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Genetic Diversity Studies using Microsatellite Markers to Analyse Genetic Variation among the Buffalo Breed Populations in Jammu Region

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ABSTRACT

The microsatellites are the markers of choice for similar genetic exploration in different buffalo breeds both in India and abroad. In the present study, the molecular genetic characterization of local germplasm within and between existing buffalo population carried out using microsatellite markers with the objectives of studying the molecular characteristics of these buffaloes and to study available genetic diversity in the existing breed population. Fifty (50) and Twenty five (25) venous blood samples were collected at random from genetically unrelated animals of different sex and age groups of local buffalo gemplasm from its natural breeding tract in J&K region. The PCR products for different microsatellite loci were resolved on 6 percent denaturing (urea) polyacrylamide gels along with 50 and 100 bp DNA ladders at 40-45W. Microsatellite alleles were visualized by silver staining. The microsatellite genotype data were analyzed using POPGENE version. The genetic distance (Ds) between two breeds as calculated according to Nei's standard genetic distance revealed Nei's genetic identity and genetic distance to be 0.8038 and 0.2184 respectively between local buffalo population and Murrah breed which shows suggests a close relationship between Murrah and local buffaloes as expected from their geographical contiguity although they are phenotypically distinct. Present findings may be useful in characterization of genetic diversity and to develop strategies for conservation and utilization of local germplasm of J&K region, within and between existing buffalo population. The two studied buffalo population in present study has shown genetically distinctness from each other.

HIGHLIGHTS

- Closeness of Local buffalo population (Gujjari buffalo) and Murrah breed on the basis of Nei's measures as expected from same geographical contiguity.
- All the 15 microsatellite loci, amplified successfully were found to be polymorphic in local Buffaloes.

Keywords: Microsatellite, popgene, buffalo, genetic distance, germplasm

The genetic variation between and within the breeds also described as genetic diversity, is the raw material for animal breeders to act upon for bringing genetic improvement in livestock. The current world scenario has glimpsed an amplified loss in wildlife diversity due to ever increasing human intrusion into ecological habitats. Although the trends of loss in livestock diversity are comparatively different, many breeds are near to losing their genetic identity due to the forced gene flow of superior traits from economically healthier populations. The commercially underestimated livestock breeds could provide great economic stimulus in the future in terms of the beneficial allele spectra they have gained as a result of adaptation to their environments. Therefore, the FAO Domestic Animal Diversity Information System (DAD-IS) and Domestic

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Animal Genetic Resources Information System (DAGRIS) have begun a worldwide campaign for the conservation of within- and between-breed genetic diversity in livestock for their sustainable future use befitting their economic and social value. The within- and between-breed genetic variability in livestock predicted by phenotypic attributes can nowadays be validated by the use of molecular markers and their analysis with sophisticated statistical techniques. (Hussain *et al*., 2017) The population structure and genetic variation among breeds have been studied using different markers including detection of polymorphism at DNA level including such markers like RFLP, RAPD, VNTR's and STR or microsatellites. Microsatellites, also known as Simple Sequence Repeats (SSRs) or Short Tandem Repeats (STRs), are tandemly repeating sequences of 2 to 6 bp of DNA, which have been demonstrated to be polymorphic in length in a number of eukaryotic genome. Since microsatellites are polymorphic, they act as extremely useful markers for comparative study of genetic variation, parentage estimation, linkage map analysis and could well be the marker of choice for analysis of population structure in both wild and domesticated species. The microsatellite loci could also prove highly informative markers for the construction of genetic linkage maps which could be used in search for quantitative trait loci associated with economically important traits. The availability of a wide range of microsatellites from livestock species has also generated interest in studies of variation and evolutionary relationships among livestock populations and a number of such studies (Kim *et al*., 2002) have appeared in the literature. So a huge amount of literature can now be reviewed for the use of microsatellite markers in the diversity assessment of various livestock species with reference to the (Cannon *et al.,* 2006; Consortium E.C.G.D., 2006; Peter *et al.,* 2007).

The aims of this study were to assess the genetic diversity within and between the Jammu buffalo population; and to identify the genetic relationship and describe geographical and genetic distinction between different water buffalo populations at different sites in Jammu. A total of (Fifteen) ISAG-FAO recommended primers were used in the study for genetic diversity analysis. These microsatellites were amplified on DNA samples extracted from 75 blood samples including 50 samples of local buffalo/Gujjari (Fig. 1) and 25 of Murrah buffaloes.

