiation despite high genetic variability in the investigated breeds.

**Key Words:** Gene diversity, genetic distance, migration, differentiation

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## INTRODUCTION

The concept of cattle breeds emerged in the 18th century in Britain primarily based upon the experiences of Robert Blackwell (Porter 1991). Since then intensive culling and inbreeding became a prevalent approach to achieve specific breeding goals. This sustained intensive breeding culminated in the development of different breeds with strikingly distinct phenotypic and production characteristics from each other. These characteristics range from those selected for intensive milk, beef production, draught purpose and few selected even for breed phenotype or coat colour or pattern. Probably more than any other region of the world, Indian zebu cattle have a global influence and many Indian zebu breeds (Brahaman, Gir, Sahiwal, Ongole etc.) have worldwide distributions.

The vast and varied reservoir of Indian cattle genetic resources is represented by 34 acknowledged breeds (including the four recently added) and a large number of hitherto uncharacterized populations (Nivsarkar et al., 2000). The origin of large number of Livestock breeds in India is the outcome of the country's enormous cultural and ecological diversity. During the thousands of years since animals were first domesticated, they have been introduced to diverse environments and subjected to breeding practices of different communities and cultural and social groups. A large variety of breeds thus developed are favorably adapted to varying ecological niches and to the divergent necessities and preferences of their breeders. The indigenous cattle breeds have been broadly classified as dairy, draft, and dual type depending upon their utility for milk production or agricultural work or both. Barring a few breeds developed for diary production (Sahiwal, Gir, Tharparkar, Kankrej, Red Sindhi), a majority was developed for draught or dual purpose, as Indian agriculture remained intensely dependant on animal draught power. Hariana and Mewati, the two dual-purpose cattle breeds of northern India were developed for draught as well as milk (dual purpose) in the predominantly agrarian states of Haryana, Rajasthan and Uttar Pradesh. Hariana has been a domineering draught breed and has been utilized as an improver breed in a larger part of the country extending from its breeding tract in the state of Hariana to Uttar Pradesh, Bihar, Orissa and west Bengal. As a result the grey coloured cattle of the entire region (including Gangatiri, Kenkatha, Bachaur, Kherigarh, Mewati, Ghumsari and Binjharpuri cattle) are believed to have Hariana inheritance. Mewati and Hariana have similar phenotypic characteristics (Singh et al., 2007) and occasionally it is intricate to differentiate the animals of the two breeds. It is therefore, fundamental to analyze the two breeds genetically to delineate their genetic diversity and relationship, which are vital for formulating conservation and breeding strategies.

Characterization of intimately interrelated populations relies on multiple, highly informative markers. Initially, biochemical markers such as blood group polymorphisms were used to discriminate between cattle breeds worldwide (Kidd et al., 1980; Blott et al., 1998). Subsequently, microsatellite loci were found to be more variable than the biochemical markers and thus have a broad application in animal genetics, including the evaluation of inter-breed genetic similarities. They are also very useful for elucidating the evolutionary relationships between closely related populations, like human populations and livestock breeds

(Bowcock et al., 1994; MacHugh et al., 1994, Ritz et al., 2000). Recently, numerous studies on cattle breeds have utilized microsatellite markers to reveal the relationships between breeds and, where possible, the history of breeds (MacHugh et al., 1994; Kantanen et al., 2000; Arranz et al., 2001; Bjornstad and Roed 2001; and Beja-Pereira et al., 2003).

The aim of the present investigation was to utilize microsatellite markers to characterize genetic diversity levels within, and relationships between two morphologically similar breeds, i.e. Hariana and Mewati cattle.

## MATERIAL AND METHODS

*Samples:* Blood samples were collected from 46 animals of Hariana and 43 of Mewati cattle in line with MoDAD suggestions. Blood sampling was coordinated with owners and veterinary practitioners implementing regular disease testing. 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 the procedure of Sambrook and Russell (2001). 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. These markers are distributed across the genome, covering 15 of the autosomes. Amplification of the microsatellite loci was realized by multiplex PCR by combining a maximum of five markers per multiplex. PCR products were detected by capillary electrophoresis using an ABI Prism 3130 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 heterozyogote deficiency were calculated using Microsatellite Analyzer version 4.05 (Dieringer and Christian, 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 (Page 1996). Bootstrap resampling (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

Genetic diversity, along with the degree of differentiation, was evaluated using 21 microsatellite markers within and between Hariana and Mewati cattle breeds from northern states of India. The diversity parameters are presented in Table 1.

