Genomic Insights into the Population History and Adaptive Traits of Latin American Criollo Cattle

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ABSTRACT

Criollo cattle, descendants of animals brought by Iberian colonists to the Americas, have been subject to centuries of natural and human-mediated selection in novel tropical agroecological zones. Consequently, these breeds have evolved distinct characteristics such as resistance to disease and exceptional heat tolerance. In addition to European taurine (*Bos taurus*) ancestry, it has been proposed that gene flow from African taurine and Asian zebu (*Bos indicus*) cattle has shaped the ancestry of Criollo cattle. In this study, we analysed Criollo breeds from Colombia and Venezuela using whole genome sequencing data to examine population structure and admixture at very high resolution. We did not detect evidence of African taurine admixture in Criollo cattle; however, there was evidence of longstanding zebu ancestry in one breed (Hartón del Valle). In addition, we detected selection signatures associated with a myriad of adaptive traits, revealing genes linked to thermotolerance, reproduction, fertility, immunity, and distinct coat and skin coloration traits. This study underscores the remarkable adaptability of Criollo cattle and highlights the genetic richness and potential of these breeds in the face of climate change, habitat flux, and disease challenges. Further research is warranted to leverage these findings for more effective and sustainable cattle breeding programmes.

Keywords: cattle, Latin America, microevolution, population genomics, selection, thermotolerance

INTRODUCTION

The term "Criollo" is derived from the Portuguese word "crioulo" and was originally used in the colonial period to distinguish people born overseas from those born in the home country. Over time, this term was extended to domestic livestock (cattle, sheep, horses, and goats) with genetic roots in the Iberian Peninsula. The first introductions of cattle (taurine – *Bos taurus*) to the Americas were relatively recent, dating back to the end of the 15th century with animals transported during Columbus' second voyage and brought initially to the island of Hispaniola [1]. Early estimates suggest that fewer than 1,000 cattle were introduced to the Americas during these initial expeditions [2]. However, over the ensuing five centuries, increasing numbers of cattle were exported to North and South America particularly as the process of European colonization intensified during the 18th and 19th centuries [3]. A comparable narrative has been reported for sheep [4], and for horses, as both historical and extant horses in North America exhibit pronounced genetic affinities with Iberian horse populations [5]. More recently, zebu cattle (*Bos indicus*) were directly introduced from South Asia—primarily the region encompassed by present-day India and Pakistan [6].

The extent of contributions from cattle outside Europe and the Indian subcontinent to the South and Central American cattle gene pools remains uncertain. Modern Iberian cattle populations, whose ancestors likely contributed the bulk of South American Criollo cattle ancestry have an African taurine cattle genomic component [7-11]; however, this could post-date the initial introductions to the Americas. In parallel to this, it has also been posited that African cattle may have been directly introduced into South America alongside the initial imports [12]. This hypothesis is supported by the presence of the T1-c mtDNA haplotype, which is prevalent in African cattle, rarely found in Iberian animals, yet appears with an approximate frequency of 8% in Criollo cattle. [13]. Relatively few studies have explored potential autosomal contributions from African cattle. Preliminary findings, however, indicate that the degree of African ancestry in Criollo cattle is contingent upon the specific breed. Notably, the Guadeloupe breed, indigenous to the Caribbean,

exhibits a substantial African genetic influence, with almost 50% of its genetic ancestry estimated to trace back to African origins [14, 15]. The potential direct or indirect influence of zebu cattle is also an active area of research. While there is no question on whether zebu cattle have been directly imported from South Asia, it is unclear whether this accounts for all of the zebu ancestry evident in South and Central American cattle [14]. In Africa a gradient of zebu ancestry is present, escalating across the continent from West to East Africa [16-20], Therefore, it is plausible that some of the zebu ancestry for certain Criollo breeds could be attributed to historical African cattle importation [15].

The cattle that were brought to the Americas from Iberia in the 15th and 16th centuries were adapted to Mediterranean agroecologies and, over the succeeding centuries, these populations have evolved adaptations to tropical and arid environments, emerging as distinctive breeds with unique heat tolerance and disease resistance traits [11, 21-29]. Despite this, Criollo cattle are not highly valued in modern production systems and are slowly being replaced with more productive commercial breeds, which is eroding indigenous cattle genetic resources in South and Central America [30-32]. Therefore, using genome-scale data, the primary aims of this study were to evaluate *B. taurus* and *B. indicus* ancestry and detect historical gene flow from African cattle into the Blanco Orejinegro, Hartón del Valle, and Limonero Criollo breeds. In addition, we use high-resolution analyses of genetic structure, and selection signatures to provide new information about the microevolutionary histories of these populations.

MATERIALS AND METHODS

Cattle samples, library preparation, high-throughput sequencing, and variant calling

In total, 34 Criollo cattle were used to generate high-quality de novo whole-genome sequence (WGS) data sets with the following methods. Coat hair follicles obtained from 24 Colombian and Venezuelan cattle and semen straws from 10 Colombian AI bulls were used to obtain genomic DNA using standard commercial procedures for livestock DNA extraction and purification (Weatherbys Scientific, Co. Kildare, Ireland). For the coat hair follicle-derived samples, the quality

and concentration of DNA was low; therefore, library preparation was performed in-house using the Nextera DNA Flex Library Prep kit (now available as the Illumina® DNA Prep kit; Illumina, Cambridge, UK). The WGS data generation was performed by sequencing to a mean depth of 20× using two commercial service providers (Novogene, Cambridge, UK and Macrogen, Seoul, South Korea). The geographical origins of the three primary Criollo breeds used in this study are shown in figure *1*.

Publicly available WGS data was also assembled for 37 European taurine (EUT) (NCBI Bioproject IDs: PRJNA210521; PRJNA318089; PRJNA343262; and PRJNA514237), 13 American indicine (ASI) (NCBI Bioproject ID: PRJNA343262), nine African taurine (AFT), and 13 American Criollo (AMC) cattle (NCBI Bioproject IDs: PRJNA471656 and PRJNA422135). Sequence reads were mapped to the bovine reference genome (ARS-UCD1.2) [33] using the default parameters for bwa-mem2 v.2.2.1 [34]. Samtools v.1.13 [35] was used to sort bam files and create index files. Potential PCR duplicates were identified using the “MarkDuplicates” function of Picard v.2.27.1 (http://broadinstitute.github.io/picard). The “BaseRecalibrator” and “ApplyBQSR” functions of the genome analysis toolkit (GATK) v.4.2.6.1 [36] were used to perform base quality score recalibration.

