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Microsatellite-based analysis deciphers the uniqueness of three lesser-known Indian cattle populations

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ABSTRACT

Indian cattle genetic resources constitute an important global gene pool. A majority of these (59.3%) are still not characterized. Identifying unique populations can ensure their inclusion in national policies of improvement and conservation. The present research describes the uniqueness of three lesser-known cattle populations (Jhari, Kamma, and Vandharvi) of Deccan Plateau region of India. These are known for draught power but are on the decline due to changing socio-agricultural scenario. Comprehensive genetic diversity and differentiation analyses using 25 FAO-recommended microsatellite markers identified high variability in all three populations with mean allelic diversity (Na) ranging between 9.32-9.80. Similarly, high genetic variability was recorded in all three populations (Ho=0.67 to 0.71). Random mating in the populations was indicated by the small positive F value. A low but significant genetic differentiation, pairwise Nei's genetic distance, phylogenetic relationship, and genetic assignment substantiated their separate genetic identity. The phylogenetic analysis reflected the closeness of Vandharvi and Kamma populations. Substantial gene flow was evidenced by the effective number of migrants per generation (Nm=16.31±2.69 >1). Bayesian-based clustering indicated the germplasm exchange between Vandharvi and Kamma, whereas, Jhari comes out to be a separate gene pool. None of these have suffered demographic bottlenecks in the recent past. Findings are valuable for the scientific management, recognition, and conservation of the three populations that contribute to the livelihood, and economic sustainability of agro-pastoral communities.

Keywords: Cattle, Genetic diversity, Microsatellite markers, Population differentiation

India is blessed with vast diversity of animal genetic resources including zebu cattle (*Bos indicus*) with 50 registered breeds (https://nbagr.icar.gov.in/). Indigenous cattle contribute significantly to the country's food security and sustainability. The majority of these were selected for adaptation to extreme environments, resistance to tropical diseases, and to be efficient draught breeds. Characterization and inventorization of cattle genetic resources are still not complete in the country as a significant proportion (59.3%) is referred as the non-descript (Sharma *et al.* 2020a). According to the 20th Livestock Census (2019) the indigenous cattle declined by 6% as compared to the previous census (www.dahd.nic.in) which is attributed to commercialization of milk production, mechanization, and modern intensive farming practices (Srivastava *et al.* 2019). Moreover, crossbreeding program is resulting in the genetic dilution of Indian cattle. Therefore the conservation of the country's cattle genetic resources has become a priority. However, it is likely that the majority of cattle will not be covered under such programs being not recognized and registered as distinct breeds.

The Telangana state is blessed with rich cattle resources (https://www.telangana.gov.in/departments/animal-husbandry-and-fisheries) with 4.21 million cattle of which the majority (3.62 million) are indigenous (www.dahd.nic.in). Almost all are categorized as non-descript though some are referred to by local names. Recently, three lesser-known populations (Jhari, Vandharvi, and Kamma) were characterized with collaboration between ICAR-National Bureau of Animal Genetic Resources (ICAR-NBAGR), the Watershed Support Services, and Activity Network (WASSAN), Telangana. These grey or white cattle were found to be unique populations based on the physical characteristics and morphometric traits, among the mixed population of cattle and contribute significant to the livelihood of the farmers (Siripurapu *et al.* 2019, Pundir *et al.* 2020). A population receives scientific attention and government support (insurance and other schemes) only once it is recognized as a breed. Thus there is an urgent need to characterize and evaluate these cattle populations and if found suitable register them as distinct breeds.

Genetic characterization is a frequently utilized tool to find out genetic variability existing in a population and

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also for differentiating the livestock populations (Sharma *et al.* 2020b, Saravanan *et al.* 2022). Microsatellites are the markers of choice for genetic diversity studies among genomic markers and have been used extensively for cattle across the globe including in India (Sharma *et al.* 2015, 2022). Thus, microsatellite markers were employed to reveal the genetic variability, structure, and relationship of the three lesser-known cattle populations Jhari, Kamma, and Vandharvi with an aim to identify the genetically differentiated populations. Findings can give impetus to their registration and can facilitate their conservation.

MATERIALS AND METHODS

Sample collection: Animals of Jhari, Vandharvi, and Kamma cattle (n=48 per population) were selected from their distribution area (Pundir *et al.* 2020, Siripurapu *et al.* 2019), viz. Jhari from Adilabad, Asifabad/Kumar Bheem, Nizamabad, and Jagtlial districts, Vandharvi from Kamareddi, Rajanna Sircilla, Nizamabad, and Vikarabad districts of Telangana state, and Kamma from Kurnool district of Andhra Pradesh. Only 2-3 clinically healthy cattle were selected per village to minimize genetic relationships and to maximize sample representativeness. Blood samples (8-10 ml) were collected from the *vena jugularis* of animals by veterinarians in the vacutainer tubes having EDTA (Ethylene diamine tetraacetic acid) as an anticoagulant. Samples were transported at 4°C and stored in the laboratory at -20°C till further use. DNA from whole blood was isolated by the standard phenol-chloroform extraction method (Sambrook and Russel 2001) and samples having 260/280 ratio of 1.8-1.9 indicated the good quality of extracted DNA.

