Genetic Diversity and Population Structure Analysis of Nepalese Cattle Breeds Inferred from Microsatellite Markers

Ishwari Prasad Kadariya*
Department of Animal Breeding and Biotechnology, Agriculture and Forestry University, Rampur
**Corresponding author: ipkadariya@afu.edu.np*

Abstract

Purpose: This study was conducted to investigate the genetic diversity and relationship among five Nepalese indigenous and crossbreeds' cattle using microsatellite markers.
Methods: Experiments were done using blood samples taken from different geographical locations and extraction of genomic DNA, PCR and GEL electrophoresis accomplished in the Center for Biotechnology lab AFU. Amplified fragments were analyzed by gel documentation system and dara were analysed by GenAIEx 6.51software program.
Results: The study of nuclear DNA was accomplished in 119 cattle which exhibited highly genetic variability across all the sample population. Mean allele number and observed heterozygosity for all loci were 18.00±0.67 and 0.04±0.01 respectively. Moreover, moderate genetic variability (4%) was reported among the sampled population. In spite of the sufficient genetic variability high inbreeding coefficient was observed within five different cattle breeds. Nei's genetic distance analysis, genetic distinctness was observed in Achhami as compared to other cattle breeds. Findings also revealed a close relationship between indigenous Pahadi and Jersey and Holstein crossbreds' cattle which might be due to Artificial insemination program of government.
Conclusion: Findings showed that bovine DNA markers has moderate genetic variability and genetic differentiation in Nepalese cattle breeds. Based on the results concluded that Achhami breeds was distinct from other breeds. However, results showed straightforward evidence of genetic diversity and relationships of Nepalese cattle that facilitate in executing effective breeding plan for the preservation and improvement of indigenous cattle breeds of Nepal.

Keywords: Crossbred, Genetic Diversity, Indigenous cattle, Microsatellite marker,

1 Introduction

Nepal is home to the rich in animal genetic resources and ranks the 49th in the world for genetic biodiversity (Joshi et al. 2020). The overall contribution of livestock to gross domestic product (GDP) of Nepal is 5.8% (DLS 2020) where cattle contribute significantly in food security and economic perspective. The indigenous Nepalese cattle breeds are Pahadi, Siri, Lulu, Terai, Khaila, Achhami, and Yak (Gorkhali, Sapkota, et al. 2021). In the last decades, marker-based selection as an efficient selection program have augmented genetic improvement in a number of breeds. Artificial insemination (AI) and embryo transfer (ET) have enabled the diffusion of genetic material. However, Nepalese indigenous cattle breeds developed over long times without following planed selection practices. Taurine cattle (Bos taurus) and zebu (Bos indicus) are considered as separate species despite their complete interfertility (Skryabin 2023). Likewise, Nepalese cattle breeds are divided into these two-district group: Bos Taurus and Bos indicus. Nevertheless, Nepalese indigenous and cross breed cattle has high genetic potentialities to adapt harsh climate, resistance to disease and sustain to low quality fodder and pasture (Gorkhali et al.2020). A wide and divergent range of climatic variability of Nepal have the conducive environment to develop and sustain for various cattle populations. Among the Nepalese cattle breeds most of the breeds are dual-purpose breeds especially for milk and draft power in agriculture and yak mainly for milk and transportation in high altitudes. Besides, Government crossbreeding program for genetic development and human population pressure are also contributing to the erosion of valued traits of Nepalese indigenous cattle breeds. In addition, introduction of highly yielding exotic breeds is also contributing to reduce the number of indigenous traits. Thus, some breeds are under severe negligence due to low productivity causing the continuous decline the indigenous cattle population globally (FAO 2007).
No doubt, Animal genetic diversity is under threat. The stated rate of breed extinctions is of great concern. However, the genetic diversity of indigenous low-production breeds is expected to contribute to current or future traits of interest (Toro, Fernández, and Caballero 2009). Growth of new and virulent animal diseases and climate change emphasize the need to retain this adaptive capacity. Millions of poor rural households, livestock remain

a key benefit, often meeting multiple needs, and enabling livelihoods to be built in some of the world's harshest environments. Livestock production system makes a big contribution to food and livelihood security of rural people, and meeting the United Nations Millennium Development Goals (FAO 2007). In Nepal where farming is largely subsistence, no pedigree record of farm animal is maintained at farmer herd and scientific mating plans are not practiced, leading to inbreeding depression and resulting in loss of potential production. In addition, advancement in feed technology has allowed optimal nutrition, improved transportation facility and modern communication systems have led to controlled animal production settings. As a result, highly productive breeds have substituted by local ones across the world.

