Letters in Animal Biology

Journal homepage: www.liabjournal.com

Genetic diversity analysis by using Heterologous Microsatellite markers among cattle and buffalo breeds

Manpreet Kaur [1], Simarjeet Kaur [2], Puneet Malhotra [3], C.S. Mukhopadhyay *[1]

[1] *College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India*

[2] *Department of Animal Genetics and Breeding, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India*

[3] *Directorate of Livestock Farms, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab India*

Article info

Received: 07 February 2024
Received in revised form: 17 April 2024
Accepted: 19 April 2024
Published online: 21 April 2024

Keywords

Microsatellite markers
Genetic variation
Cattle
Buffalo
Bottleneck

* *Corresponding author:*

C. S. Mukhopadhyay

Email: csmbioinfo@gmail.com

Reviewed by:

Rajkumar Sah

National Bureau of Animal Genetics Resources, Karnal-132 001, Haryana, India

Abstract

Microsatellite markers have become a reliable technique for genetic diversity studies, parentage analysis, and breed characterization in animals. The 18 amplified heterologous microsatellite markers out of 20 microsatellites were used for studying the genetic variation among cattle and buffalo. The estimated mean allelic diversity for cattle breeds was 12.50 for Sahiwal, 10.94 alleles in HF crossbred, and 10.444 and 10.944 alleles for Murrah and Nili Ravi breeds of buffalo, respectively. The Sahiwal breed had the highest allelic diversity compared to other studied breeds. A high level of genetic variability was observed for the observed heterozygosity (0.857±0.027) and expected heterozygosity (0.811±0.017) between the Sahiwal and HF crossbred breed of cattle. A low level of genetic variability was observed between the Murrahand Nili Ravi breeds of buffalo. The F_{IS} values -0.156 to 0.065 depicted low inbreeding in the breeds. The Nei's genetic distance was measured for all the breeds which showed the genetic distance/divergence between the HF crossbred and Sahiwal was 1.070. The genetic difference based on Nei's genetic distance between the cattle HF crossbred and Nili Ravi breed of buffalo was 2.456. The genetic difference between the Nili Ravi breed of buffalo and the HF crossbred was the highest. The principal component analysis accurately reflected genetic distances, forming distinct groups for HF crossbred, Sahiwal, Murrah, and Nili Ravi. The HF crossbred and Sahiwal appeared in different coordinates, indicating the notable genetic distance between these breeds, while Nili Ravi and Murrah clustered together in a single coordinate. These groups showcased clear genetic distinctiveness. The bottleneck analysis exhibited the typical L-shaped pattern, implying that all breeds did not undergo a recent bottleneck and were not at risk of potential extinction.

1. Introduction

In recent years, there have been significant studies on the individual and breed identification of major livestock species across many countries. This progress has been primarily driven by the adoption of microsatellites as the preferred molecular markers for tracking the information from species-level identification down to individual animals (Ansari et al. 2016). Microsatellites are DNA segments containing repetitive sequences of nucleotides that are distributed relatively evenly throughout the genomes of eukaryotic organisms. The variability in the number of repeat units within these regions contributes to their high polymorphism (Litt and Luty 1989). The combination of this high polymorphism and the uniform coverage of microsatellite markers across the genome has rendered them indispensable markers in a wide range of genetic investigations. Microsatellite markers have found extensive application in genetic research involving humans, model organisms, wild animals, and economically important animals in agriculture. Specifically, microsatellites have played a pivotal role in the study and characterization of diverse livestock breeds, including cattle and Buffalo (Katariaet al. 2009), horses (Gupta et al. 2005; Martinez et al. 2021), and pigs (Behl et al. 2002) due to their high polymorphism, locus specificity, and compatibility with polymerase chain reaction (PCR)-based analysis. Utilizing microsatellite markers to characterize animal populations can provide valuable insights into their evolution, phylogenetic relationships, and genetic variability. The understanding of genetic diversity within and among domesticated populations is essential for designing effective breeding programs and conservation policies. The researchers have successfully employed heterologous microsatellite markers designed for related species. This approach aids in analyzing diversity and conducting population genetic studies in less-studied species like buffalo (Hussain et al. 2017), providing a cost-effective and time-saving alternative (Moore et al. 1995; Barker 2002). Utilizing microsatellite markers allows the verification and assessment of genetic diversity within livestock, providing insights into the level of genetic variation within a specific lineage or breed. The evolution, forces such as mutation, adaptation, reproductive isolation, random drift, selection, and breeding can create the vast diversity (breeds) among the livestock (Groeneveld et al. 2010). The neutrality, co-dominant inheritance, and high polymorphic information content of microsatellite markers have rendered them the markers of choice for diversity studies

