J. Dairy Sci. 108:9876–9900
https://doi.org/10.3168/jds.2024-26147

Selection signatures in Gir and Holstein cattle

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ABSTRACT

Natural and artificial selection leave footprints on the genome, known as selection signatures, that can indicate regions related to adaptive and economically important traits. The recurrent use of a limited number of sires and increased selection pressure may affect genetic diversity, potentially affecting long-term breeding programs. Among dairy cattle, the Holstein breed has been intensively selected to maximize productivity, particularly in Canada and the United States. In the dairy industry, the Gir breed plays an important role in milk production in tropical regions such as India and Latin America. Gir cattle were introduced into Brazil in the 19th and 20th centuries, and since 1985, this breed has been intensively selected for milk production. This study aimed to assess the genetic diversity and characterize the selection signatures in Holstein cattle from the United States and Canada (HOL), Gir cattle from India (GIR_IN), and Dairy Gir cattle from Brazil (GIR_BR). Genetic diversity was assessed by nucleotide diversity, single nucleotide variant density analysis, minor allele frequency, observed and expected heterozygosity, and the inbreeding coefficient. Selection signatures were identified via Tajima's D, the integrated haplotype score (iHS), the fixation index, and the cross-population extended haplotype homozygosity test for autosomes. Additionally, the analysis of selection signatures using Tajima's D and iHS was conducted for the X chromosome. Lower genetic diversity was observed in the HOL population, whereas the GIR_IN and GIR_BR populations presented greater diversity. Several genes previously related to economically important traits were identified as being under selection, including *DNAJC18*, *FSHR*, *HELB*, *HMGA2, PLAG1*, *GAB3*, and *PTEN*. In conclusion, the genes identified within the selection signatures were linked to several traits, including growth, reproduction, mastitis, milk production, heat tolerance, health, and adaptation.
Key words: milk, selection signature, selective sweep, genetic diversity

INTRODUCTION

The selection process, either natural or artificial, leaves footprints on the genome that can be detected. Unique genetic patterns or marks in the genomes of selected individuals are called selection signatures or selective sweeps (Jensen et al., 2016; Fay and Wu, 2000). Selection signature studies can identify genes and beneficial mutations linked to adaptive and economically important traits in dairy cattle, including reproduction, health, and milk production. Additionally, studying selection signatures can contribute to understanding genetic architecture diversity within and between populations.

Taurine cattle breeds have been selected for economic traits for a longer period and have been subjected to greater selection pressure than indicine breeds, especially when referring to indicine populations from developing and subdeveloping countries. As a major example, the Holstein–Friesian breed (*Bos taurus*) is the most widely distributed breed of cattle in more than 150 countries. This breed originated in the northern provinces of North Holland and West Friesland of the Netherlands (McGuffey and Shirley, 2011). It was exported to the United States in the 1870s and 1880s and was strongly selected to increase milk yield (Theunissen, 2012). After the implementation of genomic selection, there has been a notable increase in the rate of inbreeding and co-ancestry in Holstein populations (Makanjuola et al., 2020). Owing to the intense selection that has been applied, monitoring both the genetic diversity and the impact of selection on the genome of Holstein cattle is important.

The Gir breed (*Bos indicus*) is an indicine breed adapted to tropical conditions that originated in the

Received December 12, 2024.
Accepted May 22, 2025.
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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

Indian state of Gujarat. It is one of the most important local dairy breeds in India. Between 1870 and 1962, approximately 6,000 zebu cattle were imported from India to Brazil, including fewer than 700 Gir individuals (O'Brien et al., 2015). After their arrival in Brazil, the Gir cattle were initially bred for dual purposes (meat and milk). In 1985, the Brazilian Dairy Gir Breeding Program (**PNMGL**) was established, which resulted in an increase in milk production from 2,200 kg to 5,500 kg per 305 d of lactation on average. The Dairy Gir is well adapted to the harsh conditions of tropical dairy production, and it is considered the main dairy indicine breed in Brazil (Prata et al., 2015). It can also be found in most other Latin American countries (Corredor et al., 2023). Dairy Gir cattle are also used in crossbreeding with taurine breeds, such as Holstein cattle or locally adapted breeds.

A motivation for investigating selection signatures is that these regions can pinpoint genes and mutations with large phenotypic effects in a population even if they are no longer segregating. Additionally, selection signatures can complement GWAS. Genetic diversity is an important element to consider in breeding programs because selection often affects allele and genotype frequency and may affect the capacity to respond to environmental changes. Careful planning and monitoring are required to maintain diversity and offer alternatives for long-term breed improvement or the selection of animals for specific immediate purposes (Makanjuola et al., 2020; Carrier et al., 2023). Studying selection signatures and genetic diversity can provide valuable insights into the progress of selection and aid in genetic improvement in livestock populations. This study aimed to assess genetic diversity and characterize selection signatures in Holstein and Gir cattle breeds.

MATERIALS AND METHODS

The institutional research ethics board of the São Paulo State University (Jaboticabal, Brazil), University of Guelph (Guelph, Canada), and the Brazilian Agricultural Research Corporation (Embrapa; Juiz de Fora, Brazil) did not require ethics approval for this study because the data used in this investigation were assessed from previous projects.

Samples and Sequencing

For the Gir breed, 14 samples from Gir bulls from India (**GIR_IN**) and 42 samples from Dairy Gir sires from Brazil (**GIR_BR**) born between 1960 and 2007 were used. In brief, the sires that had the most progeny in PNMGL, representing most lineages in the population, were selected for whole-genome sequencing (**WGS**).

For GIR_BR, DNA was extracted via either the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA), following the recommendations of the manufacturer, or a saline buffer and phenol/chloroform purification protocol (Machado et al., 2010). The concentration and quality of the isolated DNA were evaluated via a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE) or a Qubit fluorometer 2.0 (Life Technologies, Grand Island, NY). For library preparation, the Illumina TruSeq Nano Kit (Illumina Inc., San Diego, CA) was used according to the manufacturer's recommendations. Sequencing was performed via the Illumina HiSeq2000 platform (Illumina Inc., San Diego, CA), producing reads measuring 2 × 100 bp and 2 × 200 bp, with an average sequencing coverage of 13.9X, or via the NovaSeq 6000 platform (Illumina Inc., San Diego, CA), with an average sequencing coverage of 16X and reads measuring 2 × 100 bp and 2 × 200 bp.

The GIR_IN population consisted of 14 samples of bulls and cows from Gujarat state in India raised for milk production. DNA extraction was performed by following the Sambrook and Russel adapted protocol (Sambrook and Russel, 2001). The sequencing was performed on the Illumina HiSeq2500 platform (Illumina Inc., San Diego, CA), producing reads measuring 2 × 125 bp, with an average sequencing coverage of 13.5X.

The WGS processing for the GIR_BR and GIR_IN samples followed the same pipeline, according to the recommendations of the 1000 Bull Genomes Project protocol (http://www.1000bullgenomes.com, last accessed on 11/20/2020; Hayes and Daetwyler, 2019). In summary, read quality was evaluated using the FastQC tool, and trimming was performed using SeqyClean software (Zhbannikov et al., 2017). The reads were aligned to the bovine reference genome ARS-UCD 1.2 using the mem option of the BWA algorithm (Li and Durbin, 2009). Conversion to binary format, sorting, and indexing were completed via SAMtools (Li et al., 2009). Duplicates were removed using the MarkDuplicates option of the Picard tool software (2019; https://broadinstitute.github.io/picard/). Base quality score recalibration (**BQSR**) was performed via BaseRecalibrator and PrintReads of the Genome Analysis Toolkit (**GATK**; v. 3.8-1-0-gf15c-1c3ef; https://gatk.broadinstitute.org/hc/en-us). The set of known variants provided by the 1000 Bull Genomes project (Hayes and Daetwyler, 2019) was applied for BQSR. Variant calling was performed using the HaplotypeCaller option from GATK according to the same protocol (-ERC GVCF -variant_index_type LINEAR -variant_index_parameter 128000).

Following BQSR, samples were combined using the option genotypeGVCFs from the GATK software. The variant filtering followed the variant quality score recalibration (**VQSR**) from GATK software (v. 4.4.0)

according to the parameters in the Run 9 pipeline from 1000 Bull Genomes (Hayes and Daetwyler, 2019). Variant quality score recalibration uses machine learning algorithms to learn from training datasets the annotation profile of good variants and bad variants, integrating information from multiple dimensions. The training and validation sets of the variants were provided by the 1000 Bull Genomes Project (Hayes and Daetwyler, 2019). Only variants occurring in the tranche 99% were used (Neumann et al., 2023). Duplicate samples were checked through the KING-robust estimator (Manichaikul et al., 2010) with a cutoff of 0.354 using Plink2 software (Chang et al., 2015).

Single nucleotide variants (**SNVs**) from WGS data from 307 Holstein bulls and cows from Canada and the United States (**HOL**) were accessed from the 1000 Bull Genomes project (Run 9; Hayes and Daetwyler, 2019). Only samples with a minimum coverage of 6X and autosomal SNVs occurring in the tranche 99% (from the VQSR performed by the 1000 Bull Genomes Project) were used (Neumann et al., 2023). Duplicate samples were excluded through the KING-robust estimator (Manichaikul et al., 2010) with a cutoff of 0.354 via Plink2 software. For GIR_BR, GIR_IN, and HOL, autosomes were retrieved for diversity and selection signature analysis, and the *Bos taurus* X chromosome (**BTX**) was scanned for intrapopulation selection signatures.

Genomic Diversity

Principal component analysis (**PCA**) using the genotype matrix was performed as follows. One marker of pairs with a high level of linkage disequilibrium (**LD**) was filtered out using an r^2 threshold of 0.2, a window size of 50 SNVs, and a step size of 5 SNVs (–indep-pairwise 50 5 0.2) for each population separately. Additionally, variants with missing call rates exceeding 0.05 (–geno 0.05) were filtered out. For the population differentiation metric, the ratio of averages of the fixation index ($\mathbf{F_{ST}}$) based on Hudson's method (Hudson et al., 1992) was computed via the option –fst from Plink2 between each pair of populations.

The genomic variation within populations was assessed separately using nucleotide diversity ($\boldsymbol{\pi}$), SNV density analysis, minor allele frequency (**MAF**), observed ($\mathbf{H_O}$) and expected ($\mathbf{H_E}$) heterozygosity, and inbreeding coefficient based on the methods-of-moments estimator ($\mathbf{F_{HOM}}$). Nucleotide diversity is defined as the average number of nucleotide differences per site between 2 DNA sequences sampled from a population, in which higher values indicate greater diversity (Nei and Li, 1979). The software VCFtools v. 0.1.15 (Danecek et al., 2011) was used to estimate the nucleotide diversity of each population in nonoverlapping window sizes of 50 kb with a step of 20 kb containing at least 5 SNVs. Descriptive statistics of total ($\boldsymbol{\pi_{Total}}$) and chromosome ($\boldsymbol{\pi_{Chr}}$) nucleotide diversity were subsequently calculated. Biallelic marker distribution was evaluated with an SNV density plot through the CMplot package (Yin et al., 2021) in R software (https://www.r-project.org/), which uses a bin of 1 Mb.

For heterozygosity, MAF, and inbreeding coefficient, only variants with a minimum mean depth of 10× were retrieved to avoid biased estimates (Kardos and Waples, 2024). The MAF was calculated via Plink 1.9 (–freq) for each population. SNVs with MAF greater than 0.1 (10%) were classified as polymorphic SNVs (Rajawat et al., 2024). VCFtools v. 0.1.15 was used to estimate H_O and H_E (–het). Heterozygosity is a measure often used to track the amount of genetic variation retained by a population. The F_{HOM} was computed according to the method of moments estimator (Li and Horvitz, 1953) using the following equation:

$$F_{HOM} = \frac{\left(O_{HOM} - E_{HOM}\right)}{\left(N_{VAR} - E_{HOM}\right)},$$

where O_{HOM} represents the number of observed homozygous genotypes, E_{HOM} the number of expected homozygous genotypes, and N_{VAR} the total number of variants.