*Table 1. Diversity parameters of Hariana and Mewati breeds*

| **Marker** | **Hariana** | | | | | **Mewati** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Na** | **Ne** | **Ho** | **He** | **PIC** | **Na** | **Ne** | **Ho** | **He** | **PIC** |
| CSRM60 | 12 | 3.661 | 0.652 | 0.735 | 0.695 | 8 | 2.272 | 0.628 | 0.566 | 0.513 |
| ETH10 | 9 | 4.161 | 0.696 | 0.768 | 0.726 | 8 | 3.902 | 0.605 | 0.752 | 0.705 |
| ILSTS11 | 8 | 2.366 | 0.457 | 0.584 | 0.538 | 4 | 2.142 | 0.465 | 0.540 | 0.475 |
| TGLA122 | 14 | 8.798 | 0.870 | 0.896 | 0.876 | 10 | 7.072 | 0.884 | 0.869 | 0.842 |
| INRA05 | 7 | 4.236 | 0.891 | 0.772 | 0.728 | 7 | 5.329 | 0.721 | 0.822 | 0.786 |
| INRA63 | 7 | 3.094 | 0.630 | 0.684 | 0.638 | 7 | 2.163 | 0.581 | 0.544 | 0.504 |
| TGLA227 | 10 | 3.228 | 1.000 | 0.698 | 0.641 | 9 | 2.326 | 0.907 | 0.577 | 0.491 |
| CSSM08 | 11 | 4.402 | 0.689 | 0.782 | 0.741 | 7 | 3.570 | 0.884 | 0.728 | 0.6833 |
| HEL05 | 10 | 5.153 | 1.000 | 0.815 | 0.781 | 8 | 4.772 | 0.977 | 0.800 | 0.767 |
| ILSTS05 | 13 | 6.151 | 0.870 | 0.847 | 0.819 | 9 | 5.446 | 0.977 | 0.826 | 0.803 |
| ILSTS33 | 13 | 4.093 | 0.587 | 0.764 | 0.721 | 9 | 3.284 | 0.674 | 0.704 | 0.647 |
| INRA35 | 15 | 6.771 | 0.870 | 0.866 | 0.841 | 11 | 5.313 | 0.674 | 0.824 | 0.790 |
| BM1824 | 7 | 2.622 | 0.739 | 0.625 | 0.549 | 5 | 2.535 | 0.651 | 0.613 | 0.541 |
| CSSM66 | 13 | 6.551 | 0.761 | 0.857 | 0.832 | 10 | 4.068 | 0.791 | 0.763 | 0.729 |
| ETH03 | 10 | 4.015 | 0.761 | 0.759 | 0.714 | 7 | 2.639 | 0.581 | 0.628 | 0.547 |
| ETH225 | 10 | 2.782 | 0.652 | 0.648 | 0.622 | 8 | 2.108 | 0.581 | 0.532 | 0.508 |
| MM12 | 16 | 4.422 | 0.652 | 0.782 | 0.747 | 13 | 4.050 | 0.837 | 0.762 | 0.723 |
| CSSM33 | 16 | 8.687 | 0.902 | 0.896 | 0.876 | 19 | 10.05 | 0.977 | 0.915 | 0.898 |
| HEL01 | 17 | 5.286 | 0.732 | 0.821 | 0.799 | 15 | 3.639 | 0.488 | 0.734 | 0.712 |
| HEL09 | 13 | 8.977 | 0.976 | 0.899 | 0.878 | 11 | 7.670 | 0.881 | 0.880 | 0.855 |
| ILSTS34 | 17 | 5.512 | 0.732 | 0.829 | 0.804 | 15 | 5.641 | 0.780 | 0.833 | 0.803 |
| **Mean** | 11.81 | 4.999 | 0.768 | 0.777 | 0.761 | 9.52 | 4.285 | 0.740 | 0.724 | 0.713 |

*Na= Observed number of loci, Ne= Effective number of loci, Ho= Observed heterozygosity,He = Expected heterozygosity, PIC= Polymorphism information content*

*Within Breed Variability:* A total of 251 alleles were detected across the 21 microsatellite loci investigated in the two breeds. Analysis of microsatellite data suggested that both the breeds from the northern part of India exhibited a high within-population genetic variability at the investigated loci. This is surely a desirable feature when planning conservation and improvement programs.