Microsatellite genotyping: The DNA samples were genotyped using 25 cattle-specific microsatellite markers (Table 1) recommended by the Food and Agricultural Organization (FAO) of the United Nations for bovine genetic diversity studies (http://dad.fao.org/en/refer/ library/ guideline/marker.pdf). These are highly polymorphic markers that are spread all over the genome and can co-amplify in PCR reactions (FAO 2011). Fluorescent dye (FAM-blue, NED-yellow, PET-Red & VIC-green) labeled primers for the selected loci were synthesized by Applied Biosystems (Applied Biosystems, Foster City, CA, USA). PCR amplification and genotyping on automatic sequencer was carried out as per Sharma *et al.* (2022).

Estimation of diversity and differentiation among populations: Statistical analysis of the genotypes for genetic parameters, allele frequencies at each locus, the average number of alleles per population; observed (Na) and effective numbers of alleles (Ne) and observed (Ho) and expected heterozygosity (He), Shannon information index (I), as well as heterozygote deficit (F_{IS}) per locus across breeds and markers, were computed using GenAlEx 6.5 software (Peakall and Smouse 2012). To delineate relationships and genetic differentiation genotype, data was further analyzed for the population assignment, the distribution of genetic variability between various populations by Wright's *F*-statistics (F_{IS}, F_{ST}, *and* F_{IT}), phylogeny of the populations and the Bayesian method to infer clusters or sub-populations (K) that were most appropriate for interpreting the data as described by Sharma *et al.* (2022).

Bottleneck detection: Three heterozygosity excess tests (Sign test, standardized differences test, and Wilcoxon signed-rank test) developed by Cornuet and Luikart (1996) and a Mode shift indicator test based on qualitative descriptive allele frequency distribution (Luikart and Cornuet 1996) were utilized to test any bottleneck events in the history of populations. The probability distribution was established using 1,000 simulations based on allele frequency and heterozygosity under three models that are infinite allele model (IAM), step-wise mutation model (SMM), and two-phase model of mutation (TPM) using Bottleneck v1.2.02 (http://www.ensam.inra.fr/URLB).

RESULTS AND DISCUSSION

Allelic diversity of microsatellite markers and genetic diversity among populations: Microsatellite markers that are extensively exploited for estimating genetic diversity and divergence within and among populations (Sharma *et al*. 2020b, Mohanty *et al.* 2021) detected a total of 136 alleles across the 25 loci. All the SSR markers were found to be polymorphic and alleles could be scored unambiguously. The overall mean observed number of alleles per locus was 9.56±0.35. TGLA122 amplified the highest number of alleles (17) while TGLA227 amplified a minimum number of alleles (4) across populations. Accordingly, the expected number of alleles varied from 1.53 (TGLA227) to 9.40 (TGLA122) with an overall mean of 4.43±0.21. Allelic diversity in the Indian cattle breeds has normally been observed to be superior to that reported for the European counterpart possibly due to their large effective population size and lack of artificial selection pressure. Only a few breeds had lower diversity such as Deoni (5.82) and Red Kandhari (5.86) and Hariana (6.571±0.732) (Sodhi *et al.* 2005), while allelic diversity of higher magnitude has been reported for cattle breeds such as Ghumusari (12.19) and Bhinjharpuri (11.43) by Prakash and Deepika (2014).

All the 25 microsatellite loci of the chosen panel had four or more alleles and hence they fulfilled the criterion recommended by FAO (2011) for the markers to be used in analyzing diversity within populations and evaluating genetic differences between the breeds Moreover, the linkage disequilibrium between the selected loci was not significant. The private alleles, confined to one population only, were identified but the majority were rare alleles having less than 5% allele frequencies. Ninety-nine unique alleles were detected across three populations of which only 8 alleles in Jhari (3 at Hel09, 4 at Hel01, 1 at ETH10), 3 in Kamma (1 at Hel09, 2 at Hel01), and only one (ILSTS06) in Vandharvi cattle had >5% frequency. Shannon's Information Index (I), presented an overall high value (1.68±0.05). All the markers except TGLA227 had high I values (>1), which may be ascribed to the highly

Table 1. Characteristics of 25 microsatellite markers used for cattle diversity estimation