Selection Signatures

For autosome chromosomes, intra- and between-population analyses were performed. For chromosome X, only intrapopulation analyses were conducted.

Intrapopulation.

Integrated Haplotype Score Test Statistic: The R package rehh v. 3.2.2 (Gautier et al., 2017) was used to perform the integrated haplotype score (**iHS**) test within each population with unpolarized markers. Phasing within each population was performed via Beagle v. 5.1 (Browning and Browning, 2007) with default parameters. To identify potential selection signatures, |iHS| was calculated for windows of 50 kb containing a minimum of 5 SNVs. For autosomes, the top 1%, and for chromosome X, the top 0.5% were considered potential selection signatures, and adjacent windows were merged. Integrated haplotype score is a highly sensitive test that identifies high-LD regions, and it is a standard measure of decay in extended haplotype homozygosity (**EHH**). The iHS detects selection when the selected allele has achieved intermediate frequencies, but before selection; the derived allele must have existed on a distinct background and not yet reached fixation (Voight et al., 2006; Utsunomiya et al., 2013). In the case of unpolarized markers, this test uses major and minor alleles instead (Klassmann and Gautier, 2022), which was applied in this study.

Table 1. Summary of number of samples, minimum coverage, mean and standard deviation, maximum coverage, and numbers of autosome biallelic SNVs and multiallelic SNVs after quality control, as well as transition/transversion rate (Ts/Tv) per population[1]

Population[2]	N	Min. (X)	Mean (X)	Max. (X)	Biallelic SNVs	Multiallelic SNVs	Ts/Tv
GIR_IN	14	6.60	17.77 (5.47)	29.20	31,253,205	352,760	2.26
GIR_BR	42	10.20	16.09 (3.02)	25	31,264,796	353,009	2.26
HOL	307	6.38	17.55 (8.08)	51.78	57,106,559	2,538,795	2.18

[1]N = number of samples, Min. (X) = minimum coverage, Mean (X) = mean and SD, Max. (X) = maximum coverage.
[2]GIR_IN = Gir bulls from India; GIR_BR = Dairy Gir sires from Brazil; HOL = Holstein bulls and cows from Canada and the United States.

Tajima's D Test Statistic: Tajima's D statistic was computed within the population in window sizes of 50 kb via VCFtools software. Only windows with at least 5 markers were considered. For autosomes, the bottommost 1%, and for chromosome X, the bottommost 0.5% were retrieved, and adjacent selection signatures were merged. Tajima's D statistic compares 2 measures of genetic diversity, the number of segregating sites (S) and the nucleotide diversity, which is the average number of pairwise nucleotide differences between sequences in a sample (Tajima, 1989). The purpose of Tajima's D test is to distinguish between a DNA sequence evolving randomly and one evolving under a nonrandom process, which may include, for instance, selection, demographic expansion, contraction, or introgression. Tajima's D statistic value is expected to be zero under the standard neutral evolutionary model. However, deviations from zero may indicate nonneutral forces or selection. Negative Tajima's D values suggest the fixation of alleles or rare alleles, whereas positive values denote balancing selection, which reflects an abundance of intermediate allele frequencies (Tajima, 1989; Korneliussen et al., 2013).

Between Populations.

Fixation Index: Pairwise autosomal Wright's F_{ST} values were estimated based on Hudson's method (Hudson et al., 1992) using the scikit-allel v1.3.7 library (Miles et al., 2023) in Python with windows of 50 kb and a minimum of 5 markers. Only the top 1% of windows were selected as signature candidates. The fixation index (Wright, 1949) is a test that measures population genetic differentiation and can detect actual genetic variants under selection. The F_{ST} values vary from zero, which implies no differences in allelic frequencies between 2 populations, to one, meaning that each population is fixed for a different allele. Hudson's method performs better than traditional F_{ST} calculation when there is a large variation in sample size across populations and accounts for changes in the demographic history of the populations being compared (Bhatia et al., 2013).

Cross-Population Extended Haplotype Homozygosity (XP-EHH): Phasing within each population was performed using Beagle v. 5.1 (Browning and Browning, 2007), and selection signatures between populations were subsequently investigated via the XP-EHH test with windows of 50 kb containing a minimum of 5 SNPs via the rehh (Gautier et al., 2017) R package. The top and bottom 0.5% extreme windows were considered potential selection signatures, and adjacent windows were merged. Cross-population EHH compares the integrated EHH between 2 populations at the same SNP and detects selected alleles that have approached or achieved fixation in one population but not in the other. This method is indicated for detecting ongoing or nearly fixed selection signatures because this test is based on LD and identifies alleles that have rapidly increased to high frequency and are still associated with nearby polymorphisms (Sabeti et al., 2007).

Functional Analysis

The GALLO package (Fonseca et al., 2020) from R software was used to identify genes and QTL within selection signatures. Genes and QTL were retrieved from the Ensembl Genes database (Ensembl Release 110, ARS_UCD1.2; https://www.ensembl.org) and the Animal Genome database (https://www.animalgenome.org/cgi-bin/QTLdb/BT/index, ARS_UCD1.2), respectively. Additionally, colocalized genes were also evaluated for the top 10 windows with the highest nucleotide diversity and the regions with the highest and lowest SNV density. For genes within selection signatures, terms from the Gene Ontology (**GO**) database and metabolic pathways predicted by the Kyoto Encyclopedia of Genes and Genomes (**KEGG**) database were enriched (false discovery rate [**FDR**] <0.05) using the WebGestaltR package (Wang and Liao, 2020) in R software. The enrichment analysis was performed via hypergeometric overrepresentation analysis. Gene Ontology terms are divided into 3 groups: Cellular Components, Biological Processes, and Molecular Functions. Metabolic pathways predicted by the Reactome database (https://reactome.org/) were also enriched (FDR <0.05) using the website of the repository. Gene under selection were used to perform protein-protein interaction network using STRING database (Szklarczyk et al., 2023). The networks were clustered using the k-mean algorithm to improve visualization.

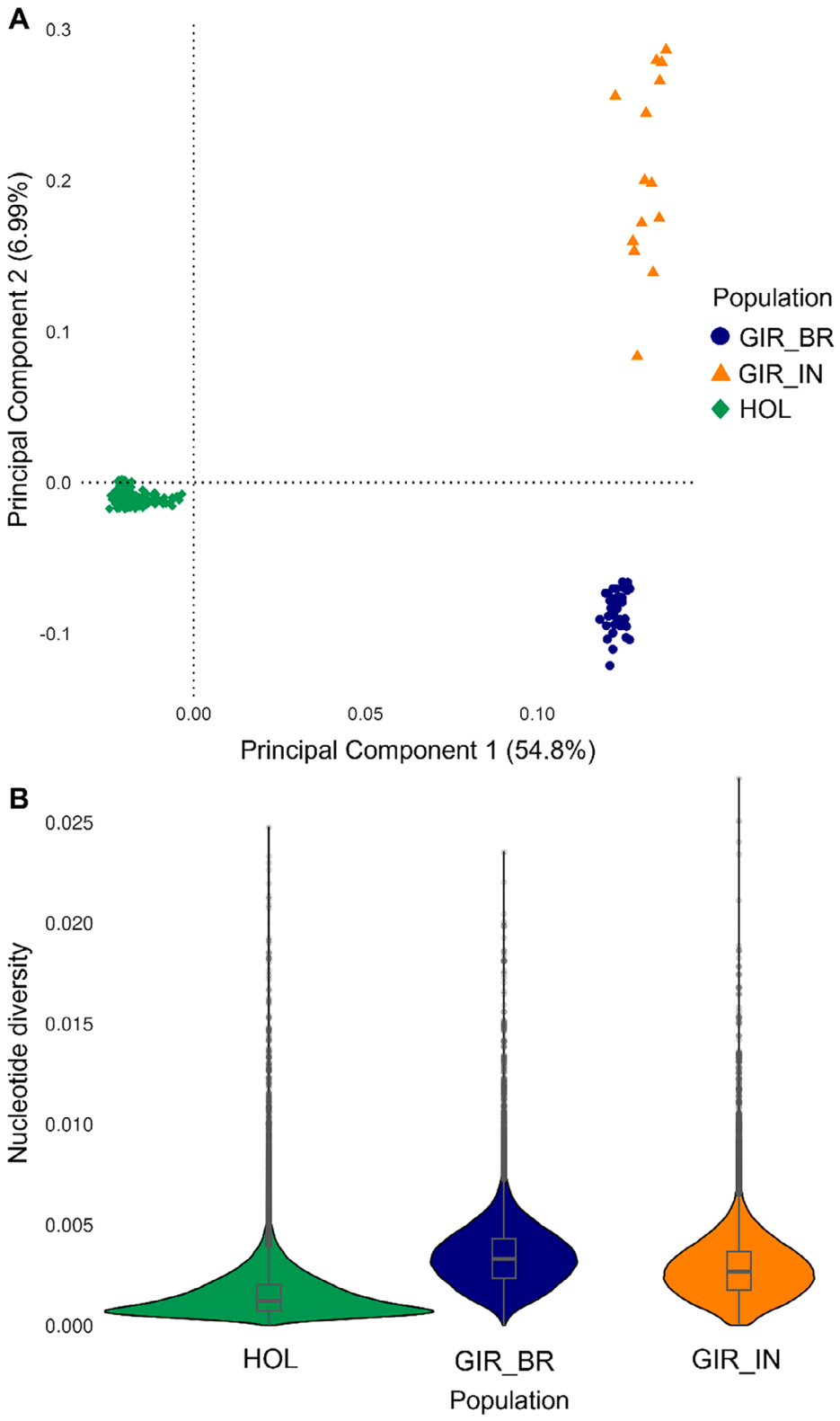

Figure 1. (A) Principal component analysis of individuals. (B) Violin and boxplot graphs of nucleotide diversity for populations of Holstein bulls and cows from Canada and the United States (HOL), Dairy Gir sires from Brazil (GIR_BR), and Gir bulls from India (GIR_IN). The midline indicates the median; the upper and lower edges of the box represent the first and third quartiles; the whiskers extend to 1.5 times the interquartile range; and the dots represent outliers. The shape of each violin illustrates the kernel density estimation of the distribution of nucleotide diversity for each population.

RESULTS

Alignment and Variant Calling

Table 1 summarizes the number of samples and descriptive statistics and metrics for the WGS samples. Overall, the coverage ranged from 6.38X to 51.78X. The alignment metrics and coverage per sample are presented in Supplemental Table S1 (see Notes). The number of consensus markers for the HOL and GIR_IN pair was 31,229,604, for the HOL and GIR_BR pair, 37,296,579, and for the GIR_IN and GIR_BR pair, 30,395,111.

Genomic Diversity

The PCA results are shown in Figure 1A, in which the 3 populations exhibit distinct clusters. The ratio-of-average F_{ST} values obtained via the Hudson method can be found in Table 2.

The GIR_BR population exhibited the highest π_{Total}, whereas the HOL population presented the lowest mean value despite having a larger sample size (Table 3; Figure 1B; Supplemental Figure S1; see Notes). For the GIR_IN population, a higher mean π_{Chr} was observed in BTA 28, whereas the lowest was noted in BTA2 and 11 (Supplemental Table S2; see Notes). In the GIR_BR population, the highest mean π_{Chr} was observed in BTA27 and 28, whereas the lowest was found in BTA3, 5, 13, and 19 (Supplemental Table S3; see Notes). For the HOL population, the highest mean π_{Chr} was observed in BTA23, with the lowest in BTA13 and 22 (Supplemental Table S4; see Notes). The top 10 windows with the highest nucleotide diversity and colocalized genes across the 3 populations are reported in Supplemental Table S5 (see Notes).

The SNV density plots for each population are displayed in Supplemental Figures S2–S4 (see Notes). There was an absence of SNVs in BTA10:23.78–24.78 Mb across all populations. No gene is described in the ARS_UCD1.2 bovine annotation, and no variants are reported for this region (https://www.ensembl.org/). We hypothesize that this region is highly conserved in bovines. The region BTA23:24.00–30.00 Mb presented a greater density of SNVs for all populations. The annotated genes for this region are presented in Supplemental Table S6 (see Notes). The H_O, H_E, F_{HOM}, MAF, and proportion of polymorphic SNVs results are reported in Table 4 and Figure 2A.