(Sharma et al. 2013). In this study, the heterologous microsatellites have been used for the genetic diversity analysis in cattle and buffalo breeds of the Punjab.

2. Materials and methods

2.1 Experimental samples

The blood samples were collected from the female and semen samples from the bulls. The samples were collected from 41 HF crossbreed, 37 Sahiwal breed of cattle; and 35 Murrah and 41 Nili Ravi breeds of buffalo from the ICAR-CIRB Nabha Farm, NDRI, Karnal, Directorate of Livestock Farm Ludhiana, and Veterinary Polytechnic Kaljharani were collected.

2.2 DNA extraction

The genomic DNA was isolated from whole blood stored at -20 ^{0}C using the phenol-chloroform-isoamyl alcohol method (Green and Sambrook 2018).

2.3 Genotyping of Microsatellite Primers

The 20 microsatellite primer pairs were selected from the published research papers based on the high polymorphism (Table 1). The fluorescently labeled 5′-end of the forward primer with FAM, HEX, and Texas Red was used in the multiplex for the amplification. The simple sequence length polymorphism was done by Barcode Biosciences, Bangalore.

2.4 Statistical analysis

Statistical analysis was done to check the genetic variation of the markers among the cattle and buffalo breeds. GenAlEx 6.5

Table 1 Details of Heterologous microsatellite primer pairs (sequence, annealing temperature) used for the study

