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 indicine (*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 (WGS) and single-nucleotide polymorphism (SNP) array data to examine population structure and admixture at high resolution. Analysis of genetic structure and ancestry components provided evidence for African taurine and Asian indicine admixture in Criollo cattle. In addition, using WGS data, 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", with origins in the Portuguese "Crioulo", historically distinguished people born in the New World from those native to Iberia and was subsequently extended to livestock (cattle, sheep, horses, and goats). European livestock production in the Americas can be traced back to Columbus' second voyage in 1493, where cattle, among other animals, sourced from La Gomera in the Canary Islands were brought to the island of Hispaniola [1-3]. Adoption and growth of cattle agriculture on Hispaniola was rapid; however, the spread of animals to the South American continent was more gradual. This was documented by early colonial accounts, illustrating a transformative ecological and economic process that started in the Caribbean, then Mexico, and eventually reaching the Ilanos of Colombia and Venezuela, where by 1600, cattle ranching had become a significant industry [4, 5]. 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 [6]. Evidence for a similar Iberian origin narrative has been described for sheep [7], pigs [8], and horses. For example, recent work has shown that historical and extant North American horse populations exhibit pronounced genetic affinities with Iberian horse populations [9]. The ancestry of modern South American cattle has also been shaped by indicine (zebu) cattle (*Bos indicus*) that were directly introduced from South Asia—primarily present-day India and Pakistan [10].

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 Latin American Criollo cattle ancestry have an African taurine cattle genomic component [11-13]. In parallel to this, it has also been posited that African cattle may have been directly introduced into South America alongside imports from Europe [14]. This hypothesis is supported by the presence of the T1-c mtDNA haplotype at a frequency of 8% in Criollo cattle; T1-c is prevalent in African cattle and found at low frequencies in Iberian populations [15]. It is also supported by recent findings of colonial era mitogenomes which reveal the presence of African

71 haplogroups in 17th century cattle from Mexico [16]. Recent work suggests that the degree of African
72 ancestry in Criollo cattle depends on the breed. Notably, the Guadeloupe breed, indigenous to the
73 Caribbean, traces almost 35% of its genetic ancestry back to African taurine cattle [17].

74 The cattle that were brought to the Americas from Iberia in the 15th and 16th centuries were
75 adapted to Mediterranean agroecologies and, over the following centuries, these populations have
76 evolved adaptations to tropical and arid environments, emerging as distinctive breeds with unique
77 heat tolerance and disease resistance traits [12, 18-21]. However, Criollo cattle are undervalued in
78 modern production systems and face gradual replacement by more productive commercial breeds,
79 which is eroding indigenous cattle genetic resources in Latin America [22]. Using genome-scale data,
80 this study aims to evaluate *B. taurus* and *B. indicus* ancestry and detect historical gene flow from
81 African cattle into the Blanco Orejinegro, Hartón del Valle, and Limonero Criollo breeds.
82 Additionally, we use high-resolution analyses of genetic structure, and selection signatures to provide
83 new information about the microevolutionary histories of these populations.

84 MATERIALS AND METHODS

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

86 In total, 34 Criollo cattle were used to generate high-quality de novo whole-genome sequence
87 (WGS) data sets. Coat hair follicles obtained from 24 Colombian and Venezuelan cattle and semen
88 straws from 10 Colombian AI bulls were used to obtain genomic DNA using standard commercial
89 procedures for livestock DNA extraction and purification (Weatherbys Scientific, Co. Kildare,
90 Ireland). The WGS data generation was performed by sequencing to a mean depth of 20× using two
91 commercial service providers (Novogene, Cambridge, UK and Macrogen, Seoul, South Korea).
92 Figure 1 displays the geographical origins of the three main Criollo breeds in this study.

93 Publicly available WGS data was also assembled for 37 European taurine, 13 American
94 indicine, and 13 American Criollo cattle. Sequence reads were mapped to the bovine reference
95 genome (ARS-UCD1.2) [23] using the default parameters for bwa-mem2 v.2.2.1 [24]. Samtools

v.1.13 [25] 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 [26] were used to perform base quality score recalibration.