Selection Signatures and Genes Under Selection

Supplemental Table S7 (see Notes) lists the top 10 extreme windows from each test and population. No metabolic pathway from the Reactome database was enriched for the list of genes under selection in all 3 populations.

Table 2. Pairwise ratio of averages of F_{ST} based on Hudson's method among Holstein bulls and cows from Canada and the United States (HOL), Dairy Gir sires from Brazil (GIR_BR), and Gir bulls from India (GIR_IN) populations

Population 1	Population 2	Ratio of averages F_{ST} value
HOL	GIR_IN	0.5411
HOL	GIR_BR	0.4671
GIR_IN	GIR_BR	0.1062

Protein-protein interactions networks are listed in Supplemental Figures S5–S19 (see Notes).

Intrapopulation

iHS Test Statistic for Autosomes. The Manhattan plots of autosomal windows are shown in Figure 3, and for BTX, in Supplemental Figure S20 (see Notes). In GIR_IN, after the top windows were merged, 326 nonoverlapping selection signatures were revealed. The largest length of a selection signature was BTA22:23.55–23.95 Mb. Across 252 selection signatures, a total of 354 genes and 1,315 QTL were found, with 39 enriched QTL (FDR <0.01; Supplemental Tables S8 and S9; see Notes).

For the GIR_BR population, the largest selection signature in length was BTA11:11.65–12.4 Mb. After merging, 320 nonoverlapping selection signatures were identified. A total of 884 QTL and 314 genes were under selection in 231 selection signature regions in the functional analysis (Supplemental Table S10; see Notes). Furthermore, QTL enrichment revealed 27 enriched QTL (FDR <0.01; Supplemental Table S9).

For the HOL population, the largest length of a selection signature was found in BTA20:26.75–27.1 Mb. After merging, 385 nonoverlapping selection signatures were identified (Supplemental Table S11; see Notes). For the functional analysis, 274 regions revealed 374 candidate genes and 1,342 QTL in selection signature regions, 22 of which were enriched (FDR <0.01; Supplemental Table S9).

Tajima's D Statistic Test for Autosomes. The Manhattan plots of autosomal windows are shown in Figure 3, and for the BTX, in Supplemental Figure S21 (see Notes). In GIR_IN, the autosomal mean Tajima's D was 1.24, and the SD was 1.12 (Figure 4). After merging overlapping windows, 292 selection signatures were identified. The largest length of a selection signature was found in BTA14:39.25–39.70 Mb. In the functional analysis, 378 candidate genes and 1,203 QTL were under selection across 232 selection signature regions (Supplemental Table S12; see Notes). Furthermore, QTL enrichment analysis revealed 32 enriched QTL (FDR <0.01; Supplemental Table S9). The KEGG term "axon guidance" was significantly enriched (FDR <0.05) for the genes

Table 3. Minimum, first quartile, median, mean, third quartile, and SD of π_{Total}[1] for HOL, GIR_BR, and GIR_IN populations[2]

Population	Minimum	1st quartile	Median	Mean	3rd quartile	Maximum	SD
GIR_IN	2.17e-04	0.175	0.267	0.278	0.366	2.716	0.143
GIR_BR	1.07e-04	0.233	0.330	0.338	0.431	2.351	0.146
HOL	2.34e-05	0.073	0.122	0.152	0.203	2.475	0.112

[1]All π_{Total} measures are shown as percentages (%).
[2]GIR_IN = Gir bulls from India; GIR_BR = Dairy Gir sires from Brazil; HOL = Holstein bulls and cows from Canada and the United States.

PPP3R1, *ROCK2*, *ABLIM3*, *TRPC4*, *PARD3*, *CFL2*, *PARD6G*, *NTN3*, *PRKCZ*, and *NFATC4* (Supplemental Table S13; see Notes).

For GIR_BR, the autosomal Tajima's D mean was 1.31 (SD: 0.82). A total of 304 nonoverlapping selection signatures were observed. The selection signature with the greatest length was BTA11:11.85–12.4 Mb. We identified 1,392 QTL and 531 genes under selection in 264 selection signatures for the functional analysis (Supplemental Table S14; see Notes). Among the QTL, 33 were enriched (FDR <0.01; Supplemental Table S9).

In the HOL population, the autosomal Tajima's D mean was 1.48 (SD: 1.46). A total of 357 nonoverlapping selection signatures were detected after merging. The largest selection signature was found in BTA8:105.9–106.4 Mb. In the functional analysis, 283 regions revealed 457 candidate genes and 1,713 QTL under selection (Supplemental Table S15; see Notes), 40 of which were enriched (FDR <0.01; Supplemental Table S9).

Between Populations

Fixation Index. For the HOL and GIR_IN population pair, the F_{ST} values ranged from 0 to 0.991 (Figure 5A). By merging adjacent windows, 324 nonoverlapping selection signatures were identified. In the functional analysis, 268 putative selection signatures were colocalized with 558 genes and 1,417 QTL (Supplemental Table S19; see Notes), of which 32 QTL were enriched (FDR <0.01; Supplemental Table S9).

For the HOL and GIR_BR population pair, the F_{ST} values ranged from 0.008 to 0.988 (Figure 5B). After adjacent windows were merged, 326 nonoverlapping selection signatures were identified. The functional analysis revealed 1,962 QTL and 633 genes under selection in 288 selection signatures (Supplemental Table S20; see Notes). Furthermore, QTL enrichment revealed 37 enriched QTL (FDR <0.01; Supplemental Table S9). The KEGG terms "EGFR tyrosine kinase inhibitor resistance," "Bacterial invasion of epithelial cells," "Chronic myeloid leukemia," "Cellular senescence," and "Thyroid hormone signaling pathway" were significantly enriched (FDR <0.05) for several genes (Supplemental Table S13).

For the GIR_IN and GIR_BR pair, the F_{ST} values ranged from 0 to 0.713 (Figure 5C). Following the merging of adjacent windows, a total of 341 nonoverlapping selection signatures were identified. The functional analysis revealed that 1,268 QTL and 376 genes are potentially under selection within 269 selection signatures (Supplemental Table S21; see Notes). Quantitative trait loci enrichment revealed 29 enriched QTL (FDR <0.01; Supplemental Table S9).

Cross-Population Extended Haplotype Homozygosity. For the HOL and GIR_IN population pair, positive values denote selection in HOL, and negative values indicate selection in GIR_IN (Figure 6A). Following the merging of adjacent windows, 144 top and 185 bottom nonoverlapping selection signatures were identified for the HOL GIR_IN pair. A total of 119 genes and 464 QTL under selection in 98 selection signatures were found in the functional analysis for HOL (Supplemental Table S16; see Notes), and 8 QTL were enriched (FDR <0.01; Supplemental Table S9). The molecular function GO term "calcium ion binding" was significantly enriched (FDR <0.05) for the genes *CHP1*, *EHD4*, *PLA2G4D*, *PLA2G4F*, *S100A2*, *S100A3*, *S100A4*, *S100A5*, and *HPCA* (Supplemental Table S13). For selection signatures related to the

Table 4. Mean (SD) of observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding coefficient based on the method of moments (F_{HOM}), minor allele frequency (MAF), and proportion of polymorphic SNVs for three cattle populations

Population[1]	H_O	H_E	F_{HOM}	MAF	Proportion of polymorphic SNVs
GIR_IN	0.3727 (0.0327)	0.3412 (0.0002)	−0.0924 (0.0955)	0.1576 (0.1643)	0.5179
GIR_BR	0.2761 (0.0232)	0.2791 (0.0002)	0.0109 (0.0828)	0.1912 (0.1496)	0.6272
HOL	0.2186 (0.0189)	0.2256 (0.0003)	0.0309 (0.08)	0.0448 (0.1084)	0.1460

[1]GIR_IN = Gir bulls from India; GIR_BR = Dairy Gir sires from Brazil; HOL = Holstein bulls and cows from Canada and the United States.

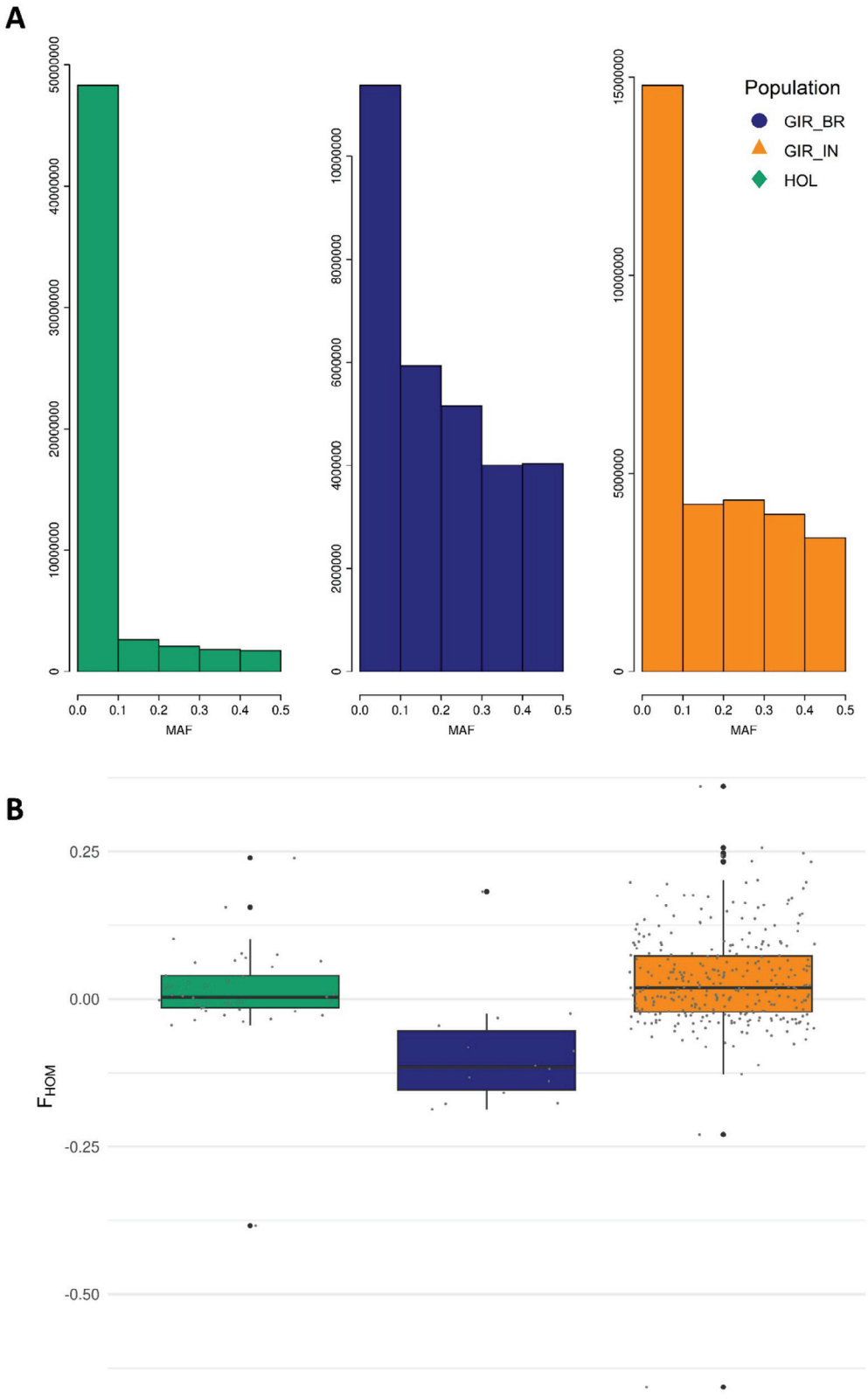

Figure 2. (A) Histogram plot of minor allele frequencies (MAF). The y-axis represents the allele count within each MAF bin. (B) Boxplots of inbreeding coefficient based on methods of moments (F_{Hom}) for Holstein bulls and cows from Canada and the United States (HOL), Dairy Gir sires from Brazil (GIR_BR), and Gir bulls from India (GIR_IN). The midline indicates the median; the upper and lower edges of the box represent the first and third quartiles; the whiskers extend to 1.5 times the interquartile range; and the dots represent individual animals.