SSR Primer	Sequence (5′ to 3′)	Primer Length	Tm (°C)	5′ Forward Dye Labeled	Reference
CSRM060	AAGATGTGATCCAAGAGAGAGGCA	24	61.37	Texas Red	Moore et al. 1995
	AGGACCAGATCGTGAAAGGCATAG	24	62.25		
ETH225	GATCACCTTGCCACTATTTCCT	22	58.11	HEX	ISAG conference 2006
	ACATGACAGCCAGCTGCTACT	21	61.79		
HEL09	CCCATTCAGTCTTCAGAGGT	20	56.54	FAM	Sodhi et al. 2007
	CACATCCATGTTCTCACCAC	20	56.43		
ILSTS006	TGTCTGTATTTCTGCTGTG	20	58.37	Texas Red	Kemp et al. 1995
	ACACGGAAGCGATCTAAACG	20	59.02		
ILSTS034	AAGGGTCTAAGTCCACTGGC	20	59.02	HEX	Kemp et al. 1995
	GACCTGGTTTAGCAGAGAGC	20	57.98		
INRA035	ATCCTTTGCAGCCTCCACATTG	22	61.47	FAM	Vaiman et al. 1994
	TTGTGCTTTATGACACTATCCG	22	56.53		
TGLA122	CCCTCCTCCAGGTAAATCAGC	21	56.53	Texas Red	Zhang et al. 2010
	AATCACATGGCAAATAAGTACATA	24	59.86		
HEL 5	GCAGGATCACTTGTTAGGGA	20	56.92	HEX	Vaiman et al. 1994
	AGACGTTAGTGTACATTAAC	20	50.31		
TGLA53	GCTTTCAGAAATAGTTTGCATTCA	24	56.75	HEX	Georges & Massey 1992
	ATCTTCACATGATATTACAGCAGA	24	55.75		
CSSM047	TCTCTGTCTCTATCACTATATTGC	24	55.12	FAM	Moore et al. 1995
	CTGGGCACCTGAAACTATCATCAT	24	60.69		
ILSTS028	TCCAGATTTTGTACCAGACC	20	54.66	Texas Red	Kemp et al. 1995
	GTCATGTCATACCTTTGAGC	20	54.1		
ILSTS029	TGTTTTGATGGAACACAGC	19	54.11	FAM	Kemp et al. 1995
	TGGATTTAGACCAGGGTTGG	20	56.81		
ILSTS030	CTGCAGTTCTGCATATGTGG	20	56.87	HEX	Kemp et al. 1995
	CTTAGACAACAGGGGTTTGG	20	55.95		
ILSTS056	GCTACTGAGTGATGGTAGGG	20	56.81	Texas Red	Kemp et al. 1995
	AATATAGCCCTGGAGGATGG	20	55.81		
ILSTS058	GCCTTACTACCATTTCCAGC	20	56.19	FAM	Kemp et al. 1995
	CATCCTGACTTTGGCTGTGG	20	58.83		
ILSTS061	AAATTATAGGGGCCATACGG	20	54.63	Texas Red	Kemp et al. 1995
	TGGCCTACCCTACCATTTCC	20	58.78		
ILSTS033	TATTAGAGTGGCTCAGTGCC	20	56.72	FAM	Kemp et al. 1995
	ATGCAGACAGTTTTAGAGGG	20	55.05		
CSSM033	CACTGTGAATGCATGTGTGTGAGC	24	63.12	HEX	Moore et al. 1995
	CCCATGATAAGAGTGCAGATGACT	24	60.2		
CSSM045	TAGAGGCACAAGCAAACCTAACAC	24	61.28	Texas Red	Moore et al. 1995
	TTGGAAAGATGCAGTAGAACTCAT	24	58.19		
CSSM066	ACACAAATCCTTTCTGCCAGCTGA	24	63.05	FAM	Moore et al. 1995
	AATTTAATGCACTGAGGAGCTTGG	24	62.08		

is a Microsoft Excel add-in that runs on Windows. GenAlEx 6.5 was used to perform the genetic diversity analysis of the population, such as the number of alleles, number of private alleles, allele frequencies and observed and expected heterozygosities, F-statistics, principal coordinate analysis, and Nei's genetic distance. Bottleneck analysis was done using the bottleneck v1.2.02 software which calculates the IAM (Infinite Allele Model), SMM (Stepwise Mutation Model), and TPM (Two Phases Model) mutation drifts.

3. Results and Discussion

The genetic variation has been depicted from the number of alleles, the effective number of alleles, and the heterozygosity for each breed of cattle and buffalo (Table 2, Fig. 1). The 18 amplified markers have been used for the study of genetic variation among cattle and buffalo. The mean number of alleles was highest in the Sahiwal breed having a 12.500±0.781 average number of alleles while the number of alleles in HF crossbred, Murrah, and Nili Ravi breeds varied from 10.444±0.923 to 10.944±0.574 alleles. The mean effective number of alleles varied from 4.604±0.528 in Nili Ravi to 6.120±0.624 in the Sahiwal breed of cattle. There was a difference in the effective number of alleles within the breed of the same species. The expected heterozygosity was 0.759±0.027 for Murrah and 0.725±0.033 for the Nili Ravi breed of buffalo breeds whereas in cattle breeds the mean expected heterozygosity was 0.7740±0.019 for the HF crossbred and 0.811±0.017 for the Sahiwal breed of the cattle (Table 2).