Molecular characterization of indigenous breeds is one of the best ways for assessment of genetic variability which is essential and getting popular for genetic conservation in recent years. Molecular markers have indeed played a transformative role in the field of genetics. They provide a way to identify and analyze specific sequences in an organism's DNA, allowing for a more detailed understanding of genetic variation and facilitating more targeted and efficient genetic selection (Shi et al. 2010). Thus, recently molecular markers are extensively used for marker-assisted selection in livestock species. This technique allows breeders to identify and select individuals with desirable traits more efficiently, speeding up the process of developing improved livestock. A thorough understanding of variation within and between breed populations is required for the sustainable management of animal genetic resources (Groeneveld et al. 2010). The utilization of microsatellite markers stands out as an exceptionally potent approach for examining genetic diversity, estimating genetic distances, identifying bottlenecks and admixture.(Lyu et al. 2023) This is attributed to their pronounced polymorphism, widespread dispersion across the genome, codominant nature, heritable, locus specific and impartiality concerning selection pressures (Sodhi et al. 2008). Cattle play significant role in Nepalese economy though limited attempts have been undertaken to assess the molecular analysis among different cattle breeds through the application of microsatellite markers.

The objective of this study was to investigate the genetic diversity and breed relationships among five Nepalese indigenous and crossbred cattle. The utilization of molecular data from this study is expected to design and execute effective conservation strategies for government and concern stakeholders.

2 Methods

2.1 Breed identification, Sample Collection and DNA extraction

In total 119 animals were selected from three indigenous and two crossbred cattle breeds which constitutes of 22 Achhami, 20 Lulu, 25 Pahadi, 30 Jersey Crossbreed and 22 Holstein crossbred. Animal samples were randomly selected from their respective pocket areas, breeding tract and from an organized population maintained at National Cattle Research Program, NARC whereas the crossbred animal samples were selected from an organized farm as well as from various small farmers. The samples of Achhami cattle were collected from Bajhang district, Lulu cattle from Manang district and from at NCRP-NARC whereas samples of Pahadi cattle were collected from different hilly districts of Nepal; Okhaldhunga, Pyuthan, Arghakhachi and Uppardangadhi of Chitwan. Samples of Jersey crossbred cattle were collected from NCPR, NARC, Nawalparasi, Arghakhachi, Lahan, Rupandehi and samples of Holstein crossbred cattle from NCPR, NARC, Nawalparasi, Arghakhachi, Lahan. Morphometric data like, body length, height at withers, heart girth, ear length, head length, tail length and muzzle circumference were taken to be compared with the available phenotypes for breed confirmation in indigenous population. To confirm a random sample, blood sample were collected from different geographical area within their habitat, with a deliberate effort to ignore closely related individuals, determined through inclusive interviews with owners. Blood samples were drawn aseptically from jugular vein in 10 ml vacutainer tubes with EDTA as anticoagulant and were stored at–20 °C until for DNA extraction. Genomic DNA was extracted from blood using the protocols of Qiagen DNA extraction Minikit (Mihailova 2021). A commercial kit was used to extract genomic DNA from white blood cells for all samples. A spectrophotometer was used to determine the purity and concentration of each sample (Nano Drop 2000, Thermo Scientific, and Wilmington, Delaware, USA). The concentration of DNA was calculated spectrophotometrically by taking optical density (OD) at 260 nm. The ratio of optical densities at 260 nm and 280 nm was used to determine the purity of DNA. The samples with an OD ratio (OD260/OD280) ranging from 1.7 to 2.0 were used in subsequent experiments.