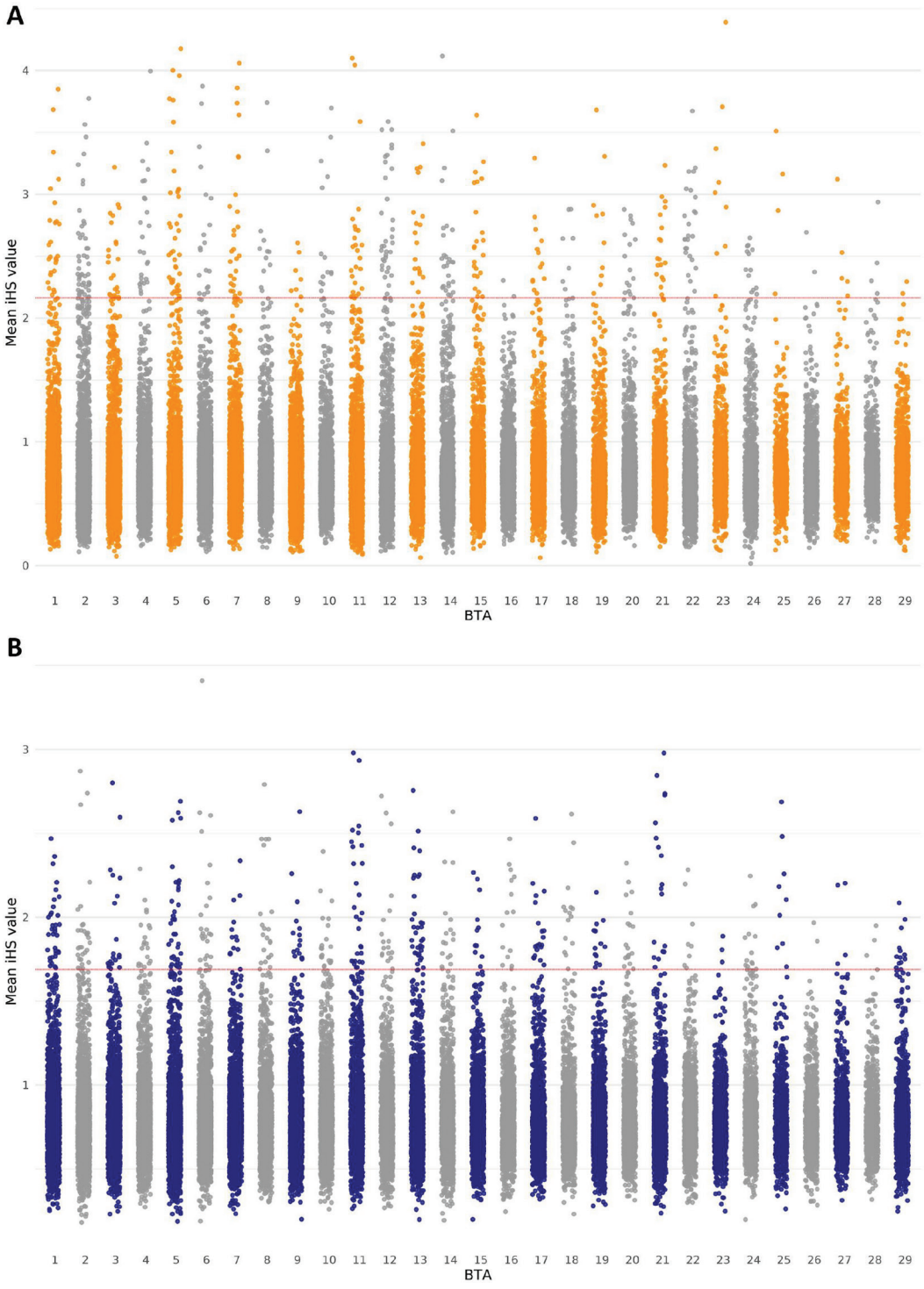

Figure 3. Manhattan plots of the mean |iHS| values for windows in 3 dairy cattle populations: (A) Gir bulls from India (GIR_IN), (B) Dairy Gir sires from Brazil (GIR_BR), and (C) Holstein bulls and cows from Canada and the United States (HOL). The red dashed line indicates the threshold values of 2.16, 1.69, and 1.72 for the identification of potential selection signatures in each population, respectively.

GIR_IN population, 166 genes and 390 QTL were under selection in 131 windows (Supplemental Table S16). The results of the QTL enrichment analysis revealed that 21 QTL were significant (FDR <0.01; Supplemental Table S9). The biological process GO term "regulation of lipase activity" was significantly enriched (FDR <0.05) for the

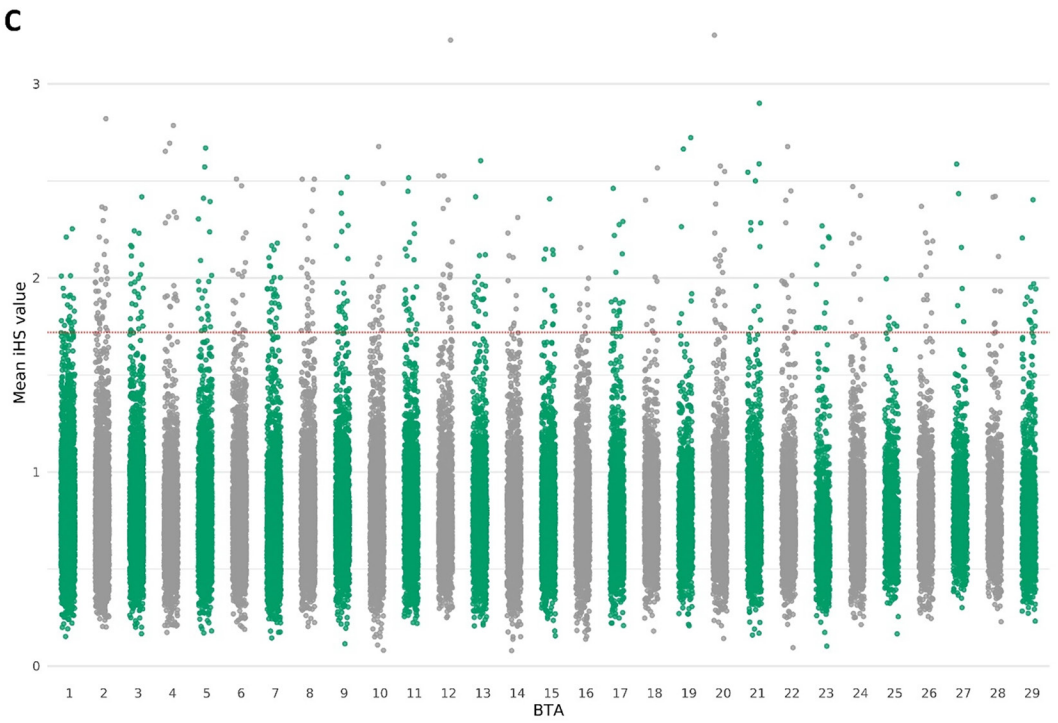

Figure 3 (Continued). Manhattan plots of the mean |iHS| values for windows in 3 dairy cattle populations: (A) Gir bulls from India (GIR_IN), (B) Dairy Gir sires from Brazil (GIR_BR), and (C) Holstein bulls and cows from Canada and the United States (HOL). The red dashed line indicates the threshold values of 2.16, 1.69, and 1.72 for the identification of potential selection signatures in each population, respectively.

genes *PLA2G5*, *SORT1*, *LPAR1*, and *ANXA8L1* (Supplemental Table S13).

For the HOL and GIR_BR population pair, positive values indicate selection in HOL, and negative values indicate selection in GIR_BR (Figure 6B). Following the cutoff and combination, 153 top and 198 bottom nonoverlapping selection signatures were observed. A total of 110 putative selection signatures overlapped with 160 genes and 631 QTL (Supplemental Table S17, see Notes), 24 of which were enriched (FDR <0.01) in the HOL population (Supplemental Table S9). The molecular function GO term "calcium ion binding" was significantly enriched (FDR <0.05) for the genes *S100A1*, *S100A13*, *S100A14*, *S100A16*, *S100A2*, *S100A3*, *S100A4*, *S100A5*, *EHD4*, *PLA2G4D*, and *PLA2G* (Supplemental Table S13). For the GIR_BR population, 160 genes and 587 QTL were located on 160 putative selection signatures (Supplemental Table S17), and 15 QTL were enriched (FDR <0.01; Supplemental Table S9).

For the GIR_IN and GIR_BR population pair, positive values indicate selection in GIR_IN, and negative values indicate selection in GIR_BR (Figure 6C). After combination, 101 top and 201 bottom nonoverlapping selection signatures were found. The functional analysis revealed 83 selection signature regions harbored 125 genes and 492 QTL (Supplemental Table S18; see Notes), with 6 enriched QTL (FDR <0.01) for GIR_IN (Supplemental Table S9). For the GIR_BR population, 146 selection signatures with 151 genes and 402 QTL (Supplemental Table S18), of which 10 were enriched (FDR <0.01) for GIR_BR (Supplemental Table S9).

Overlapping Selection Signatures

Figure 7 illustrates the overlapping autosomal selection signatures among populations and tests. In the intrapopulation analyses (Figure 7A), GIR_IN and GIR_BR shared more selection signatures with each other than with the HOL population, as expected, given that they are different populations of the same breed. However, in the interpopulation analyses (Figure 7B), the highest number of shared selection signatures was observed between GIR_BR and HOL and between GIR_IN and HOL.

DISCUSSION

This study assessed genetic diversity and selection signatures in 3 cattle populations: a Holstein population from the United States and Canada, Gir from India, and Dairy Gir from Brazil, which were originally imported from India and have been selected for milk production in Brazil over the past 40 years. We assessed several di-