Table 2 Table depicting the mean and standard error for the Number of alleles (Na), Effective number of alleles (Ne), Shannon's information index (I), Observed heterozygosity (Ho), Expected heterozygosity (He), Unbiased expected heterozygosity (uHe), and Fixation indices (F_{IS})

Parameters	HF crossbred	Sahiwal	Murrah	Nilli Ravi
Na	10.94±0.574	12.50±0.781	10.56±0.971	10.44±0.923
Ne	4.95±0.393	6.12±0.624	5.17±0.582	4.60±0.528
I	1.85±0.069	2.00±0.085	1.80±0.115	1.70±0.118
Ho	0.822±0.034	0.857±0.027	0.868±0.027	0.695±0.059
He	0.774±0.019	0.811±0.017	0.759±0.027	0.725±0.033
uHe	0.783±0.019	0.821±0.017	0.770±0.027	0.734±0.033
F_{IS}	-0.058±0.029	-0.057±0.030	-0.156±0.038	0.065±0.062

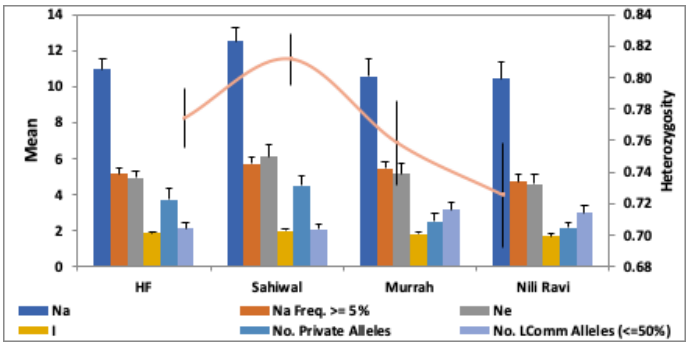

Fig. 1: Allelic patterns depicting the Number of alleles (Na), Effective number of alleles (Ne), and Shannon's information index (I) across the breeds

The Wright fixation index or F-statistics was observed to examine the variance in gene frequency between subpopulations. F-statistics measures the degree of gene differentiation within the population and is useful in evolutionary research as well as in the analysis of the population's genetic makeup (Whitlock 1999). Higher levels of inbreeding in an animal population result in a positive F_{IS} statistic; outbreeding causes a negative F_{IS} statistic. An F_{IS} of zero indicates inbreeding that is in line with the predicted amount based on allele frequencies in the population. Under the Hardy-Weinberg Equilibrium, inbreeding is indicated by a positive F_{IS}, while avoiding it is shown by a negative F_{IS} (Kardos et al. 2016). The mean F_{IS} values for the cattle breeds was -0.058±0.029 in HF Crossbred and -0.057±0.030 in Sahiwal; and for buffalo breeds it was -0.156±0.038 in Murrah, and 0.065±0.62 in Nili Ravi. The F_{IS} values point towards the low to moderate inbreeding values as the mean F_{IS} values ranged from -0.156±0.038 to 0.065±0.062. The Nili Ravi breed exhibited a positive F_{IS} value, indicating the presence of inbreeding within the population. The coefficient of genetic differentiation F_{ST} remained at zero for all loci across all breeds, indicating an absence of notable genetic subdivisions between these cattle populations (Karthickeyan et al. 2009). The gene flow across the breeds was negligible. In the study done by Radhika et al. (2023) in cattle breeds of Kerala state, the negative value of F_{IS} (-0.055) indicated a low level of inbreeding whereas the F_{ST} value of 0.1442 indicated genetic differentiation in the cattle breed of Kerala.

Nei's genetic distance is a measure of the genetic differentiation or distance between populations based on genetic markers. Nei's genetic distance estimates ranged from 1.070 to 2.456 for the cattle and buffalo breeds (Table 3). The Nei's genetic distance between the Holstein Friesian (HF crossbred) and Sahiwal was 1.070 whereas the genetic distance between the HF crossbred and Murrah and Nili Ravi was 2.397 and 2.456, respectively. The Nei's genetic distance from Murrah to Nili Ravi was 0.104. A higher genetic distance indicates greater genetic differentiation between the breeds, while a lower value suggests a closer genetic relationship. For instance, a higher genetic distance between Holstein Friesian (HF crossbred) and Nili Ravi implies a greater genetic differentiation as HF crossbred and Nili Ravi breeds are from different species. The genetic distance between Murrah and Nili

Table 3 Pairwise population matrix of Nei genetic distance

HF crossbred	Sahiwal	Murrah	Nilli Ravi	
0.000				**HF crossbred**
1.070	0.000			**Sahiwal**
2.397	1.772	0.000		**Murrah**
2.456	1.730	0.104	0.000	**Nilli Ravi**

Ravi is relatively lower as these breeds are from the same species.

