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Genome wide locus-specific ancestry analysis revealed adaptive admixtures in crossbred cattle of India

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Crossbreeding in India has been widely adopted to address low sustainability and poor productivity in non-descript cattle. This study analyzed Vrindavani (VRI) crossbred cattle and their parental populations (Holstein Friesian (HOL), Jersey (JER), Brown Swiss (BSW), Hariana (HAR) using SNP data to characterize locus-specific ancestry in VRI's genome along with admixture proportions and population stratification. Admixture analysis showed VRI have 67.3% HOL, 20.1% HAR, 8.5% JER, and 4% BSW ancestry. Locus-specific ancestry estimation identified regions with adaptive admixtures, which can be defined as admixed genomic regions favored by evolutionary forces and increased their frequencies, revealed 79.7% *Bos taurus* and 20.3% *Bos indicus* ancestry. Notably, regions on chromosome (chr) 2, 3, 4, 5, 7, 10, 12, 13, 14, 16, 17, 19, 20, 21, 22, 23, and 24 were associated with disease resistance contributed by indicine ancestry and chr 1, 6, 9, 11, 15, 18, 27, and 28 related to production which were contributed by taurine ancestry. The study concluded that increased taurine ancestry contributes to higher milk yield in VRI crosses, while indicine ancestry confers disease resistance and adaptability to tropical climates. This comprehensive genomic analysis suggests that while taurine inheritance enhances milk yield, a balance with indicine traits is essential for resilience. Understanding locus-specific ancestry patterns can aid in refining breeding strategies by selectively promoting beneficial alleles. Future advancements in genomic tools may enable controlled inheritance of desirable traits, maximizing heterosis in structured breeding programs for sustainable cattle production.

Keywords Vrindavani, Locus-specific ancestry, *Bos taurus*, *Bos indicus*, Heterosis

Cattle ranked as the important domesticated animal in terms of economic impact, playing a key role in the development of human societies and civilizations. Genomic studies of domestic cattle have shown that cattle across the globe can be categorized into five main groups based on their continent of origin i.e., European taurine, Eurasian taurine, East Asian taurine, Chinese indicine, and Indian indicine[1]. Among them, Indian indicine has made a notable contribution to the global cattle genetic pool, with a total cattle population of 193.46 million. Of these, 51.36 million (26.5%) are well characterized and recognized indigenous breeds, while 142.11 million (73.5%) are non-descript or crossbred[2]. India also holds the title of the largest milk-producing nation, with a total milk production of 198.44 million metric tons in 2019-20, and cattle account for approximately 51% of this output[3]. Over the past years, interest on crossbreeding has increased in many countries due to its well documented advantages, largely attributed to heterosis or hybrid vigor and complementarity of the traits[4,5]. As a result, structured crossbreeding programs have been implemented in various nations, with Denmark, United States, and New Zealand leading the way[6]. In India, crossbreeding programs in cattle began in the 1970s, promoted by Indian Council of Agricultural Research (ICAR) and National Dairy Development Board (NDDB)[7].

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Vrindavani (VRI) cattle is one such crossbred developed at the ICAR-Indian Veterinary Research Institute, by combining four breeds viz., Holstein Friesian (HOL), Hariana (HAR), Jersey (JER), and Brown Swiss (BSW)[8,9].

Recent technological developments have enabled the cost-effective use of high-density DNA micro-arrays, allowing for the examination of thousands of genome-wide markers in all major livestock species[10,11,13]. This information has been employed in numerous studies to successfully trace fine-scale gene flow, identify selection signatures, and associate specific allelic variants with quantitative traits in various species[14]. Gene flow events, whether occurs from natural causes or human activities causes admixing of genomes. If such admixed genomic regions contribute adaptability to dynamic environment and genetic fitness, their frequency gets increased by natural or artificial selection. Such regions are known as adaptive introgression or adaptive admixtures[15,16]. High-resolution genome data has facilitated conceptualization of local ancestry inference methods that can determine the ancestry of specific chromosomal segments and all the chromosomal segments in the genome and pinpoints ancestry blocks[17]. When selection acts within an admixed population, the frequency of selected alleles tends to increase over several generations, leading to variations in locus-specific ancestry[18]. Consequently, adaptive introgression can be identified by detecting regions in the genome where ancestry is fixed or nearly fixed from a particular source population[19]. These deviations from expected patterns in the genomes of admixed individuals, whether excesses or deficiencies, serve as indicators of recent selective pressures. Because the effect on these regions accumulates over multiple generations, they can be interpreted as signs of selection following admixture[19,20].

The examination of admixture signatures based on variations in local admixture levels has garnered significant interest in human[18,19] and livestock genetic research[21–24]. In Indian cattle there are no locus-specific ancestral studies have been observed, hence to bridge this gap, the present investigation was taken to see the chromosome wide ancestry of the VRI crossbred cattle and impact of evolutionary forces.

Materials and methods

Vrindavani cattle ($n=96$) genome wide markers were retrieved from[25] Singh et al. (2020) available at https://figshare.com/ (https://doi.org/10.6084/m9.figshare.12808343.v2). Its parental populations i.e., HOL ($n=30$), JER ($n=24$), BSW ($n=21$) and HAR ($n=10$) genotyped using BovineSNP50 chip were obtained from WIDDE database.

Preparation of dataset and pruning for quality control

Genotype quality control (QC) was performed using PLINK v1.9 software[26]. All the variants with mapped coordinates in the UMD_3.1.1 bovine reference genome assembly ((https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000003055.6/)) were included for analysis. Only autosomal markers were considered and markers on sex chromosomes and mitochondrial DNA markers were removed in order to avoid any potential bias[12,27]. Markers with call rate (CR) exceeding 95% were kept for subsequent analysis. Individuals with missing genotype data with threshold (--mind) of 0.1 was also taken into consideration. Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) criteria were intentionally omitted, as they could potentially eliminate loci under selection and will influence the effects of population intermixing[5,28–31].

Principal component and genome wide ancestry analysis

After QC analyses on the genomic dataset, Principal Component Analysis (PCA) was used for population structure study. PLINK v1.9 along with the ggplot package[32] in R Studio was used for PCA and visualize its results[33]. To identify key components with the highest loadings for PC1 and PC2, we conducted Principal Component Analysis (PCA) using PLINK with variance-weighted SNP loadings (--pca var-wts). The SNP loadings from plink.eigenvec.var were extracted and ranked by absolute values to determine the top 20 SNPs contributing to PC1 and PC2. These SNPs were then mapped to their chromosomal positions using the .bim file. The identified SNPs were further analyzed for their association with known gene groups to assess their biological relevance using annotated UMD_3.1.1 genome reference assembly (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000003055.6/) with the help of in house developed tool called snp2feature (https://github.com/kkokay07/pq-genetics/tree/main/Annotation_of_features). Admixture analysis at genome level (global) was done to estimate ancestry proportions using ADMIXTURE software[34]. The analysis included 20,000 MCMC iterations following 10,000 burn-in steps. The optimal predefined number of ancestral groups (K) value, was determined by cross-validation errors. Admixture frequencies for all animals were calculated and plotted using Microsoft excel.

Inferring genome wide locus specific ancestry and adaptive admixtures

Local Ancestry in adMixed Populations (LAMP) is a software tool designed to estimate locus-specific ancestry in admixed individuals by analyzing the allele frequencies of reference populations[35]. Local ancestry for admixed animals (VRI in this study) was calculated using LAMP-ANC mode. In the LAMP configuration, a constant recombination rate of 1e-8 based on the assumption that 0.01 recombination's occur per Mb (equivalent to 1 cM) was considered, given that no accurate genetic map is currently available for cattle. Furthermore, the excess and deficiency of local ancestry ('Δ ancestry') with respect to the reference panel breeds was estimated using the approach proposed by[19,21]. The 'Δ ancestry' captures significant shifts in ancestry variations across the genome and it is calculated by subtracting the genome-wide baseline ancestry from the average locus-specific ancestry for each of the two ancestral components[19].