Panel	Locus*	Bovine chromosome no.	Primer sequences (5′-3′)	Fluorescent dye	T_m (°C)	Observed allele size range (bp)		
						Kamma	Vandharvi	Jhari
Panel 1	CSSM66	14	F-acacaaatcctttctgccagctga R-aatttaatgcactgaggagcttgg	FAM	60	179-221	179-221	179-221
	ETH10	5	F-gttcaggactggccctgctaaca R-cctccagcccactttctcttctc	NED	55	209-219	209-219	207-221
	ILSTS06	7	F-tgtctgtatttctgctgtgg R-acacggaagcgatctaaacg	FAM	58	291-303	291-305	287-301
	TGLA122	21	F-ccctcctccaggtaaatcagc R-aatcacatggcaaataagtacatac	VIC	58	135-163	135-173	129-167
	TGLA227	18	F-cgaattccaaatctgttaatttgct R-acagacagaaactcaatgaaagca	PET	55	83-103	83-87	83-135
Panel 2	BM1824	1	F-gagcaaggtgtttttccaatc R-cattctccaactgcttccttg	VIC	58	172-196	170-194	172-196
	CSRM60	10	F-aagatgtgatccaagagagaggca R-aggaccagatcgtgaaaggcatag	PET	55	90-120	88-116	90-116
	ILSTS11	14	F-gcttgctacatggaaagtgc R-ctaaaatgcagagccctacc	NED	58	251-275	249-271	249-271
	INRA05	12	F-caatctgcatgaagtataaatat R-cttcaggcataccctacacc	FAM	55	128-150	128-150	136-148
	INRA63	18	F-atttgcacaagctaaatctaacc R-aaaccacagaaatgcttggaag	PET	55	170-188	170-188	170-186
Panel 3	HEL05	21	F-gcaggatcacttgttaggga R-agacgttagtgtacattaac	VIC	55	90-118	100-124	100-136
	ETH03	19	F-gaacctgcctctcctgcattgg R-actctgcctgtggccaagtagg	NED	64	149-193	147-193	121-201
	ILSTS33	12	F-tattagagtggctcagtgcc R-atgcagacagttttagaggg	PET	55	113-149	113-149	113-157
	ILSTS05	10	F-ggaagcaatgaaatctatagcc R-tgttctgtgagtttgtaagc	NED	55	100-126	100-124	102-160
	INRA35	16	F-atcctttgcagcctccacattg R-ttgtgctttatgacactatccg	FAM	55	180-220	180-220	180-242
Panel 4	CSSM08	-	F-cttggtgttactagccctggg R-gatatatttgccagagattctgca	VIC	55	176-200	176-200	166-200
	CSSM33	17	F-cactgtgaatgcatgtgtgtgagc R-cccatgataagagtgacgatgact	NED	65	150-200	154-200	146-186
	TGLA53	16	F-gctttcagaaatagtttgcattca R-atcttcacatgatattacagcaga	FAM	58	152-186	152-178	150-188
	CSSM45	2q(2)	F-tagaggcacaagcaaacctaacac R-ttggaaagatgcagtagaactcat	PET	60	54-114	102-114	54-114
Panel 5	HEL09	8	F-cccattcagtcttcagaggt R-cacatccatgttctcaccac	FAM	59	136-200	138-198	138-168
	ILSTS54	21	F-gaggatcttgattttgatgtcc R-agggccactatggtacttcc	VIC	55	120-172	124-168	128-158
	MM08	2	F-cccaaggacagaaaagact R-ctcaagataagaccacacc	NED	55	102-144	102-152	106-142
	MM12	9	F-caagacaggtgtttcaatct R-atcgactctggggatgatgt	PET	55	102-136	102-166	102-126
Panel 6	HEL01	15	F-caacagctatttaacaagga R-aggctacagtccatgggatt	PET	55	152-198	152-172	104-166
	ILSTS34	5	F-aagggtctaagtccactggc R-gacctggtttagcagagagc	VIC	57	152-214	152-220	152-202

*Additional information concerning the bovine microsatellite markers can be acquired from http://dad.fao.org/en/refer/library/guidelin/marker. F, Forward primer; R, Reverse primer; T_m (°C), Annealing temperature.; I, Shannon's information index; Na, Number of alleles; Ne, Number of effective alleles; Ho, Observed heterozygosity; He, Expected heterozygosity; F_{IS}, Heterozygore deficiency/inbreeding coefficient.

polymorphic SSR markers and also to the higher genetic diversity present in cattle populations. It indicated that all the 25 SSR markers used in this diversity study were very informative and can potentially be used for performing diverse population genetics applications including linkage mapping, individual identification, and parentage testing in Indian cattle populations. Higher I estimate for selected SSRs were also reported in various Indian cattle breeds (Sharma *et al.* 2015) including the non-descript cattle (Sharma *et al.* 2022). The higher rate of SSR polymorphism might be contributed by the high diversity existing in these populations. Indeed the three lesser-known populations had sufficient diversity as evidenced by the observed number of alleles and heterozygosity (Table 2).