The population assignment (Fig. 2 & 3), performed using GenAIEx, revealed that the HF crossbred and Sahiwal were at a distance in the quadrants whereas Nili Ravi and Murrah were clustered closely. The Nili Ravi and Murrah breeds were clustered in one quadrant depicting the least genetic difference between the buffalo breeds as shown by the Nei's genetic distance. The Principal Coordinates analysis (PCoA) showed that markers could correctly represent the genetic distances for the populations. PCoA forms a distinct group for the HF crossbred, Sahiwal, Murrah, and Nili Ravi which illustrates that they are separated from all the populations and thereby genetically distinguishable. In PCoA, the HF crossbred and Sahiwal are in different coordinates (Fig. 4) which depicts that the genetic distance between the breeds is significant, whereas Nili Ravi and Murrah are clustered in one coordinate.

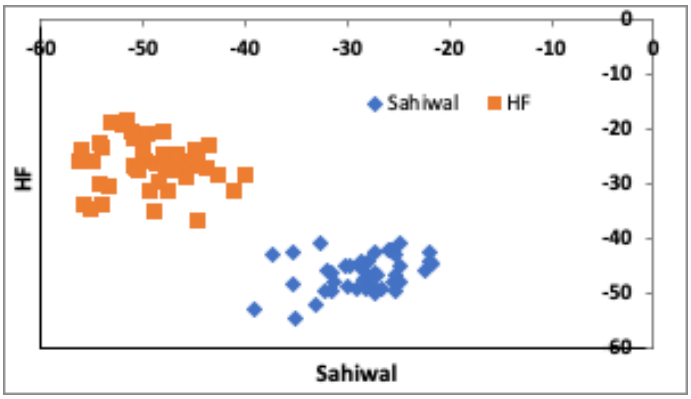

***Fig. 2**: Population assignment of HF crossbred and Sahiwal breed of cattle*

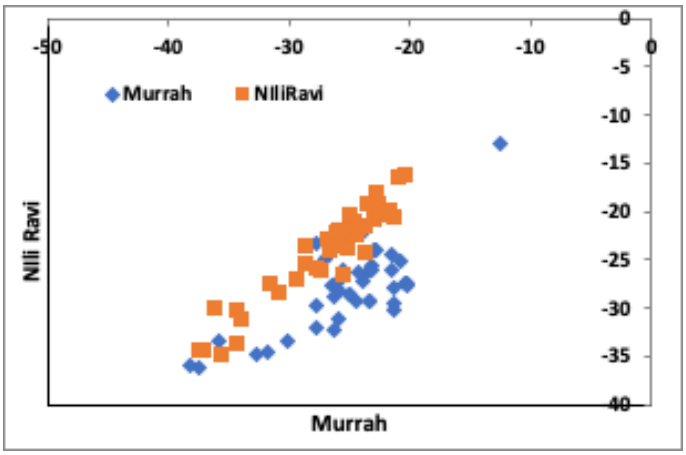

***Fig. 3**: Population assignment of Murrah and Nilli Ravi breed of buffalo*

The UPGMA based on Nei's genetic distance tree was constructed which splits the breeds based on the genetic distance between the studied breeds of cattle and buffalo (Fig. 5). In the UPGMA analysis of Ethiopian indigenous cattle Bonga, Jimma, and Kerayu cattle, it was observed that Bonga and Jimma clustered closely together, indicating a similarity in their characteristics. However, the Kerayu cattle stood out as a distinct group. Subsequent analyses using Principal Coordinates Analysis (PCOA) supported these findings by demonstrating that individuals were grouped differently, underscoring the influence of varied geographical origins and unregulated mating in creating a mixture of ecotypes (Bora et al. 2023).