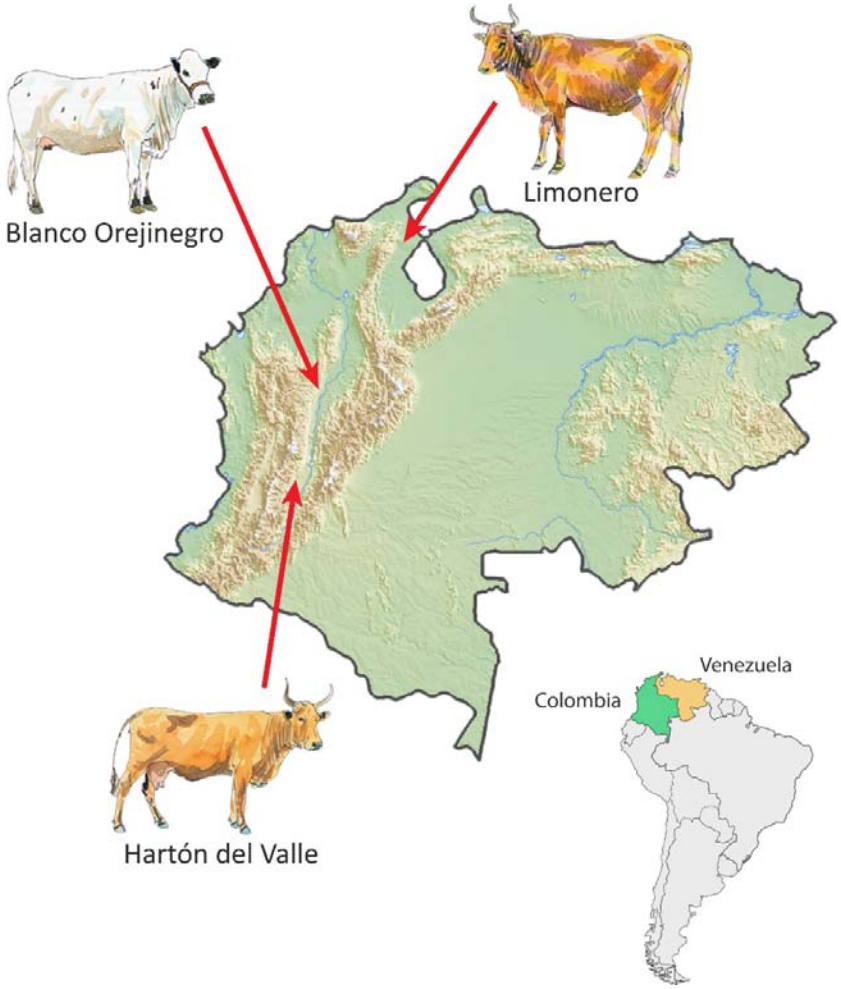

Figure 1. Approximate geographical origins of the animals from three Criollo breeds that were sampled in Colombia and Venezuela and used to generate new whole-genome sequence data. Map modified from original by Mapswire (https://mapswire.com) (Creative Commons Attribution 4.0 International License CC-BY 4.0). Individual cattle art images modified from Felius [37] with permission of the author.

Candidate SNPs were called from the bam files using the "HaplotypeCaller" function in GATK with the "--emit-ref-confidence GVCF" option. Individual GVCF files were merged using the "GenomicsDBImport" function. SNPs from the genomics database were called and selected using "GenotypeGVCFs" and the "SelectVariants" function. The "VariantFiltration" function of GATK was used to avoid possible false-positive calls according to GATK best practices: SNP clusters with "--cluster-size 3" and "--cluster-window-size 10", quality by depth "QD <2", read position rank sum test "ReadPosRankSum < -8", phred-scaled variant quality score "QUAL < 30",

Fisher strand "FS > 60", mapping quality "MQ < 40", mapping quality rank sum test "MQRankSum < -12.5" and strand odds ratio "SOR > 3". Triallelic SNPs and those with a minor allele frequency < 0.01 and/or missing genotype rates over > 0.1 were filtered out. The SNPs remaining in each WGS data set were then annotated according to their positions using SnpEff [38]. The combined panel of 106 cattle samples with WGS data are detailed in electronic supplementary material, table S1.

BovineHD 777k array data

For broader comparative population genomics analyses, publicly available BovineHD 777k SNP array data for 45 Iberian cattle representing 14 breeds [39], and 12 Criollo cattle representing one breed, the Senepol were obtained from the WIDDE database [40]. This SNP panel was originally mapped to the UMD3.1 assembly [41]; therefore, we updated SNP positions to ARS-UCD1.2 following a protocol detailed by Riggio et al. [42]. Firstly, BovineHD data were converted from the TOP coding format to the FOR coding format using the SNPchiMp v.3 suite of tools [43]. Secondly, SNP positions from UMD3.1 were updated to the ARS-UCD1.2 genome using updated BovineHD array coordinates available from the NAGRP Data Repository [44] and SNPchiMp v.3 data for the BovineHD array with positions updated using the liftOver implementation of the rtracklayer R package [45]. Thirdly, reference and alternative alleles for the data were updated using BovineHD array SNPs that were extracted from WGS data aligned to the ARS-UCD1.2 genome and files of updated alleles also available from the NAGRP Data Repository. Processing of these data was conducted using PLINK v1.90b6.25 [46] and R version 4.1.3 [47]. The combined panel of 57 cattle samples with BovineHD 777k SNP data are detailed in electronic supplementary material, table S2.

Population differentiation and genetic structure analyses

Principal component analysis (PCA) for all animals (WGS and imputed BovineHD genotype data) were performed using PLINK v1.90b6.25 and the results were plotted using ggplot2 v3.4.0

[48] in the R v4.1.3 environment. The genetic structure of each population was visualized using fastSTRUCTURE v1.0 and ggplot2 v3.4.0 with K = 2–5 unsupervised modelled ancestries [49]. We used VCFtools v.0.1.16 [50] to calculate nucleotide diversity (π) in windows of 10 Mb.