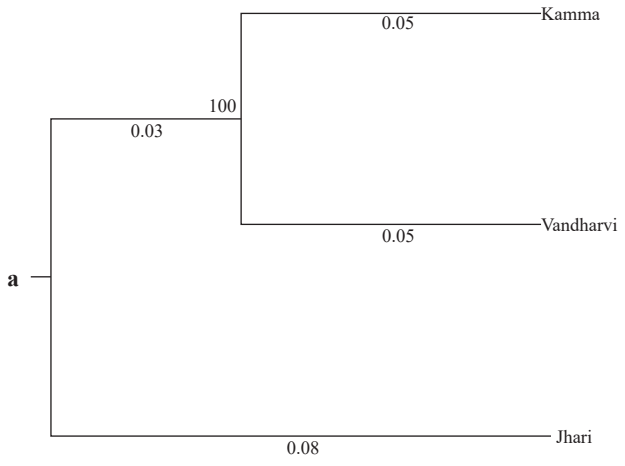
The overall mean values of observed and expected heterozygosity were 0.70±0.19 and 0.73±0.01, respectively. These values varied within a narrow range among the three populations. Mean observed heterozygosity was 0.67±0.03, 0.72±0.03, and 0.71±0.04 for Kamma, Vandharvi, and Jhari, respectively. Corresponding values for expected diversity were 0.72±0.03, 0.74±0.02, and 0.73±0.03. The highest heterozygosity was observed at the ILSTS54 locus in both the Kamma (0.96) and Vandharvi (0.94) populations while TGLA227 presented the lowest heterozygosity (Kamma=0.32 and Vandharvi=0.35). Whereas, TGLA122 and HEL05 had the maximum (0.96) and minimum (0.30) values in the Jhari cattle. The expected heterozygosity values ranged from 0.37 (TGLA227) to 0.88 (ILSTS34), 0.35 (TGLA227) to 0.89 (TGLA122), and 0.42 (TGLA227) to 0.89 (TGLA122) in Kamma, Vandharvi and Jhari cattle, respectively (Table 2). The indices of genetic diversity for the three populations were in the perspective of parameters reported previously for the registered breeds (Sharma *et al.* 2015) and lesser-known (Sharma *et al.* 2020a, 2020c, 2022) Indian cattle. Observed heterozygosity was more than that reported for several registered breeds of Indian cattle (0.61 to 0.66) as well as exotic such as Creole (0.61) and Chinese cattle (0.62) (McHugh *et al.* 1998, Radhika *et al.* 2017). Previously a higher genetic diversity in the native cattle breeds of Algeria was attributed to the extensive or semi-extensive breeding system (Rahal *et al.* 2021). Since all the populations investigated here also thrive under an extensive system of management, random mating might be one of the reasons for their substantial diversity status.

The majority of Indian cattle suffer from inbreeding and

Table 2. Genetic diversity indices for the three indigenous cattle populations of India