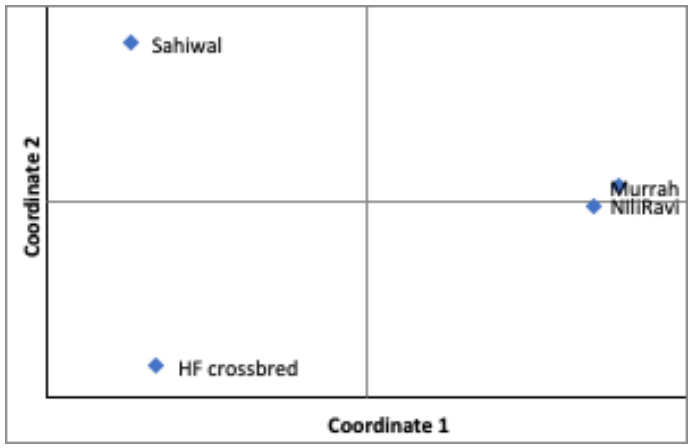

***Fig. 4**: Principal Coordinates (PCoA) assignment for the HF crossbred, Sahiwal, Murrah, and Nili Ravi breeds of cattle and buffalo*

***Fig. 5**: UPGMA-method-based dendrogram splitting the breeds into distinct clusters (Where HF = HF crossbred, SW = Sahiwal, MU = Murrah, NR = Nili Ravi)*

The Hardy Weinberg equilibrium was analysed for all the breeds which showed the departure for some of the markers used for the study depicting that there are some mechanisms such as evolutionary or non-random mating which could influence the allele and genotype frequencies. In the study of Kathiravan et al. (2010) the average observed and expected heterozygosity and inbreeding estimate (F_{IS}) of South Kanara buffaloes was calculated. Notably, the within-population F_{IS} showed a significant positive value across the majority of examined genetic loci which led to the departure from the Hardy-Weinberg principle. Microsatellite data were analyzed to assess the bottleneck of the population displaying a significant number of loci with an excess of gene diversity. The BOTTLENECK program analysis utilized three mutation models: the Infinite Allele Model (IAM), the Two-Phase Model of mutation (TPM), and the Stepwise Mutation Model (SMM) to evaluate the presence of population bottlenecks (Du et al. 2022). The probability values were obtained using the aforementioned models and statistical tests in the BOTTLENECK program. The expected number of loci with heterozygosity excess was 10.88, 10.71, and 10.49 for the IAM, TPM, and SMM, respectively, for HF crossbred. In Sahiwal, the expected number of loci with heterozygosity excess was 10.98, 10.87, and 10.63 for the IAM,

TPM, and SMM, respectively. The expected number of loci with heterozygosity excess was 10.85, 10.67, and 10.57 for the Murrah and 10.73, 10.72, and 10.57 for the Nili Ravi breed for the IAM, TPM, and SMM, respectively. The distribution followed the normal L-shaped form which suggests that none of the breeds encountered the bottleneck in the recent past and did not experience any potential risk of extinction. The absence of a shift in allele frequency distribution and the presence of a typical L-shaped curve in the Nagpuri buffalo of India (Kataria et al. 2009) suggest that there is no evidence of a bottleneck event in their genetic history.

4. Conclusions

The study shows the genetic variation between the cattle and buffalo breeds using the microsatellite markers. The number of alleles per locus (k) varied for Sahiwal, HF crossbred, Murrah, and Nili Ravi breeds of cattle and buffalo, respectively. The effective number of alleles also varied between the species and breeds. The microsatellite markers showed the genetic distance between the breeds which were in distinct quadrants for population analysis whereas Nei genetic distance also showed the difference between the breeds of cattle and buffalo. The microsatellite markers did not show the bottleneck as the markers followed the normal L-shaped distribution. The markers were highly informative for the genetic diversity and characterization of the cattle and buffalo breeds. The studied markers can be used as heterologous / cross-hybridization markers for both the cattle and buffalo species for genetic characterization, genetic diversity, and parentage studies.