Hariana cattle had higher total number of alleles (242; range 7-17) across the 21-microsatellite markers evaluated. The corresponding allelic count in Mewati cattle was 204 (4-19) (Table 1). The mean allelic diversity (number of alleles per locus) varied from 9.52 in Mewati (range 4-20) to 11.81 in Hariana (range 7-18). As a general observation the gene frequencies and the evenness of allele frequencies varied greatly between the two 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 two investigated breeds is, in general, higher than those previously reported for a majority of the acknowledged indigenous breeds and 4 hitherto uncharacterized populations from India, which varied from 3.88 to 9.60 using microsatellite markers (Table 2) as well as majority of exotic cattle breeds studied worldwide (Groeneveld et al., 2010).

*Table 2. Summary of diversity parameters of Indian cattle breeds reported by various workers*

| **Breed** | **Na** | **Ne** | **Ho** | **He** | **PIC** | **$F_{IS}$** | **$F_{ST}$** | **Ref** |
|---|---|---|---|---|---|---|---|---|
| Tharparkar | 6.2 | 3.23 | 0.57 | 0.67 | 0.60 | 0.140 | -- | Sodhi et al., 2006 |
| Ongole | 4.5 | -- | -- | 0.46 | -- | 0.36 | 0.117 | Metta et al., 2004 |
| Deoni | 4.1 | -- | -- | 0.50 | -- | 0.18 | | |
| Gir | 5.29 | 3.55 | 0.679 | -- | -- | -- | 0.086 | Kale et al., 2010 |
| Deoni | 5.71 | 3.24 | 0.674 | -- | -- | -- | | |
| Kankrej | 6.00 | 3.30 | 0.674 | -- | -- | -- | | |
| Gir | 8.4 | -- | 0.694 | 0.717 | 0.678 | | -- | Singh *et al.*, 2006 |
| Kangayam | 4.04 | 2.90 | -- | 0.618 | 0.563 | -0.084 | | Karthickeyan et al., 2009 |
| Kenkatha | 9.52 | 3.99 | 0.535 | 0.689 | 0.65 | 0.212 | 0.022 | Chaudhari et al., 2009 |
| Gaolao | 7.92 | 3.53 | 0.47 | 0.62 | 0.59 | 0.225 | | |
| Punganur | 6.00 | -- | 0.684 | 0.666 | 0.628 | -0.001 | -- | Kesvulu Chenna et al., 2009 |
| Umblachary | 4.00 | 2.91 | 0.658 | 0.614 | 0.563 | -0.049 | -- | Karthickeyan et al., 2007 |
| Ongole | 3.88 | 3.00 | 0.645 | 0.608 | 0.558 | -0.062 | -- | Karthickeyan et al., 2008 |
| Hallikar | 6.38 | -- | 0.752 | 0.785 | -- | -- | -- | Naveen Kumar et al., 2006 |
| Kankrej | 8.2 | 4.2 | 0.694 | 0.717 | 0.684 | -0.013 | -- | Sodhi et al., 2007 |
| Kenkatha | 5.95 | 3.410 | 0.540 | 0.685 | 0.640 | 0.214 | -- | Pandey et al., 2006a |
| Kherigarh | 6.24 | 3.77 | 0.574 | 0.617 | 0.669 | 0.188 | -- | Pandey et al., 2006b |
| Ponwar | 5.88 | 3.43 | 0.497 | 0.682 | 0.632 | 0.262 | -- | Sharma et al., 2006a |
| Krishna Valley | 4.72 | -- | 0.668 | 0.657 | 0.623 | -- | -- | Karthickeyan et al., 2006 |
| Sahiwal | 5.2 | -- | 0.420 | 0.61 | 0.550 | -- | 0.113 | Mukesh et al., 2004 |
| Hariana | 6.5 | -- | -- | 0.66 | -- | -- | | |
| Deoni | 5.9 | -- | 0.590 | 0.70 | -- | -- | | |
| Red Kandhari | 5.82 | 3.12 | 0.47 | 0.64 | -- | 0.278 | 0.110 | Sodhi et al., 2005 |
| Deoni | 5.86 | 3.53 | 0.57 | 0.69 | -- | 0.179 | | |
| Tharparkar | 9.0 | 4.2 | 0.643 | 0.716 | -- | 0.103 | 0.065 | Sodhi et al., 2008 |
| Rathi | 9.6 | 5.0 | 0.694 | 0.789 | -- | 0.121 | | |
| Gangatiri | 6.27 | 3.36 | 0.464 | 0.678 | 0.625 | 0.318 | -- | Sharma et al., 2006b |
| Siri | 6.13 | 3.48 | 0.53 | 0.69 | -- | 0.221 | -- | Sharma et al., 2008 |
| Bachaur | 6.30 | -- | 0.534 | 0.694 | - | -- | -- | Sharma et al., 2007 |
| Vatakara | 5.54 | -- | 0. 769 | 0. 691 | 0.679 | -- | -- | Tantia et al., 2004 |
| Iduki | 5.54 | -- | 0.757 | 0.699 | 0.684 | -- | -- | |
| Kasargod | 4.58 | -- | 0.717 | 0.661 | 0.632 | -- | -- | |