Population	Kamma						Vandharvi						Jhari					
Locus	Na	Ne	I	Ho	He	F	Na	Ne	I	Ho	He	F	Na	Ne	I	Ho	He	F
CSSM66	8	4.25	1.66	0.64	0.76	0.17	8	4.14	1.67	0.83	0.76	-0.10	8	4.18	1.58	0.80	0.76	-0.05
ETH10	5	3.00	1.26	0.42	0.67	0.37	5	3.20	1.26	0.71	0.69	-0.03	8	3.99	1.56	0.58	0.75	0.23
ILSTS06	6	3.58	1.42	0.65	0.72	0.10	8	4.36	1.72	0.54	0.77	0.31	7	3.21	1.45	0.61	0.69	0.11
TGLA122	13	6.03	2.12	0.81	0.83	0.02	15	8.78	2.37	0.68	0.89	0.24	17	9.40	2.46	0.96	0.89	-0.07
TGLA227	5	1.60	0.78	0.32	0.37	0.15	4	1.53	0.65	0.41	0.35	-0.18	8	1.72	0.93	0.44	0.42	-0.04
BM1824	8	3.55	1.49	0.73	0.72	-0.02	8	3.64	1.56	0.83	0.73	-0.14	6	2.77	1.22	0.65	0.64	-0.01
CSSM60	12	6.43	2.04	0.81	0.84	0.04	11	4.44	1.91	0.77	0.78	0.01	10	5.10	1.88	0.79	0.80	0.02
ILSTS11	10	2.84	1.46	0.71	0.65	-0.09	11	4.75	1.83	0.85	0.79	-0.08	9	2.28	1.25	0.60	0.56	-0.08
INRA05	11	4.89	1.81	0.83	0.80	-0.05	11	5.88	2.01	0.94	0.83	-0.13	6	4.52	1.58	0.81	0.78	-0.04
INRA63	7	2.67	1.32	0.65	0.63	-0.03	9	4.33	1.75	0.83	0.77	-0.08	6	2.69	1.23	0.53	0.63	0.15
ETH03	7	2.08	1.09	0.51	0.52	0.02	6	2.61	1.16	0.63	0.62	-0.02	7	2.45	1.26	0.55	0.59	0.07
HEL05	12	5.96	2.11	0.45	0.83	0.46	13	5.00	2.01	0.41	0.80	0.49	13	4.50	1.92	0.30	0.78	0.62
ILSTS33	8	3.31	1.39	0.78	0.70	-0.12	6	4.00	1.56	0.83	0.75	-0.10	9	3.80	1.63	0.85	0.74	-0.15
INRA35	8	5.18	1.83	0.91	0.81	-0.13	9	5.92	1.93	0.71	0.83	0.14	10	5.44	1.89	0.78	0.82	0.04
ILSTS05	7	4.27	1.65	0.72	0.77	0.06	9	6.88	2.03	0.80	0.86	0.06	11	6.54	2.02	0.92	0.85	-0.08
CSSM08	7	1.82	0.95	0.51	0.45	-0.13	7	1.97	1.09	0.42	0.49	0.14	10	1.98	1.17	0.46	0.50	0.07
CSSM33	12	3.99	1.84	0.72	0.75	0.04	15	5.63	2.21	0.84	0.82	-0.03	15	8.42	2.37	0.98	0.88	-0.11
TGLA53	14	2.61	1.62	0.70	0.62	-0.14	11	2.85	1.60	0.66	0.65	-0.01	17	3.56	1.96	0.73	0.72	-0.01
CSSM45	8	4.00	1.61	0.60	0.75	0.21	7	4.07	1.58	0.77	0.75	-0.02	9	4.67	1.79	0.90	0.79	-0.14
HEL09	14	7.23	2.26	0.84	0.86	0.03	14	7.13	2.27	0.76	0.86	0.12	12	7.76	2.24	0.75	0.87	0.14
ILSTS54	14	7.54	2.24	0.96	0.87	-0.11	12	6.22	2.07	0.94	0.84	-0.12	10	4.89	1.82	0.89	0.80	-0.12
MM08	10	3.55	1.61	0.63	0.72	0.13	9	3.09	1.42	0.83	0.68	-0.22	10	3.63	1.58	0.80	0.72	-0.10
MM12	10	3.44	1.67	0.79	0.71	-0.12	10	4.21	1.78	0.85	0.76	-0.11	9	3.91	1.69	0.77	0.74	-0.04
HEL01	10	6.02	2.01	0.35	0.83	0.58	5	3.60	1.40	0.43	0.72	0.41	6	2.46	1.17	0.63	0.59	-0.05
ILSTS34	13	8.08	2.25	0.71	0.88	0.20	11	5.45	1.95	0.82	0.82	-0.01	12	6.60	2.15	0.57	0.85	0.33
Mean	9.56	4.32	1.66	0.67	0.72	0.07	9.3	4.55	1.71	0.72	0.74	0.02	9.8	4.42	1.67	0.71	0.73	0.03
SE	0.57	0.36	0.08	0.03	0.03	0.04	0.6	0.34	0.08	0.03	0.02	0.04	0.63	0.40	0.08	0.04	0.03	0.03

N, Number of animals; Na, Number of observed alleles; Ne, number of effective alleles; I, Shannon information index for polymorphism content; Ho, observed heterozygosity; He, expected heterozygosity; F (F_{IS}), heterozygote deficiency/ Inbreeding coefficient.



b

Cattle population	Kamma	Vandharvi	Jhari
Kamma	0.000	0.019	0.024
Vandharvi	0.114	0.000	0.026
Jhari	0.157	0.168	0.000

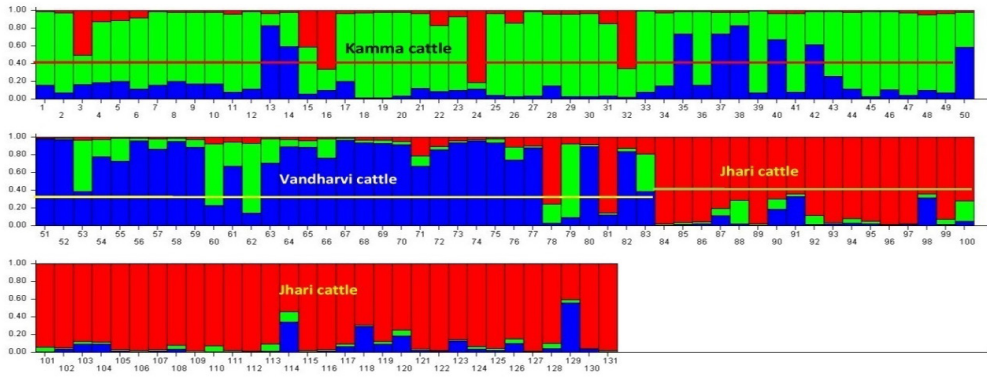
0.020

Fig. 1. a) Dendrogram (neighbor-joining tree) depicting genetic relationships among cattle populations based on Nei's genetic distance; b) Pair-wise population matrix of Nei's unbiased genetic distance (D_A) below diagonal and population differentiation (F_{ST}) above diagonal.