The Δ ancestry for ancestral population k at each SNP m is defined as:

$$\delta_k^m = \frac{1}{I}\sum_{i=1}^{I}\left(q_k^{i,m} - q_k^{-i}\right)$$

Where, $q_k^{i,m}$—locus-specific ancestry of animal i at SNP m; q_k^{-i}—mean of the locus-specific ancestry for individual I.

Finally, the outlier approach is used to find adaptive admixtures from local ancestry information. The regions which exceeded the 3 ± SD (standard deviation) were considered as adaptive admixtures[21]. Finally, structural annotation was done using the *snp2feature* (https://github.com/kkokay07/pq-genetics/tree/main/Annotation_of_features) and genomic differences between these populations were established through the functional genomic studies by carrying out functional annotation using DAVID, g:Profiler[36] and Animal QTL database[37]. Also, phenotypic annotation was done using OMIA (Online Mendelian Inheritance in Animals) (https://www.omia.org/home/).

Results

Prior to quality control (QC), the dataset comprised 181 individuals and 51,998 single nucleotide polymorphism (SNP) markers. Following QC procedures, approximately 9,553 SNPs were removed, resulting in the retention of 42,445 SNPs for downstream analyses. Principal component analysis revealed that the first two principal components effectively separated the data into distinct groups based on geographic origins. Taurine breeds viz., HOL, JER, and BSW, formed a distinct cluster, whereas the indicine breed, HAR, formed a separate cluster. The VRI cattle population was positioned between these two clusters but exhibited a greater affinity toward the taurine group, particularly HOL, indicating a higher proportion of taurine ancestry. The first and second principal components accounted for 20.27% and 11.833% of the total genetic variation, respectively (Fig. 1). The top 20 SNPs were identified across different chromosomes each for PC1 and PC2. Several SNPs were linked to genes with important functions (Table 1).

Genome wide ancestry estimation

The individual admixture proportions, estimated using the full set of SNPs for both parental and admixed populations, are presented in Fig. 2. The admixture analysis with **K = 4** revealed the composition proportions of the parental populations—BSW, HOL, JER, and HAR—at 4%, 67.3%, 8.5%, and 20.1%, respectively (Supplementary file S1 and S2).

Locus specific ancestry estimation

Locus-specific ancestry (local ancestry) estimation was conducted using the delta ancestry method by classifying all exotic breeds as part of the *Bos taurus* group (HOL, JER, and BSW) and HAR in the *Bos indicus* group. Regions exhibiting adaptive admixture were identified using an outlier approach, wherein loci exceeding 3 ± SD were considered significant. These noteworthy regions are highlighted in red (Figs. 3 and 4). Within the genomic landscape, adaptive admixtures contributed by *Bos indicus* were detected on Chr 2, 3, 4, 5, 7, 10, 12, 13, 14, 16, 17, 19, 20, 21, 22, 23, and 24. In contrast, those attributed to *Bos taurus* were localized to Chr 1, 6, 9, 11, 15, 18, 27, and 28.

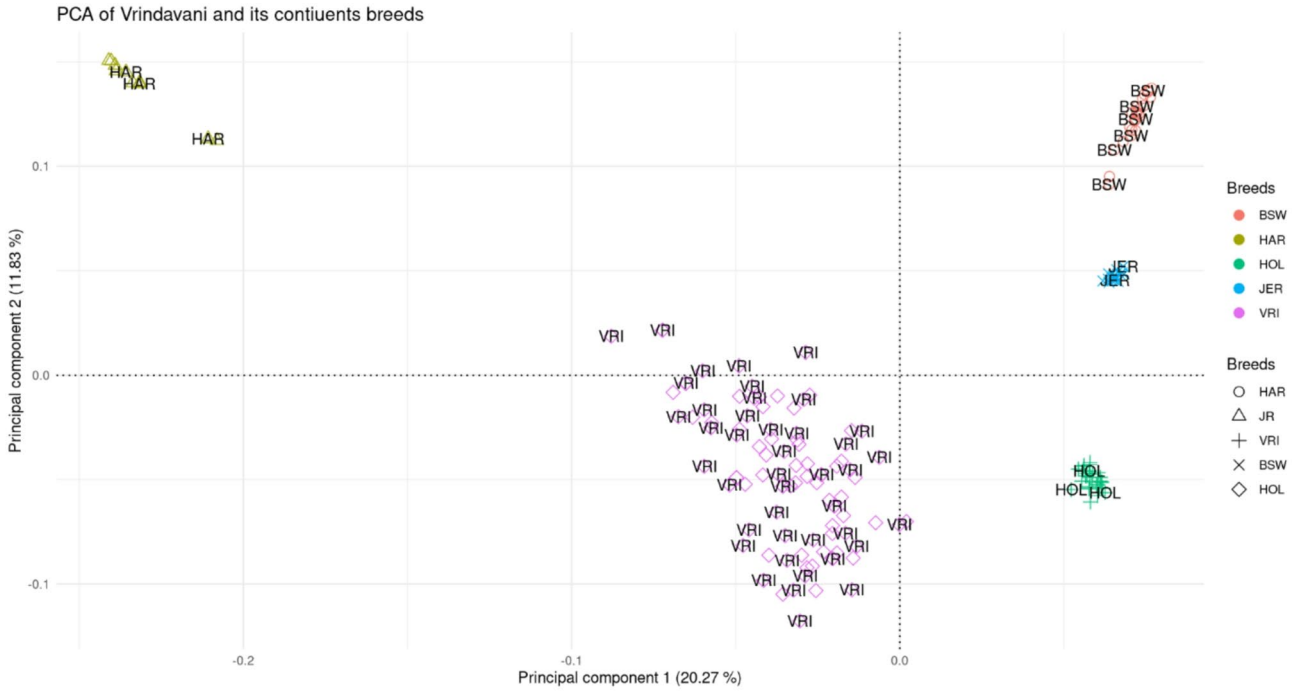


Fig. 1. The principal component analysis (PCA) plot illustrates how various cattle breeds cluster based on their geographic locations. The first and second principal components explained 20.27% and 11.83% of the total variation, respectively. BSW-Brown Swiss; HAR-Hariana; HOL-Holstein Friesian; JER-Jersey; VRI-Vrindavani.

Principal component 1				Principal component 2			
Chr	SNP Position	Genes linked to SNP	Feature loading score	Chr	SNP position	Genes linked to SNP	Feature loading score
3	15,818,013	KCNN3	2.77819	3	110,272,602	MAP7D1, TRAPPC3, COL8A2	3.58398
3	32,316,119	DENND2D, CEPT1	2.77441	3	62,196,445	-	3.25334
4	53,050,980	-	2.82218	3	90,692,920	-	3.21352
4	34,669,189	LOC781830	2.75179	4	77,635,835	NUDCD3, LOC104972146	3.43166
6	77,145,240	-	2.77703	5	66,157,408	LOC104972477, CCDC53	3.43206
8	20,364,722	ELAVL2	2.79931	5	24,451,576	TMCC3	3.28886
10	72,846,830	-	2.74764	6	94,589,635	LOC107131184	3.87454
10	25,907,293	RPGRIP1, LOC107132837	2.74107	6	70,320,611	SCFD2	3.47473
11	85,097,357	LOC104973452	2.78714	6	52,371,211	-	3.32266
13	77,635,899	PREX1	2.7456	6	71,421,017	PDGFRA	3.25643
15	82,335,513	CTNND1, ZDHHC5	2.7975	7	45,383,502	CIRBP, C7H19orf24, MUM1, LOC104969177	3.37343
16	4,061,366	SRGAP2	2.74541	7	48,376,442	H2AFY	3.33401
17	36,339,293	LOC107131163	2.83632	8	104,971,314	ZNF618	3.21094
18	16,070,657	PHKB	2.91285	9	65,564,012	TBX18	3.60243
20	14,290,727	ADAMTS6	2.76776	14	19,290,077	LOC100139328	3.48735
21	33,439,863	LINGO1	2.73207	16	27,894,801	TP53BP2	3.49402
22	53,470,362	TDGF1, LRRC2	2.7348	16	32,681,586	KIF26B	3.31581
22	53,457,282	RTP3	2.73451	22	19,168,846	GRM7	3.39106
24	34,876,101	MIB1	2.75379	26	37,797,893	VAX1	3.2925
24	37,194,377	-	2.74442	29	22,853,469	ANO5, LOC104976236	3.47477

Table 1. Genes in linkage disequilibrium with the top 20 SNPs (features) in principal component 1 and 2 of principal component analysis involving Vrindavani cattle and its parental breeds.