2.2 Marker Selection, PCR Amplification and Microsatellite Genotyping

Genomic DNA samples were amplified by PCR with the selected panel of bovine specific loci. The loci were chosen, according to ISAG/FAO recommendation aiming to analyze high polymorphic markers distributed all over the genome and with the ability to amplify in PCR reactions. A total of four microsatellite markers/loci present on the bovine genome were selected for genetic diversity analysis were designated to be amplified in cattle suggested by FAO Measurement of Domestic Animal Diversity (MoDAD), (FAO 2011). However, these markers were selected on the basis of their chromosomal location and their usage in previous studies. The details of these microsatellite markers along with their corresponding primer sequences, chromosomal location, annealing

temperature, allele range and GenBank accession numbers are summarized in Table 1.
The microsatellite marker primers pairs were synthesized at Bio Serve Biotechnologies (India) Pvt. Ltd., Hyderabad as recommended by FAO (ISAG). Initial additions of 100 µl of TE buffer (pH 8) were made to all of the primers provided by the manufacturer. Then, each primer was reconstituted in nuclease-free water that had been sterilized to a final concentration of 10 pmoles/µl. PCR was carried out in a total volume of 20 µl containing 7 µl of nuclease free water, 10 µl of master Mix, 185ng of forward primer, 185ng of reverse primer and 100 ng of template DNA. For the final PCR mixture preparation, the concentration of the genomic DNA was maintained at 90 µg/ml. The amplification of the microsatellite was performed in a BIO-RAD PTC 100 thermal cycler using the following temperature profile: initial denaturation at 95 ˚C for 4 min, denaturation at 94 ˚C for 30 sec, annealing 50-65 ˚C (Temp for different marker is in Table 1) for 45 sec, Extension 72 ˚C for 45 sec followed by 35 cycles and the final extension for 10 min at 72 ˚C. DNA fragments were divided by electrophoresis using UVP Gel documentation system. During electrophoresis, PCR products size was marked by running the 50bp/100bp DNA ladder as per expected size of primer. ABI PRISM 3100 automatic sequencer using agarose gel documentation (1.5-2%), the PCR products' amplification was validated. The gel was stained with Ethidium Bromide (EtBr). Under UV light, agarose gel documentation was used to record the alleles and likely genotypes of each sample for each microsatellite locus. The scoring of molecular size of microsatellite alleles in agarose gel was analyzed using Nine alliance gel documentation system by comparing the band size with standard 50bp/100bp DNA ladder. For a certain microsatellite locus, a score of one allele was regarded as homozygous, whilst a score of two alleles was regarded as heterozygous. The generated genotypic data was then assembled for statistical analysis.

Table 1: Details of ISAG-FAO recommended microsatellite markers selected for diversity study of Nepalese indigenous and exotic crossbred cattle and buffaloes.

S.N.	**Marker Name**	**Chromo -some**	**Primer Sequence Forward Reverse**	**Annealing temp(˚C)**	**Gene bank accession number**	**Allele Range (bp)**
1	BM1818 (D23S21)	23	AGCTGGGAATATAACCAAAGG AGTGCTTTCAAG GTCCATGC	56-60	G18391	248-278
2	BM1824 (D1s34)	1	GAG CAA GGT GTT TTT CCA ATC CAT TCT CCA ACT GCT TCC TTG	55-60	G18394	176-197
3	MM12 (D9s20)	9	CAAGACAGGTGTTTCAATCT ATCGACTCTGGGGATGATGT	50-55	Z30343	101-145
4	ETH225 (D9s1)	9	GATCACCTTGCCACTATTTCCT ACATGACAGCCAGCTGCTACT	55-65	Z14043	131-159

2.3 Statistical Analysis

Genetic polymorphism parameters including the allele frequencies, observed or number of alleles (N_a) per locus, effective (N_e) number of alleles, observed (H_o) and expected (H_e) heterozygosity and Hardy-Weinberg equilibrium tests (HWE) were calculated for each microsatellite locus using GenAIEx 6.5 program (Peakall and Smouse 2012). The Excel Power Marker 3.25 software calculated the polymorphic information content (PIC) for each microsatellite. Population structure was analyzed through unbiased Wright's F statistics (F_{is}, F_{st}, and F_{st}) and the Analysis of Molecular Variance (AMOVA) were obtained from the GenAIEx 6.5. The degree of genetic differentiation between the breeds was established by estimating Nei's standard genetic distance using GenAIEx 6.5 programs in the three breeds analyzed. Pairwise F_{ST} values among pairs of populations and Principal Coordinate Analysis (PCoA) exploring the relationship were also computed by using the GeneAlex. Based on the genetic distances obtained, MEGA 11.0.13 software was used to construct a phylogenetic/consensus tree using the neighbor-joining algorithm (Tamura et al. 2013).