Declarations

Funding: The research work has been funded by RKVY-12 (2018-19) project: "Enhancement of Production and Productivity Potential of Livestock, poultry, and Fisheries Sector for Socio-economic Upliftment of the Farmers of Punjab" B-5 "Identification of the Species-Specific Molecular Markers for Parentage Determination in Livestock.

Conflict of interest: The authors declare no conflict of interest

Ethical approval: Institutional Animal Ethics Committee (GADVASU/2021/IAEC/61/07; Dated 13.10.2021 & GADVASU/2023/ IAEC/67/02; Dated 14.01.2023.

Acknowledgements: Authors are thankful to the funding agency and Dr. Subhash Chandra Director of Animal Husbandry Punjab, Dr. Gurcharan Singh, Deputy Director of Rauni farm, Dr. Fateh Chand Tuteja ICAR-CIRB Nabha Farm, Director NDRI, Karnal, Directorate of Livestock Farm and the Veterinary Polytechnic Kaljharani staff for their support in sample collection.

References

Ansari A, Sikarwar P, Lade S, Yadav H, Ranade SH. (2016). Genetic diversity clusters in germplasm of Cluster Bean (*Cyamopsis tetragonoloba* L., Taub), an important food and an industrial legume crop. Journal of Agricultural Science and Technology 18(5): 1393-1406.

Barker GC. (2002). Microsatellite DNA: a tool for population genetic analysis. Transactions of the Royal Society of Tropical Medicine and Hygiene 96: S21-S24. https://doi.org/10.1016/S0035-9203(02)90047-7

Behl R, Sheoran N, Behl J, Tantia MS, Vijh, RK. (2002). Microsatellite sequences of mammals and their applications in genome analysis in pigs - A review. Asian-Australasian Journal of Animal Sciences 15(12): 1822-1830.

Bora SK, Tessema TS, Girmay G. (2003). Genetic diversity and population structure of selected Ethiopian indigenous cattle breeds using microsatellite markers. Genetics Research 2023: 1106755. https://doi.org/10.1155/2023/1106755

Du J, Hou C, Chen X, Xiao J, Gul Y, Wang H. (2022). Morphometric analysis and fluorescent microsatellite markers to evaluate the genetic diversity of five populations of *Penaeus japonicus* in China. Aquaculture and Fisheries 7(3): 321-327. https://doi.org/10.1016/j.aaf.2020.10.005

Georges M, Massey JM. (1992). Polymorphic DNA Markers in Bovidae. (World Intellectual Property Organization, Geneva) WO Publ. No. 92/13102.

Green M R, Sambrook J. (2018). Isolation and Quantification of DNA. Cold Spring Harbor Protocols 2018(6). https://doi.org/10.1101/pdb.top093336

Groeneveld LF, Lenstra JA, Eding H, Toro MA, Scherf B, Pilling D, Negrini R, Finlay EK, Groeneveld JE, Weigend S, Globaldiv Consortium. (2010). Genetic diversity in farm animals – a review. Animal Genetics 41: 6-31. https://doi.org/10.1111/j.1365-2052.2010.02038.x

Gupta AK, Chauhan M, Tandon SN, Sonia. (2005). Genetic diversity and bottleneck studies in the Marwari horse breed. Journal of Genetics 84(3): 295–301. https://doi.org/10.1007/bf02715799

Hussain T, Babar ME, Ali A, Nadeem A, Rehman ZU, Musthafa MM, Marikar FM. (2017). Microsatellite-based genetic variation among the buffalo breed populations in Pakistan. Journal of Veterinary Research 61(4): 535-542. https://doi.org/10.1515/jvetres-2017-0057

ISAG Conference. (2006). Cattle Molecular Markers and Parentage Testing Workshop, Porto Seguro, Brazil. http://www.isag.org.uk/ISAG/all/ISAG2006_CMMPT.pdf

Kardos M, Taylor HR, Ellegren H, Luikart G, Allendorf FW. (2016). Genomics advances the study of inbreeding depression in the wild. Evolutionary Applications 9(10): 1205–1218. https://doi.org/10.1111/eva.12414

Karthickeyan SMK, Sivaselvam SN, Selvam R, Thangaraju P. (2009). Microsatellite analysis of Kangayam cattle (*Bos indicus*) of Tamil Nadu. Indian Journal of Science and Technology 2(10): 38-40.