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 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 lower utilization of AI under filed conditions and thus implies the existence of larger effective population sizes of the explored Indian cattle breeds. The mean effective number of alleles (Ne) estimated in the two breeds varied from 4.29 in Mewati to 5.00 in Hariana (Table 1). The mean effective number of alleles in both the breeds is far lower (even less than 50%) than the actual mean observed number of alleles per locus. This suggests that we have a set of alleles with very diverse frequencies. Alleles with frequencies away from the even 'average' contribute very little to the effective number of alleles.

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 2 breeds was very high ranging from 0.740 in Mewati to 0.768 in Hariana (Table 1). Correspondingly, the expected heterozygosity or gene diversity in the investigated breeds was high, varying from 0.724 in Mewati to 0.777 in Hariana. The observed as well as expected heterozygosity estimates in both breeds are in general higher than those assessed for Indian (Table 3) 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. Similarly, of the 29 diversity values reported for different Indian cattle breeds, 3.45% were below 0.50, 3.45% varied from 0.50 to 0.60, 75.9% from 0.60 to 0.70 and 17.24% were above 0.70. The observed and expected heterozygosities in both 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 2 breeds are indicative of low inbreeding, which is also supported by close to zero mean inbreeding coefficients ($F_{IS}$) across the 21 loci in both breeds (ranged from –0.025 in Mewati to 0.007 in Hariana) (Table 3).

*Hardy-Weinberg Equilibrium:* Hardy Weinberg equilibrium (HWE) calculations were determined for each population, combining the allele frequency data for all 21 markers used. The results of the HWE test are shown in Table 3. There were a total of 42 HWE tests (21 loci in 2 populations). A total of 12 locus-population combinations were statistically significant (P <0.05). These deviations consisted of 8 loci in Hariana and 4 in Mewati. In almost all of the locus-population combinations deviating from HWE frequencies, the observed hetrozygosities were lower than the expected heterozygosities. Significant departure from HWE is a common occurrence and has been identified in a number of cattle breeds from diverse parts of the world (Vallejo et al., 2003, Hansen et al., 2002, Sifuentes-Rincón et al., 2007, Kantenen et al., 2000, Beja-Pereira et al., 2003, Martin-Burriel et al., 2007, Sodhi et al., 2005, Kale et al., 2010, Chaudhari et al., 2009; Ginja et al., 2010, Sharma et al., 2010). It is, though, tricky to predict the precise source of this departure, however, the presence of low-frequency null alleles segregating at these loci may be a probable reason. Other factors, which play a role in HWE deviations, include inbreeding, genetic hitchhiking, null alleles (nonamplifying alleles) and occurrence of population substructure (Wahlund effect), which result in heterozygote deficiencies (Nei 1987).

*Table 3.* $F_{IS}$ *and HWE (p-values) and pair wise* $F_{ST}$, $F_{IT}$ *and* $F_{IS}$ *values in Hariana and Mewati cattle*