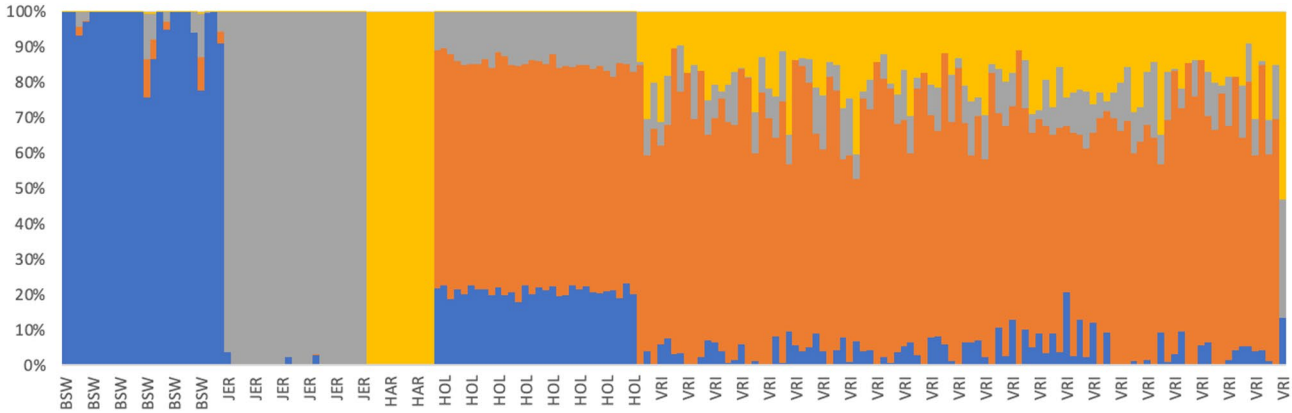


Fig. 2. Individual-wise bar plot results for dataset for *K* value of 4, separating the populations/breeds from their lineages. BSW-Brown Swiss; HAR-Hariana; HOL-Holstein Friesian; JER-Jersey; VRI-Vrindavani.

Adaptive admixture with excess of indicine or taurine ancestry in Vrindavani cattle

Haplotypes of indicine and taurine ancestry may confer VRI a relative adaptive advantage under selection pressures. To identify haplotypes associated with selection in VRI cattle, an outlier approach was applied. The analysis identified 609 adaptive regions of indicine ancestry and 4,868 adaptive regions of taurine ancestry, encompassing approximately 534 and 4,321 genes, respectively (Supplementary file S6, S7, S9 and S10). The highest number of regions under selection from indicine ancestry (105 regions) was observed on chr 5, whereas the highest number from taurine ancestry (1,000 regions) was detected on chr 18, followed closely by chr 11 (845 regions) and chr 1 (826 regions) (Fig. 5). Gene ontology (GO) enrichment analysis revealed several biological processes and pathways associated with both indicine and taurine ancestry (Supplementary file S3 and S4).

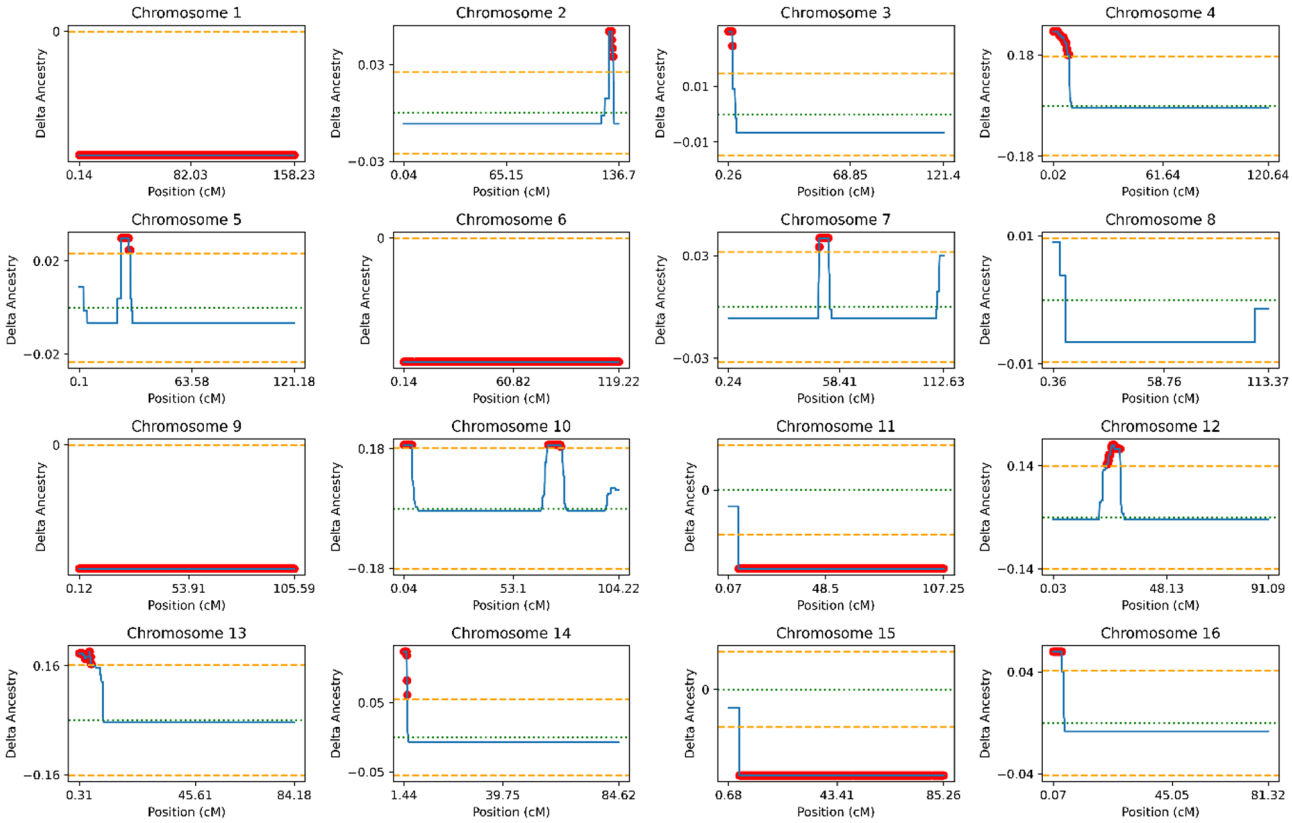


Fig. 3. Local ancestry analysis of chromosome 1–16 in Vrindavani cattle population. Portions above the green broken line in the middle of each plot are contributed by *Bos indicus* ancestry (Hariana cattle) and those below were contributed by *Bos taurus* ancestry (Holstein Friesian, Jersey and Brown Swiss). Those regions identified with red color are adaptive admixtures.

QTL enrichment analysis

A total of 69 and 107 quantitative trait loci (QTLs) were identified within the adaptive admixtures of VRI contributed by *Bos indicus* and *Bos taurus*, respectively, associated with exterior traits, production, reproduction, milk, and carcass traits (Supplementary file S5 and S8.

). The majority of these QTLs were of *Bos taurus* origin, constituting more than 50% in each category. For milk production *Bos indicus* QTLs accounted for approximately 51% (Fig. 6). Chromosome 1 and 14 harbored the most important QTLs, originating from *Bos taurus* and *Bos indicus*, respectively, with both predominantly associated with milk production. QTLs of taurine ancestry were distributed across chr 1, 6, 9, 11, 15, 18, 27, and 28, whereas those of indicine ancestry were located on chr 2, 3, 4, 5, 10, 12, 13, 14, 16, 19, 21 and 23. The chromosome-wise distribution of QTLs of taurine and indicine ancestry in *Bos taurus* and *Bos indicus* is presented in Fig. 7.