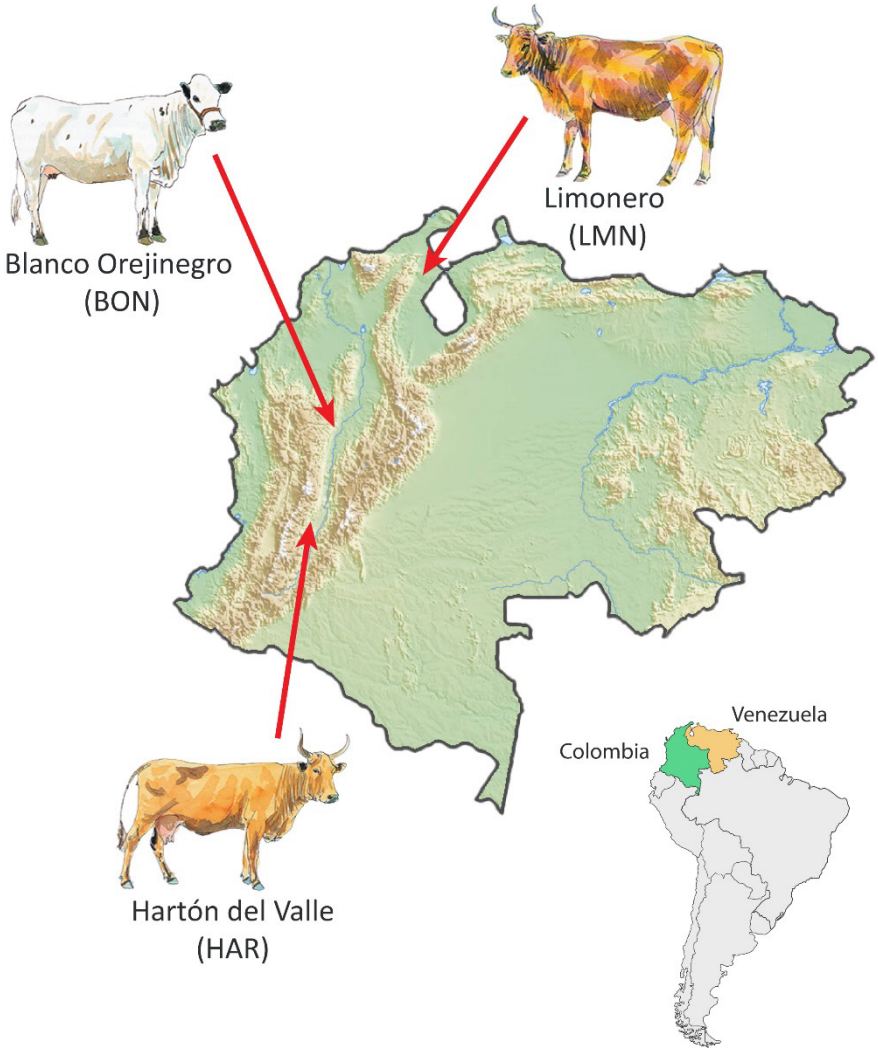

Figure 1. Approximate geographical origins of the animals from three Criollo breeds that were used to generate new whole-genome sequence data. Map modified from original by Mapswire (https://mapswire.com) (Creative Commons Attribution 4.0 International License CC-BY 4.0). Individual cattle art images modified from Felius [27] with permission of the author.

108 Candidate SNPs were called from the bam files using the "HaplotypeCaller" function in GATK
109 with the "--emit-ref-confidence GVCF" option. Individual GVCF files were merged using the
110 "GenomicsDBImport" function. SNPs from the genomics database were called and selected using
111 "GenotypeGVCFs" and the "SelectVariants" function. The "VariantFiltration" function of GATK
112 was used to avoid possible false-positive calls according to GATK best practices: SNP clusters with
113 "--cluster-size 3" and "--cluster-window-size 10", quality by depth "QD <2", read position rank sum
114 test "ReadPosRankSum < -8", phred-scaled variant quality score "QUAL < 30", Fisher strand "FS >
115 60", mapping quality "MQ < 40", mapping quality rank sum test "MQRankSum < -12.5" and strand
116 odds ratio "SOR > 3". Triallelic SNPs and those with a minor allele frequency < 0.01 and/or missing
117 genotype rates over > 0.1 were filtered out. The SNPs remaining in each WGS data set were then
118 annotated according to their positions using SnpEff [28]. The combined panel of 97 cattle samples
119 with WGS data are detailed in electronic supplementary material, table S1.

120 **BovineHD 777k array data**

121 For broader comparative population genomics analyses, publicly available BovineHD
122 777k SNP array data were used. These were 91 European taurine cattle representing two breeds, 82
123 Asian and American indicine cattle representing four breeds [29, 30], 45 Iberian cattle representing
124 13 breeds [31], and 31 African taurine cattle representing two breeds [29, 32]. In addition, 12 Criollo
125 cattle representing the Senepol breed were obtained from the WIDDE database [33]. SNP positions
126 were updated from the UMD3.1 assembly [34] to ARS-UCD1.2 following Riggio et al. [35].
127 Subsequent processing of these data was conducted using PLINK v1.90b6.25 [36] and R version 4.1.3
128 [37]. The combined panel of 261 cattle samples with BovineHD 777k SNP data are detailed in
129 electronic supplementary material, table S2.

130 **Population differentiation, genetic structure, and demographic analyses**

131 Principal component analysis (PCA) for all animals (WGS and BovineHD genotype data) were
132 performed using PLINK v1.90b6.25 and the results were plotted using ggplot2 v3.4.0 [38] in the R

133 v4.1.3 environment. The genetic structure of each population was visualized using ADMIXTURE
134 v1.3.0 [39] with K = 3–6 unsupervised modelled ancestries and ggplot2 v3.4.0. Demographic
135 modelling of current and historical effective population size (N_e) trends was conducted using the
136 SNeP software tool [40] with WGS data for the Blanco Orejinegro, Hartón del Valle, Limonero,
137 N'Dama, and Holstein populations.