heterozygote deficiency and values as high as 11% and 22.1% have been reported for the Jharkhandi cattle and Ongole cattle, respectively (Sharma *et al.* 2022). Negative F_{IS} values are rare in literature and have been reported for Gangatiri cattle (F_{IS} = -0.01) (Sharma *et al.* 2015). However, in the present case, heterozygote deficiency as reflected by the coefficient of within-population inbreeding (F_{IS}) was not significant (P<0.05) (Table 2). A low positive F_{IS} value reflected a strong possibility that these are randomly mating populations. Some degree of outcrossing cannot be ruled out as the number of loci presenting negative F_{IS} values, 40% in Kamma, 64% in Vandharvi, and 60% in Jhari. Most likely it is arising from unplanned breeding as these are not registered populations and hence are not covered under the state breeding policy. Moreover, authorities recommend the upgradation of the low milk producing nondescript cattle with the semen of high milk producing Indian breeds or exotic or crossbred cattle that is resulting in the genetic dilution of the indigenous populations.

Genetic differentiation among the populations: *F*-statistics were applied to decipher the level of heterogeneity within and between the studied Indian cattle populations and results for each locus, across all the populations, are summarized in Supplementary Table 1. The global deficit of heterozygotes across populations (F_{IT}) amounted to 6.6 %. Heterozygote deficit (F_{IS}) in the analyzed loci due to the inbreeding within the populations amounted only to 3.9%. Cattle populations depicted an overall small (F_{ST} < 0.05), but significant genetic differentiation as the multi-locus F_{ST} value of breed differentiation indicated that 3% of the total genetic variation was due to the unique allelic differences between the populations. Similarly, AMOVA (analysis of molecular variance), revealed that 3.1% of total genetic variance resulted from genetic differentiation between the populations that differed significantly from zero at P<0.001 (Supplementary Table 2). Most of the genetic variation is within the breed and this variation could be a valuable tool for genetic improvement and conservation of these cattle populations.

Genetic differentiation of similar magnitude has been reported among cattle breeds of Odisha and hill cattle of Kumaun (0.044) (Sharma *et al.* 2012), Badri cattle (Dar *et al.* 2020), and cattle of eastern India (4.8%) (Sharma *et al.* 2013). Much higher F_{ST} value has been reported in other indigenous cattle (Malik *et al.* 2018, Sharma *et al.* 2015, 2022). The low value of genetic differentiation in these populations may be attributed to the lack of high selection



K	Reps	Mean LnP (K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
2	10	-10916.170000	4.960970	–	–	–
3	10	-10998.210000	296.365467	-82.040000	86.070000	0.290418
4	10	-10994.180000	270113535	4.030000	–	–

Fig. 2. Bayesian clustering of cattle populations under the assumption of *K* = 2-4 clusters. Different groups were represented by different colours. Each individual is represented by a vertical bar displaying membership coefficients for each genetic cluster. Optimum *K* was 3 as derived from the ΔK value (Highlighted).

Test/ Model		Kamma cattle			Vandharvi cattle			Jaari cattle		
		I.A.M.	T.P.M.	S.M.M.	I.A.M.	T.P.M.	S.M.M.	I.A.M.	T.P.M.	S.M.M.
Sign rank test (Number of loci with heterozygosity excess)	Exp	15.08	14.83	14.77	14.95	14.84	14.71	15.06	14.77	14.67
	Obs	17	11	3	18	11	5	17	12	2
	P- value	0.28418	0.08887	0.00000*	0.14815	0.08809	0.00009*	0.28196	0.17739	0.00000*
Standardized differences test	T2 value	0.917	-3.836	-12.519	1.633	-2.127	-8.764	0.760	-4.116	-13.514
	P- value	0.17969	0.00006*	0.00000*	0.05118	0.01671*	0. 00000*	0.22356*	0.00002*	0.00000*
Wilcoxon rank test (one tail for heterozygosity excess)	P- value	0.05997	0.95743	1.00000	0.01275*	0.89001	0.99999	0.05675	0.87395	1.00000

*Rejection of null hypothesis (p<0.05)

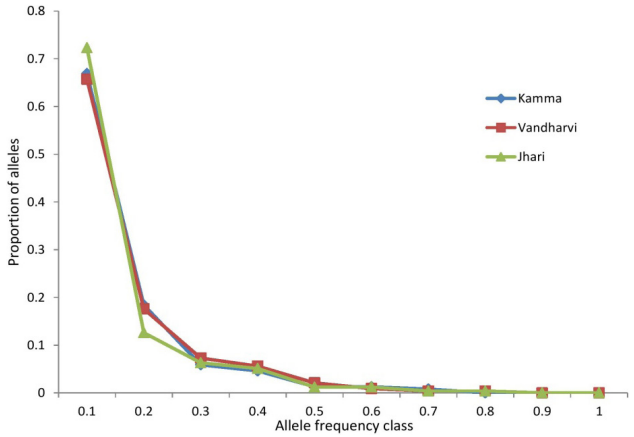


Fig. 3. Population bottleneck analysis under different mutation models and corresponding mode shift curves depicting lack of bottleneck for the three cattle populations.

pressure as compared to established breeds of India.