Phenotype enrichment through online Mendelian inheritance in animal (OMIA)

The Online Mendelian Inheritance in Animals (OMIA) database (https://omia.org/home/) was queried to identify variants previously reported as causal or associated with cattle diseases, coat color, or other phenotypic traits. Genes play a crucial role in determining various observable traits across different organisms, and their functional implications in cattle are summarized in Supplementary file S11.

Functional enrichment analysis

The functional enrichment analysis of genes linked to adaptive admixtures in VRI cattle, derived from *Bos indicus* and *Bos taurus* ancestry, identified significant molecular functions (MF), biological processes (BP), and cellular components (CC) associated with environmental adaptation (Figs. 8 and 9). In the molecular functions category, the most significant term from *Bos indicus* ancestry were the structural constituent of the skin epidermis (GO:0030280, $P_{adj} = 5.99 \times 10^{-21}$), transcription cis-regulatory region binding (GO:0000976) and DNA-binding transcription factor activity (GO:0003700). From *Bos taurus* ancestry, protein binding (GO:0005515, $P_{adj} = 7.71 \times 10^{-7}$), sequence-specific double-stranded DNA binding (GO:1990837), transcription regulator activity (GO:0140110), catalytic activity (GO:0003824) and small molecule binding (GO:0036094) were significantly enriched.

In the biological processes category, *Bos indicus*-derived genes were significantly enriched in pathways related to keratinization (GO:0031424, $P_{adj} = 3.73 \times 10^{-17}$), intermediate filament cytoskeleton organization

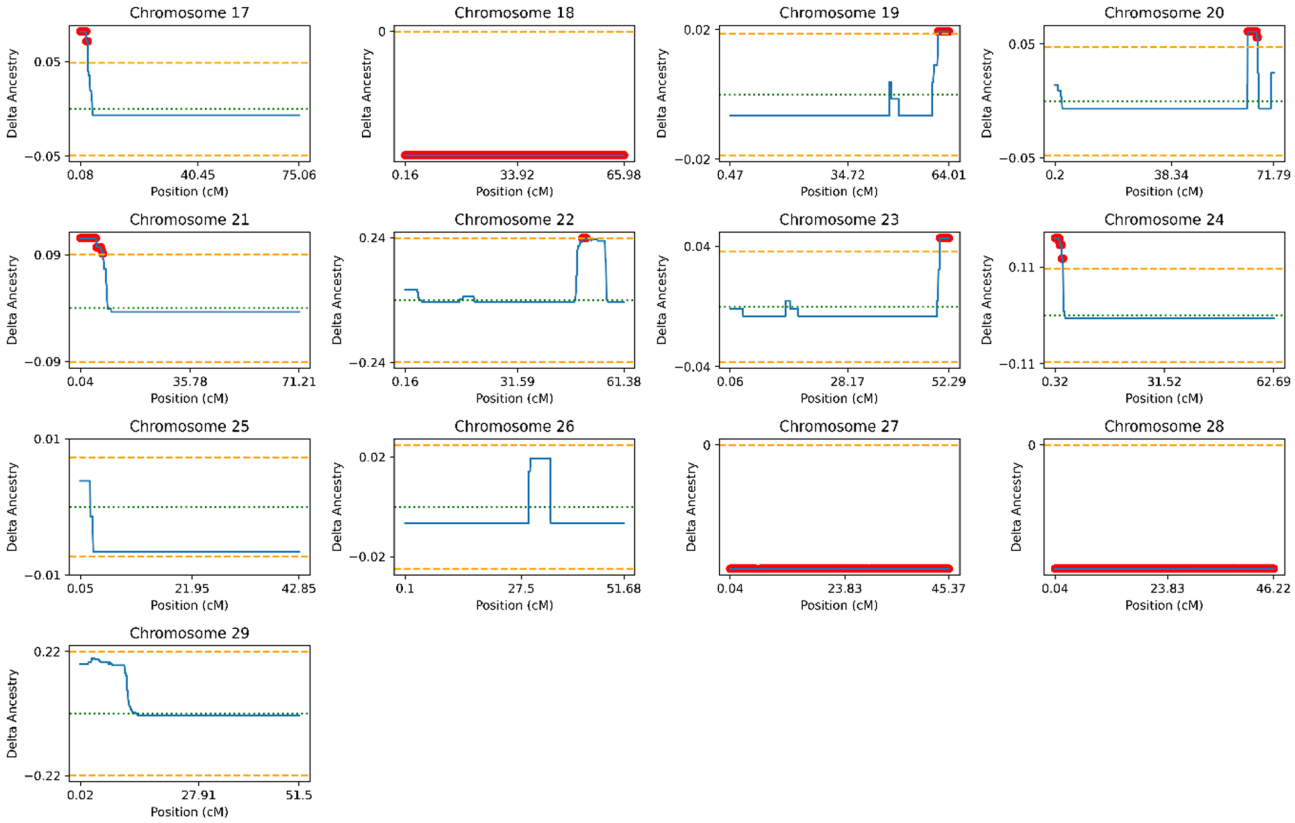


Fig. 4. Local ancestry analysis of chromosome 17–29 in Vrindavani cattle population. Portions above the green broken line in the middle of each plot are contributed by *Bos indicus* ancestry (Hariana cattle) and those below were contributed by *Bos taurus* ancestry (Holstein Friesian, Jersey and Brown Swiss). Those regions identified with red color are adaptive admixtures.

(GO:0045104), system development (GO:0048731) and anterior/posterior pattern specification (GO:0009952). *Bos taurus*-derived genes showed significant enrichment in anatomical structure development (GO:0048856, $P_{adj} = 1.35 \times 10^{-13}$), cell surface receptor signalling pathway (GO:0007166), plasma membrane-bounded cell projection organization (GO:0120026) and ovulation cycle process (GO:0022606).

In the cellular component category, *Bos indicus*-derived genes showed significant enrichment in keratin filament (GO:0045095, $P_{adj} = 7.13 \times 10^{-19}$) and transcription regulator complex (GO:0005667). *Bos taurus*-derived genes were significantly enriched in extracellular matrix organization (GO:0030198, $P_{adj} = 3.54 \times 10^{-3}$), response to lipid (GO:0071398), and cell adhesion (GO:0007155), cytoplasm (GO:0005737), nucleoplasm (GO:0005654), and cell junctions (GO:0030054, GO:0005911), very-low-density lipoprotein particles (GO:0034361) and extracellular vesicles (GO:0031012). These findings highlight the genetic contributions of both *Bos indicus* and *Bos taurus* ancestry in shaping adaptive traits in VRI cattle, with enriched pathways linked to thermotolerance, immune function, metabolic regulation, reproductive efficiency, and structural integrity.

Discussion

To address low sustainability and poor productivity, crossbreeding has been widely adopted in India. Despite its importance, the genomic attributes contributing to the advantages of crossbreeding have not been thoroughly explored across the entire genome. Previous studies on VRI cattle have mainly focused on various diversity parameters, neglecting the estimation of local ancestry. Vrindavani cattle is a composite breed[8,9,25,38] designed to exploit breed complementarity and heterosis (hybrid vigor)[16]. Understanding the admixture proportions of individuals helps estimate heterozygosity, comprehend the breeding history of the population, and make informed management decisions for crossbreeding programs[39]. Historically, breed origins were estimated using microsatellite markers[40] and more recently using SNPs[8]. DNA markers are accurate for estimating animal ancestry because they measure realized parental contributions at the genomic level[41]. This can correct pedigree errors and estimate kinships when pedigree data are incomplete or missing[42,43].