138 **Detection of historical admixture**

139 Using ADMIXTOOLS [41] with default parameters, we calculated f_3 statistics using the
140 combined WGS and BovineHD genotype data set to test for evidence that three Criollo breeds
141 (Blanco Orejinegro, Hartón del Valle, and Limonero) and three Iberian breeds (Limia, Pajuna, and
142 Sayaguesa) are derived from the admixture of two sets of populations (European taurine populations
143 and indicine cattle, and European taurine populations and African taurine cattle). A significant
144 negative f_3 value is considered evidence of historical admixture in the target population (Criollo or
145 Iberian). For visualization of the f_3 statistics, we plotted the f_3 values against the population trios with
146 error bars indicating the standard errors using ggplot2 v3.4.0 [38].

147 **Test statistics for detection of selection signatures**

148 For the selection signatures analysis, only the WGS data set was used, which included
149 approximately 9.7 million SNPs genotyped in 97 cattle. SNP-by-SNP F_{ST} values were calculated for
150 each Criollo breed versus a panel of Holstein cattle using VCFtools v.0.1.16 [42]. XP-EHH statistics
151 were calculated using the program selscan v.1.2.0 [43] with default parameters, where each Criollo
152 breed was also compared to the Holstein panel. The unstandardized XP-EHH values were then
153 normalized using the --norm function in selscan. We calculated the ΔSAF (directional change in
154 selected allele frequency) using the --freq function of VCFtools to generate allele frequencies, then
155 using a custom R script we calculated the ΔSAF for each Criollo breed versus the Holstein panel.
156 Similarly to the XPEHH values, ΔSAF values were standardized to $Z \sim N(0,1)$ as described by
157 Randhawa et al. [44].

158 **Implementing the composite selection signals (CSS) methodology**

159 To obtain composite selection signals (CSS) statistics [44] for each Criollo breed, the F_{ST}, XP-
160 EHH, and ΔSAF statistics were combined by SNP ID (chromosome:position), which produced final
161 sets of SNPs for the CSS analysis of 9,344,833, 9,465,007 and 9,660,647 million SNPs, for the Blanco
162 Orejinegro, Hartón del Valle, and Limonero breeds, respectively. Following this, the MINOTAUR R
163 package [45] was used to calculate CSS statistics, which were then averaged over 20 kb windows to
164 reduce spurious signals. In addition, a signal was only considered significant if at least one SNP in
165 the 0.1% of CSS scores was flanked by at least five other SNPs in the top 1% of CSS values [44, 46].

166 **Identification of genomic regions under selection**

167 From the top 0.1% of SNPs identified, genomic intervals in the format chromosome:start-end
168 were extracted and the BovineMine resource (v1.6) [47] was then used to find genes ± 500 kb from
169 the start and end of these regions. The functional relevance of these CSS-derived gene sets was then
170 evaluated using the scientific literature and the g:Profiler software tool [48], which was used to
171 identify overrepresented gene ontology (GO) terms for each of the CSS-derived gene sets by breed.
172 Following this, BovineMine v1.6 was used to systematically identify previously described
173 quantitative trait loci (QTLs) for production, health, and welfare traits that are located within the CSS
174 cluster regions for each breed.

175

176 # RESULTS

177 ## Population differentiation, genetic structure, and admixture in Criollo cattle

178 The merged WGS and remapped BovineHD 777K BeadChip datasets yielded 302,012 shared
179 SNPs for 308 individual animals. A PCA of these SNPs demonstrated that PC1 and PC2 accounted
180 for 44.7% and 12.9% of the variation observed across the first 20 PCs, with PC1 representing the split
181 between the *B. taurus* and *B. indicus* lineages and PC2 the split between the European and African
182 taurine lineages (figure 2*a*). Genetic structure was analysed using ADMIXTURE, with cluster
183 numbers (*K*) ranging from 3 to 6 (figure 2*b*). At $K = 3$, we recovered the three clusters observed in
184 the PCA, which delineated European *B. taurus*, African *B. taurus*, and *B. indicus* ancestry
185 components, respectively. The subsequent split at $K = 4$ captured an additional cluster within the
186 European taurine populations that differentiated Jersey cattle. At $K = 5$ a Holstein cattle ancestry
187 component was evident and at $K = 6$ a split was observed that divided *B. indicus* ancestry into two
188 clusters.