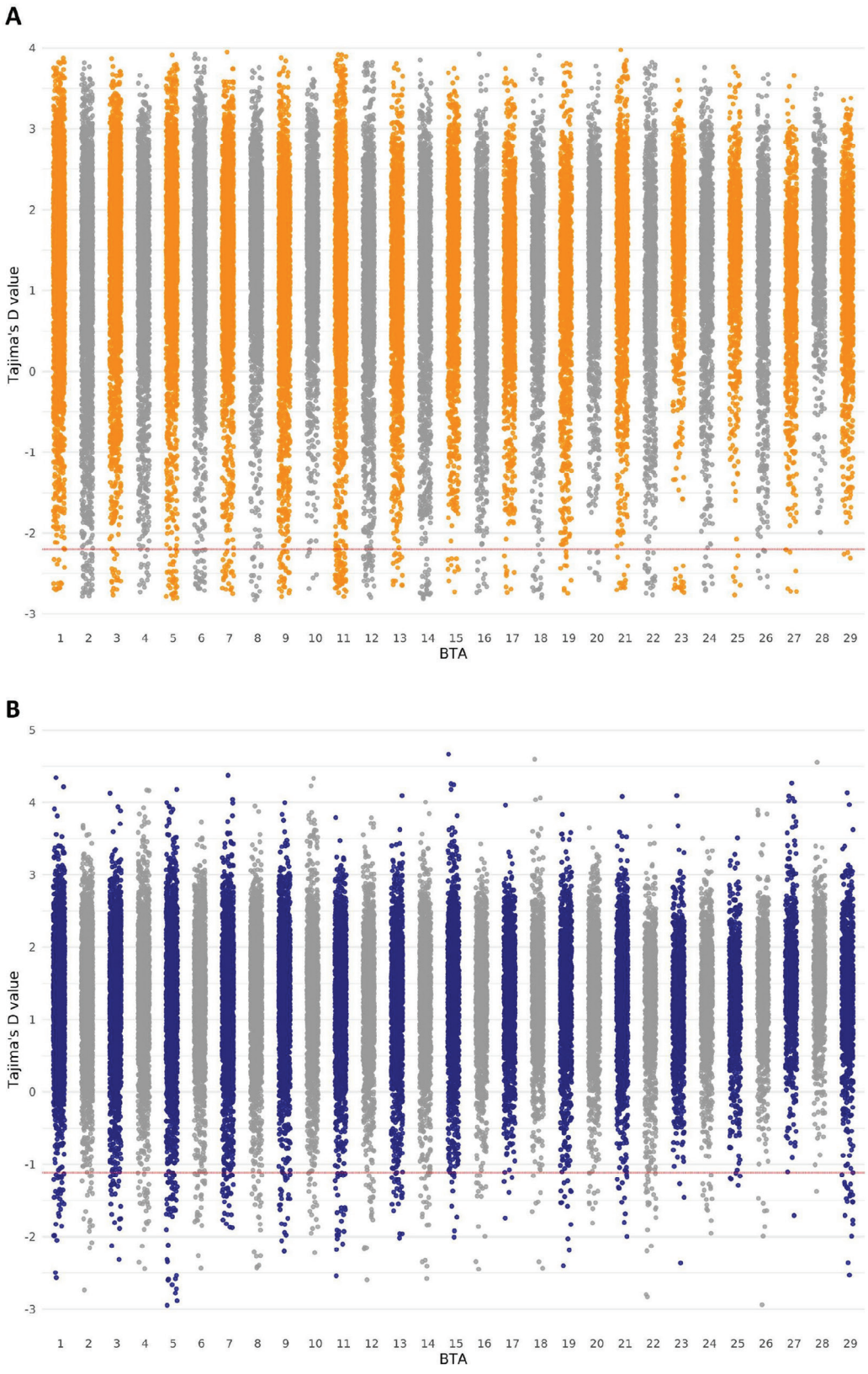

Figure 4. Manhattan plot of Tajima's D values for 3 dairy cattle populations: (A) Gir bulls from India (GIR_IN), (B) Dairy Gir sires from Brazil (GIR_BR), and (C) Holstein bulls and cows from Canada and the United States (HOL). The red dashed line indicates the thresholds of −2.20, −1.11, and −1.74 used to identify potential selection signatures in each population, respectively.

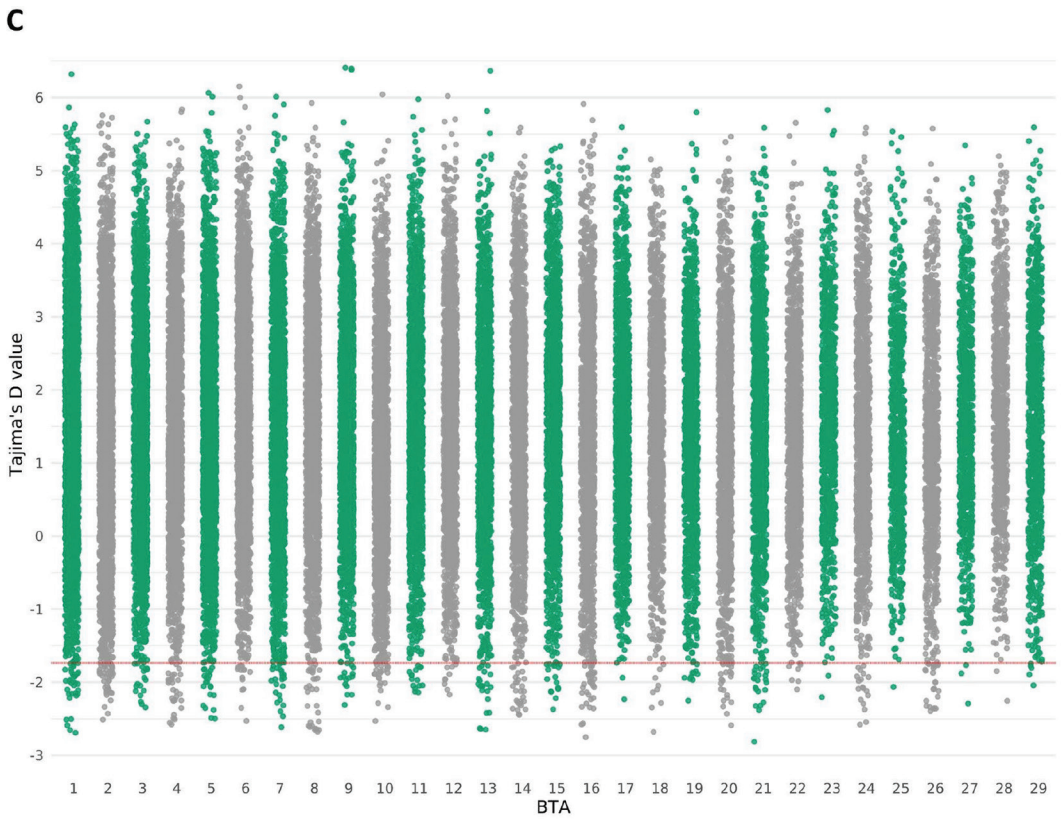

Figure 4 (Continued). Manhattan plot of Tajima's D values for 3 dairy cattle populations: (A) Gir bulls from India (GIR_IN), (B) Dairy Gir sires from Brazil (GIR_BR), and (C) Holstein bulls and cows from Canada and the United States (HOL). The red dashed line indicates the thresholds of −2.20, −1.11, and −1.74 used to identify potential selection signatures in each population, respectively.

versity metrics and detected regions under selection via intrapopulation and interpopulation tests.

Population Differentiation and Genetic Diversity

Population Differentiation. As expected, a high ratio of averages of F_{ST} based on Hudson's method was observed when the HOL population was included in the pair. Specifically, we observed a similar genetic differentiation between the HOL and GIR_BR populations (0.4671) compared with the findings (0.436) reported by Tijjani et al. (2022) when Holstein cows from Europe and Dairy Gir cows from the same GIR_BR population that were studied in our research. Moderate (0.1062) genetic differentiation was observed between the GIR_BR and GIR_IN pair. Despite belonging to the same breed, these populations have diverse backgrounds and have experienced distinct selection processes, with greater selection pressure on the GIR_BR population.

Nucleotide and SNV Diversity. The Gir populations, particularly GIR_BR, presented higher values of mean π_{Total} than did the HOL population, indicating considerably greater genetic diversity for Gir populations. In contrast to HOL, GIR_BR, and GIR_IN populations experienced weaker selection intensity, likely resulting in more alleles segregating and sequences more divergent, creating more different sites per sequence (Nei and Li, 1979). Masharing et al. (2023) reported a greater nucleotide diversity value (0.356) for Gir from India using ddRAD sequencing than our study did (mean π_{Total} = 0.278). Differences in sampling methods and the density of the molecular sequencing technique used may have contributed to this discrepancy. The HOL population showed the lowest mean π_{Total} (0.152%), likely due to the stronger selection pressure for maximizing productivity. Neumann et al. (2023) reported a similar value for nucleotide diversity in the Holstein population (Mean π_{Total} = 0.147%). The authors used a set of Holstein samples from Run 9 of the 1000 Bull Genomes project, including 60 individuals from Canada and the United States.

The regions with the highest levels of nucleotide diversity across all populations were BTA4 (113.1–113.17 Mb, 105.56–105.63 Mb), BTA12 (71.16–71.27 Mb, 72.8 - 72.85 Mb), BTA15 (45.96–46.01 Mb, 46.2–46.25 Mb), and BTA23 (26.04–26.17 Mb, 27.1–27.15 Mb). The top 10 regions with relatively high nucleotide diversity

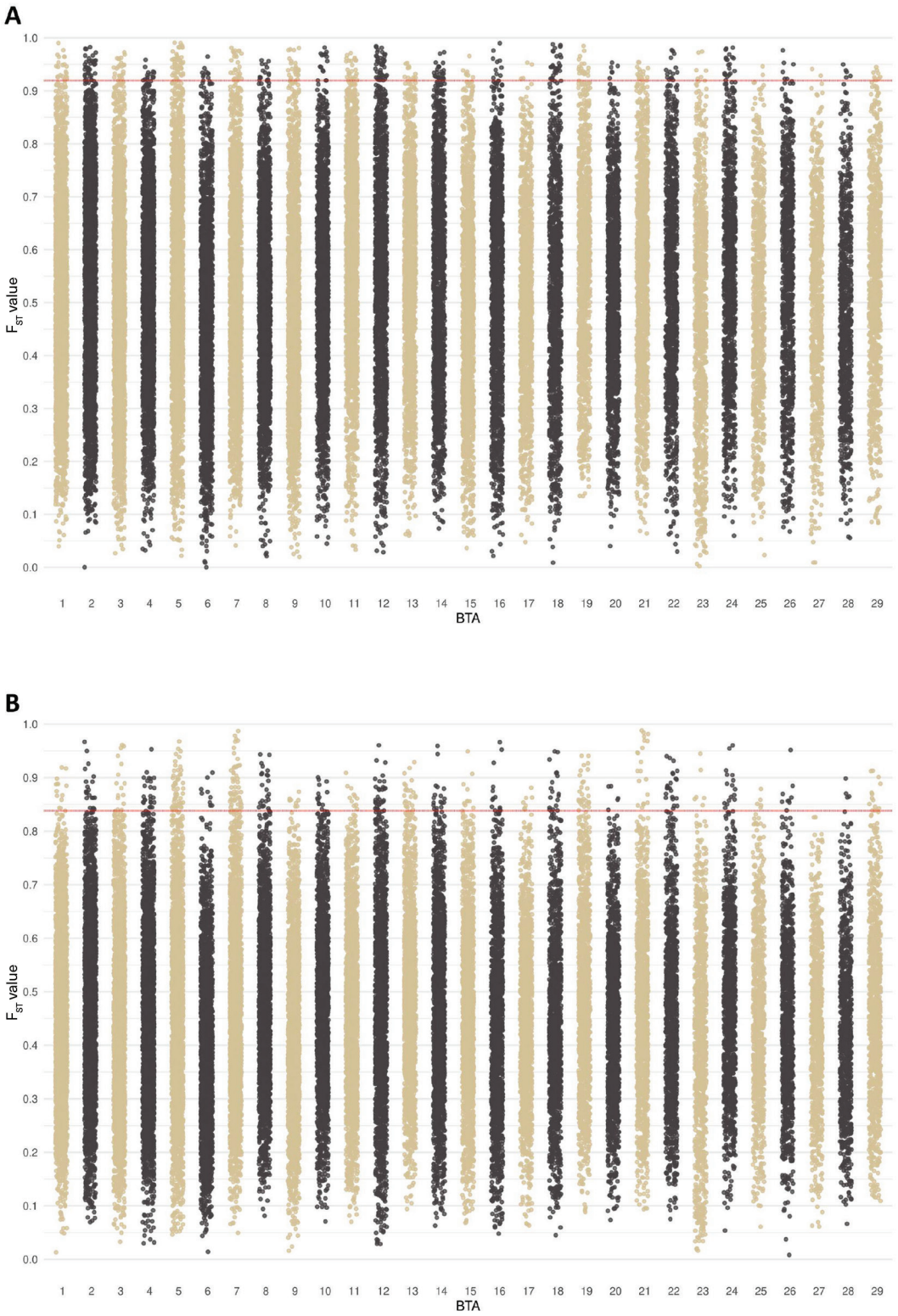

Figure 5. (A) Manhattan plot of F_{ST} values for HOL (Holstein bulls and cows from Canada and the United States) and GIR_IN (Gir bulls from India) population pair; the red dashed line represents the threshold of 0.92 to consider potential selection signatures. (B) Manhattan plot of F_{ST} values for HOL and GIR_BR (Dairy Gir sires from Brazil) population pair; the red dashed line represents the threshold of 0.84 to consider potential selection signatures. (C) Manhattan plot of F_{ST} values for GIR_IN and GIR_BR population pair; the red dashed line represents the threshold of 0.37 to consider potential selection signatures.