Detection of historical admixture

Using ADMIXTOOLS [51] with default parameters, we calculated f_3 statistics using the whole genome data set to test for evidence that the Blanco Orejinegro, Hartón del Valle, and Limonero breeds are derived from the admixture of two sets of populations (European taurine populations and American zebu cattle, and European taurine populations and African taurine cattle). A significant negative f_3 value is considered evidence of historical admixture in the target population (Criollo). For visualization of the f_3 statistics, we plotted the f_3 values against the population trios with error bars indicating the standard errors using ggplot2 v3.4.0 [48].

Test statistics for detection of selection signatures

For the selection signatures analysis, only the WGS data set was used, which included approximately 9.7 million SNPs genotyped in 106 cattle. We implemented three selection tests to investigate if genomic regions were under selection. SNP-by-SNP F_{ST} values [52] were calculated for each Criollo breed versus a panel of Holstein cattle using VCFtools v.0.1.16 [50]. XP-EHH statistics were calculated using the program selscan v.1.2.0 [53] using the default parameters, where each Criollo breed was also compared to the Holstein panel. The unstandardized XP-EHH values were then normalized using the --norm function in selscan. We calculated the ΔSAF (directional change in selected allele frequency) using the --freq function of VCFtools to generate allele frequencies, then using a custom R script we calculated the ΔSAF for each Criollo breed versus the Holstein panel. Similarly to the XPEHH values, ΔSAF values were standardized to $Z \sim N(0,1)$ as described by Randhawa et al. [54].

Implementing the composite selection signals (CSS) methodology

To obtain composite selection signals (CSS) statistics [54] for each Criollo breed, the F_{ST}, XP-EHH, and ΔSAF statistics were combined by SNP ID (chromosome:position), which produced final sets of SNPs for the CSS analysis of 9,344,833, 9,465,007 and 9,660,647 million SNPs, for the Blanco Orejinegro, Hartón del Valle, and Limonero, respectively. Following this, the MINOTAUR package in R [55] was used to calculate CSS statistics as described by Randhawa et al. [54]. The resulting statistics were then averaged over 20 kb windows to reduce spurious signals. To further reduce the likelihood of spurious signals, a signal was only considered significant if at least one SNP in the 0.1% of CSS scores was flanked by at least five other SNPs in the top 1% of CSS values [54, 56-58].

Identification of genomic regions under selection

From the top 0.1% of SNPs identified, genomic intervals in the format chromosome:start-end were extracted and the BovineMine resource (v1.6) [59] was then used to find genes ± 500 kb from the start and end of these regions. The functional relevance of these CSS-derived gene sets was then evaluated using the scientific literature and the g:Profiler software tool [60] was used to identify overrepresented gene ontology (GO) terms for each of the CSS-derived gene sets by breed. Following this, BovineMine v1.6 was used to systematically identify previously described quantitative trait loci (QTLs) for production, health, and welfare traits that are located within the CSS cluster regions for each breed.

RESULTS

Population differentiation, genetic structure, and admixture in Criollo cattle

Merging of the WGS and remapped BovineHD 777K BeadChip data sets resulted in a shared set of 367,339 SNPs for 163 individual animals. A PCA of these SNPs demonstrated that PC1 and PC2 accounted for 22.5% and 9.5% of the variation observed, with PC1 representing the split between the *B. taurus* (taurine) and *B. indicus* (zebu) lineages and PC2 the split between the

European and African taurine (figure *2a*). The genetic structure of the cattle populations was analysed using fastSTRUCTURE, wherein the inferred number of clusters (*K*) was set to range between two and five (*K*=2–5) (figure *2b*). At *K* = 2, the initial partition delineated the taurine and zebu lineages, which mirrored PC1 in the PCA. At *K* = 3, a second partition differentiated the European and African taurine lineages, which reflects PC2 in the PCA. The subsequent split at *K* = 4 discerned an additional cluster within the European taurine populations, and *K* = 5 primarily segregated the Iberian taurine and Criollo populations from the other groups.