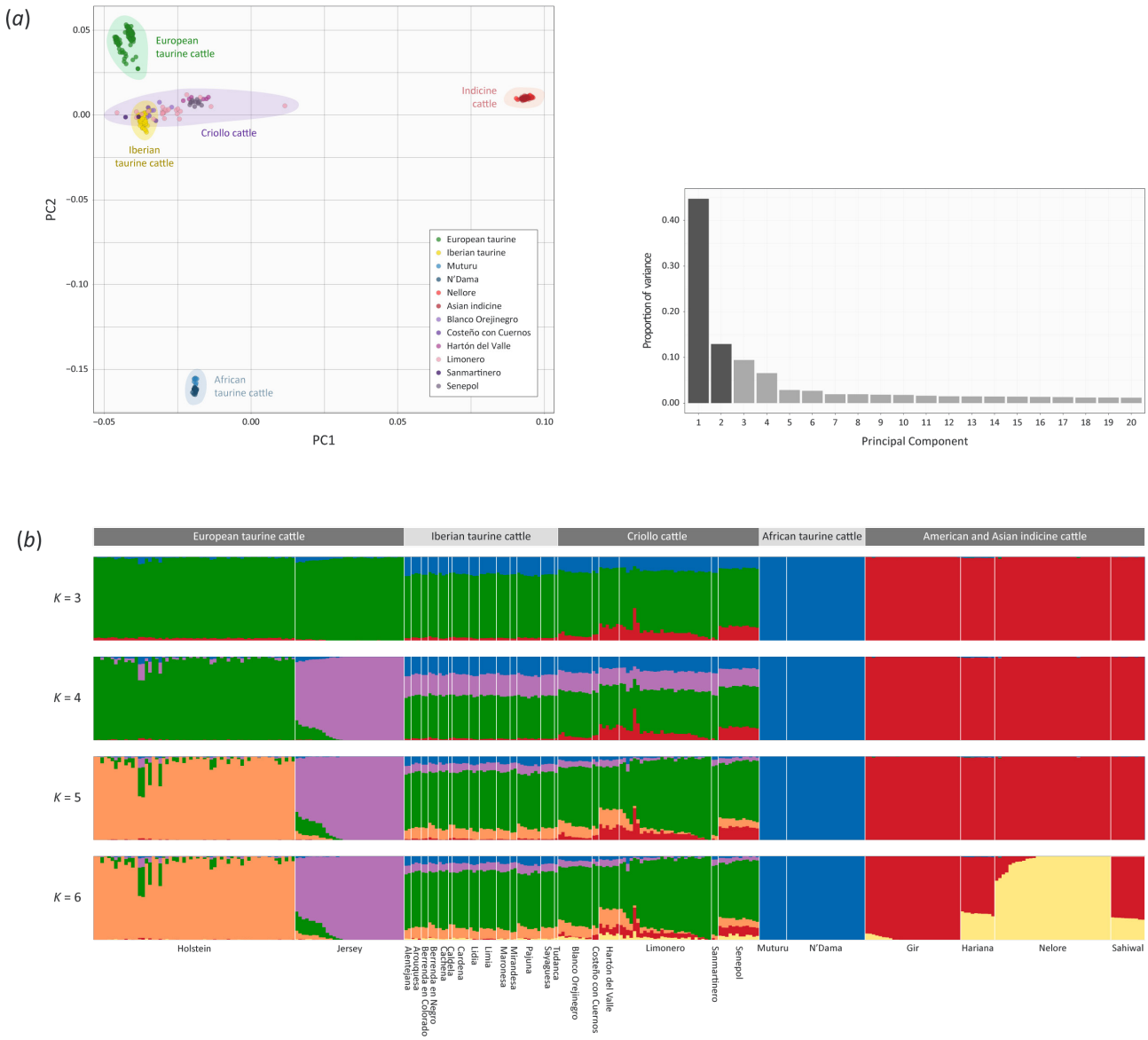

Figure 2. Genetic diversity and admixture in Criollo and comparator cattle populations. (*a*) Results of the principal component analysis (PCA) for 308 animals (47 from whole-genome sequence data and 261 from high-density SNP array data) with a shared set of 302,012 SNPs. The PCA plot shows the coordinates for each animal based on the first two principal components with the major breed groupings highlighted. Principal component 1 (PC1) differentiates the *Bos taurus* and *Bos indicus* lineages, whereas PC2 separates the African and European taurine groups. A histogram plot also shows the variance contributions for the first 20 PCs, with PC1 and PC2 accounting for 44.7% and 12.9% of this variance, respectively. (*b*) Unsupervised genetic structure plot for European and Iberian taurine, Criollo cattle, African taurine, and American and Asian indicine populations. Results for ancestry clusters ranging from K = 3–6 are shown.

201 Further examination of the PCA and structure plots in figure 2*a* and 2*b* showed that the Criollo
202 cattle samples cluster with the Iberian taurine cattle samples, all of which show relatively uniform
203 levels of African taurine admixture at $K = 3$ and $K = 4$. In addition, the Senepol and Hartón del Valle
204 Criollo breeds display a consistent and relatively uniform ancestry component from the *B. indicus*
205 lineage, while the Blanco Orejinegro, Costeño con Cuernos, Limonero, and Sanmartinero breeds
206 exhibit more heterogeneous patterns of indicine admixture. Conversely, f_3 statistics [41] indicate that
207 there is no evidence of admixture from either African taurine or indicine cattle in the Blanco
208 Orejinegro and Limonero breeds when using any of the European taurine reference populations and
209 African taurine or indicine breeds as the second reference (electronic supplementary material, figure
210 S1*a* and *c*). However, there is some evidence of indicine admixture in the Hartón del Valle as the f_3
211 statistics for all population trios that used any European taurine reference population with an indicine
212 breed as the second reference population produced negative f_3 values for this breed (electronic
213 supplementary material, figure S1*b*). This is also evident from the PCA (figure 2*a*), and the structure
214 plot (figure 2*b*).