3 Results

3.1 Microsatellite genetic variability in population

Genetic diversity status of Nepalese indigenous and crossbred cattle was established using microsatellite markers. All five breeds of cattle were able to successfully amplify in all four bovine specific microsatellite markers, and it declared that these markers were all polymorphic and can be utilized for the genetic studies on cattle species. Across all populations, a significant proportion of the markers followed to Hardy-Weinberg Equilibrium (HWE). The four microsatellite loci studied in the three native breeds, and two crossbreed cattle of Nepal found a total of 225 distinct alleles. The minimum number of alleles for selected microsatellite loci to be considered useful for

the evaluation of genetic diversity was four according selective standard of the microsatellite given by FAO (FAO 2011). Based on this measure, the observed allele number per locus ranged 15.75±1.89 Lulu to 20.25±1.11 Jersey crossbreed with mean number of alleles 18.00±0.67 confirming the selection of these microsatellite loci suitable for population genetic analysis in the current study (Table 2). While effective alleles per locus (A^e) varied from 12.62±0.96 Acchami to 16.63±1.65 Pahadi with overall mean number of 15.02±0.69. Genetic differences showed by number of alleles in each locus demonstrations direct consequence through breeds differentiation within a species (Fiona. C. Buchaman, Linda J. Adams Roger P. Littlejohn, Jillian F. Maddox 1994).
Observed (Ho) and expected (He) heterozygosity per locus are also presented in **(Table 2)**. Expected heterozygosity (H_e) varied from 0.92±0.01 (Lulu and Acchchami) to 0.94±0.01 (Pahadi, Jersey CB and Holstein CB) with an overall mean of 0.93±0.00, whereas observed heterozygosity (H_o) ranged from 0.10±0.05 (Holstein CB) to 0.05±0.03 (Achchcami) with an overall mean of 0.04±0.01. Lower the observed heterozygosity than expected across all the loci suggested that small number of samples used for analysis and low frequencies of allele in the populations (Demir and Balcioglu 2019). Heterozygote deficiency (F_{is}) found highest 98.1 % in Pahadi and lowest 89.06% in Holstein CB

Table 2: Genetic diversity indices across five Nepalese cattle breeds with four SSR markers

Cattle population	N_a	N_e	H_o	H_e	F_{is}
Pahadi	19.75±1.11	16.63±1.65	0.02±0.01	0.94±0.01	0.98±0.01
Lulu	15.75±1.89	13.52±2.03	0.03±0.01	0.92±0.01	0.97±0.02
Achhami	16.25±1.03	12.62±0.96	0.05±0.03	0.92±0.01	0.95±0.03
Jersey CB	20.25±1.11	16.61±1.08	0.03±0.02	0.94±0.00	0.97±0.02
Holstein CB	18.00±1.29	15.70±1.23	0.10±0.05	0.94±0.00	0.89±0.06
Mean±SE	18.00±0.67	15.02±0.69	0.04±0.01	0.93±0.00	0.95±0.01

N_a: Number of alleles; Ne: Effective number of alleles H_o: observed heterozygosity; H_e: expected heterozygosity; y, F_{is}- Inbreeding coefficient, *(p ¡0.05)

3.2 Population differentiation

Results of Wright's F-statistics for 4 microsatellite loci across populations are shown in **(Table 3)**. The deficit of heterozygotes across populations (F_{it}) accounted to 95.02% $*p < 0.05$. An overall significant deficit of heterozygotes (F_{is}) of 95.2 % occurred in the analyzed loci might be due to inbreeding within populations. The multi-locus F_{st} values of breed differentiation indicated that 5 % of the total genetic variation was due to unique allelic differences between the breeds, with the remaining 95 % corresponding to differences among individuals within the breed across the 4 markers. All loci contributed to the differentiation with the highest values found for ETH225 and BM1818 (5.5%).