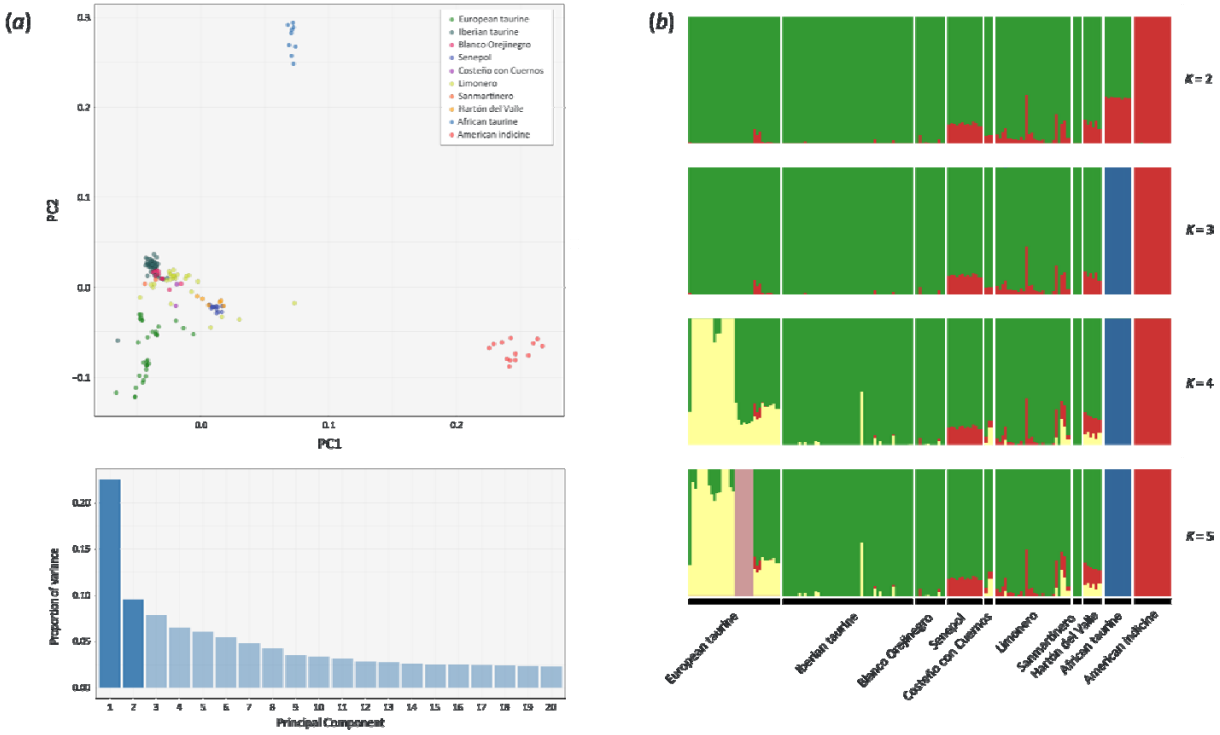

Figure 2. Genetic diversity and admixture in African, Asian, Criollo, and European cattle. (*a*) Results of the principal component analysis (PCA) for 163 animals (106 from whole-genome sequence data and 57 from high-density SNP array data) with a shared set of 367,339 SNPs. The PCA plot shows the coordinates for each animal based on the first two principal components. Principal component 1 (PC1) differentiates the *Bos taurus* and *Bos indicus* lineages, whereas PC2 separates the African and European taurine groups. A histogram plot shows the variance contributions for the first 20 PCs, with PC1 and PC2 accounting for 22.5% and 9.5% of the variance, respectively. (*b*) Unsupervised genetic structure plot for European and Iberian taurine populations, Criollo cattle populations, African taurine, and Asian zebu populations. Results for ancestry clusters ranging from *K*=2-5 are shown.

Further examination of the PCA and structure plots in figure *2a* and *2b* showed that the Criollo cattle samples tend to cluster with the Iberian taurine cattle samples. However, some breeds, notably the Senepol and the Hartón del Valle, display a consistent and relatively uniform ancestry component from the *B. indicus* zebu lineage. The other Criollo breeds (Blanco Orejinegro, Costeño con Cuernos, Limonero, and Sanmartinero) are more heterogeneous with some individual animals showing substantial zebu admixture and other animals with an exclusively taurine genetic composition. Calculation of f_3 statistics, which used the WGS data, indicate that there is no strong evidence of admixture in the Blanco Orejinegro (figure *3a*) and Limonero (figure *3c*) breeds when using any of the European taurine reference populations and the American indicine as the second reference. However, there is some evidence of admixture in the Hartón del Valle breed (figure *3b*) as the f_3 statistics for all population trios that used any European taurine reference population with the American indicine as the second reference population produced negative f_3 values. This result is reflected in the positions of the Hartón del Valle animals on the PCA plot (figure *2a*), and their genetic composition shown in the structure plot (figure *2b*).

Selection signals across Criollo cattle breed genomes

The CSS test was used to identify selection signals across the genomes of three cattle breeds that had WGS data and sufficient sample sizes: the Blanco Orejinegro, Limonero, and Hartón del Valle breeds. For each breed, the test highlighted several selection signals spanning multiple chromosomes. For the Blanco Orejinegro breed, the CSS test detected 31 clustered regions spanning 17 chromosomes, with 442 genes located within these regions and extended ±500 kb (figure *4a*). Similarly, for the Hartón del Valle breed, the CSS test revealed 31 distinct clusters distributed over 17 chromosomes, encompassing 410 genes (figure *4b*). Lastly, for the Limonero breed, the CSS test detected 38 cluster regions, also extending across 17 chromosomes, and that contained 451 genes in total (figure *4c*). When these results were consolidated, 76 genes within selection signature regions are shared across the three breeds, and BTA18 was the chromosome

with the largest number of these genes (28 genes; 36.8% of the total). The CSS cluster regions and the genes within these regions are detailed by breed in electronic supplementary material, table S3).