215 **Selection signals across Criollo cattle breed genomes**

216 The CSS test identified selection signals for the three Criollo cattle breeds that had WGS data
217 and sufficient sample sizes (Blanco Orejinegro, Hartón del Valle, and Limonero). For each breed, the
218 test highlighted several selection signals spanning multiple chromosomes. For the Blanco Orejinegro
219 breed, the CSS test detected 31 cluster regions spanning 17 chromosomes, with 442 genes located
220 within these regions and extended ±500 kb (figure 3*a*). Similarly, for the Hartón del Valle breed, the
221 CSS test revealed 31 distinct clusters distributed over 17 chromosomes, encompassing 410 genes
222 (figure 3*b*). Lastly, for the Limonero breed, the CSS test detected 38 cluster regions, also extending
223 across 17 chromosomes, and that contained 451 genes in total (figure 3*c*). When these results were
224 consolidated, 76 genes within selection signature regions are shared across the three breeds, and
225 BTA18 was the chromosome with the largest number of these genes (28 genes; 36.8% of the total).

226 The CSS cluster regions and the genes within these regions are detailed by breed in electronic
227 supplementary material, tables S3 and S4).

228 **Gene ontology (GO) term enrichment analysis**

229 The functional significance of the genes within the CSS cluster regions for each breed was
230 evaluated using GO term overrepresentation analysis with the g:Profiler software tool. After adjusting
231 for multiple tests and using a *P*-value adjusted for the false discovery rate (FDR-$P_{adj.} < 0.05$),
232 overrepresented GO terms were catalogued for each breed. The results of the GO term
233 overrepresentation analysis are shown in the electronic supplementary material, tables S5–S7 and
234 figure 4. For both the Blanco Orejinegro (figure 4*a*) and Limonero (figure 4*b*) breeds, the GO terms
235 related primarily to immunobiology, encompassing functions such as immunoglobulin receptor
236 binding, phagocytosis, complement activation, and antigen binding. Conversely, for the Hartón del
237 Valle breed (figure 4*c*), the predominant GO terms were associated with skin epidermis and
238 keratinization processes.