Kataria RS, Sunder S, Malik G, Mukesh M, Kathiravan P, Mishra BP. (2009). Genetic diversity and bottleneck analysis of Nagpuri buffalo breed of India based on microsatellite data. Russian Journal of Genetics 45(7): 826-832. https://doi.org/10.1134/S1022795409070102

Kathiravan P, Mishra BP, Kataria RS, Goyal S, Tripathy K, Sadana DK. (2010). Short tandem repeat-based analysis of genetic variability in Kanarese buffalo of South India. Russian Journal of Genetics 46: 988-993. https://doi.org/10.1134/S1022795410080119

Kemp SJ, Hishida O, Wambugu J, Rink A, Teale AJ, Longeri ML, Teale AJ. (1995). A panel of polymorphic bovine, ovine, and caprine microsatellite markers. Animal Genetics 26(5): 299-306. https://doi.org/10.1111/j.1365-2052.1995.tb02663.x

Litt M, Luty JA. (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. American Journal of Human Genetics 44(3): 397 – 401.

Martinez MM, Costa M, Corva PM. (2021). Analysis of genetic

variability in the Argentine polo horse with a panel of microsatellite markers. Journal of Equine Veterinary Science 96: 103320. https://doi.org/10.1016/j.jevs.2020.103320

Moore SS, Evans D, Byrne K, Barker JSF, Tan SG, Vankan D, Hetzel DJS. (1995). A set of polymorphic DNA microsatellites useful in swamp and river buffalo (*Bubalus bubalis*). Animal Genetics 26(5): 355-359. https://doi.org/10.1111/j.1365-2052.1995.tb02674.x

Radhika G, Aravindakshan T V, Anilkumar K, Manoj M, Thomas S. (2023). Genetic diversity analysis of cattle genetic groups of Kerala state using microsatellite data. Animal Biotechnology 34(4): 1154-1162. https://doi.org/10.1080/10495398.2021.2014857

Sharma PN, Diaz LM, Blair MW. (2013). Genetic diversity of two Indian common bean germplasm collections based on morphological and microsatellite markers. Plant Genetic Resources 11(2): 121-130. https://doi.org/10.1017/S1479262112000469

Sodhi M, Mukesh M, Prakash B, Mishra BP, Sobti RC, Singh KP, Ahlawat SPS. (2007). Microsatellite marker-based characterization of genetic diversity in Kankrej cattle. Journal of Applied Animal Research 31(2): 153-158. https://doi.org/10.1080/09712119.2007.9706651

Vaiman D, Mercier D, Moazami-Goudarzi K, Eggen A, Ciampolini R, Lepingle A, Guerin G. (1994). A set of 99 cattle microsatellites: characterization, synteny mapping, and polymorphism. Mammalian Genome 5: 288-297. https://doi.org/10.1007/BF00389543

Whitlock MC. (1999). Neutral additive genetic variance in a metapopulation. Genetics Research 74(3): 215-221.

Zhang Y, Wang Y, Sun D, Yu Y, Zhang Y. (2010). Validation of 17 microsatellite markers for parentage verification and identity test in Chinese Holstein cattle. Asian-Australasian Journal of Animal Sciences *23*(4): 425-429.

Citation

Kaur M, Kaur S, Malhotra P, Mukhopadhyay CS. (2024). Genetic diversity analysis by using Heterologous Microsatellite markers among cattle and buffalo breeds. Letters in Animal Biology 04(1): 23 – 28.