Our study characterized admixed genome of VRI cattle using their parental genomes viz., HOL, BSW, JER and HAR. According to[44], the sample size of our study is sufficient for admixture studies and aligns with previous research[9,25,45]. Only the markers present on autosomes are generally included in population genetic studies to remove the biases and ambiguities arising from sex chromosomes[5]. After quality control, 181 individuals and 42,445 SNPs left for subsequent analysis. The quality SNPs obtained were in agreement with other mid density genotyping viz.[46–48], wherein 40,492, 54,404, and 54,609 SNPs were used, respectively, and were relatively higher

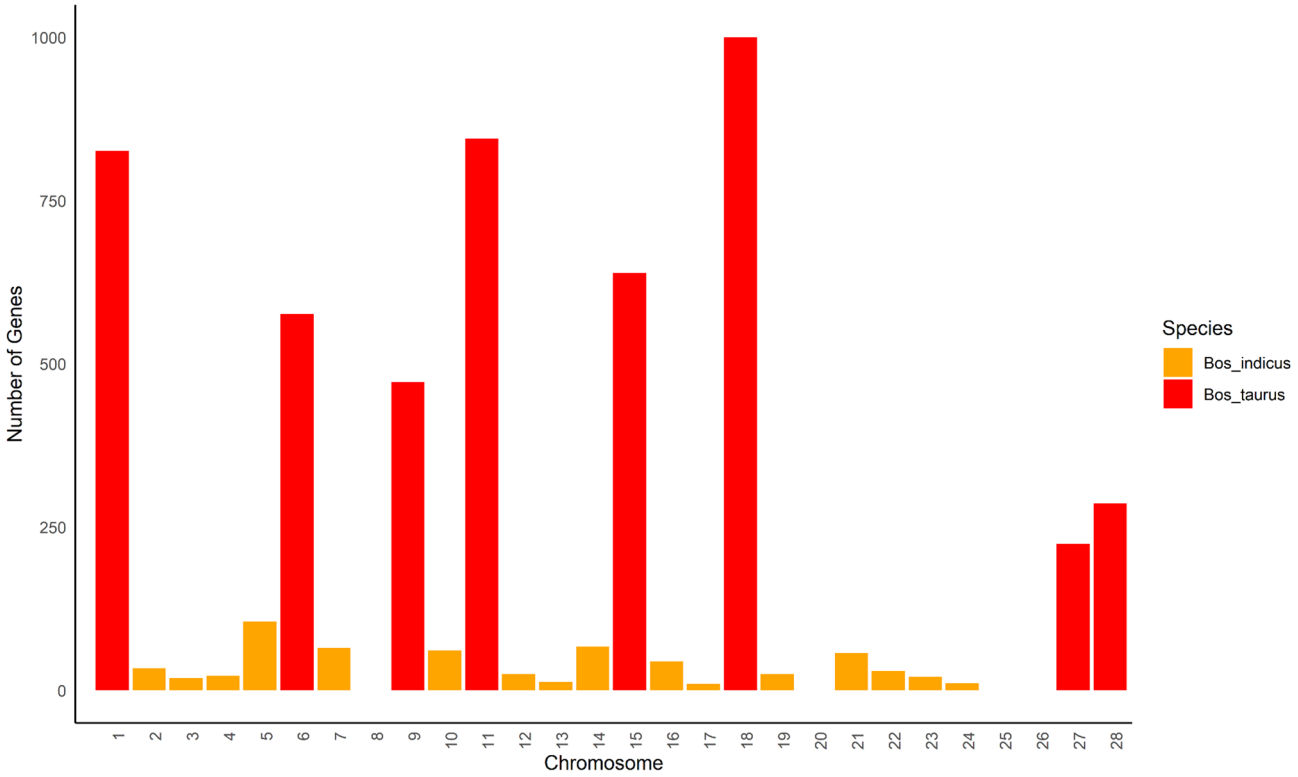


Fig. 5. Chromosome-wise distribution of genes in linkage disequilibrium with adaptive admixtures from *Bos taurus* and *Bos indicus* in Vrindavani cattle.

compared to previous studies on VRI cattle[8,25]. This comparison suggests good quality genotypes used in the present study.

Population stratification

Using PCA, we observed that the first two principal components distinctly separated the data based on geographic origins. Taurine breeds (HOL, JER and BSW) formed one cluster, while the Indicine breed (HAR) formed another (Fig. 1). Interestingly, Vrindavani cattle was positioned between the taurine and indicine lineages, leaning more towards the taurine breeds as it can be attributed to their more than 50% taurine ancestry, consistent with previous findings[8,25]. Similarly, Frieswal cattle, a crossbred developed in India, exhibited a comparable pattern, positioned between the Taurine (HOL) and Indicine (Sahiwal) breeds, with an 61.5% taurine ancestry[45]. Also observed some individuals from the Red Sindhi breed clustering towards the JER breed[49].

The PCA revealed significant SNPs associated with key genomic regions that potentially influence various biological functions in cattle. In PC1, the top 20 SNPs were identified with feature loading scores ranging from 2.73207 to 2.91285, emphasizing regions of functional relevance. The highest feature loading score (2.91285) was observed for SNP Hapmap27031-BTA-161,389 on chr 18, linked to PHKB, a gene crucial for glycogen metabolism and energy balance in muscle function[50]. Other associations include KCNN3 (chr 3: 15818013), which encodes a calcium-activated potassium channel involved in neural signaling and muscle contractility[51], and PREX1 (chr 13: 77635899), which influences meat quality traits in Simmental cattle[52]. SRGAP2 (chr 16: 4061366) has been identified as a potential regulator of milk production in indicine cattle[53]. ADAMTS6 (chr 20: 14290727), a gene associated with extracellular matrix remodeling and skeletal muscle development, regulating myoblast proliferation and differentiation in cattle-yak[54]. Additionally, MIB1 (chr 24: 3476101) was associated with the fatty acid profile in Belgian Blue cattle[55]. TDGF1 (chr 22: 53470362) plays a critical role in embryonic development, the estrous cycle, and early pregnancy[56], while LRRC2 (chr 22: 53470362) is involved in epigenetic modifications in skeletal muscle[57].

In PC2, the top 20 SNPs were identified across several chromosomes, with feature loading scores ranging from 3.21094 to 3.87454. The highest feature loading score (3.87454) was observed for SNP ARS-BFGL-NGS-41,209 on chr 6, linked to the unannotated locus LOC107131184. Several SNPs were associated with genes playing essential roles in physiology, development, and adaptation. MAP7D1, TRAPPC3, and COL8A2 (chr 3: 11027602) are involved in cytoskeletal organization, vesicle trafficking, and extracellular matrix composition, which are vital for muscle structure and tissue integrity in cattle[58–60]. NUDCD3 (chr 4: 77635835) may play a role in cell division and developmental pathways[61]. PDGFRA (chr 6: 71421017) encodes a receptor involved in cell proliferation and differentiation, making it a strong candidate for growth traits in cattle[62]. H2AFY (chr 7: 4876442) encodes a histone variant crucial for chromatin remodeling and gene regulation, potentially impacting epigenetic modifications in cattle[63]. TBX18 (chr 9: 65564012) is involved in embryonic development, particularly