Pair-wise F_{ST} coefficients between the populations revealed that the Jhari cattle were most differentiated (0.026) from the Vandharvi followed by Kamma cattle (Fig. 1b). The smallest differentiation was observed among Vandharvi and Kamma cattle (0.019). Visualization of breed relationship through NJ tree separated all the cattle populations with Jhari cattle having the largest genetic distance (Fig. 1a). Based on the Nei's genetic distance (D_A) Jhari cattle were distant from both Vandharvi (D_A = 0.168) and Kamma (D_A = 0.157) cattle populations. These results were in unison with the *F*-statistics interpretation. Moreover, all the individuals of Jhari cattle were assigned correctly to their group which once again reiterated the discrete genetic differentiation from the other two populations. However, a large proportion of Vandharvi animals (26%) were assigned to the Kamma population. Similarly, 17% Kamma animals were assigned to the Vandharvi group (Supplementary Fig. 1).

Intermixing among the populations was evidenced by the exchange of germplasm among populations (Nm = 16.31±2.69) (Supplementary Table 1). The largest gene flow between pairs of the populations was among Kamma and Vandharvi (Nm = 13.02) followed by Jhari and Vandharvi (Nm = 10) and Jhari and Kamma (Nm = 9.42).

Further, Bayesian approach-based clustering was used to study the population structure in these three populations. The highest ΔK value that is likely to best capture the variation present in the data was found at K = 3. Therefore it was chosen to demonstrate the genetic structure (Fig. 2). Populations partitioned into individual clusters (presented in different colours) indicated that the three populations are distinct but intermixing among Kamma and Vandharvi. Probable factors contributing to the gene flow can be a consequence of the past and existing management practices, totally natural breeding, ecological factors, and lack of breeding policies.

Genetic bottleneck estimation: A population that has recently suffered a bottleneck has the preponderance of loci with an excess of heterozygotes (beyond the heterozygosity expected in a population at mutation drift equilibrium). Thus, the Sign, Standardized differences, and Wilcoxon sign rank tests were applied across all three models (IAM, TPM, and SMM) to estimate the excess of heterozygosity (Fig. 3). For the Wilcoxon rank test, heterozygosity excess was not significant across all three mutation models in both Kamma and Jhari cattle and except IAM in Vandharvi cattle. Similarly, the heterozygosity excess under two models (IAM and TPM) was not significant (P>0.05) for the Sign rank test in all three populations. Thus the null hypothesis that these three populations are at present in the mutation-drift equilibrium was accepted similar to other Indian cattle breeds reported in the literature (Sharma *et al.* 2020a, 2020c). A second method to identify the potential bottleneck, the Mode-shift indicator test was also applied. Due to the abundance of alleles with the lowest frequencies (0.01-0.1) a normal 'L' shaped distribution between allelic class and proportion of alleles was observed across the three populations (Fig. 3). It can be concluded that the demographic bottlenecks were not identified in the recent history of any of these populations in spite the use of data generated with the microsatellite markers.

Molecular characterization identified plentiful genetic diversity and uniqueness of the three lesser-known populations of India in accordance with the previous findings based on morphometric characterization. High genetic diversity infers that these populations can respond to the future challenges imposed by diseases, environmental variations, and changing market scenarios. These are also precious gene pools for the selection (natural or artificial) of adaptive traits. Unfortunately, animals of these populations are continuously decreasing due to

changes in agricultural practices as mechanization is fast replacing the traditional, crossing with available exotic or crossbred semen, and exclusion from government policies. Apathy of young generation under the influence of socio-economic aspirations and shrinking common grazing areas and forest restrictions are also adversely affecting them. Thus, it is critical to register Jhari, Vandharvi, and Kamma as registered cattle breeds of India and include them in the national conservation program where maintenance of genetic diversity is the major objective.

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REFERENCES

Cornuet J M and Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001–14.

Dar A H, Kumar S, Mukesh M, Ahmad S F, Singh D V, Sharma R K, Ghosh A K, Singh B, Rahman J U and Sodhi M. 2020. Genetic characterization and population structure of different coat colour variants of Badri cattle. *Molecular Biology Reports* **47**: 8485–97.

FAO. 2011. Guideline for molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Commission on Genetic Resources for Food and Agriculture. Food and Agriculture Organization of the United Nations.