| Marker | $F_{IS}$ | | HWE (p-values) | | Pair wise F-Statistics values | | |
|---|---|---|---|---|---|---|---|
| | Hariana | Mewati | Hariana | Mewati | $F_{ST}$ | $F_{IT}$ | $F_{IS}$ |
| CSRM60 | 0.108 | -0.116 | 0.000 | 0.3870 | 0.024 | 0.043 | 0.020 |
| ETH10 | 0.090 | 0.193 | 0.180 | 0.0013* | -0.009 | 0.137 | 0.145 |
| ILSTS11 | 0.214 | 0.134 | 0.001* | 0.0842 | -0.010 | 0.174 | 0.183 |
| TGLA122 | 0.024 | -0.023 | 0.305 | 0.6400 | 0.007 | 0.014 | 0.007 |
| INRA05 | -0.161 | 0.118 | 0.772 | 0.0128* | 0.006 | -0.010 | -0.016 |
| INRA63 | 0.074 | -0.076 | 0.052 | 0.8858 | 0.022 | 0.037 | 0.016 |
| TGLA227 | -0.444 | -0.587 | 1.000 | 1.0000 | 0.011 | -0.486 | -0.502 |
| CSSM08 | 0.114 | -0.222 | 0.207 | 0.9958 | 0.015 | -0.023 | -0.038 |
| HEL05 | -0.236 | -0.230 | 1.000 | 1.0000 | 0.011 | -0.214 | -0.228 |
| ILSTS05 | -0.033 | -0.191 | 0.047 | 1.0000 | 0.001 | -0.102 | -0.103 |
| ILSTS33 | 0.228 | 0.036 | 0.000* | 0.4974 | -0.000 | 0.145 | 0.145 |
| INRA35 | -0.010 | 0.178 | 0.636 | 0.0022* | 0.006 | 0.089 | 0.084 |
| BM1824 | -0.190 | -0.070 | 0.033* | 0.3494 | -0.001 | -0.128 | -0.127 |
| CSSM66 | 0.107 | -0.043 | 0.046* | 0.7368 | 0.008 | 0.053 | 0.045 |
| ETH03 | -0.008 | 0.070 | 0.023* | 0.3765 | 0.013 | 0.045 | 0.032 |
| ETH225 | -0.013 | -0.100 | 0.374 | 0.9399 | 0.007 | -0.037 | -0.045 |
| MM12 | 0.163 | -0.106 | 0.000* | 0.1701 | 0.005 | 0.046 | 0.041 |
| CSSM33 | -0.014 | -0.074 | 0.072 | 0.9876 | -0.002 | -0.040 | -0.039 |
| HEL01 | 0.104 | 0.333 | 0.000* | 0.0000* | 0.007 | 0.224 | 0.218 |
| HEL09 | -0.093 | -0.007 | 0.985 | 0.5183 | -0.001 | -0.045 | -0.044 |
| ILSTS34 | 0.112 | 0.057 | 0.000* | 0.2006 | 0.001 | 0.092 | 0.091 |
| Mean | 0.007 | -0.035 | | | 0.006 | 0.002 | -0.003 |

**Deviate significantly from Hardy Weinberg equilibrium frequencies*

*Between Breed Variability and Genetic Differentiation:* Mean estimates of F-statistics from Jackknifing over loci were: FIT (total inbreeding estimate) =0.0023, $F_{IS}$ (within-population inbreeding estimate) = -0.0034 and $F_{ST}$ (population differentiation) = 0.0056 (Table 3). These values were not significantly different from zero ($p > 0.05$). The non-significant values of $F_{IS}$ (-0.0034), $F_{IT}$ (0.0023) and $F_{IT} > F_{ST}$ (0.0056) suggested random mating in the two populations. Genetic differentiation between the two populations was very low ($F_{ST}$ = 0.0056) implying that only 0.56% of the total variation corresponded to between breed differences and 99.44% of the total genetic variation corresponded to within-breed differences. This appears logical in view of the fact that the two breeds dwell in close proximity to each other and there is very high migration between the two breeds irrespective of their respective breeding tracts, as there is no breed society or breeding policy to check such indiscriminate breeding. Consequently there is considerable morphological similarity in the two breeds (Nivsarkar et al., 2000).

In order to further investigate the inter-breed relationship between Hariana and Mewati cattle, different approaches were employed. Neighbor Joining tree derived from inter-individual Nei's genetic distances between two cattle breeds exhibited wide-ranging admixture of animals of the two breeds (Fig. 1). This was further corroborated through Factorial Component Analysis (FCA), which has the advantage of concurrent manifestation of genetic differences that are contributed by each allele. The first three components accounted for only 32.25% 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 Hariana and Mewati cattle in an overlapped state and thus reflected insignificant breed-specific clustering (Figure 2).

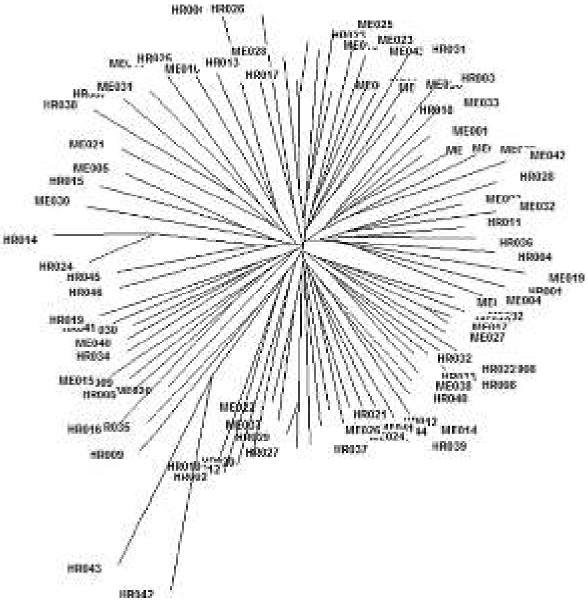

*Figure 1. NJ tree based on inter-individual Nei's genetic distance between two cattle breeds (M= Mewati, H= Hariana)*