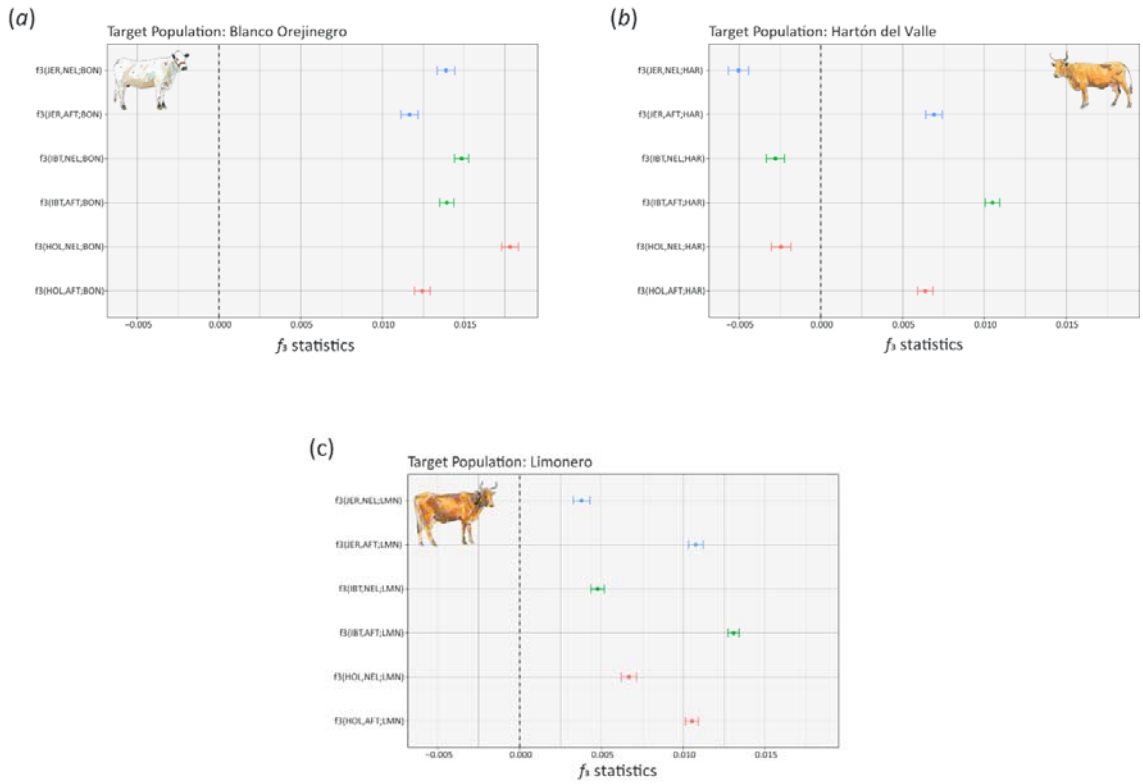

Figure 3. Three-population tests using Blanco Orejinegrio (*a*), Hartón del Valle (*b*) and Limonero (*c*) as target populations. Each coloured data point shows the f_3 statistics value and the horizontal line represents plus or minus the standard error. Three-letter breed/population codes are detailed in Table S1. All tests were conducted on whole-genome sequencing data. Individual cattle art images modified from Felius [37] with permission of the author.

Gene ontology (GO) term enrichment analysis

The functional significance of the genes within the CSS cluster regions for each breed was evaluated using GO term overrepresentation analysis with the g:Profiler software tool [60]. After adjusting for multiple tests and using a *P*-value adjusted for the false discovery rate (FDR-$P_{adj.}$ < 0.05), overrepresented GO terms were catalogued for each breed. The results of the GO term overrepresentation analysis are shown in electronic supplementary material, tables S4–S6 and figure *5*. For both the Blanco Orejinegro (figure *5a*) and Limonero (figure *5b*) breeds, the GO terms related primarily to immunobiology, encompassing functions such as immunoglobulin receptor binding, phagocytosis, complement activation, and antigen binding. Conversely, for the Hartón del

Valle breed (figure *5c*), the predominant GO terms were associated with skin epidermis and keratinization processes.

Figure 4. Chromosome-wide circular plots of the smoothed composite selection signals (CSS) scores for Blanco Orejinegrio (*a*), Hartón del Valle (*b*) and Limonero (*c*). The dashed red lines indicate the genome-wide 0.1% thresholds of the empirical CSS scores. Green coloured dots are those deemed significant by being surrounded at least five SNPs among the top 1% of CSS scores. Individual cattle art images modified from Felius [37] with permission of the author.

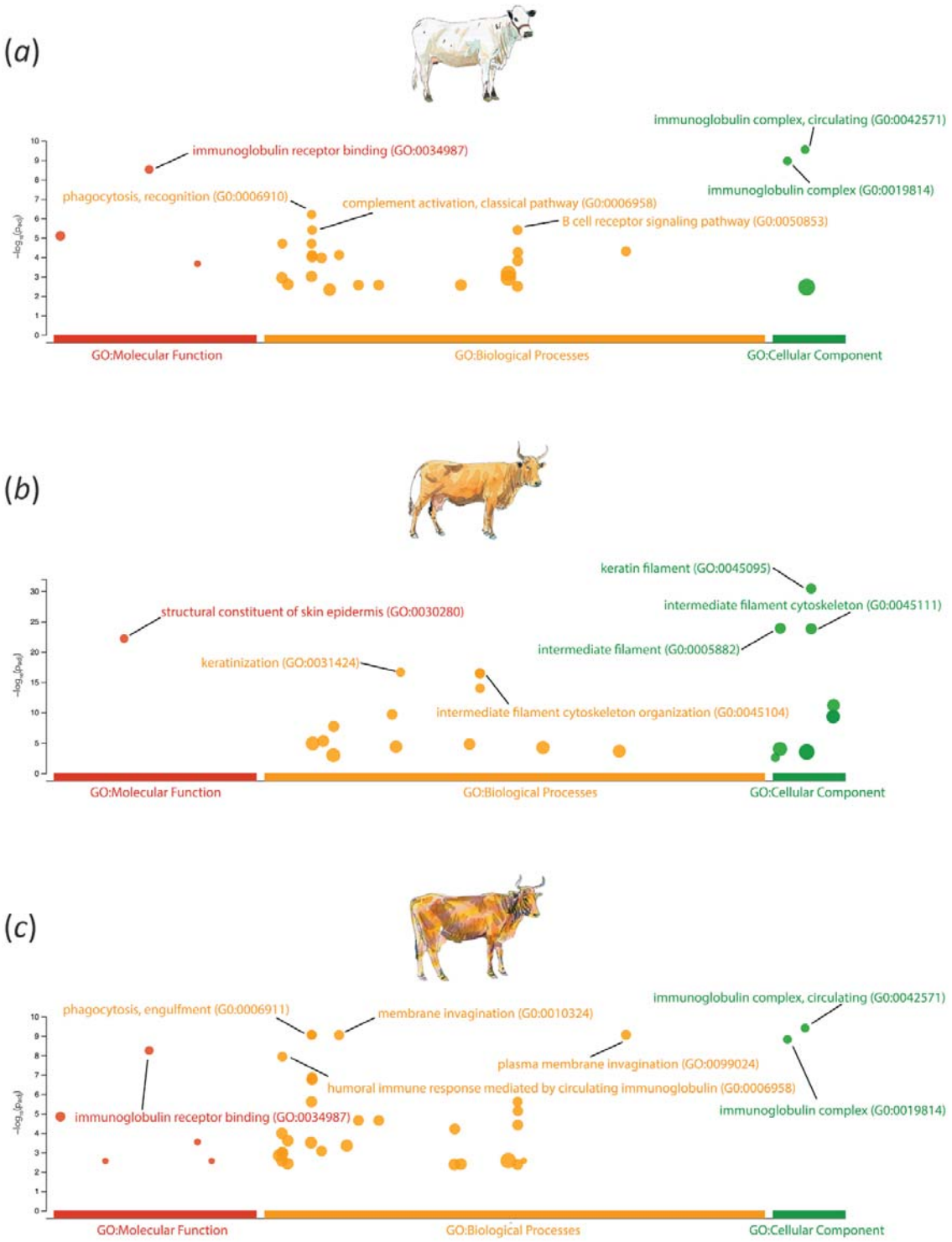

Figure 5. Gene ontology (GO) overrepresentation analysis results for the Blanco Orejinegrio (*a*), Hartón del Valle (*b*) and Limonero (*c*). The y-axis represents the negative $\log_{10}$ FDR-$P_{adj.}$ from the overrepresentation analysis. Individual cattle art images modified from Felius [37] with permission of the author.