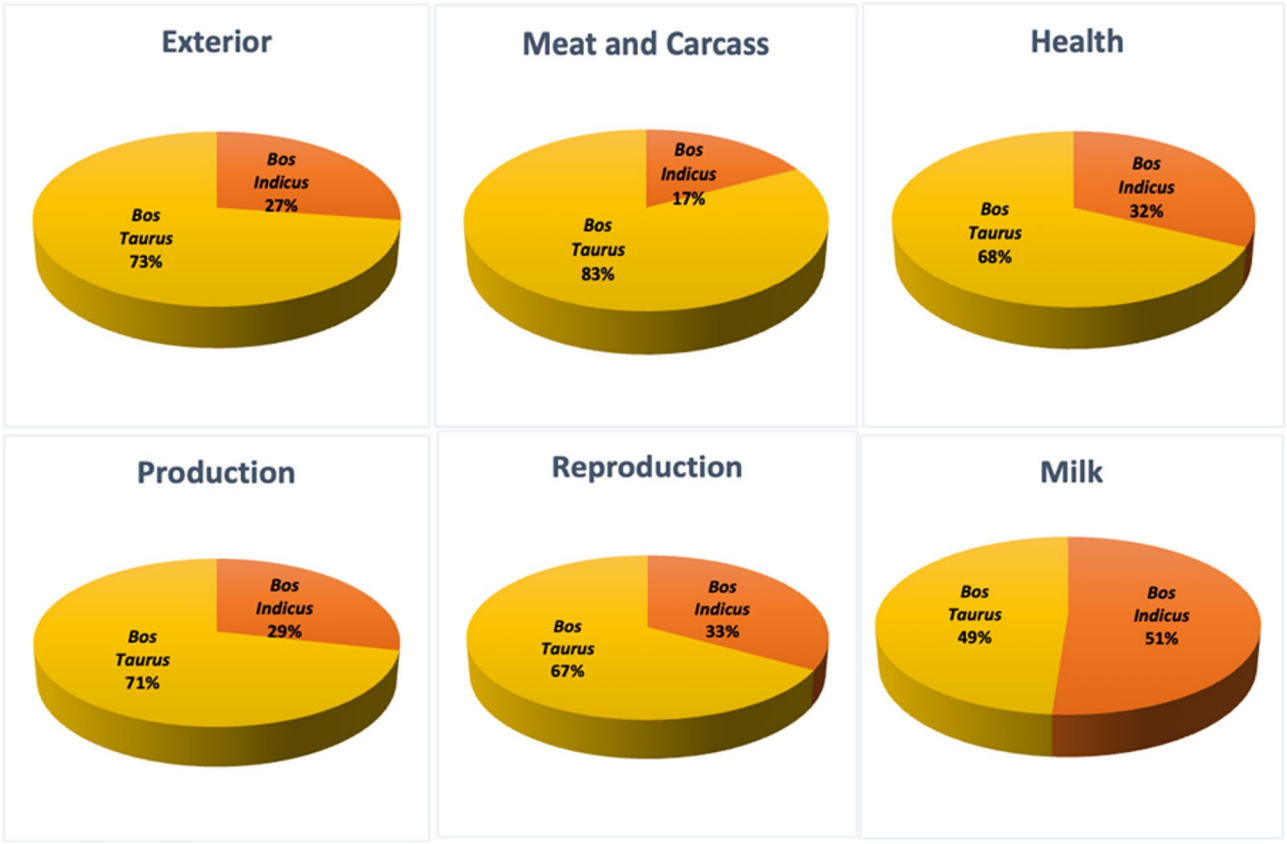


Fig. 6. Proportion quantitative trait loci (QTL) related to different categories in linkage disequilibrium with adaptive admixtures in Vrindavani cattle contributed by *Bos taurus* and *Bos indicus* ancestry.

in cardiovascular and urogenital system formation, with potential implications for reproductive traits. It has been associated with development in the dual-purpose Simmental breed but not in other specialized breeds[64]. TP53BP2 (chr 16: 27894801) plays a critical role in apoptosis and cell cycle control, suggesting its potential involvement in immune response and disease resistance[65]. Another significant gene, GRM7 (chr 22: 19168846), a metabotropic glutamate receptor, may influence nervous system function and behavioral traits in cattle[62].

Genome-wide ancestry estimation

Admixture analysis using parental populations determined the ancestry proportions in VRI cattle at K = 4 (Fig. 2). The genetic contributions were identified as 67.3% from HOL, 8.5% from JER, 4% from BSW and 20.1% from HAR, indicating a strong taurine ancestry. These findings are consistent with earlier studies on VRI cattle[8,25] and Frieswal cattle[38], though with different proportions which could be due to quality control parameters that we incorporated in our study, mainly the Hardy-Weinberg equilibrium and linkage disequilibrium criteria were not applied as they may remove loci under selection and also remove influence of intermixing of populations with different gene frequencies[5,28–30]. Similarly, crossbred cattle in Maharashtra, India, have an average exotic ancestry of 70.3%[66]. Additionally, one more study on Indian cattle showed that while breeds such as Sahiwal, Tharparkar, Gir, Ongole, Kangayam, and Hariana formed distinct genetic clusters; Vechur exhibited significant admixture, likely due to crossbreeding[67]. Higher levels of taurine ancestry might not be suitable for tropical climate, as optimal production and economic traits are generally observed at exotic inheritance levels between 50% and 62.5%. Higher levels of exotic inheritance often result in decreased performance in both production and reproduction traits[68]. Research on Sahiwal and Holstein crossbreds[69] revealed no improvement in production beyond 50% exotic inheritance. Conversely, lower exotic inheritance in crossbred cattle suggests better adaptation to tropical conditions and less emphasis on selecting for exotic traits. Given that crossbred cows contribute to 54% of India's total milk production[3], it is crucial to address any discrepancies in inheritance levels by refining breeding strategies.

Locus-specific ancestry

Adaptive admixture regions, resulting from hybridization between distinct parental populations, were investigated to understand their role in evolutionary adaptation. These genomic segments emerge in hybrid progeny and, when conferring fitness advantages, are subject to positive selection. Regions exhibiting significantly elevated frequencies toward fixation were identified using an outlier detection approach[21]. Identifying distinct ancestral origins of genomic segments has numerous applications, such as boosting milk production, mapping

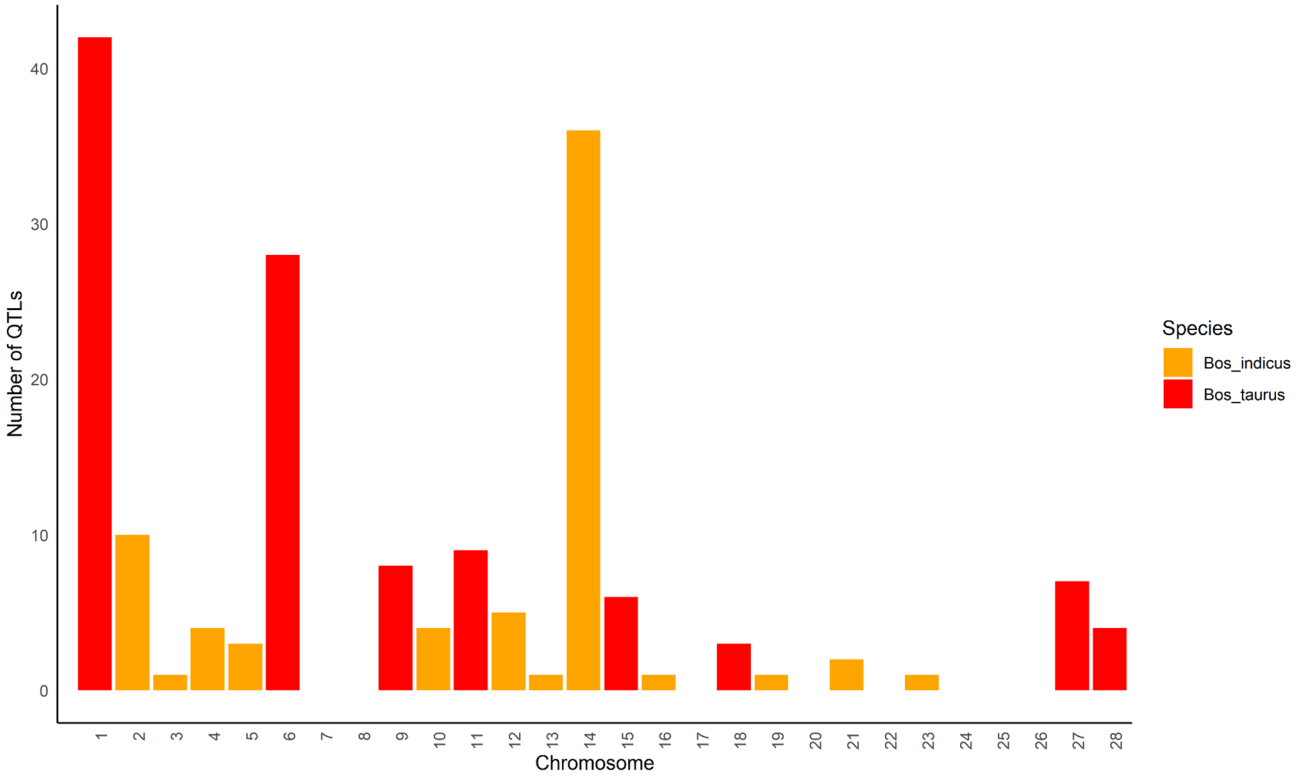
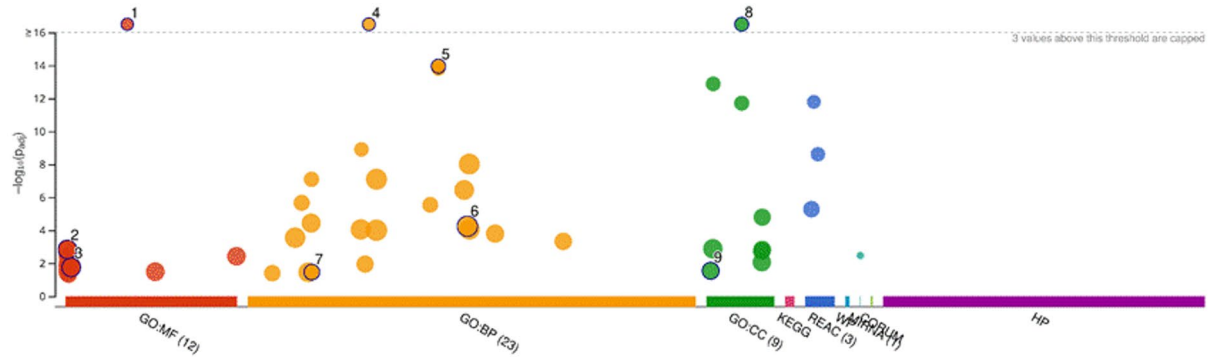