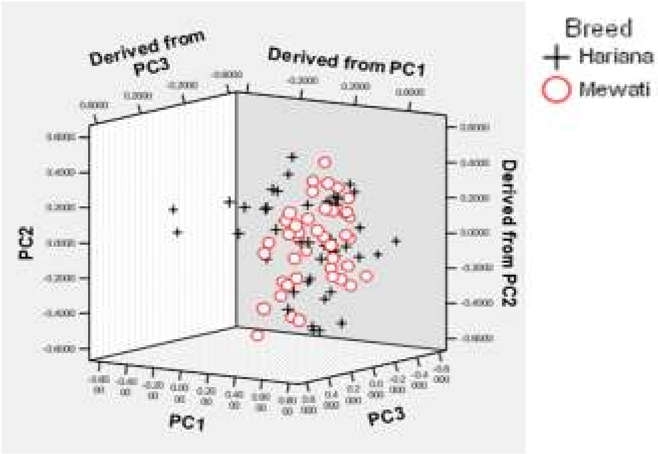

*Figure 2. Scattergram showing relative positions of different individuals in a geometric space (First three principal components together explain 32.25% of the total variance)*

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, 99.44% of the total variation was found to be within breeds and a mere 0.56% was found to be among breeds. Present day subdivision of Hariana and Mewati cattle into distinct breeds based on microsatellite markers is, thus, minimal and much smaller than that reported for Sahiwal, Hariana and Deoni cattle (11.3%; Mukesh et al., 2004); Gir, Deoni and Kaknrej cattle (8.6%; Kale et al., 2010); Ongole and Deoni cattle (11.7%; Metta et al., 2004); Deoni and Red Kandhari cattle (11%, Sodhi et al., 2005) and Tharparkar and Rathi (6.5%; Sodhi et al., 2008) breeds of Indian cattle; 20 Northern European breeds (10.7%; Kantanen et al., 2000); 7 European breeds (11.2%; MacHugh et al., 1998); 18 local cattle breeds from Spain, Portugal, and France (7%; Canon et al., 2001); 7 French breeds with 23 loci (8%; Maudet et al., 2002); eight Southwest European beef cattle breeds (6.8%; Jordana et al., 2003); 5 Korean native breeds (11%; Chung et al., 2006) and 10 Brazilian breeds (9.8%; Egito et al., 2007). However, lower differentiation between Kenkatha and Gaolao breeds of Indian cattle has been lately described (2.19%; Chaudhari et al., 2009).

The genetic differentiation observed between the two breeds is far less than observed even between three populations of Charolais breed of cattle (7.9%) located in northeast Mexico (Sifuentes-Rincón et al., 2007). Similarly Chikhi et al. (2004)) also detected a significant but limited amount of genetic differentiation between parishes (1.3%), or even between farms (3.5%) of Jersey cattle breed despite an apparent lack of movement. 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). Low genetic differentiation (3.4%) was also detected in 8 Indian riverine buffalo breeds inhabiting broadly varying ecological regions of the country and having distinctly diverse morphology and production characteristics (Kumar et al., 2006). Virtual Lack of genetic differentiation between Hariana and Mewati cattle, in this study, is exciting in view of the fact that each breed exhibited very high genetic diversity. Lack of genetic structuring in the two closely related breeds is, in all probability, due to very high rate of migration between the breeds which is practically operative in their breeding tracts.

*Table 4. Analysis of Molecular variance*

| Source of variation | Sum of Squares | Variance components | Percentage variation |
|---|---|---|---|
| Among populations | 11.822 | 0.04459 | 0.56173 |
| Within Populations | 1367.383 | 7.89308 | 99.43827 |
| Total | 1379.206 | 7.93766 | |

This study, thus, presents a comprehensive diversity analysis of closely related Hariana and Mewati cattle breeds from Northern part 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 negligible differentiation between the two breeds as revealed by $F_{ST}$, AMOVA and FCA. 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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