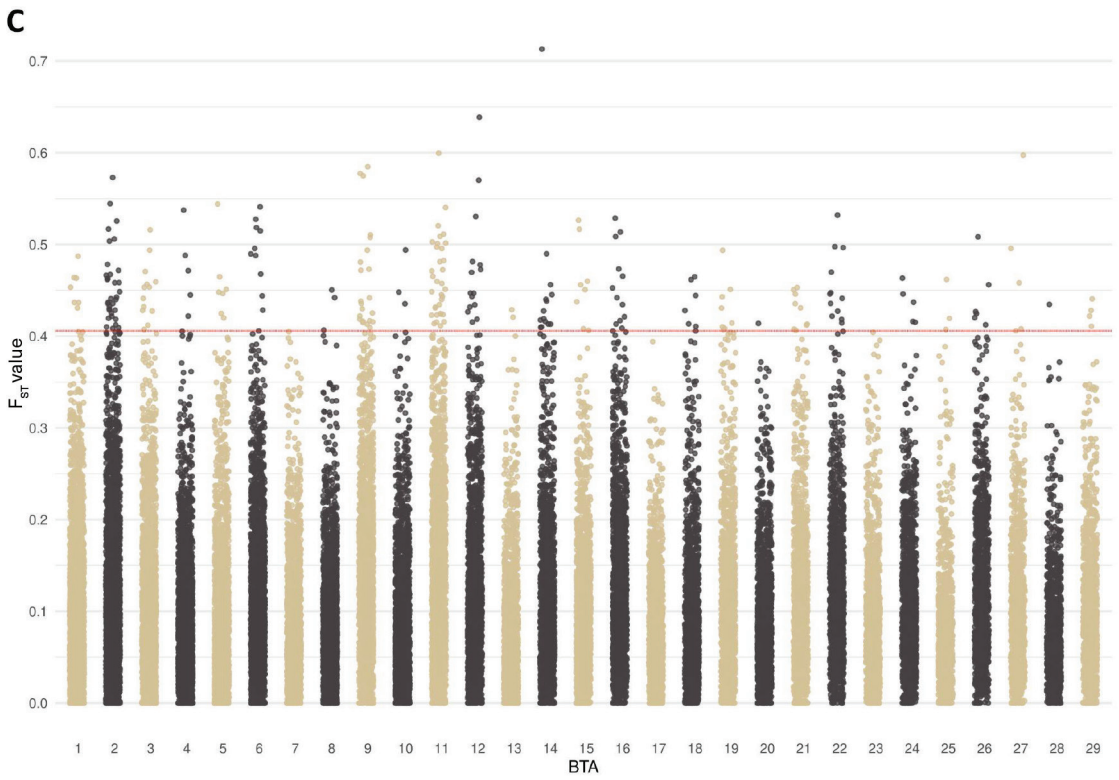

Figure 5 (Continued). (A) Manhattan plot of F_{ST} values for HOL (Holstein bulls and cows from Canada and the United States) and GIR_IN (Gir bulls from India) population pair; the red dashed line represents the threshold of 0.92 to consider potential selection signatures. (B) Manhattan plot of F_{ST} values for HOL and GIR_BR (Dairy Gir sires from Brazil) population pair; the red dashed line represents the threshold of 0.84 to consider potential selection signatures. (C) Manhattan plot of F_{ST} values for GIR_IN and GIR_BR population pair; the red dashed line represents the threshold of 0.37 to consider potential selection signatures.

were associated with uncharacterized genes (LOC genes; Supplemental Table S5). Among the characterized genes with higher nucleotide diversity, *GIMAP7*, on BTA4, is a member of the GTPase family that is conserved in both plants and vertebrates (Krücken et al., 2004). Regions with high genetic diversity are known to be responsible for regulating immunity (Li et al., 2023). Future research could further unravel the genomic architecture and impact of regions with high nucleotide diversity.

The region with the highest SNV density (Supplemental Figures S2–S4) aligns with our analysis of nucleotide diversity on BTA23 (regions 26.04–26.17 Mb and 27.1–27.15 Mb) across all populations. When a region has a high density of SNVs and exhibits much higher-than-average nucleotide diversity, this suggests that the region has accumulated many mutations over time. These mutations contribute to increased genetic variation within each population, because nucleotide diversity measures the degree of variation between the sequences of different alleles (Nei and Li, 1979). Several gene families related to immunity (i.e., major histocompatibility complex, *BOLA*), olfaction (olfactory receptor family), and reproduction (i.e., glutathione S-transferase α; Rabahi et al., 1999) were annotated in this region (Supplemental Table S6).

MAF. The GIR_BR cows showed the highest mean MAF (0.191) among the studied populations. This may be because the animals sampled for this population were strategically chosen to represent the different lineages of the Brazilian Dairy Gir. A previous study including a sample of 5 Peruvian Gir descendants from Brazilian Dairy Gir reported a lower MAF of 0.13 (Corredor et al., 2023). An MAF score higher than 0.1 indicates sufficient polymorphism in a population for effective genomic selection (Rajawat et al., 2024). The proportions of polymorphic markers (markers with an MAF >0.1) in GIR_IN and GIR_BR were 0.52 and 0.63, respectively. The GIR_IN individuals were not part of an animal breeding program. The lower MAF (0.044) observed in the HOL population corroborates the intense selection pressure applied on this breed in Canada and the United States.

Heterozygosity and Inbreeding Coefficient. The H_O and H_E in the HOL population (0.219 and 0.226, respectively) were lower than those reported for other Holstein populations. Previous studies reported H_O ranging from 0.36 to 0.38 and H_E between 0.31 and 0.37 (Makina et

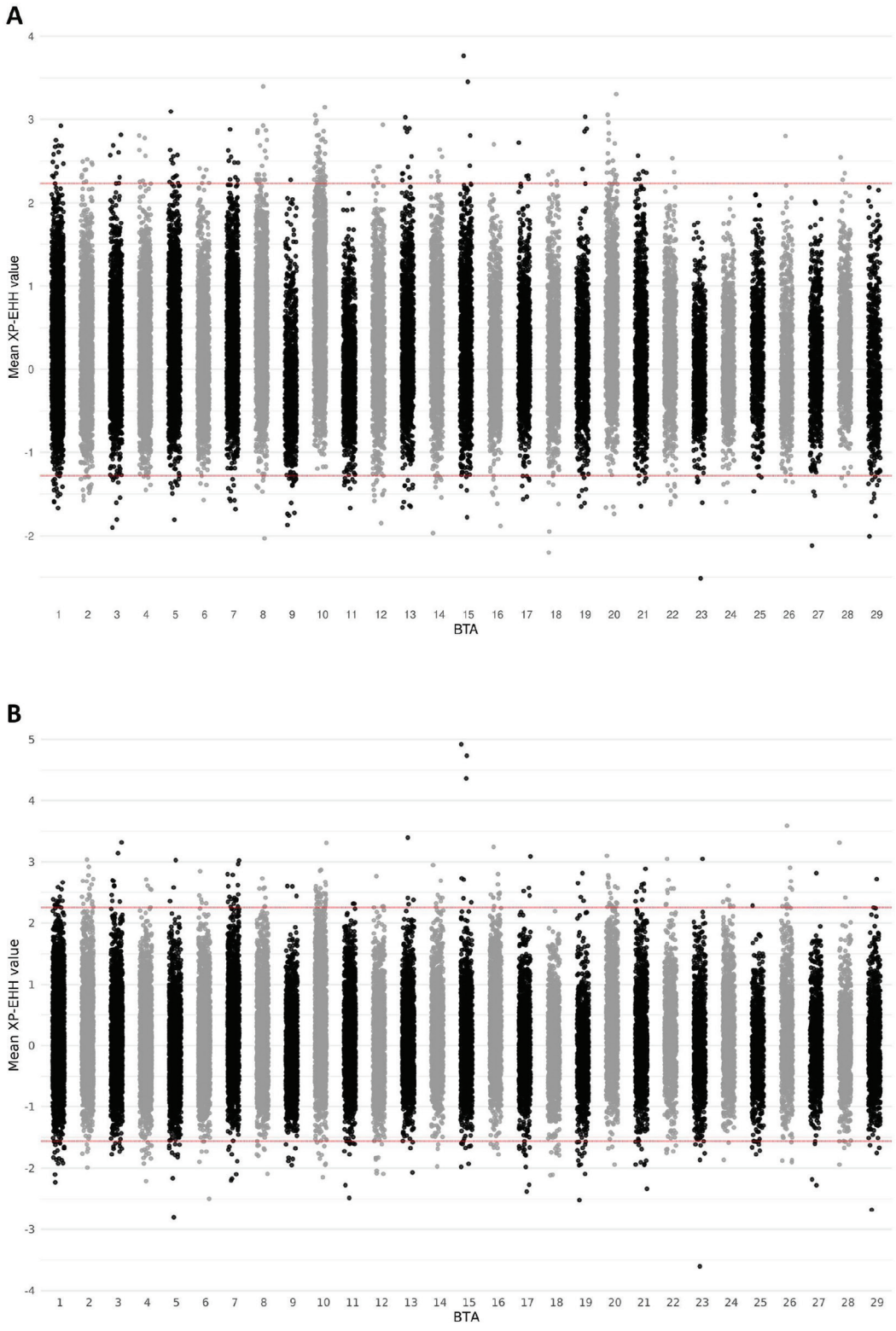

Figure 6. (A) Manhattan plot of XP-EHH value for HOL (Holstein bulls and cows from Canada and the United States) and GIR_IN (Gir bulls from India) pair. The red dashed lines represent thresholds of 2.232 and −1.280 to consider potential selection signatures for HOL and GIR_IN populations, respectively. (B) Manhattan plot of XP-EHH value for HOL and GIR_BR (Dairy Gir sires from Brazil) pair. The red dashed lines represent thresholds of 2.252 and −1.561 to consider potential selection signatures for HOL and GIR_BR populations, respectively. (C) Manhattan plot of XP-EHH value for GIR_IN and GIR_BR pair. The red dashed lines represent thresholds of 2.550 and −1.486 to consider potential selection signatures for GIR_IN and GIR_BR populations, respectively.

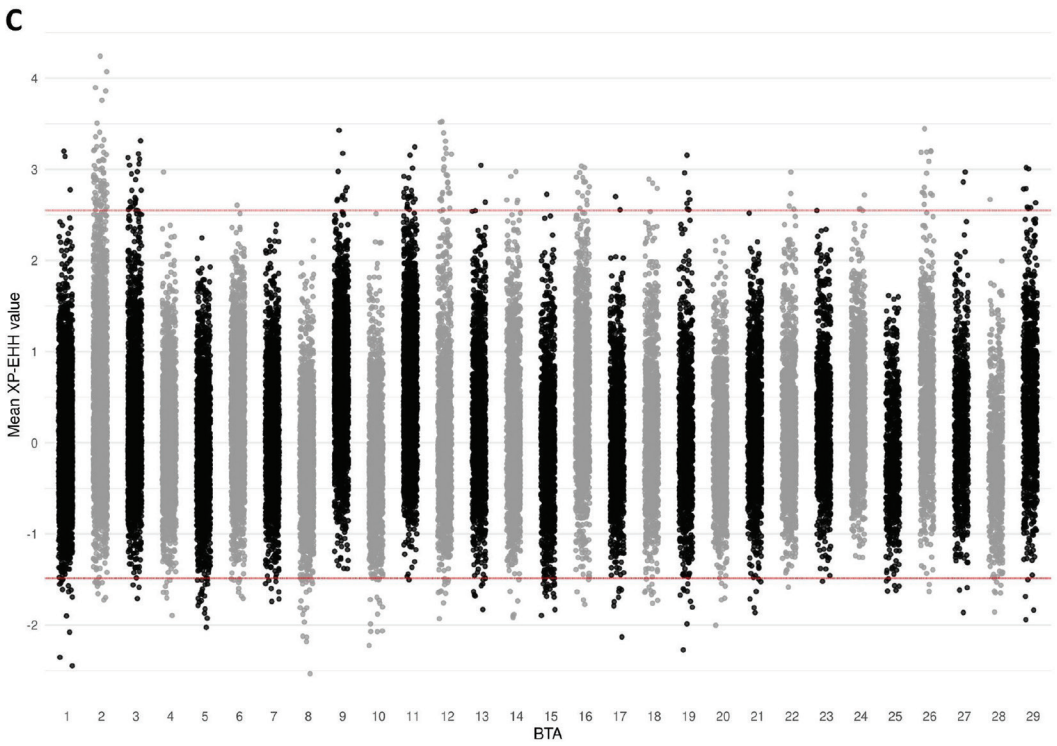

Figure 6 (Continued). (A) Manhattan plot of XP-EHH value for HOL (Holstein bulls and cows from Canada and the United States) and GIR_IN (Gir bulls from India) pair. The red dashed lines represent thresholds of 2.232 and −1.280 to consider potential selection signatures for HOL and GIR_IN populations, respectively. (B) Manhattan plot of XP-EHH value for HOL and GIR_BR (Dairy Gir sires from Brazil) pair. The red dashed lines represent thresholds of 2.252 and −1.561 to consider potential selection signatures for HOL and GIR_BR populations, respectively. (C) Manhattan plot of XP-EHH value for GIR_IN and GIR_BR pair. The red dashed lines represent thresholds of 2.550 and −1.486 to consider potential selection signatures for GIR_IN and GIR_BR populations, respectively.

al., 2014; Vostry et al., 2023). The inbreeding coefficient in HOL was 0.031, similar to values previously reported for Holsteins from Canada and the United States (Vostry et al., 2023). Our results suggest that, despite the lower levels of heterozygosity, the overall level of inbreeding remains relatively low. The reduced genetic diversity is likely associated with the intensive selection pressure applied to Holsteins, which increased after the implementation of genomic selection (Makanjuola et al., 2020). The average inbreeding coefficient increased by 0.26% between 2010 and 2020 in Canadian Holsteins (Van Doormaal, 2024) while maintaining high productivity, which agrees with our findings.