Fig. 7. Chromosome-wise distribution of quantitative trait loci (QTL) in linkage disequilibrium with adaptive admixtures in Vrindavani cattle contributed by *Bos taurus* and *Bos indicus* ancestry.

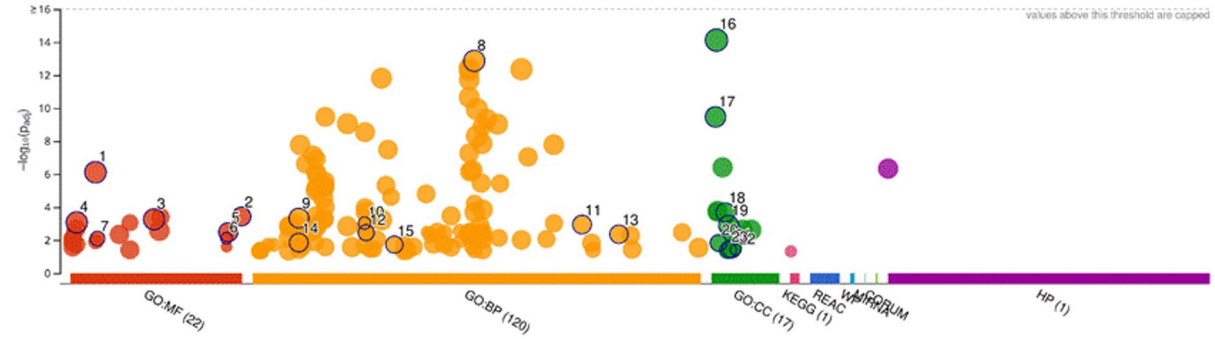


ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:MF	GO:0030280	structural constituent of skin epidermis	5.992×10^{-21}
2	GO:MF	GO:0000976	transcription cis-regulatory region binding	1.449×10^{-3}
3	GO:MF	GO:0003700	DNA-binding transcription factor activity	1.787×10^{-2}
4	GO:BP	GO:0031424	keratinization	3.732×10^{-17}
5	GO:BP	GO:0045104	intermediate filament cytoskeleton organization	1.107×10^{-14}
6	GO:BP	GO:0048731	system development	5.731×10^{-5}
7	GO:BP	GO:0009952	anterior/posterior pattern specification	3.451×10^{-2}
8	GO:CC	GO:0045095	keratin filament	7.132×10^{-19}
9	GO:CC	GO:0005667	transcription regulator complex	2.891×10^{-2}

version	e112_eg59_p19_25aa4782
date	2/18/2025, 5:06:04 PM
organism	btaurus

g:Profiler

Fig. 8. Gene ontology enrichment of genes in linkage disequilibrium with the adaptive admixtures in Vrindavani cattle derived from *Bos indicus* ancestry.



ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:MF	GO:0005515	protein binding	7.713×10^{-7}
2	GO:MF	GO:1990837	sequence-specific double-stranded DNA binding	3.693×10^{-4}
3	GO:MF	GO:0036094	small molecule binding	5.513×10^{-4}
4	GO:MF	GO:0003824	catalytic activity	8.446×10^{-4}
5	GO:MF	GO:0140110	transcription regulator activity	3.299×10^{-3}
6	GO:MF	GO:0120015	sterol transfer activity	7.624×10^{-3}
7	GO:MF	GO:0008201	heparin binding	8.083×10^{-3}
8	GO:BP	GO:0048856	anatomical structure development	1.350×10^{-13}
9	GO:BP	GO:0007166	cell surface receptor signaling pathway	4.634×10^{-4}
10	GO:BP	GO:0022602	ovulation cycle process	9.246×10^{-4}
11	GO:BP	GO:0120036	plasma membrane bounded cell projection organ...	1.136×10^{-3}
12	GO:BP	GO:0030198	extracellular matrix organization	3.547×10^{-3}
13	GO:BP	GO:1901700	response to oxygen-containing compound	4.400×10^{-3}
14	GO:BP	GO:0007155	cell adhesion	1.415×10^{-2}
15	GO:BP	GO:0033993	response to lipid	1.879×10^{-2}
16	GO:CC	GO:0005737	cytoplasm	7.553×10^{-15}
17	GO:CC	GO:0005654	nucleoplasm	3.414×10^{-10}
18	GO:CC	GO:0030054	cell junction	2.122×10^{-4}
19	GO:CC	GO:0031090	organelle membrane	1.265×10^{-3}
20	GO:CC	GO:0005911	cell-cell junction	1.489×10^{-2}
21	GO:CC	GO:0031984	organelle subcompartment	3.395×10^{-2}
22	GO:CC	GO:0034361	very-low-density lipoprotein particle	3.695×10^{-2}
23	GO:CC	GO:0031012	extracellular matrix	3.945×10^{-2}

version	e112_eg59_p19_25aa4782
date	2/18/2025, 5:08:00 PM
organism	btaurus

g:Profiler

Fig. 9. Gene ontology enrichment of genes in linkage disequilibrium with the adaptive admixtures in Vrindavani cattle derived from *Bos taurus* ancestry.

diseases, and uncovering historical genetic events[16,35]. This allows us to detect beneficial local genetic traits from specific breeds in mixed livestock populations. For instance, genomic sequence analysis has shown that local ancestry segments from Angus, Brahman, and Wannan breeds might enhance rapid growth, immune resistance, and indigenous adaptation in Yunling cattle[70]. Furthermore, research has found evidence of Mito-nuclear co-evolution in hybrid African cattle populations, with a notable increase in taurine ancestry at nuclear genes targeted by mitochondria[71].

Local ancestry estimation on Vrindavani cattle was performed using the delta ancestry method, classifying all exotic breeds (HOL, JER and BSW) and indicine breed (HAR) parental breed as part of the *Bos taurus* group and *Bos indicus* group respectively. Previously Local ancestry analysis has been effective in identifying recent selection in admixed populations, such as Swiss Fleckvieh cattle[72] and Zebu introgressed regions in Colombian creole taurine cattle[73]. Used a similar approach to detect significant differences in local admixture levels, identifying five chromosomes with notable deviations from average ancestries[74]. This included an excess of Baoulé ancestry, potentially linked to higher trypanosomiasis tolerance, particularly on chr 6, 8, and 19 in trypanosome-negative individuals. Additionally, higher Baoulé ancestry on Chr 8 (35–50 Mb) in trypanosome-positive cattle suggests beneficial Baoulé haplotypes unrelated to trypanosomiasis tolerance. Similarly, on Chr 2, 5, 7, 12 and 14 of VRI cattle we found the genes and QTLs related to disease resistance which is attributed to the HAR breed (known for tropical adaptation). For instance, region on chr 7 of Vrindavani cattle overlaps with a previously reported region for Mycobacterium paratuberculosis susceptibility in U.S. Holsteins[75] and encompasses the SPOCK1 gene, which has been shown to be associated with cancer in humans[76]. Discovered that genes such as VAV1, PIK3R5, RAC1, VAV2, GAB2, and INPP5D on Chr 8 are under selection in Muturu and N'Dama cattle breeds in response to trypanosome infection[77].