239

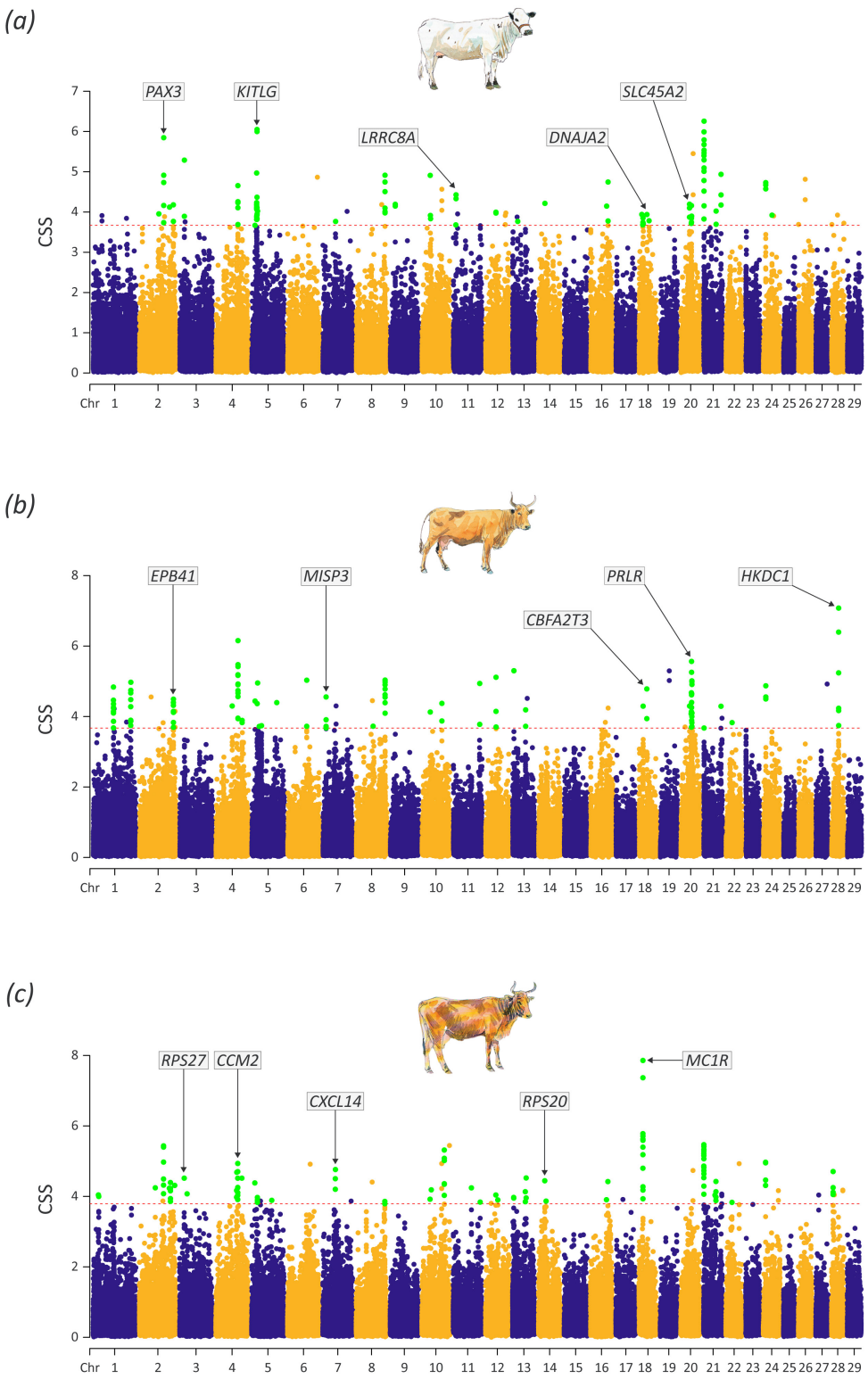

Figure 3. Chromosome-wide Manhattan plots of the smoothed composite selection signals (CSS) scores for Blanco Orejinegrio (*a*), Hartón del Valle (*b*) and Limonero (*c*). The dashed red lines indicate the genome-wide 0.1% thresholds of the empirical CSS scores. Green data points represent SNPs classified as significant and flanked by at least five additional SNPs among the top 1% of CSS scores. Notable candidate genes are indicated and discussed further in the text. Individual cattle art images modified from Felius [27] with permission of the author.