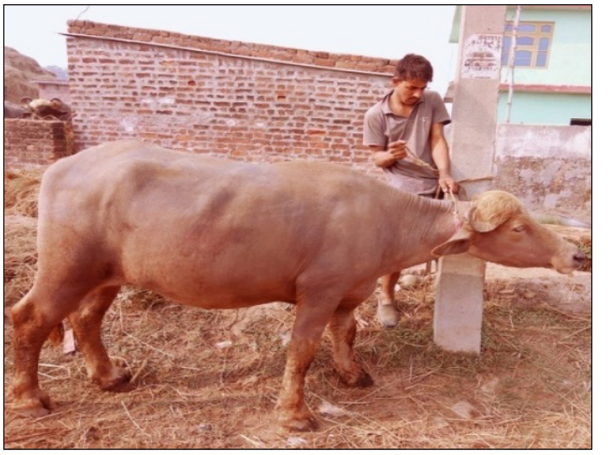

Fig. 1: Local Buffalo Population of Jammu region in their natural breeding tract

All the 15 microsatellite markers amplified from DNA samples were resolved on 1.5% agarose gel. PCR products from positive samples were resolved on 6% urea-PAGE (Denaturing Poly Acrylamide Gel Electrophoresis) and the alleles were visualized by silver staining. Total number of alleles for DNA samples was screened for their allelic combination and size of alleles. The samples were then analyzed for total number of alleles for a particular microsatellite, their frequency, F-stat, Nei's measures of genetic identity and genetic distances, level of inbreeding, etc. The data were also used to determine Genetic bottleneck and linkage disequilibrium within and between the population and Smouse's multilocus analysis for single populations.

MATERIALS AND METHODS

Ethical regulations

Attention had been paid to minimize pain to the animals and all the samples collection was carried out in accordance with the guidelines laid down by the International Animal Ethics Committee and prevailing local laws/regulations during collection of blood samples from local buffalo population.

Sample collection and DNA extraction

A total of 75 animals (50 local/gujjari buffaloes and 25

murrah buffaloe) were selected from different locations of the natural breeding tract (Miran sahib, Kathua, Akhnoor, RS Pura, Samba, Udhampur) and adjoining areas of Jammu region and other districts of Jammu and Kashmir as shown in Fig. 2. Blood samples 10 mL in volume were aseptically collected from the jugular vein of 50 local buffalo (Gujjari) and 25 Murrah respectively. The coagulation of blood samples was blocked with the use of K3 EDTA and DNA was isolated from the peripheral leukocytes using organic extraction.

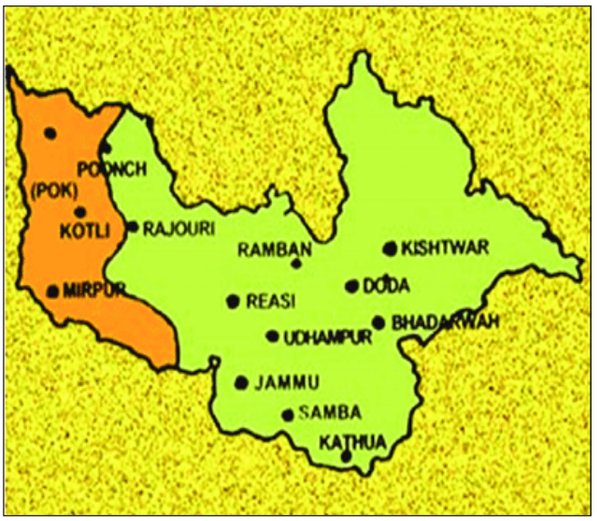

Fig. 2: Migratory route along with distribution of Local Buffalo Population/Gujjari Buffalo in Jammu region

Microsatellite typing

DNA was extracted using a phenol-chloroform standard protocol (Sambrook and Russel, 2001) from blood samples of 50 local buffalo (Gujjari) and 25 Murrah population respectively. DNA quality was assessed using 0.7 % horizontal mini-submarine agarose gel electrophoresis. The purity of DNA was assessed by calculating ratio of optical densities at 260 nm and 280 nm. 15 FAO (DADIS MoDAD) recommended buffalo specific microsatellite markers namely CSSM033, CSSM038, BRN, CSSM032, CSSM013, ETH003, CSSM061, BMC1013, CSSM062, ILSTS030, ILSTS008, HMH1R, ETH121, ILSTS033, RM099 which gave amplification were utilized in the study.

DNA Sample processing

The microsatellite loci were amplified in programmable thermal cycler (Bio-Rad, S 1000) after optimization. The polymerase chain reaction (PCR) program used involved initial denaturation at 94°C for 5 min and 30 cycles of denaturation at 94°C for 30 s, annealing for 45s, extension at 72°C for 45 s and final extension at 72°C for 10 min. Documentation of PCR product was done in 1.5% agarose gel electrophoresis at 2-5 v/cm. The PCR products for different microsatellite loci were resolved on 6% denaturing (urea) polyacrylamide gels along with 50 and 100 bp DNA ladders at 40-45w. Microsatellite alleles were thereafter visualized by silver staining.