Identification of quantitative trait loci (QTLs) within CSS cluster regions

Quantitative trait loci (QTLs) within the CSS cluster regions were systematically identified using BovineMine v1.6 [59]. The number of QTLs identified varied across the breeds, with 519 QTLs found in Blanco Orejinegro, 332 in Hartón del Valle, and 370 in Limonero. Interestingly, only 11 QTLs were common to all three breeds, of which a QTL associated with tick resistance [61] was particularly noteworthy. In the case of Blanco Orejinegro, most of the identified QTLs (291 out of 519) in the CSS cluster regions were associated with calving ease. Additionally, 46 QTLs were linked to milk protein percentage, 40 to milk yield, and 24 to coat texture. For the Limonero breed, there were 292 QTLs located in the CSS cluster regions related to calving ease; however, no other traits had more than five SNP markers among the QTLs. In the case of the Hartón del Valle breed, the most notable traits represented among the CSS cluster region QTLs were milk related. Specifically, 86 QTLs were associated with milk protein percentage, 45 with milk yield, and 35 with milk fat percentage. Additionally, 27 QTLs in Hartón del Valle were associated with coat texture. The results of the QTL search using BovineMine v1.6 are detailed in electronic supplementary material, table S7.

DISCUSSION

Population structure and admixture

The differentiation of cattle samples across PC1 of the PCA plot (figure *2a*) and the primary split in the structure plot (figure *2b*) for $K = 2$ reflects the evolutionary divergence between the *B. taurus* and *B. indicus* lineages, which occurred 150–500 kya [62-64]. The second division evident for PC2 in the PCA plot (figure *2a*) and for $K = 3$ in the structure plot differentiates the European and African taurine groups, a separation that has been well documented in several previous studies using genome-wide SNPs and other autosomal genetic markers [10, 17-19, 65, 66]. Extending these observations further, complex patterns of admixture due to human-mediated and unmanaged crossbreeding between taurine and zebu cattle have also been described in cattle populations across several geographical regions, particular in sub-Saharan Africa [10, 18, 19, 65].

Previous studies have also highlighted the presence of African taurine autosomal admixture in the Criollo cattle breeds of the Caribbean [11, 12, 14, 15]. Results from the present study, which are captured in the PCA plot (figure *2a*) and structure analysis (figure *2b*) suggest this is not the case for the Criollo breeds and individual animals analysed here (Blanco Orejinegro, Costeño con Cuernos, Hartón del Valle, Limonero, Sanmartinero, and Senepol). Therefore, the presence of a significant proportion of African taurine autosomal ancestry is highly breed-specific and may be confined to the Caribbean region [14, 15]. With regard to *B. indicus* ancestry, all of the South American Criollo cattle populations examined here do exhibit varying degrees of zebu admixture (figure *2b*). Interestingly, however, the Hartón del Valle and the Blanco Orejinegro, which both originate in Colombia, do not cluster together, which is a consequence of the relatively modest zebu ancestry in the Blanco Orejinegro versus a significantly larger zebu component in the Hartón del Valle. The Limonero breed samples in this study largely cluster together except for four animals that exhibit a significant proportion of indicine (zebu) ancestry. These results, taken together with the f_3 statistics for the Blanco Orejinegro, Hartón del Valle, and Limonero (figure *3*), and the histories of these breeds (the Blanco Orejinegro breed has been in a conservation programme since

1940 [67]), suggest that zebu gene flow has had a long-term impact on breeds such as the Hartón del Valle and Senepol, which exhibit relatively uniform levels of zebu admixture (figure *2b*). This has implications for understanding the selection signatures that are described in the following sections (detailed in figure *4* and electronic supplementary material, table S3). In some cases, the selection signals detected may be caused by adaptive introgression of *B. indicus* haplotypes. Details for several of the genes identified through the CSS analysis are provided in supplementary material, table S8.

Genomic signatures of human-driven selection in Criollo cattle

Blanco Orejinegro, translating to "white with black ears" designates a breed of cattle predominantly found in the Andean region of Colombia. They stand out among cattle in the Americas due to their distinctive coat and skin coloration; their coats are typically white, often speckled with black spots, and their skin is highly pigmented, as exemplified by their black ears. This coat-skin pattern also occurs in British White and Chillingham/White Park cattle [68], and the White Fulani cattle of West Africa [69]. For White Park cattle, it has been demonstrated that their coat colour variation is caused by a duplication in the *KIT* gene and an aberrantly inserted *KIT* gene on BTA29 [70]. Other genes that harbour cattle coat colour polymorphisms include *MC1R*, *TWIST2*, *MITF*, *PAX3*, *SLC45A2*, *COPA*, *TYR*, *TYRP1*, *KITLG*, and *ASIP*; however, in addition to this group of genes, there are more than 100 other genes that have been shown to determine coat colour traits in mammals [71-73]. For the Blanco Orejinegro breed, the genes *PAX3*, *SLC45A2*, and *KITLG* are in genome regions with evidence for directional selection based on the CSS results. Mutations in *PAX3* have been shown to result in a spectrum of phenotypic outcomes in horses from white spotting to a uniform white coat trait [74]. Similarly, mutations in the bovine *SLC45A2* gene have been shown to cause oculocutaneous albinism [75], while the *KITLG* gene has been associated with the roan coat type in cattle [76].