Table 3: Wright's F-statistics for each of four SSR loci analyzed across five cattle populations

Locus	F_{is}	F_{it}	F_{st}	N_m
BM1818	0.99	0.99	0.04	5.54
BM1824	0.99	0.99	0.05	4.52
MM12	0.92	0.92	0.04	6.05
ETH225	0.91	0.92	0.05	4.91
Mean±SE	0.95±0.02	0.95±0.02	0.05±0.00	5.26±0.34

F_{is}: Inbreeding estimate; F_{st}: Level of genetic differentiation; F_{it}: Heterozygotes across populations N_m: Gene flow *** P¡0.001

The pair-wise F_{st} values of breeds **(Table 4)** ranged between 0.003 to 0.01, thereby revealing the least differentiation between Lulu and Jersey crossbred (0.03) and Jersey and Holstein cross breed (0.01). Similarly, AMOVA revealed that percent of variation among the populations was 2 % while within the population it was 94 % **(Table 5)** which is in line with the results of high expected heterozygosity than observed heterozygosity in all loci. while the remaining 4% of the variation is accounted for individual variation** Significant at P≤0.01.
In order to further visualization of the breed relationship and differentiation between the Nepalese indigenous

and crossbred cattle, associated phylogenetic tree was generated using the neighbor-joining tree on the basis of Nei's genetic distance. **(Figure 1)**. The dendrogram revealed two distinct clusters Achhami and other cattle breeds with more than 95 % bootstrap value. The first sub-cluster consists of Achhami breed which is uniquely separated from the rest of the cattle breeds. The second sub-cluster constituted of Lulu, Pahadi, Holstein and Jersey crossbred cattle. Similarly, the second cluster which is further divided into two different sub clusters constituting Lulu and Pahadi cattle breed. Whereas, another sub cluster constituted of crossbreds which are further well divided into Holstein and Jersey crossbreeds. The dendrogram indicated clear genetic separation of cattle with designating more distinct genetic uniqueness in Achhami cattle among cattle population as it was evidently demarcated from both indigenous and exotic cattle breeds. Phylogenetic relationship based on genetic distance revealed the clustering of breeds in two major clades, according to their geographic locations. The breeds form central regions were phylogeographically separated from achhami breed of western region. This demography of population expansion was in accordance with the mismatch distribution.

Table 4: F_{st} estimates between each pair of five Nepalese cattle populations

Pahadi	Lulu	Achhami	Jersey	Holstein	Cattle population
0.00					Pahadi
0.01	0.00				Lulu
0.02	0.02	0.00			Achhami
0.02	0.03	0.02	0.00		Jersey
0.02	0.02	0.02	0.01	0.00	Holstein

Table 5: Distribution of genetic diversity among Nepalese indigenous and cross bred according to Analysis of Molecular Variance

Sources of variation	Sum of squares	Variance components	Percentage variation(%)	F-statistics over all loci
Among Population	22.58	0.04	2%	Fst =0.02**
Among Individuals	436.72	1.88	94%	Fis =0.96**
Within individuals	0.029.50	0.08	4%	Fit =0.96**
Total	468.81	1.99	100%	

** Significant at P≤0.01

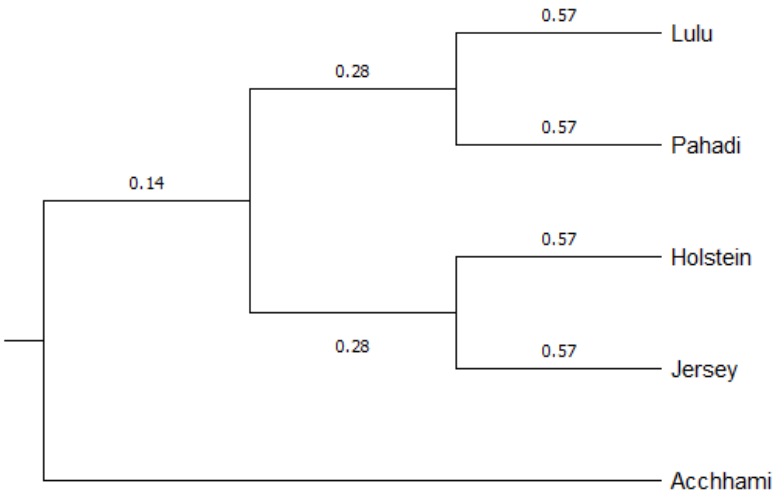

Figure 1: Dendogram (UPGMA) showing genetic relationship among five cattle populations based on Nei's (1972) distance. The numbers at the nodes are branch distance.