Statistical analysis

PopGene version (1.3.1) software Yeh *et al.* (1999) were used to analyse microsatellite genotype data. It was used to compute summary statistics (e.g., allele frequency, gene diversity, genetic distance, F-statistics, multilocus structure etc.) for single-population and multi-population.

Bottleneck analysis

The BOTTLENECK program (Cornuet and Luikart, 1996) was applied to determine if there had been past bottlenecks in population size at any locality. It tests for the departure from mutation drift equilibrium based on heterozygosity deficient or excess. The bottleneck compares heterozygosity expected at Hardy-Weinberg equilibrium to the heterozygosity expected at mutation drift equilibrium in same sample that has the same size and same number of alleles.

Nei's measures of genetic identity and genetic distance:

Genetic distance is a measure of the genetic divergence between species or between populations within a species, whether the distance measures time from common ancestor or degree of differentiation. Populations with many similar alleles have small genetic distances. This indicates that they are closely related and have a recent common ancestor. Genetic distance is useful for reconstructing the history of populations. Nei's genetic distance was calculated based on Dendogram based

UPGMA-Modified method from NEGHBOR of PHYLIP Version 3.5 and Nei's Unbiased Measures of Genetic Identity and Genetic distance (Nei's 1978).

Smouse's multilocus analysis for single populations

In Smouse's multilocus analysis for single populations the average correlation and chi square value for both local population and murrah breed was estimated. Hardy Weinberg disequilibrium was also calculated. The WHD measures non-random union of gametes. The significant Hardy Weinberg disequilibrium (WHD) suggests that systematic and dispersive forces acts in the population.

RESULTS AND DISCUSSION

In the study of livestock breeds where the primary focus is on estimating breed relationships, it is assumed that the markers are neutral to selection and populations are in equilibrium under drift and migration (Barker *et al.,* 1999). Therefore, our understanding of genetic structure of Local Buffalo and Murrah Population of Jammu region for estimation of within and between breed relationships will benefit from analysis of microsatellite markers and population structure of investigated population by estimation of diversity variables (Arora *et al.,* 2004; Singh *et al.,* 2015). Several genetic variability measures namely number of alleles, heterozygosity levels and values of gene diversity for each marker and mean diversity indices for the investigated population were calculated from allele frequency data assuming the population to be in Hardy-Weinberg equilibrium. These parameters were studied using FAO-DADIS Buffalo primers which have shown adequate polymorphism in other Buffalo breeds earlier studied in India.

All the 15 microsatellite loci, which have been identified to be polymorphic in a variety of domestic Buffalo breeds amplified successfully in the local buffalo and Murrah Population of Jammu region and produced definite banding patterns from which individual genotypes could be ascertained (Singh *et al.,* 2017) as shown in the Fig. 3 and Fig. 4 for the Microsatellite (CSM032).

Genetic bottleneck effect

Microsatellite data were also subjected to statistical analysis to test whether the populations have undergone recent genetic bottleneck. Cornuet and Luikart (1996) described the quantitative methods suitable for analysis of microsatellite data for detection of recent bottlenecks in 100-200 generations. Any population that experienced a recent bottleneck will show higher than expected (equilibrium) heterozgosity for a large number of loci. The qualitative graphical method was employed to visualize the allele frequency spectra. The microsatellite alleles were categorized into 10 frequency classes, which permits checking whether the scattering followed the normal L-shaped form, where alleles with low frequency (0.01 – 0.1) were the most abundant.

The mode shift analysis exhibited no distortion of allelic frequency and L-shaped curve with the L-shaped distribution of allelic frequencies indicating no mode-shift in the frequency distribution confirming that both the local buffalo population and Murrah breed had not experienced genetic bottleneck in the recent past and is in mutation-drift equilibrium as shown in Fig. 5.