Interestingly, for the Hartón del Valle and Limonero breeds, *MC1R* was part of a BTA18 CSS cluster region. The protein receptor encoded by *MC1R* regulates tyrosinase levels in melanocytes,

an enzyme critical to melanin synthesis. High concentrations of tyrosinase facilitate the production of eumelanin, contributing to darker brown or black shades, whereas lower concentrations drive the synthesis of phaeomelanin, yielding lighter red or yellow tones [77]. It is therefore noteworthy that Limonero and the Hartón del Valle cattle generally possess a “bayo” or bay coat, which is a yellow to light brown/red colour [78-80]. Though not in a CSS cluster region in the Blanco Orejinegro, a notable peak close to *MC1R* is present on BTA18. This gene has been observed to be under positive selection in *B. indicus* cattle resulting in a light grey to white coat in the Brahman and Nelore breeds [81, 82]. The adaptive significance of this observation is that lighter coat colours, determined by functional polymorphisms at M*C1R*, reflect a significant portion of incident solar radiation [83], which makes these cattle more suited to tropical environments [84].

Selection for heat tolerance in Criollo cattle

Criollo cattle breeds such as the Blanco Orejinegro, Hartón del Valle, and Limonero have evolved over five centuries to thrive in the challenging tropical agroecologies of Colombia and Venezuela. In the context of this environmental adaptation, a key finding for all three breeds is the presence of a CSS peak on BTA20 that encompasses the *PRLR* gene, most notably in Blanco Orejinegro and Hartón del Valle breeds, where it was statistically significant. Mutations in this gene have been shown to cause the “slick” phenotype in in Criollo breeds such as the Blanco Orejinegro, Harton del Valle, Limonero, Romosinuano, Costeño con Cuernos, Mexican Criollo Lechero Tropical, and also Criollo-influenced composite breeds such as the Carora and Senepol [27, 28, 85]. Of particular interest for understanding how convergent evolution can act in livestock populations is the existence of several different *PRLR* mutations that can produce the "slick" phenotype in Criollo cattle [85]. Mutations in the bovine *PRLR* gene can have major effects on the length and the structure of hair coats providing improved thermotolerance and concomitant increase on fertility and milk yields in cattle populations that inhabit dry and tropical conditions [22, 78, 86-88]. In addition, it has been shown that these mutations can act pleiotropically and cause other physiological changes [89].

Variation at other genes may also confer enhanced thermotolerance in Criollo cattle; for example, *MVD*, previously implicated as being under selection in North African cattle [90], was found in a BTA18 CSS cluster region for all three breeds. In addition, *CCM2* which regulates heart and blood vessel formation and integrity, is located in a Limonero BTA4 CSS cluster region and was also highlighted by Ben Jemaa and colleagues in North African cattle [90]. This may represent an adaptation that increases heat transfer from the interior to the skin, which is also supported by the enhanced vascularisation of the dermis that has been documented in Limonero cattle [78]. Another gene, *SESN2*, located in a BTA2 CSS cluster detected in the Hartón del Valle and the Blanco Orejinegro breeds may also be associated with adaptation to tropical environments. This gene was observed to be downregulated in a functional genomics study of heat-stress responses in dermal fibroblast cells from zebu and zebu × taurine crossbred cattle [91].

Heat-induced stress is a form of proteotoxicity that can cause protein denaturation; if these denatured proteins aggregate, they can become cytotoxic [92]. Many heat shock proteins (HSPs) serve as molecular chaperones, aiding in the proper folding of proteins, and thereby preventing such potentially harmful protein aggregation [93]. In this regard, *FBXO4* located in a BTA11 Limonero CSS cluster region encodes a protein that regulates body temperature through interactions with HSPB6 (previously HSP20) [84, 94]. We also observed that *DNAJA2* was in a Blanco Orejinegro BTA18 CSS cluster region. This gene encodes a member of the DNAJ/HSP40 family of proteins and shows increased expression in peripheral blood from Holstein calves exposed to heat stress [95]. Similarly, *HIGD1A* (BTA22 CSS cluster) and *CBFA2T3* (a BTA18 CSS cluster distinct from that containing *DNAJA2*), encode proteins associated with cellular responses to hypoxia [96-98], which could be indicative of adaptation to oxidative stress caused by high temperatures [99].

Genomic signatures of selection for fertility and reproductive traits in Criollo cattle

Compared to taurine breeds from temperate zones, Criollo cattle are renowned for high fertility and excellent reproductive performance under tropical conditions [21, 29, 100, 101] and using the CSS method we detected several genes associated with these traits. For example, in the

Blanco Orejinegro breed a BTA7 CSS cluster region contains the *CATSPER3* gene exclusively expressed in the testis, which encodes a sperm-specific ion channel protein directly linked to sperm motility and male fertility in many species, including cattle [102]. Another CSS cluster region in the Blanco Orejinegro on BTA20 contained *SPEF2*, which is involved in the formation and functionality of sperm flagella [103]. Additionally, the *CREM* gene, implicated in spermatogenesis, was located within a Blanco Orejinegro CSS cluster region on BTA13 [104]. Other genes related to reproductive physiology were located within CSS cluster regions for the Hartón del Valle breed including *HKDC1* on BTA28 that exhibits high expression in the testes [105], and *MISP3* on BTA7, which is implicated in spermatogenesis [106]. For the Limonero breed, CSS cluster regions included one on BTA10 that contains *FSIP1*; this gene is crucial for normal spermiogenesis and flagella development [107]. In addition, the *PTGES* gene within a Limonero CSS cluster region on BTA11 encodes an enzyme involved in the synthesis of prostaglandin E2 (*PGE2*), which is a signalling molecule with a crucial role in reproductive processes [108]. In parallel to this, the QTL analysis (electronic supplementary material, table S7) for the Blanco Orejinegro breed identified QTLs predominantly associated with reproduction, notably calving ease, and lactation. These findings corroborate previous results obtained using SNP array data for Blanco Orejinegro cattle [25, 109]. The results of the QTL analysis in the CSS cluster regions for the Limonero breed were similar to those for the Blanco Orejinegro breed.