4 Discussion

Utilization of molecular data are the key for analyzing genetic diversity and preventing the undesirable loss of alleles. By this study genetic diversity and population structure of Nepalese cattle was estimated using nuclear genetic polymorphism. Allele frequencies were used to estimate Nei's genetic distances and Wright F-statistics was calculated using F_{is}, F_{it} and F_{st} between the Nepalese indigenous and crossbreed cattle.

Overall genetic variation of the five breeds populations found medium considering allele numbers and heterozygosity values of the microsatellite loci (Table 2). A total of 225 different alleles were detected for 4 microsatellite loci in all five cattle breed populations. The mean observed number of alleles in all the microsatellite loci were 18.00±0.67 and were higher than that observed by Sharma et al. in other Indian indigenous cattle breeds (Sharma et al. 2015). Similarly, mean allele numbers from this study were also higher than previous study of turkey's indigenous cattle (Demir and Balcioglu 2019). This may be indicative due lack of artificial selection pressure and also suggests, the substantial effective population size within the studied cattle populations (Bataillon, David, and Schoen 1996). Determination of genetic diversity based on allelic richness are important consideration for preservation of genetic potential through marker-assisted animal selection methods. For the long-term perspective selection limits are influenced by the initial allelic composition for future utilization genetic material (Sun et al. 2008).

observed heterozygosity (H_o) ranged from 0.10±0.05 (Holstein CB) to 0.05±0.03 (Achchcami) with an overall mean of 0.04±0.01. Expected heterozygosity (He) ranged from 0.92±0.01 (Lulu and Acchchami) to 0.94±0.01 (Pahadi, Jersey CB and Holstein CB) with an overall mean of 0.93±0.00 confirm the remarkable level of diversity in the populations Mostly, observed heterozygosity was lower than expected heterozygosity, this might be due to the existence of more homozygous individuals in the studied samples. Genetic variation from introduction of new alleles occurred by the random and natural process of mutation in cattle population contribute to adapt in the increasing climate change context (Sharma et al. 2015). The modest heterozygosity in cattle population might be low selection and lack of organized breeding programs.

Finding of this study showed significant genetic differentiation (F_{st} = 0.05±0.00). A shortfall in observed heterozygosity at the level of the population structure is shown by the fact that all F-statistics are positive. The F_{it}, F_{is} and F_{st} values were statistically significant at 1% levels $P < 0.001$. F-statistics results revealed an average shortfall of heterozygotes Fis of 94% $P < 0.001$ for each of the examined breeds and 4% (P¡0.001) between indigenous and crossbred exotic cattle population (F_{it}). Whereas, the F_{st} score of 2% suggested that there was a medium level of significant differences among the entire populations. Significant heterozygote deficit (F_{is}) 0.95±0.02 was observed for all breeds. Positive F_{is} value estimate for the populations indicates either the presence of inbreeding and /or Wahlund effect (existence of substructure within breed). However, blood samples were collected from dispersed geographical locations though existence genetic substructure cannot be controlled. It is evident that these native and crossbred cattle are genetically differentiated at some level and crossed the criterion for the differentiation of livestock breeds (i.e. F_{st} between 0.06 to 0.12 (Hall 2022). The relatedness of Pahadi cattle to both the exotic crossbreds and Lulu breeds might be due to the closer the geographical area and crossbreeding breeding practices for genetic improvement along with the nationwide spread of exotic germplasm via artificial insemination programs. Besides that, the gene flow through time and crossbreeding could be one of the reason to attained the results of differentiation (Bakae et al. 2022). Our finding is in line with F_{st} values reported in Legendary Vechur Cattle and Crossbred Cattle (F_{st}= 0.06) of Kerala State, India (Radhika et al. 2018).

Among cattle populations, the gene differentiation values ranged from 2% for Lulu-Pahadi pair and 1% for jersey cross -Pahadi pair, and 2% for Holstein Pahadi pair based on pairwise F_{st} estimates, thus showing lowest degree of genetic differentiation between Lulu-Pahadi pair and Holstein cross-Pahadi pair (Table 4). Some of the genetic closeness with Holstein and Jersey cross cattle with indigenous population could be accredited to the presence of gene flow among the indigenous populations during artificial breeding. However, all the values were unalike from zero $p < 0.01$ demonstrating that breeds are genetically distinct from one another. Open grazing practice in rural area may enhance the breeding for genetic mix-up in common pasture land. It could be inferred that Lulu and Pahadi breeds are more closely related genetically which could because of their geographical proximity and some level of genetic flow among these population. Using same bull for certain geographical area could be the reason of genetic resemblance between lulu and Pahadi cattle breeds. All indigenous cattle have higher genetic distance with exotic crossbred cattle as compared to indigenous cattle. Crossbreeding program and haphazard breeding practiced by farmers of study area might be the reason of higher inbreeding coefficient.

