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# Genetic Characterization and Diversity Study of Indigenous Cattle Breeds through Microsatellite Markers

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**Abstract**

The present study aims to understand the existing genetic diversity and structure of Six native cattle breeds (Rathi, Sahiwal, Kankrej, Nagori, Gir, and Tharpakar) adapted to the north-western arid and semi-arid region of India based on fourteen microsatellite markers. The mean number of observed and effective alleles in all the six population was overall in the range of Rathi was 3.667 and 2.926, respectively wherein the mean Shannon Index for the overall six populations showed the value of 1.114. The average expected heterozygosity values (0.622) indicated high diversity for this set of markers in all selected populations. High PIC values were observed foremost of the markers with an average of 0.5775.The fixation indices (FIS, FIT, and FST) values for each microsatellite locus for the overall population were found to be 0.118, 0.833, and 0.859, respectively. The high FIS and FIT values indicated a high level of inbreeding within and among the populations. The allele diversity (mean observed number of alleles 9.0, mean effective number of alleles 6.670) and gene diversity (0.845) values imply a substantial amount of genetic variability in all the populations. Reasonably high PIC values observed for most of the markers, with an average PIC value of 0.5609 across all the loci imply that this set of microsatellites is very informative for the evaluation of genetic diversity and characterization in all the breeds.

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**Keywords**: Indigenous breed, six population, PIC, Shannon Index, microsatellite markers, Fixation indices

India is one of the mega bio-diversity centers of the world. Livestock are domesticated animals mainly contributing income to farmers. For arriving at the total livestock count, the species namely Cattle, Buffaloes, Sheep, Goats, Pigs, Horses & Ponies, Mules, Donkeys, Camels, Mithun, and Yak were considered in the 20$^{th}$Livestock Census. As per the 20$^{th}$ Livestock Census, the total Livestock population is 536.76 million in the country showing an increase of 4.8% over the Livestock Census 2012. The total livestock population in rural and urban areas is 514.11 million and 22.65 million respectively with a percentage share of 95.78% for rural and 4.22% for urban areas. Mechanization, unplanned and indiscriminate breeding among native stocks as well as human bias in favor of certain breeds have directly or indirectly lead to the dilution of indigenous germplasm. Hence, there is an urgent need to prevent the rapid erosion of animal genetic resources. Indian agriculture is an economic symbiosis of crop and livestock production with cattle as the foundation. There is a decline of 6% in the total Indigenous (both descript and non-descript) Cattle population over the previous census. However, the pace of decline of the Indigenous Cattle population during 2012-2019 is much less as compared to 2007-12 which was about 9%. and which requires a need to conserve the Indigenous germplasm with immediate scientific management. Genetic characterization is the first step in the development of proper management strategies for preserving genetic diversity and preventing undesirable loss of alleles.

Livestock conservation is carried out at breed level. The genetic variation both between and within breeds is described as the diversity within each species. The genetic variation exists between two groups, which separate them genotypically. Genetic variation of the animal is the basic material, which is utilized for changing the genetic makeup or genetic potentiality of domestic species to suit our needs. Mechanization, unplanned and indiscriminate breeding among native stocks and human bias in favor of certain breeds are directly or indirectly responsible for the dilution of Indian livestock germplasm. Hence, characterization of indigenous germplasm is essential for their conservation.

Genetic characterization can be done by various methods e.g. cytogenetic/biochemical and molecular techniques. Cytogenetic and biochemical methods are less sensitive, less accurate and reveal less polymorphism. Recent trend is to use molecular techniques for characterization which detect the genetic variation at DNA level. These techniques explore molecular markers such as RFLP, RAPD, AFLP and microsatellites.

In recent years, a range of innovations in molecular genetics has been developed for the study of genetic variation and evolution of populations using DNA genotyping information. The most utilized DNA marker for population genetics of livestock is microsatellite. Microsatellite markers, also called short tandem repeats (STRs) or simple sequence repeats (SSRs), are a relatively new class of genetic marker. Over a few years they have become a tool of choice to address population genetics and demographic questions (Sunnucks, 2000). The application of microsatellite markers is currently considered to be useful in the analysis of genetic diversity as they are numerous, randomly distributed in the genome, highly polymorphic, and they show codominant mode of inheritance (Ellegren, 1993). They allow the study of genetic diversity and differentiation of closely related populations. Microsatellite as genetic markers, have been applied successfully in the study of genetic variation of livestock 4including between and among European and African cattle breeds (Machugh *et al*., 1997; Okoma *et al*., 1998).