The GIR_IN population displayed the lowest mean of the inbreeding coefficient (−0.092) and the highest difference between H_O (0.373) and H_E (0.341). The lower levels of selection pressure, compared with the other populations, may have contributed to these results. However, this comparison should be taken with caution due to the GIR_IN population sample size. A small sample size (<20 animals) can lead to underestimation of H_E and, thus, alter the genetic distances to other populations (Ajmone-Marsan et al., 2023).

The GIR_BR population displayed an F_{HOM} of 0.011. Using a part of the population used in this study, Peripolli et al. (2020) reported an inbreeding coefficient based on runs of homozygosity ($\mathbf{F_{ROH}}$) of 0.04, and Garcia et al. (2023) reported an F_{ROH} of 0.19; however, those coefficients rely on distinct methods, and low to high correlations were observed between F_{ROH} and F_{HOM} in Dairy Gir cattle (Peripolli et al., 2018).

Selection Signatures and Genes Under Selection

This study used several tests to identify selection signatures within and between Holstein and 2 populations of Gir cattle. The genes colocalized with the selection signatures highlight positive selection pressure related to adaptation and the selection criteria applied, especially for Holstein and Dairy Gir cattle. More specifically, the genes under selection were related to immunity, milk production, mammary gland function, embryogenesis, reproduction, and environmental adaptation.

We assumed the extremes of the 1% windows as candidate selection signatures, and we merged adjacent windows to identify signs of potential recent selection.

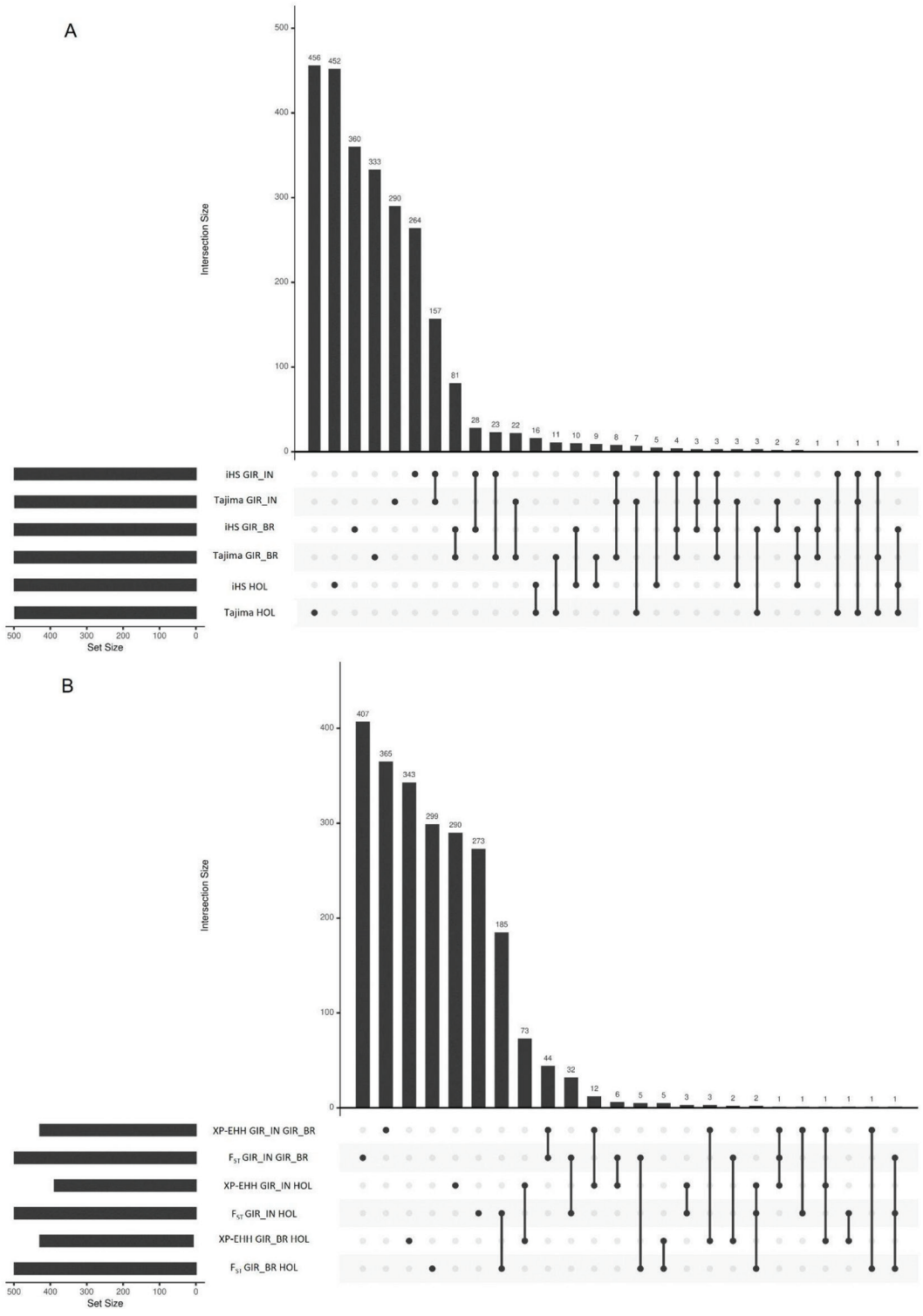

Figure 7. (A) UpSet plot of overlapping autosomal selection signatures among Gir bulls from India (GIR_IN), Dairy Gir sires from Brazil (GIR_BR), and Holstein bulls and cows from Canada and the United States (HOL) populations and Tajima's D and iHS tests. (B) UpSet plot of overlapping selection signatures among pairwise population analyses and XP-EHH and F_{ST} tests. Single dots indicate the total number of selection signatures identified by each test and population; connected dots represent regions shared across methods or populations.

This is because there has not been enough time for crossover events during meiosis to disrupt and recombine the chromosomal segments exhibiting these signatures. The positive mean Tajima's D value observed in the genome across all genome regions is expected because only approximately 5% of the mammalian genome is under selection (Mouse Genome Sequencing Consortium, 2002), and selection is applied for numerous traits in breeding programs. Although the 3 population sets had a large difference in number of individuals, the chosen tests for interpopulation analyses (XP-EHH and F_{ST}) were suitable for unbalanced sample sizes.

Employing multiple methods can enhance the probability of identifying selection signatures (Qanbari and Simianer, 2014) and increase confidence in signatures detected by different methods, highlighting specific signatures that could be further explored in the future. However, because these strategies are grounded in varying statistical assumptions, they may generate different findings that can complement one another (Rodrigues et al., 2025).

Environmental Adaptation. Selection signatures between Holstein (*Bos taurus taurus*) and Gir (*Bos taurus indicus*) populations may reflect the long-term evolutionary divergence that led to differentiation between the 2 subspecies. We detected selection signatures through multiple tests (iHS GIR_IN, iHS GIR_BR, Tajima's D GIR_BR, F_{ST} GIR_IN and HOL, and F_{ST} GIR_BR and HOL) that coincided with the regions reported by Naval-Sánchez et al. (2020) and Balbi et al. (2023). These regions are also colocalized with the *HELB* gene. Naval-Sánchez et al. (2020) identified exclusive indicine variants and a selection signature in BTA5:47.67–48.1 Mb, whereas Balbi et al. (2023) identified a main peak on BTA5:46.94–48.03 Mb in a GWAS for coat score and hair length in Brangus animals. The *HELB* gene is an ATP-dependent DNA helicase involved in DNA replication, damage repair, and replication stress responses (Hazeslip et al., 2020). Thus, the *HELB* gene may have contributed to the adaptation of indicine cattle to the environmental conditions of tropical regions (Naval-Sánchez et al., 2020).

Members of the heat shock protein 70 (HSP70) family were found in the selection signatures for the Gir populations. This family is the largest and most abundant family of heat shock proteins (**HSP**). These proteins are chaperones that provide protection against cellular stress by maintaining protein folding and decreasing apoptosis (Velichko et al., 2013). The *HSPA4* gene was found in the GIR_IN population through the iHS test. *HSPA4* also regulates the progression of spermatogenesis, directly affecting male fertility (Held et al., 2011). Additionally, the *HSPA12A* gene was detected in the analysis between GIR_IN and GIR_BR via the F_{ST} test, which could be explained by the particular differences between Brazil and India, because the expression of this gene may be linked to environmental stressors and adaptation between Brazil and India.

Members of the heat shock protein 40 (HSP40) family were also found in our analyses, especially for Gir populations. This protein collaborates with other HSP to maintain protein homeostasis, forming an important chaperone system (Liu et al., 2020). The *DNAJC18* gene was found in the GIR_IN and HOL pair and in the GIR_BR and HOL pair in the F_{ST} analysis and in the GIR_BR population through the iHS test. This gene is involved in thermogenesis and cold and heat adaptation (Huang et al., 2023). The *DNAJC3* gene was observed under selection pressure in the HOL population via the iHS test and in HOL (GIR_IN and HOL pair) and GIR_BR (GIR_IN and GIR_BR pair) through the XP-EHH test. This gene was previously implicated in endoplasmic reticulum stress in hepatocytes exposed to nonesterified fatty acids (**NEFA**), indicating that this gene might be directly related to negative energy balance (Fang et al., 2022).

Many members of the olfactory family were identified via intra- and interpopulation analyses. Olfaction is a primary sense in cattle and is essential for the recognition of toxic plants; behavioral interactions, such as mother–offspring and social relationships; and the communication and recognition of fear, danger, estrus, disease, and death (Salesse, 2017). Several olfactory genes are subjected to copy number variation in Holstein and Dairy Gir cattle (Butty et al., 2020; Braga et al., 2023). For example, the gene *OR2AJ9* was found in the GIR_IN and GIR_BR pairwise analyses and was previously found to be subjected to deletion in Dairy Gir animals from the same population as in this study (Braga et al., 2023).