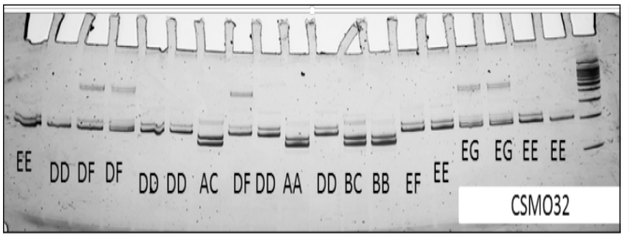

Fig. 3: Microsatellite (CSM032) alleles resolved on 6% Urea PAGE (Denaturing) and visualized by silver staining

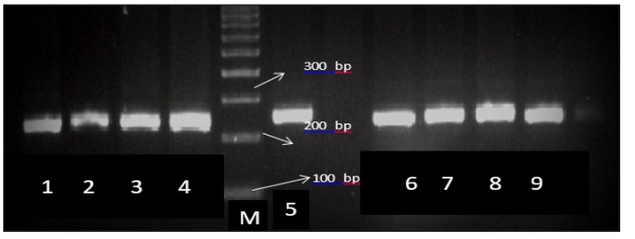

Fig. 4: UV illumination of PCR product (Microsatellite CSM032 (208-224 bp) run on agarose gel electrophoresis. M=100 bp DNA marker; Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9 = PCR products of CSM032 lying between range of 208-224 bp

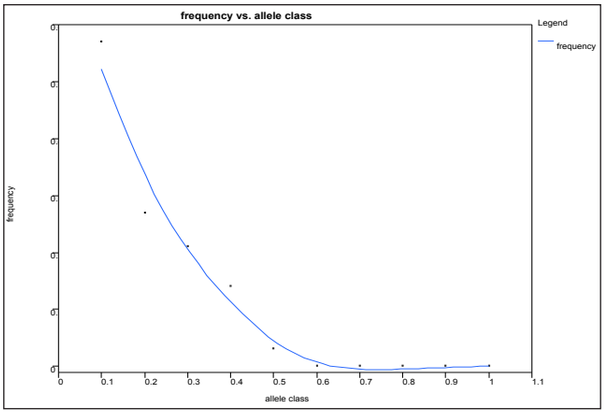

Fig. 5: Mode shift analysis in Local buffalo

Nei's measures of genetic identity and genetic distance

Genetic distance is a measure of the genetic divergence between species or between populations within a species, whether the distance measures time from common ancestor or degree of differentiation. Populations with many similar alleles have small genetic distances. This indicates that they are closely related and have a recent common ancestor. Genetic distance is useful for reconstructing the history of populations. Nei's genetic distance was calculated based on Dendogram based UPGMA-Modified method from NEGHBOR of PHYLIP Version 3.5 and Nei's Unbiased Measures of Genetic Identity and Genetic distance (Nei's 1978). The length was same (10.91883) in both Local buffalo and Murrah breed as shown in Table 1. Further Nei's genetic identity (above diagonal) and genetic distance (below diagonal) was 0.8038 and 0.2184 (Table 2) between local buffalo population and Murrah breed which shows closeness between Murrah and local buffaloes as expected from same geographical contiguity.

Smouse's multilocus analysis for single populations

In Smouse's multilocus analysis for single populations the average correlation was found as 0.2783 in Local buffalo population and 0.4322 in Murrah breed and significant chi square value for both local population and murrah breed as shown in Table 3.

Table 1: Dendrogram Based Nei's (1978) Genetic distance: Method = UPGMA (Modified from NEIGHBOR procedure of PHYLIP Version 3.5)

Between	And	Length
—	—	—
1	pop1	10.91883
1	pop2	10.91883

+----------pop1
--1
+----------pop2

Table 2: Nei's Unbiased Measures of Genetic Identity and Genetic distance

Popln Id	1	2
Local	****	0.8038
Murrah	0.2184	****

Whereas for Hardy Weinberg disequilibrium the value was significant in case of local buffalo population but non-significant in case of Murrah breed. The WHD measures non random union of gametes. The significant Hardy Weinberg disequilibrium (WHD) suggests that systematic and dispersive forces were acting in the population whereas non-significant Hardy Weinberg disequilibrium (P>0.05) value appeared in Murrah breed.

Table 3: Smouse's Multilocus Analysis for Single Populations

Pop ID	INDVD.	Ave. Corr	df	Chi-square	Probability	WHD*	df	Chi-square	Probability
Local Buffao	50	0.2783	105	155.37	0.0010	0.2012	15	25.68	0.0415
Murrah	25	0.4322	105	190.83	0.0000	0.1491	15	10.03	0.8178

* Measuring non random union of gametes (i.e., Hardy-Weinberg disequilibrium).

CONCLUSION

Overall the present study revealed that the use of microsatellite loci can be effectively used for genetic characterization and diversity studies in buffalo population. Present findings may be useful in characterization of genetic diversity and to develop strategies for conservation and utilization of local germplasm of J&K region, within and between existing buffalo population. The two studied buffalo population in present study has shown genetically distinctness from each other and thus there is good scope for bringing effective genetic improvement, conservation and designing future breeding policies for Buffaloes.

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