Some nationally recognized breeds having home tracts in Rajasthan are Gir (milch), Kankrej, Hariana (dual purpose), Rathi, Tharparker, Nagauri and Malvi(draught). Tharparker, Rathi, Kankrej, Nagauri and Hariana breeds have suffered decline in their population due to cross breeding with exotic breeds and mechanization of agriculture (Singh, 2008). While cross-bred cattle have increased heavily by 120.10%, indigenous cattle have decreased by 12.9% in 2003. Conservation of these breeds is essential as they have unusual adaptability towards nutritional, climatic and disease stress (Chopra, 1997) and possibility of long term use in cross- breeding programmes as well as more widespread use as pure breeds (Bhat *et al*., 1987). There is loss of genetic diversity due to genetic improvement programme (William, 2002) and breeding and conservation programmes can be determined by characterizing genetic variation of livestock (Notter 1999). The breed has got excellent reputation as beef animal abroad and large numbers have been exported to Brazil from where they have been introduced by other countries of American continent.

Population size of these indigenous cattle breeds is declining due to introduction of exotic cattle breeds hence molecular characterization and diversity study among them using molecular markers is required (Mathur. 2005).Owing to the extreme importance of conservation of these indigenous breeds. Genetic characterization of breeds using molecular markers (Hetzel and Drinkwater, 1992; Erhardt and Weimann, 2007 & FAO 2007) provides information for conservation making decisions for livestock populations (Sunnucks, 2000). Breeds distinctiveness by microsatellite markers calls for variation in allele frequencies between test breeds. (Hanotte and Jianlin, 2005).

Therefore, to utilize the cattle genetic resources effectively, it is necessary to characterize Indian indigenous cattle populations genetically. Such characterization would provide a comprehensive database of genetic variation among the cattle populations in India. It would provide furthermore information as to which of the populations represent homogenous populations and which of them are genetically distinct. It would also provide information on the risk status of the goat populations. The information generated would contribute to the understanding of the evolutionary history of cattle in India. It will also contribute to the conservation and management of genetic resources.

## Materials and Methods

### DNA samples

Microsatellite analysis was performed on a total sample of 180 unrelated Gir, Tharpakar, Nagori, Sahiwal, Rathi, and Kankrej cattle collected from their breeding site. Genomic DNA was isolated using the DN easy Kit method with minor mutations. Measurement of purity and filtering of DNA samples was for UV exposure. The level of DNA samples was also assessed by agarose gel (1%) electrophoresis.

### Microsatellite markers and PCR enhancement

As recommended by FAO and the Interna-

**Table I**. List of Microsatellite markers.

| Locus | Primer Sequences 5'-3' | Base Pair Count | Annealing Temprature |
|---|---|---|---|
| BM1818 | F:AGC TGG GAA TAT AAC CAA AGG | 21 | 58 |
| | R:AGT GCT TTC AAG GTC CAT GC | 20 | |
| CSRM60 | F:AAG ATG TGA TCC AAG AGA GAG GCA | 24 | 55-60 |
| | R:AGG ACC AGA TCG TGA AAG GCA TAG | 24 | |
| ETH10 | F:GTT CAG GAC TGG CCC TGC TAA CA | 23 | 55-65 |
| | R:CCT CCA GCC CAC TTT CTC TTC TC | 23 | |
| ETH225 | F:GAT CAC CTT GCC ACT ATT TCC T | 22 | 55-65 |
| | R:ACA TGA CAG CCA GCT GCT ACT | 21 | |
| INRA005 | F: CAA TCT GCA TGA AGT ATA AAT AT | 23 | 55 |
| | R: CTT CAG GCA TAC CCT ACA CC | 20 | |
| ILSTS006 | F:TGT CTG TAT TTC TGC TGT GG | 20 | 55 |
| | R:ACA CGG AAG CGA TCT AAA CG | 20 | |
| HEL5 | F: GCA GGA TCA CTT GTT AGG GA | 20 | 52-57 |
| | R: AGA CGT TAG TGT ACA TTA AC | 20 | |
| BM2113 | F:GCT GCC TTC TAC CAA ATA CCC | 21 | 55-60 |
| | R:CTT CCT GAG AGA AGC AAC ACC | 21 | |
| ETH3 | F: GAT CAC CTT GCC ACT ATT TCC T | 22 | 55-65 |
| | R: ACA TGA CAG CCA GCT GCT ACT | 21 | |
| ETH152 | F:AGG GAG GGT CAC CTC TGC | 18 | 55-60 |
| | R:CTT GTA CTC GTA GGG CAG GC | 20 | |
| HEL1 | F: AGT CCA TGG GAT TGA AAG AGT TG | 23 | 54-57 |
| | R: CTT TTA TTC AAC AGA TAT TTA ACA AGG | 27 | |
| ILSTS022 | F:AGT CTG AAG GCC TGA GAA CC | 20 | 55-57 |
| | R:CTT ACA GTC CTT GGG GTT GC | 20 | |
| INRA035 | F: ATC CTT TGC AGC CTC CAC ATT G | 22 | 55-60 |
| | R: TTG TGC TTT ATG ACA CTA TCC G | 22 | |
| INRA063 | F:ATT TGC ACA AGC TAA ATC TAA CC | 23 | 55-58 |
| | R: AAA CCA CAG AAA TGC TTG GAA G | 22 | |