Several phospholipase A2 (PLA2) family genes were found under selection across the 3 populations. *PLA2G4E*, *PLA2G4D*, and *PLA2G4F* were identified via the XP-EHH statistic for HOL with both Gir populations. Phospholipase A2 is involved in several biological events, such as the production of lipid mediators, the immune response, membrane remodeling, and skin barrier maintenance (Murakami et al., 2020). The *PLA2G* gene family was previously reported to be subjected to copy number variation in cattle and related to the immune response (Golik et al., 2006; Stothard et al., 2011; Seroussi et al., 2013). The *PLA2R1* gene was identified via intrapopulation tests for the GIR_IN population and interpopulation tests for the GIR_IN and GIR_BR pair, highlighting that this gene is under selection in the GIR_IN population. This gene has been linked to stress, the immune response, and oxidative stress in heat-stressed Holstein cows (Zheng et al., 2014), and may be related to heat tolerance in this population

Milk Production and Mammary Gland Function in Relation to Embryogenesis. The greater selection

pressure for milk traits in GIR_BR led by PNMGL, compared with GIR_IN, may have left a distinct genetic footprint related to milk production. In the F_{ST} analysis, the window with the highest value in the GIR_BR and GIR_IN pair was 0.713 (BTA 14:0.05–0.1 Mb). This region harbors QTL related to milk yield (243502) and *ACER3*, *OR5D70P*, and *U6* genes. Further studies are encouraged to take a closer look at this region in Gir cattle because this region may be related to increased milk production. The QTL colocalized with ACER3, insulin level (193405), was significantly enriched (FDR <0.01) in our analysis. Insulin levels are directly related to milk yield (Horst et al., 2021). However, no study comparing insulin levels in Brazilian Gir and Gir from India has been published to our knowledge. Another gene that might affect lactation is *PTEN*. This gene was found in the HOL population via the iHS test. *PTEN* regulates fatty acid metabolism (Fu et al., 2012) and can inhibit mammary gland development and lactation in dairy cows (Wang et al., 2014).

Genes previously found to affect embryogenesis and mammary gland function were found under selection. The *CAV1* gene is downregulated in Holstein cows with hemorrhagic mastitis (Zhang et al., 2022). The authors also observed strong detection of *CAV1* protein in vascular cells of healthy mammary glands and minor evidence of the same protein in hemorrhagic mastitis. Moreover, this gene was found to be upregulated in bovine oviduct epithelial cells exposed to elevated NEFA compared with basal levels (Jordaens et al., 2017), as well as in granulosa cells during ovulation and peri-ovulation (Lussier et al., 2017). These findings suggest that the *CAV1* gene influences fertility and mammary gland health.

The *AQP1* gene was found in a Tajima's D selection signature in HOL, and *AQP8* was found in an iHS selection signature in GIR_BR. Aquaporins (**AQP**) are integral transmembrane proteins that regulate water transport across cell membranes (Laloux et al., 2018). *AQP1* and *AQP8* play essential roles in early bovine embryo development. It was previously found to be expressed in the cumulus–oocyte complex and blastocyst embryo stage (Petano-Duque et al., 2022). The AQP are expressed in the bovine mammary gland and might be responsible for increasing permeability and, to a lesser extent, milk secretion (Mobasheri et al., 2011).

Fertility. Several genes and QTL related to reproduction were under selection in the BTX. The *GAB3* gene was found to be under selection via Tajima's D in the HOL population. This gene has been previously associated with spontaneous abortion in Holstein cattle (Oliver et al., 2019). *GAB3* mutant mice exhibited defects in their ability to successfully complete pregnancies (Sliz et al., 2019). *DACH2* was found in the GIR_BR population via iHS, and it is associated with premature ovarian failure in humans (Bione et al., 2004) and puberty in cattle (Cánovas et al., 2014).

The *FSHR* gene was found to be under selection in the GIR_IN population in the XP-EHH, GIR_IN and HOL pair analysis. This gene was previously related to early reproductive traits in the Brahman breed (Tahir et al., 2021). It encodes the follicle-stimulating hormone receptor, which controls follicle development, spermatogenesis, steroidogenesis, ovulation, and hormonal balance (Ulloa-Aguirre et al., 2018). Several genes of the family spermatogenesis-associated proteins (*SPATA2L*, *SPATA13*, *SPATA24*, and *SPATA33*) were identified via iHS for the HOL population and interpopulation pairwise analyses with the HOL population.

Genes previously related to reproduction traits and embryogenesis were also identified via GIR_IN and GIR_BR pair analyses. The *SAMD5* and *SNORA70* genes were found to be under selection pressure in the F_{ST} analysis. The *SAMD5* gene is related to the sire-conception rate in Holstein cattle (Fang et al., 2019), and *SNORA70* is linked to scrotal circumference at 18 mo of age in Nelore, Brahman, and Tropical Composite breeds (Melo et al., 2019), underscoring the importance of reproductive traits under selection pressure. In pairwise XP-EHH analysis, the *WDR36* gene was found to be under selection pressure. The *WDR36* gene is related to blastocyst development in cattle through miR-146b regulation (Pavani et al., 2024). Knockdown of *WDR36* can lead to preimplantation embryonic lethality in mice, and its knockout causes the apoptotic death of blastomeres (Gallenberger et al., 2011).

Growth Traits and Development. Selection signatures overlapping genes affecting growth traits and milk production were found in this study. The *PLAG1* gene was found to be under selection via the F_{ST} test in the GIR_IN and HOL pair and in the HOL population via Tajima's D test. The region harboring the *PLAG1* gene is fixed or near fixation in Holstein cattle, as supported by our findings and its identification under selection in multiple studies (Signer-Hasler et al., 2017; Taye et al., 2017; Zinovieva et al., 2020; Bertolini et al., 2022; Jin et al., 2022). *PLAG1* regulates embryonic and fetal development in cattle (D'Occhio et al., 2024) and is ubiquitously and highly expressed in fetuses compared with adult cattle (Li et al., 2020). In Holstein cattle, this gene was previously associated with milk composition traits (Fink et al., 2017), stature (Karim et al., 2011; Littlejohn et al., 2012; Bouwman et al., 2018), growth traits (Littlejohn et al., 2012; Fink et al., 2017), and heifer livability (Gao et al., 2023).

Genes related to stature in cattle, such as *IGF2*, *HMGA2*, *CCND2*, and *NCAPG*, were found in selection signatures for the GIR_BR population (Bouwman et al., 2018). *HMGA2* regulates *IGF2*, a paralog of *IGF1*

known to regulate growth (Abi Habib et al., 2018). The *HMGA2* gene is associated with ovarian length and height in various cattle breeds (Shen et al., 2024). An indicine-specific copy number variant in the *HGMA2* gene was previously associated with navel length in Nelore cattle (Aguiar et al., 2018).

Health and Disease Resistance. Our research also revealed numerous genes from the solute carrier family across different populations and tests. Interestingly, a selection signature region harboring *SLC11A1* (also known as *NRAMP1*) was close to fixation in the HOL population. This gene plays an important role in host innate immunity by modulating iron metabolism in macrophages and influencing early-phase macrophage activation (Wyllie et al., 2002). Polymorphisms in the *SLC11A1* gene were previously related to susceptibility to tuberculosis in Holstein cattle (Liu et al., 2017) and resistance to several intracellular pathogen infections, including brucellosis (Cortés and González, 2015), paratuberculosis (Pinedo et al., 2009), and tuberculosis (Kadarmideen et al., 2011; Liu et al., 2017), in various breeds of cattle. Compared with indicine and crossbred cattle, Holstein cattle are more susceptible to tuberculosis (Ameni et al., 2007), whereas indicine and crossbred cattle exhibit a greater degree of diversity in the *SLC11A1* gene (Cortés and González, 2015; Soares Fioravanti et al., 2020). Therefore, increasing variability within the *SLC11A1* gene might benefit Holstein cattle health.

Another iron-related gene was found under selection in the BTX for the GIR_IN population via iHS analysis. *TMEM164* has a key role in ferroptosis, an iron-dependent regulated cell death implicated in health and disease. This gene was previously linked to *Mycobacterium bovis* infection (Correia et al., 2022) and SCS (Sanchez et al., 2023).

CONCLUSIONS

We investigated genetic diversity and selection signatures in a Holstein cattle population from the United States and Canada, Gir cattle from India, and Gir cattle imported from India to Brazil and selected for milk production over the past 40 years (Dairy Gir cattle). We observed that genetic diversity was lower in the Holstein population than in the Dairy Gir population. Genes linked to major economic traits in dairy cattle, including growth traits, reproduction, mastitis, milk production, heat tolerance, health, temperament, and adaptation, were found to be under selection pressure. Additionally, we also recommended target areas for increasing diversity and areas that should be further assessed as advantageous for marker-enhanced selection.

NOTES

Author L. G. Braga was supported by Coordination for the Improvement of Higher Education Personnel (CAPES, Brasília, Brazil—001 and 88887.802720/2023-00). Author M. V. G. B. da Silva has received grants from Embrapa (Juiz de Fora, Brazil) SEG 02.13.05.011.00.00 and the National Council for Scientific and Technological Development (CNPq, Brasília, Brazil) 310199/2015-8, "Detecting signatures of selection from Next Generation Sequencing Data"; the Ministry of Science, Technology and Innovations (MCTI, Brasília, Brazil)/CNPq/National Institute of Science and Technology in Animal Science (INCT–Ciência Animal, Viçosa, Brazil) and the Minas Gerais State Agency for Research and Development (FAPEMIG, Belo Horizonte, Brazil) CVZ PPM 00606/16 "Detecting signatures of selection in cattle from Next Generation Sequencing Data"; and FAPEMIG APQ-02750-23 appropriated projects. Author D. P. Munari has received a grant from CNPq (431629/2016-1). Author J. L. Rodrigues was supported by CNPq (130045/2022-5), the São Paulo Research Foundation (FAPESP, Alto da Lapa, Brazil; 2022/13986-0), and the Government of Canada (Ottawa, Canada). The authors acknowledge Leandro Carrijo Cintra and Adhemar Zerlotini Neto of the Multiuser Bioinformatics Laboratory of Brazilian Agricultural Research Corporation (Embrapa) Digital Agriculture for computational and Information Technology resources. We also acknowledge 1000 Bulls Genome Consortium, ABCGIL (Uberaba, Brazil), and Embrapa Dairy Cattle Research Center for providing the data used in this study. Restrictions apply to the availability of the data underlying our findings, which were used under license for this study. Data from the Holstein breed was provided within the frame of the 1000 Bull Genomes Project Consortium (Run 9). Some Holstein samples are publicly available on SRA (see Supplementary Table S1 for accession codes). Samples from the two Gir populations were obtained in partnership with Embrapa Dairy Cattle. The data are available upon reasonable request by contacting Marcos Vinicius Gualberto Barbosa da Silva, marcos.vb.silva@embrapa.br. Supplemental material for this article is available at https://doi.org/10.17632/vsw2s5c3cg.1. The institutional research ethics board of the São Paulo State University (São Paulo, Brazil), University of Guelph (Guelph, ON, Canada), and the Brazilian Agricultural Research Corporation (Embrapa; Minas Gerais, Brazil) did not require ethics approval for this study because the data used in this investigation were accessed from previous projects. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: AQP = aquaporins; BQSR = base quality score recalibration; BTX = *Bos taurus* X chromosome; chr = chromosome; EHH = extended haplotype homozygosity; FDR = false discovery rate; F_{HOM} = inbreeding coefficient based on methods-of-moments estimator; F_{ROH} = inbreeding coefficient based on runs of homozygosity; F_{ST} = fixation index; GATK = Genome Analysis Toolkit; GIR_BR = Dairy Gir sires from Brazil; GIR_IN = Gir bulls from India; GO = Gene Ontology; H_E = expected heterozygosity; H_O = observed heterozygosity; HOL = Holstein bulls and cows from Canada and the United States; HSP = heat shock protein; iHS = integrated haplotype score; KEGG = Kyoto Encyclopedia of Genes and Genomes; LD = linkage disequilibrium; MAF = minor allele frequency; NEFA = nonesterified fatty acids; PCA = principal component analysis; π = nucleotide diversity; π_{Chr} = chromosome nucleotide diversity; π_{Total} = total nucleotide diversity; PNMGL = Brazilian Dairy Gir Breeding Program; ROH = runs of homozygosity; SNVs = single nucleotide variants; Ts/Tv = transition/transversion rate; VQSR = variant quality score recalibration; WGS = whole-genome sequencing; XP-EHH = cross-population EHH.

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