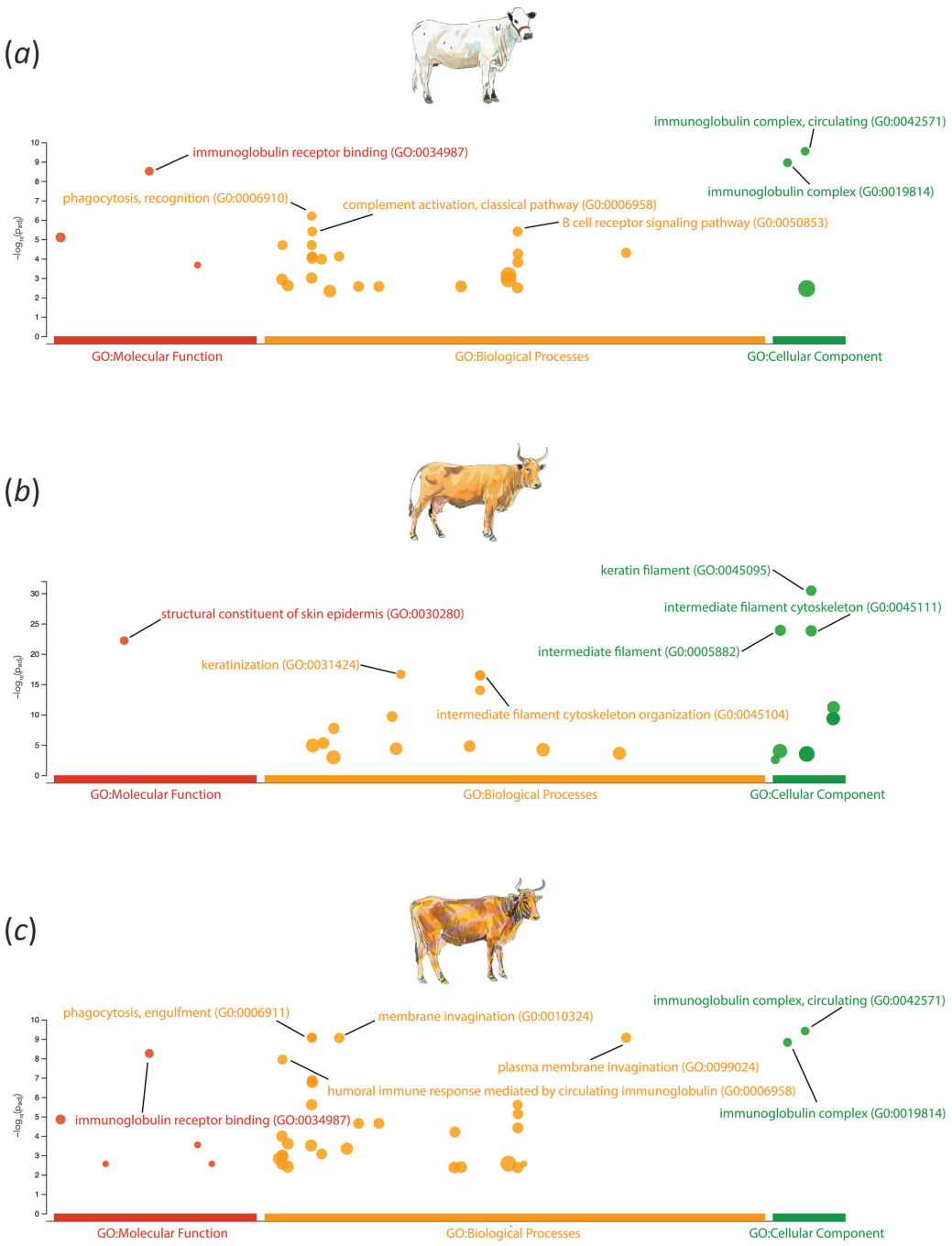

Figure 4. Gene ontology (GO) overrepresentation analysis results for the Blanco Orejinegrio (*a*), Hartón del Valle (*b*) and Limonero (*c*). The y-axis represents the negative $\log_{10}$ FDR-$P_{adj.}$ from the overrepresentation analysis. Individual cattle art images modified from Felius [27] with permission of the author.

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

253 QTLs within the CSS cluster regions were systematically identified using BovineMine v1.6.
254 The number of QTLs identified varied across the breeds: 519 in Blanco Orejinegro, 332 in Hartón
255 del Valle, and 370 in Limonero. Interestingly, only 11 QTLs were common to all three breeds and a
256 QTL associated with tick resistance [49] was particularly noteworthy. In the case of Blanco
257 Orejinegro, most of the identified QTLs (291 out of 519) in the CSS cluster regions were associated
258 with calving ease. Additionally, 46 QTLs were linked to milk protein percentage, 40 to milk yield,
259 and 24 to coat texture. For the Limonero breed, there were 292 QTLs located in the CSS cluster
260 regions related to calving ease; however, no other traits had more than five SNP markers among the
261 QTLs. In the case of the Hartón del Valle breed, the most notable traits represented among the CSS
262 cluster region QTLs were milk related. Specifically, 86 QTLs were associated with milk protein
263 percentage, 45 with milk yield, and 35 with milk fat percentage. Additionally, 27 QTLs in Hartón del
264 Valle were associated with coat texture. The results of the QTL search using BovineMine v1.6 are
265 detailed in electronic supplementary material, table S8.

266

267 # DISCUSSION

268 ## Population structure and admixture

269 The differentiation of cattle samples across PC1 of the PCA plot (figure 2*a*) reflects the
270 evolutionary divergence between the *B. taurus* and *B. indicus* lineages, which occurred 150–500 kya
271 [50, 51] The second division evident for PC2 in the PCA plot (figure 2*a*) differentiates the European
272 and African taurine groups, a separation that has been well documented in several previous studies
273 using genome-wide SNPs and other autosomal genetic markers [52-55]. These PCs are mirrored by
274 the clusters observed at $K = 3$ in the structure analysis (figure 2*b*), which differentiate the European
275 taurine, African taurine, and indicine groups and reveal patterns of shared ancestry among the major
276 groups. Extending these observations further, complex patterns of admixture due to human-mediated
277 and unmanaged crossbreeding between taurine and indicine cattle have also been described in cattle
278 populations across several geographical regions, particular in sub-Saharan Africa [53-55].

279 Previous studies have noted African taurine autosomal admixture in Criollo cattle breeds [12,
280 17]. Results from the present study, which are captured in the PCA plot (figure 2*a*) and structure
281 analysis (figure 2*b*) suggest this is also the case for the Criollo breed population samples analysed
282 here (Blanco Orejinegro, Costeño con Cuernos, Hartón del Valle, Limonero, Sanmartinero, and
283 Senepol), albeit at lower levels compared to that observed for Guadeloupe cattle [17]. Conversely,
284 the positive f_3 statistics observed for the three Criollo breeds examined using this method (Blanco
285 Orejinegro, Hartón del Valle, and Limonero) do not support this (electronic supplementary material,
286 figure S1*a*–*c*). It should be noted, however, that a positive f_3 value does not necessarily imply a lack
287 of admixture, since excessive genetic drift in the target population can obscure an admixture signal
288 [41]. In this regard, demographic modelling of historical effective population sizes (N_e values) and
289 comparison with the Holstein, a commercial dairy population with a markedly low N_e [56], indicates
290 that founder effects and subsequent genetic drift may have impacted the Blanco Orejinegro, Hartón
291 del Valle, and Limonero breeds (electronic supplementary material, figure S2).

292 For *B. indicus* ancestry, all the Latin American Criollo cattle populations examined here exhibit
293 varying degrees of indicine admixture (figure 2*b*). Interestingly, however, the Blanco Orejinegro and
294 Hartón del Valle both originate in Colombia but do not cluster together, which is presumably a
295 consequence of the relatively modest indicine ancestry in the Blanco Orejinegro. The Limonero breed
296 samples in this study largely cluster together except for four animals that exhibit a significant
297 proportion of indicine ancestry. These results, taken together with the f_3 statistics for the Blanco
298 Orejinegro, Hartón del Valle, and Limonero (electronic supplementary material, figure S1*a*–*c*), and
299 the histories of these breeds (the Blanco Orejinegro breed has been in a conservation programme
300 since 1940 [57]), suggest that indicine gene flow has only had a long-term impact on breeds such as
301 the Hartón del Valle which exhibits relatively uniform levels of indicine admixture compared to the
302 Limonero, where it is more heterogeneous (figure 2*b*). This has implications for understanding the
303 selection signatures that are described in the following sections: in some cases, the selection signals
304 detected may be caused by adaptive introgression of *B. indicus* haplotypes.