Luikart G and Cornuet J M. 1998. Empirical evaluation of a test for identifying recently bottlenecked population form alleles frequency data. *Conservation biology* **12**: 228–37.

Malik A, Sharma R, Ahlawat S, Deb R, Negi M and Tripathi S. 2018. Analysis of genetic relatedness among Indian cattle (*Bos indicus*) using genotyping-by-sequencing markers. *Animal Genetics* **49**: 242–45.

McHugh D E, Loftus R T, Cunningham P and Bradley D G. 1998. Genetic structure of seven European cattle breeds assessed using 20 microsatellite markers. *Animal Genetics* **28**: 333–40.

Mohanty S, Donde R, Das S, Panda D, Mishra B, Pradhan S K, Dash S K, Swain P and Behera L. 2021. Utilization of genetic diversity and population structure to reveal prospective drought-tolerant donors in rice. *Gene Reports* **23**, https://doi.org/10.1016/j.genrep.2021.101151.

Peakall R and Smouse P E. 2012. GenAlEx 65: genetic analysis in Excel. Population genetic software for teaching and research – An update. *Bioinformatics* **28**: 2537–39.

Prakash B and Deepika. 2014. Assessment of genetic diversity and admixture in two newly recognized cattle breeds of Eastern India. *Indian Journal of Animal Sciences* **84**: 752–56.

Pundir R K, Singh P K, Dangi P S and Das S. 2020. Characterisation of Jhari cattle-A new cattle germplasm from Telangana. *Indian Journal of Animal Sciences* **90**: 1494–98.

Radhika G, Aravindakshan T V, Jinty S and Ramya K. 2017. Evaluation of genetic diversity, population structure, and relationship between legendary Vechur cattle and crossbred cattle of Kerala state, India. *Animal Biotechnology* **29**: 50–58.

Rahal O, Aissaoui C, Ata N, Yilmaz O, Cemal I, Ameur A and Gaouar S B S. 2021. Genetic characterization of four Algerian cattle breeds using microsatellite markers. *Animal Biotechnology* **32**: 699–707.

Sambrook J, Russell D. 2001. *Molecular Cloning: A Laboratory Manual* (M), 3rd Cold Spring Harbor Laboratory Press, New York.

Saravanan K A, Panigrahi M, Kumar H, Nayak S S, Rajawat D, Bhushan B and Dutt T. 2022. Progress and future perspectives of livestock genomics in India: A mini review. *Animal Biotechnology* DOI: 10.1080/10495398.2022.2056046

Sharma R, Ahlawat S, Sharma H, Bhagat R L, Singh P K and Tantia M S. 2020a. Identification of a new potential native Indian cattle breed by population differentiation based on microsatellite markers. *Molecular Biology Reports* **47**: 6429–34.

Sharma R, Ahlawat S, Sharma H, Sharma P, Panchal P, Arora R and Tantia M S. 2020b. Microsatellite and mitochondrial DNA analyses unveil the genetic structure of native sheep breeds from three major agro-ecological regions of India. *Scientific Reports* **10**: 6050 https://doi.org/10.1038/s41598-020-77480-6

Sharma H, Sharma R, Ahlawat S, Raja K N, Jain A and Tantia M S. 2020c. Genetic diversity status of only registered cattle breed of Chhattisgarh- Kosali. *Indian Journal of Animal Sciences* **90**: 873–78.

Sharma R, Ahlawat S, Sehrawat R, Aggarwal R A K, Chandran P C, Kamal R K, Dey A and Tantia M S. 2022. Morphometric characteristics and microsatellite markers based diversity and differentiation recognizes the first prospective cattle breed from the Jharkhand state of India. *Animal Biotechnology*, DOI: 10.1080/10495398.2022.2064866

Sharma R, Kishore A, Mukesh M, Ahlawat S, Maitra A, Pandey A K and Tantia M S. 2015. Genetic diversity and relationship of Indian cattle inferred from microsatellite and mitochondrial DNA markers. *BMC Genetics* **16**: 73–84.

Sharma R, Maitra A and Pandey A K. 2012. Genetic structure and differentiation of four Indian autochthonous cattle populations. *Russian Journal of Genetics* **48**: 611–17.

Sharma R, Maitra A, Singh P K and Tantia M S. 2013. Genetic diversity and relationship of cattle populations of East India: Distinguishing lesser known cattle populations and established breeds based on STR markers. *Springer Plus* **2**: 359–68.

Siripurapu Kanna K, Das S, Pundir R and Tantia M S. 2019. Network Project of AnGR of ICAR-NBAGR for identification and characterization of yet undefined indigenous cattle populations of India. DOI: 10.13140/RG.2.2.32608.02564

Srivastava A K, Patel J B, Ankuya K J, Chauhan H D, Pawar M M and Gupta J P. 2019. Conservation of indigenous cattle breeds. *Journal of Animal Research* **9**: 1–12.