The depiction of breed association through a Neighbor-Joining (NJ) tree derived from Nei's genetic distance reveals a clustering pattern of breeds that aligns with the geographic distribution of populations from allelic frequencies is given in figure 1. Among the cattle populations, the greatest genetic distance found between Achhami and other breeds, while the least genetic distance was found between Lulu and Pahadi which could be clearly attributed to the lineage differences. The extensive sharing of diversity indicates a common ancestry or historical gene pool among the populations or breeds studied. It suggests that these groups have a shared genetic history and may have diverged relatively recently. The lack of genetic substructure implies a high level of gene flow or genetic exchange between the populations or breeds in close proximity. This could be due to factors such as migration, human-assisted movement of individuals and uncontrolled breeding practices. Our finding revealed that far western genotype achchami breed has distinct genotype compared to central regions genotype. Our findings are consistent and in agreement with previous work where nucleotide and haplotypes varied in three Nepalese indigenous breed where Achammi cattle shared higher haplotype diversity among three native Nepalese breeds obtained from nuclear DNA (Gorkhali, Sherpa, et al. 2021).

5 Conclusion

All the markers employed in the study were highly polymorphic and informative. The present study shows higher genetic diversity in Pahadi cattle as compared to that of Achhami, Lulu and other crossbred among population which could be attributed to the gene flow. It is important to report the moderate genetic diversity was observed which is lesser known in Nepalese indigenous cattle populations using molecular data. Besides that, non-random mating due to limited bulls within certain geographical region along with the absence of mating designs and registration could have resulted the deficiency of heterozygotes or increase the inbreeding coefficient. Unfortunately, the true to type native breeds are decreasing the numbers so that it may disappear in future. The analysis of genetic relationship and differentiation, it can be concluded that a medium level of genetic differentiation is revealed among the cattle breeds. The results also showed the genetic uniqueness Achhami from remaining exotic cattle and indigenous cattle which suggested that Nepalese indigenous breed has distinct breed-specific genetic characteristics. The results of the current study on Nepalese cattle breeds can be utilized for conservation and development of new breed to make it economically sustainable in the transforming agricultural scenario of the country. It also can serve as a foundation for further research and management of Nepalese cattle and buffalo breeds.

Acknowledgments

The authors acknowledge Directorate of research and Extension, AFU, for funding to accomplish the research and the Centre for Biotechnology, Agriculture and Forestry University, Rampur, Nepal for providing laboratory facilities for molecular genetics analyses. I thank National Cattle Research Program, NARC, cattle farmers and all the helping hands who supported this study through the collection of sample and information in the field.

Conflict of interest

The author declares that there is no conflict of interest.

References

Bakae, T., P. I. Monau, S. J. Nsoso, and P. M. Kgwatalala. 2022. "Assessment of Genetic Diversity and Relationship of the Two Sanga Type Cattle of Botswana Based on Microsatellite Markers." Tropical Animal Health and Production 54(4). doi:10.1007/s11250-022-03212-9.

Bataillon, Thomas M, Jacques L David, and Daniel J Schoen. 1996. "Neutral Genetic Markers." Genetics 144: 409–17.

Demir, Eymen, and Murat Soner Balcioglu. 2019. "Genetic Diversity and Population Structure of Four Cattle Breeds Raised in Turkey Using Microsatellite Markers." Czech Journal of Animal Science 64(10): 411–19. doi:10.17221/62/2019-CJAS.

DLS. 2020. "Livestock Statistics of Nepal 2077/78 (2020/21)." : 15–16. file:///C:/Users/ACER/Downloads/Livestock_Statistics_of_Nepal_2077_78_1659524236.pdf.

FAO. 2007. Food and Agriculture Organization of the United Nations The State of the World's Commission on Genetic Resources for Food and Agriculture.———. 2011. Recommended Microsatellite Markers. papers2://publication/uuid/1815F6E0-03DF-4283-ADEE-8EA900C802C2.