In VRI cattle, chr 6, 9, 11, 15, 27, 28 harbored genes and QTLs related to milk production as these regions are mostly of taurine ancestry, while QTLs and genes on chr 2, 10 and 14 are contributed by the HAR breed. Because of higher level of taurine ancestry, the milk production in VRI cattle (9.9 kg/day) is usually high when compared to the HAR cattle (2.4Kg/day)[7,78], suggesting VRI have been benefited of positive heterosis. Indicine

alleles introgression also be responsible for increase milk yield, as their genes are involved in environmental adaptation that allows expression of the milk production potential of crossbred[79].

Adaptive admixtures contributed by *Bos indicus*

The annotation of adaptive admixtures has unveiled intriguing genetic insights of *Bos indicus* in VRI cattle. YTHDC2 gene on chr 10 associated with the iron content in milk[25]. Chromosome 14 harbored the gene DGAT1, a well-known enzyme is responsible for production of milk triglycerides in HOL cattle[80–82]. Gene HSF1 on chr 14 binds to heat shock elements in HSP genes and is activated by stress, controls the transcription of HSP genes[83] and this regulatory role is crucial under conditions of elevated temperature as the VRI cattle are in tropics. Similarly, TONSL and VPS28 genes on are involved in cellular response to heat stress, where VPS28 gene was involved in heat shock resistance while TONSL maintains genomic stability during increased heat conditions[82,84,85]. Polymorphisms on gene PLCB1 on Chr 13 is a candidate gene for milk production in Indicine cattle[86] which is inherited to VRI cattle by HAR cattle.

Adaptive admixtures contributed by *Bos taurus*

Within the adaptive admixtures region attributed to *Bos taurus* on the Chr 1 in VRI cattle, genes like UMPS, ITGB2, IFT80, DGKG, CLDN16 and ATR which are related to disease resistance and genetic adaptations, were found. Chromosome 11 harbored genes related to reproduction like TTF1, SNAPC4, RPIA, and MED22. Chr 6 harbored important genes like KIT, HMX and PRKG2 genes. KIT gene is responsible for white spotting (coat color) in VRI cattle[87]. Also, variations in KIT gene are responsible for the coat color patterns in Hereford cattle, the spotting patterns in Holstein, Simmental cattle[88], and Fleckvieh cattle[89], as well as the degree of black coat color in Holstein cattle[90]. It also facilitates the migration of melanocytes from the neural crest to the skin, which is essential for melanogenesis[91]. HMX gene on chr 6 also plays a crucial in external ear development[92,93]. PRKG2 gene is responsible for growth and carcass traits in cattle[94], chicken[95] and has been reported in humans that PRKG2 gene deletion is associated with growth restriction[96].

Other important genes having taurine ancestry were ADAM2, ADAM32, CHST8, ITGB5, PLAT, TGFA and UPK1B. These genes have been previously reported in genome wide association studies (GWAS) that are associated with reproductive traits[97]. APOB and STK33 gene were associated with milk production[25], whereas DDX1 gene was found to be associated with the involution of bovine mammary glands under environmental stress[98] and it was also been linked to viral resistance and the amount of linoleic acid in Nellore cattle[99]. Previously reported genes in Vrindavani cattle in selection signature studies[25] were also detected in present study i.e., DENND5A, NBAS, NRIP3, SCUBE2, TRIM66.

As the milk production in VRI cattle was higher when compared to its indicine parent (HAR), several genes associated with the milk protein percentage (CENPN, CMIP, NECAB2, OSGIN1, CLNK), milk mineral like Copper (PPA2, PPP2R2C), iron (RHPN2, DPY19L3, FAAP24, LRP3) and Phosphorus (NAT10) were enriched. Other genes such as ALB and PDE9A involved in variation in milk constituents such as milk fat, protein and milk production respectively, were also reported in Holstein cattle and Murrah buffaloe[100–102]. Apart from KIT gene, BDNF was also responsible for coat coloration and was reported previously through GWAS studies in Vrindavani cattle[87]. ESR1 and FSHR gene previously identified in Gir and Tharparkar cattle, plays a crucial role in sexual development and fertility in livestock [14, 104, 86[104], . ATP2C1 and CAPN5 had a substantial relationship with cattle meat tenderness, marbling score, and proteolytic activity in cells in Gir[86] and Chinese Wagyu cattle[105]. As VRI cattle is tropical cattle, HSPB6 gene plays a crucial role as it was strongly correlated with thermo-tolerance in cattle breeds like Sahiwal, Gir and South African Zebu cattle[86,106,107].

Functional enrichment of adaptive admixtures in Vrindavani cattle

The molecular functions enriched in *Bos indicus*-derived genes primarily reflect adaptations to environmental stressors, particularly thermotolerance and epidermal integrity. The significant enrichment of structural constituents of the skin epidermis (GO:0030280) highlights the critical role of keratin-associated proteins in protecting against heat stress, UV radiation, and parasite load[108]. The presence of transcription cis-regulatory region binding (GO:0000976) and DNA-binding transcription factor activity (GO:0003700) suggests a strong regulatory adaptation, likely facilitating gene expression changes in response to environmental cues[109].

In contrast, *Bos taurus*-derived genes were significantly enriched for protein binding (GO:0005515) and sequence-specific double-stranded DNA binding (GO:1990837), indicating the importance of protein-protein interactions and regulatory networks in metabolic adaptation[110]. The enrichment of catalytic activity (GO:0003824) and small molecule binding (GO:0036094) further suggests a crucial role in enzymatic processes and ligand-mediated biochemical pathways, which are essential for metabolic efficiency, nutrient utilization, and immune resilience[111]. These findings suggest that *Bos taurus* ancestry enhances metabolic regulation, providing VRI cattle with improved physiological adaptability under variable environmental conditions.

Conclusion

The analysis of Vrindavani cattle revealed a predominantly taurine ancestry, with significant genetic contributions from *Bos taurus* breeds such as Holstein Friesian, Jersey, and Brown Swiss. However, the substantial taurine inheritance may not be optimal for tropical climates, where a balance between exotic and indigenous genetic traits is crucial. Local ancestry analysis highlighted adaptive admixtures from *Bos indicus* breeds, contributing to traits like disease resistance and environmental adaptation. Specific genomic regions and genes associated with these traits were identified, offering insights into the genetic basis of Vrindavani cattle's performance. The findings highlight the importance of maintaining genetic diversity to optimize production and adaptation traits. Crossbreeding strategies should aim for heterozygosity to harness the benefits of both taurine and indicine ancestries. Currently, in a crossbreeding program, because of random recombination and independent

assortment of genetic material, we can't control the inheritance of desirable alleles from the different ancestry. However, by analyzing local ancestry we can get to know the inheritance patterns of alleles. In future, certain technology can be developed in order to control inheritance of desirable combinations of alleles, hence it may maximize heterosis in crossbreeding programs and also investigating how external environmental factors, particularly India's diverse geographical landscapes and climatic conditions, influence the selective pressures on genes in Vrindavani cattle.

Data availability

The datasets presented in this study were retrieved from public repository/repositories and link was https://figshare.com/ (https://doi.org/10.6084/m9.figshare.12808343.v2).

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Author contributions

RCG, KM, KKK: conceived and designed the experiments. KK and NS: performed the experiments. KC, SC, PR, CSC, OSS and VD: analyzed the data. RCG, KM, NS, KKK, AK, GNA: wrote the paper. KC, SC, PR, CSC, IG, SS and SPD: edited the paper. All authors contributed to the article and approved the submitted version.

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