Genomic adaptations to infectious diseases in Criollo cattle

Comparable to reproductive and fertility traits, desirable disease resistance and tolerance traits have been highlighted as important features of Criollo cattle populations. However, empirical evidence for the genetic basis of these traits is often lacking; instead, much of the evidence base is anecdotal and derived from practical knowledge and experiences of veterinarians, farmers and breeders [79]. In the present study, however, we have begun to address this knowledge gap through identification of several immune genes in CSS cluster regions for the Blanco Orejinegro, Hartón del Valle, and Limonero breeds. For example, the *CBFA2T3* gene located in the BTA18 CSS cluster

region detected for all three breeds and discussed in the context of hypoxia, has a role in the bovine immune response to mammary gland inflammation [110]. Similarly, *CXCL14*, located in a BTA7 CSS cluster region for the Blanco Orejinegro and Limonero breeds has also been implicated in the immune response to bovine mastitis caused by *Staphylococcus aureus* [111]. In addition, for the Limonero, a BTA11 CSS cluster region contains the *LRRC8A* gene, which is essential for development and function of T lymphocytes [112].

In Colombia and the surrounding tropical and subtropical regions, there is a significant prevalence of vector-borne haemoparasitic infections [113] that induce anaemia, fever, wasting, and reproductive dysfunction [114]. These diseases are primarily caused by eukaryotic parasites within the *Babesia* [115] and *Trypanosoma* [116] genera, and bacterial pathogens from the *Anaplasma* genus [117]. It is noteworthy, therefore, that the *EPB41* gene located within a Blanco Orejinegro and Hartón del Valle BTA2 CSS cluster region is associated with resistance to anaemia and trypanotolerance in African cattle [118]. Also, for the Blanco Orejinegro and Limonero, *RPS27* and *RPS20* are located in CSS cluster regions on BTA3 and BTA14, respectively and mutations in these genes can cause Diamond-Blackfan anaemia [119, 120]. Furthermore, in the Blanco Orejinegro breed, the *ZFPM1* gene within a BTA18 CSS cluster region, has been shown to play a role in cardiac and hematopoietic development. In a murine *Trypanosoma congolense* infection model, mice exhibiting decreased *ZFPM1* expression recovered more effectively from anaemia [121].

Gene GO term analysis in the Criollo CSS cluster regions revealed a marked overrepresentation of immunobiological processes for the Blanco Orejinegro and Limonero breeds, many of which overlapped between the two breeds (figure *5a* and *5c* and electronic supplementary material, tables S4 and S5). The statistically significant overrepresented biological process GO terms in the Blanco Orejinegro and Limonero included *phagocytosis* (GO:0006909), *phagocytosis, engulfment* (GO:0006911), *phagocytosis, recognition* (GO:0006910), *complement activation, classical pathway* (GO:0006958), and the *B cell receptor signalling pathway* (GO:0050853) For molecular function, statistically significant overrepresented GO terms in the two breeds included

immunoglobulin receptor binding (GO:0034987), *antigen binding* (GO:0003823), and *RAGE receptor binding* (GO:0050786). Again, immunobiology was prominent in the statistically significant overrepresented cellular component GO terms for the two breeds, which included *immunoglobulin complex* (GO:0019814) and *immunoglobulin complex, circulating* (GO:0042571). Criollo cattle populations have adapted over several centuries to novel agroecological environments with significant parasite and pathogen challenges. With regard to specific infectious disease selection pressures and these overrepresented immunological GO terms, it is noteworthy that Blanco Orejinegro cattle have a documented resistance to brucellosis [122, 123], a disease characterized by abortion and retention of the placenta, which is principally caused by *Brucella abortus* an intracellular bacterium that subverts phagocytic pathways and processes to enter and manipulate host cells [124, 125].

CONCLUSIONS

This study provides valuable insights into the genomic basis of microevolutionary change in Criollo cattle as they have adapted to the tropical environments of South America. Evidence of strong selective pressure was apparent, particularly for the distinct coat and skin coloration traits observed in these breeds, which are advantageous in cattle populations exposed to significant levels of incident solar radiation. Notably, we also discovered genomic selection signatures that may be associated with thermotolerance, again underscoring adaptation to hot climates. In addition, some of the selection signatures we identified align with documented fertility traits in Criollo cattle. Moreover, functional overrepresentation analysis revealed many genes related to immune function, which could reflect resilience to multiple infectious disease challenges. Taken together, our results, show the remarkable adaptability of Criollo cattle, which has been driven by natural and human-mediated selection and that underscores the genetic richness and value of these breeds for future breeding programmes.

SUPPLEMENTARY MATERIAL

Table S1: Cattle samples with whole-genome sequence (WGS) data.

Table S2: Cattle samples with BovineHD 777K SNP array data.

Table S3: Composite selection signatures (CSS) cluster regions and genes for all breeds

Table S4: Composite selection signatures (CSS) cluster gene ontology (GO) term overrepresentation analysis for the Blanco Orejinegro breed.

Table S5: Composite selection signatures (CSS) cluster gene ontology (GO) term overrepresentation analysis for the Hartón del Valle breed.

Table S6: Composite selection signatures (CSS) cluster gene ontology (GO) term overrepresentation analysis for the Limonero breed.

Table S7: Composite selection signatures (CSS) cluster QTLs identified for all breeds.

Table S8: Composite selection signatures (CSS) genes and functional inference.

Ethics: The hair and semen samples used in this research were collected as part of a national cattle breeding programme, a process that conforms to routine animal husbandry procedures. No experimental procedure was performed on live animals.

Data accessibility: The new whole-genome sequence data generated from 34 Criollo cattle for this study is available at the European Nucleotide Archive (ENA) with the project accession ID PRJEB65887.

Authors' contributions: J.A.W.: conceptualization, data curation, formal analysis, methodology, software, visualization, and writing—original draft, and writing—review and editing; S.I.N.: data curation, formal analysis, methodology, and writing—review and editing; I.A.S.R., G.P.H., and J.F.O'G.: formal analysis, methodology, software, and writing—review and editing; J.M.F., A.M.P.O'B., and A.J.L-H.: conceptualization, resources, and writing—review and editing; J.A.B.: methodology, and writing—review and editing; T.S.S.: conceptualization, funding acquisition, project administration, resources, and writing—review and editing; L.A.F.F.: conceptualization, data curation, methodology, resources, and writing—review and editing; M.S-T.; conceptualization, methodology, supervision, and writing—review and editing; D.E.M.: conceptualization, funding acquisition, project administration, resources, supervision, writing—original draft and writing—review and editing.

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