tional Society for Animal Genetics, a total of 14 sets of microsatellite primers, specifically for cattle, were used (BM1818, CSRM60, ETH10, ETH225, INRA005, BM2113, ETH3, ETH152, HEL1, HEL5, ILSTS022, INRA035, INRA063, ILSTS002). PCR was performed with a 25 µl reaction volume containing 1.5 mM Mgcl 2,200 µM dNTPs, 50 ng of each primer, 100 ng of DNA template and 0.5 U of Taq DNA polymerase. PCR cycling conditions were: 5 min at 95 ºC, followed by 35 cycles of 45 seconds at 95 ºC, 45 seconds at the added temperature (52-64 ºC) of each primer, 45 seconds at 72 ºC, with a final extension of 10 72 ºC minutes. Amplified PCR products were tested in 2% agarose gel containing ethidium bromide and detected by UV light and microsatellite marker scores were performed using PAGE (Koreth *et al*. 1996) and genotypes obtained points by hand.

### Statistical analysis for microsatellite data

Software-assisted gel-documentation system (UVP) was used for the analysis of the genotypes of each animal received by hand. An effective number of alleles observed and expected for heterozygosity, microsatellite wavelengths & F-statistics were calculated and GenePop version 6.5 software was used to test Hardy-Weingberg equity. The content of the polymorphism (PIC) data was calculated according to Nehi (1978) using individually where the allele occurred in each location (http: // [www.genomics.liv.ac.uk/animal/pic.html](www.genomics.liv.ac.uk/animal/pic.html).)

### Results and Discussion

This study estimated allele frequency, observed (Na) and effective number of alleles (Ne), observed heterozygosity and expected heterozygosity, observed homozygosity and expected homozygosity, Shannon Index, inbreeding coefficient, and PIC value for all selected microsatellite markers.

### Genetic variability parameters for Rathi, Tharparkar, Gir, Sahiwal, Nagori and Kankrej cattle breed:

Table I summarizes observed and expected number of alleles with their sizes exhibited by various microsatellites investigated in overall, six populations, respectively. Total numbers of alleles observed across the populations were found to be 1050. Maximum number of alleles observed across the populations was 81 for CSRM60 and minimum was 31 for ILSTS-006. The mean observed allele numbers across the population for all 15 loci was 70.00 indicating