Fiona. C. Buchaman, Linda J. Adams Roger P. Littlejohn, Jillian F. Maddox, Allan M. Crawford. 1994. "Buchanan1994.Pdf." Genomics 22: 397–403.

Gorkhali, Neena Amatya, Chhiring Sherpa, et al. 2021. "Genetic Diversity of Nepalese Indigenous Cattle Breeds Based on D-Loop Mitochondrial DNA." Nepal Journal of Science and Technology 19(2): 103–8. doi:10.3126/njst.v20i1.39440.

Gorkhali, Neena Amatya, Saroj Sapkota, et al. 2021. 1 Indigenous Livestock Breeds of Nepal: A Reference Book of Government of Nepal, Nepal Agricultural Research Council.

Groeneveld, L. F. et al. 2010. "Genetic Diversity in Farm Animals - A Review." Animal Genetics 41(SUPPL. 1): 6–31. doi:10.1111/j.1365-2052.2010.02038.x.

Hall, Stephen J.G. 2022. "Genetic Differentiation among Livestock Breeds—Values for Fst." Animals 12(9). doi:10.3390/ani12091115.

Joshi, Bal Krishna et al. 2020. "Agrobiodiversity and Its Conservation in Nepal." Journal of Nepal Agricultural Research Council 6(March): 14–33. doi:10.3126/jnarc.v6i0.28111.

Lyu, Ding, Song Sun, Xiujuan Shan, and Weiji Wang. 2023. "Inbreeding Evaluation Using Microsatellite Confirmed Inbreeding Depression in Growth in the Fenneropenaeus Chinensis Natural Population." Frontiers in Genetics 14(February): 1–7. doi:10.3389/fgene.2023.1077814.

Mihailova, Yanka. 2021. "Genetic Diversity and Structure of 2 Indigenous Sheep Breeds (Kotel and Teteven) in Bulgaria Using Microsatellite Markers." Biotechnology and Biotechnological Equipment 35(1): 576–85. doi:10.1080/13102818.2021.1903339.

Peakall, Rod, and Peter E. Smouse. 2012. "GenALEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research-an Update." Bioinformatics 28(19): 2537–39. doi:10.1093/bioinformatics/bts460.

Radhika, G., T. V. Aravindakshan, S. Jinty, and K. Ramya. 2018. "Evaluation of Genetic Diversity, Population Structure, and Relationship Between Legendary Vechur Cattle and Crossbred Cattle of Kerala State, India." Animal Biotechnology 29(1): 50–58. doi:10.1080/10495398.2017.1297719.

Sharma, Rekha et al. 2015. "Genetic Diversity and Relationship of Indian Cattle Inferred from Microsatellite and Mitochondrial DNA Markers." BMC Genetics 16(1): 1–12. doi:10.1186/s12863-015-0221-0.

Shi, Zheng et al. 2010. "Analysis of Genetic Diversity and Distances in Asian Cattle Breeds Using Microsatellite Markers." 21: 795–802.

Skryabin, K.I. 2023. "Systematics and Phylogeny of Rhabditata." Key to Parasitic Nematodes (February): 132–62. doi:10.1163/9789004630437_009.

Sodhi, M. et al. 2008. "Genetic Diversity and Structure of Two Prominent Zebu Cattle Breeds Adapted to the Arid Region of India Inferred from Microsatellite Polymorphism." Biochemical Genetics 46(3–4): 124–36. doi:10.1007/s10528-007-9135-5.

Sun, Weibin et al. 2008. "Genetic Variation in Eight Chinese Cattle Breeds Based on the Analysis of Microsatellite Markers." Genetics Selection Evolution 40(6): 681–92. doi:10.1051/gse:2008027.

Tamura, Koichiro et al. 2013. "MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0." Molecular Biology and Evolution 30(12): 2725–29. doi:10.1093/molbev/mst197.

Toro, Miguel A., Jesús Fernández, and Armando Caballero. 2009. "Molecular Characterization of Breeds and Its Use in Conservation." Livestock Science 120(3): 174–95. doi:10.1016/j.livsci.2008.07.003.

Correct citation: Kadariya, I. P. (2024). Genetic Diversity and Population Structure Analysis of Nepalese Cattle Breeds Inferred from Microsatellite Markers. *Jagriti-An Official Journal of Gandaki University, 1*(1), 66-73.