**Table II.** Genetic Diversity data of fifteen microsatellites in all six cattle breeds

| LOCUS | N | Na | Ne | I | Ho | He |
|---|---|---|---|---|---|---|
| **BM1818** | 77 | 9.000 | 6.469 | 1.999 | 0.732 | 0.845 |
| CSRM60 | 81 | 7.000 | 5.416 | 1.781 | 0.791 | 0.815 |
| ETH10 | 75 | 7.000 | 4.433 | 1.630 | 0.832 | 0.774 |
| ETH225 | 78 | 11.000 | 8.155 | 2.191 | 0.678 | 0.877 |
| INRA005 | 66 | 10.000 | 6.223 | 2.006 | 0.765 | 0.839 |
| BM2113 | 80 | 9.000 | 5.953 | 1.968 | 0.713 | 0.832 |
| ETH3 | 78 | 9.000 | 6.090 | 1.946 | 0.859 | 0.836 |
| ETH152 | 75 | 10.000 | 8.152 | 2.186 | 0.855 | 0.877 |
| HEL1 | 72 | 8.000 | 5.993 | 1.912 | 0.675 | 0.833 |
| HEL5 | 71 | 7.000 | 5.473 | 1.817 | 0.568 | 0.817 |
| ILSTS022 | 71 | 11.000 | 7.791 | 2.181 | 0.821 | 0.872 |
| INRA035 | 69 | 10.000 | 8.180 | 2.192 | 0.765 | 0.878 |
| INRA063 | 68 | 12.000 | 9.285 | 2.332 | 0.657 | 0.892 |
| ILSTS002 | 58 | 8.000 | 6.259 | 1.916 | 0.821 | 0.840 |
| ILSTS006 | 31 | 7.000 | 6.180 | 1.875 | 0.768 | 0.838 |

Na = No. of Different Alleles, Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2 the high level of polymorphism of the selected microsatellites. The mean number of alleles and the expected heterozygosities detected are good indicators of the genetic polymorphism within the breed. Generally the mean number of alleles is highly dependent on the sample size because of the presence of unique alleles in populations, which occur in low frequencies and also because the number of observed alleles tends to increase with increases in population size. The number of alleles scored for each marker is an invaluable indicator of the future usefulness of the marker for genetic screening and it can become the basis for breed characterization.

## F Statistics

The fixation indices (FIS, FIT and FST) values for each locus are shown in Table-2.From jackknifing over loci the mean FIS, FIT and FST values over all the population are found to be 0.118, 0.833 and 0.859, respectively.

The high FIS and FIT values indicated high level of inbreeding within and among the populations and also point towards high genetic differentiation between the populations.

The high inbreeding values can be attributed to selective mating under field conditions. However, the high mean number of alleles and mean observed and expected heterozygosities were similar supported by FIS estimates that were not significantly different from zero .The negative values of FIS for some of the loci indicated that the mates were less related in comparison with in the average population.

## Conclusion

The current study clearly demonstrates the efficacy of 14 microsatellite markers in depicting current variety levels, population structure, and gathering the degree of inbreeding despondencies within the population. The recent study concluded the astonishing data on genetic variation in six cow breed populations, providing vital insight into their current hereditary changeability. A similarly substantial level of inconstancy was acknowledged, implying that sufficient genetic diversity exists. This reality, together with effective ecological variation (transient cows), strengthens the meaning of significant pure variety and its relevance as double-purpose cows from North India. Findings from the current study, such as polymorphism, will help develop a workable raising plan for this

**Table III.** F-statistics analysis for 15 microsatellite loci in Tharpakar, Gir, Rathi, Nagori, Sahiwal and Kankrej breeds of cattle

| Locus | Fis | Fit | Fst | Nm |
|---|---|---|---|---|
| BM1818 | 0.183 | 0.833 | 0.858 | 0.041 |
| CSRM60 | 0.226 | 0.832 | 0.863 | 0.040 |
| ETH10 | -0.291 | 0.832 | 0.870 | 0.037 |
| ETH225 | 0.111 | 0.837 | 0.853 | 0.043 |
| INRA005 | 0.191 | 0.833 | 0.859 | 0.041 |
| BM2113 | 0.202 | 0.833 | 0.861 | 0.040 |
| ETH3 | -0.196 | 0.833 | 0.860 | 0.041 |
| ETH152 | 0.140 | 0.833 | 0.853 | 0.043 |
| HEL1 | 0.200 | 0.833 | 0.860 | 0.041 |
| HEL5 | 0.224 | 0.832 | 0.863 | 0.040 |
| ILSTS022 | 0.147 | 0.833 | 0.854 | 0.043 |
| INRA035 | 0.139 | 0.833 | 0.853 | 0.043 |
| INRA063 | 0.121 | 0.833 | 0.851 | 0.044 |
| ILSTS002 | 0.190 | 0.833 | 0.859 | 0.041 |
| ILSTS006 | 0.193 | 0.833 | 0.860 | 0.041 |
| Mean | 0.118 | 0.833 | 0.859 | |

Fis = (Mean He - Mean Ho) / Mean He    Fit = (Ht - Mean Ho) / Ht    Fst = (Ht - Mean He) / Ht variety to promote hereditary improvement and establish an animal research station that is required to preserve native breeds.

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