305 Despite the inclusion of a diverse panel of European taurine, African taurine, Asian indicine,
306 and admixed cattle in the design and validation of the BovineHD SNP array [58], we acknowledge
307 that ascertainment bias could have influenced results from some of the analyses of population
308 differentiation and genetic ancestry. However, we think that this is unlikely because ascertainment
309 bias typically affects analyses such as selection signal detection that use individual SNP locus
310 frequency-based statistics (e.g., F_{ST}) substantially more than genome-wide multi-locus dimension
311 reduction tools like ADMIXTURE and PCA [59-62]. Importantly, in this regard, we used only WGS
312 data for the CSS analyses (figure 3 and electronic supplementary material, table S3).

313 **Genomic signatures of human-driven selection in Criollo cattle**

314 Blanco Orejinegro are distinctive among Criollo cattle for their white coats, often speckled with
315 black spots, and their skin is highly pigmented, as exemplified by their black ears. This coat-skin
316 pattern also occurs in British White and White Park cattle [63], and for White Park, it has been
317 demonstrated that this is caused by a duplication in the *KIT* gene and an aberrantly inserted *KIT* gene

318 on BTA29 [64]. Additional genes that harbour cattle coat colour polymorphisms include *MC1R*,
319 *TWIST2*, *MITF*, *PAX3*, *SLC45A2*, *COPA*, *TYR*, *TYRP1*, *KITLG*, and *ASIP*. For the Blanco Orejinegro
320 breed, the genes *PAX3*, *SLC45A2*, and *KITLG* are in genome regions with evidence for directional
321 selection based on the CSS results. Mutations in *PAX3* have been shown to result in a spectrum of
322 phenotypic outcomes in horses from white spotting to a uniform white coat trait [65]. Similarly,
323 mutations in the bovine *SLC45A2* gene have been shown to cause oculocutaneous albinism [66],
324 while the *KITLG* gene has been associated with the roan coat type in cattle [67].

325 Interestingly, for the Hartón del Valle and Limonero breeds, *MC1R* was part of a BTA18 CSS
326 cluster region. The protein receptor encoded by *MC1R* regulates tyrosinase levels in melanocytes, an
327 enzyme critical to melanin synthesis. High concentrations of tyrosinase facilitate the production of
328 eumelanin, contributing to darker brown or black shades, whereas lower concentrations drive the
329 synthesis of phaeomelanin, yielding lighter red or yellow tones [68]. It is therefore noteworthy that
330 Limonero and Hartón del Valle cattle generally possess a "bayo" or bay coat, which is a yellow to
331 light brown/red colour [69, 70]. Though not in a CSS cluster region in the Blanco Orejinegro, a
332 notable peak close to *MC1R* is present on BTA18. This gene has been observed to be under positive
333 selection in *B. indicus* cattle resulting in a light grey to white coat in the Brahman and Nelore breeds
334 [71]. The adaptive significance of functional polymorphisms at *MC1R* is that lighter coat colours
335 reflect a significant portion of incident solar radiation, which makes these cattle more suited to
336 tropical environments [72].

337 **Selection for heat tolerance in Criollo cattle**

338 Criollo cattle breeds such as the Blanco Orejinegro, Hartón del Valle, and Limonero have
339 evolved over five centuries to thrive in challenging tropical agroecologies. In the context of this
340 environmental adaptation, a key finding for all three breeds is the presence of a CSS peak on BTA20
341 that encompasses the *PRLR* gene, most notably in the Blanco Orejinegro and Hartón del Valle breeds,
342 where it was statistically significant. Mutations in this gene have been shown to cause the "slick"
343 phenotype in in Criollo breeds such as the Blanco Orejinegro, Hartón del Valle, Limonero,

344 Romosinuano, Costeño con Cuernos, Mexican Criollo Lechero Tropical, and also Criollo-influenced
345 composite breeds such as the Carora and Senepol [73]. Of particular interest for understanding how
346 convergent evolution can act in livestock populations is the existence of several different *PRLR*
347 mutations that can produce the "slick" phenotype in Criollo cattle [73]. Mutations in the bovine *PRLR*
348 gene can have major effects on the length and the structure of hair coats providing improved
349 thermotolerance and concomitant increase on fertility and milk yields in cattle populations that inhabit
350 dry and tropical conditions [18, 69, 74]. In addition, it has been shown that these mutations can act
351 pleiotropically and cause other physiological changes [75].

352 Variation at other genes may also confer enhanced thermotolerance in Criollo cattle; for
353 example, *MVD*, previously implicated as being under selection in North African cattle [76], was found
354 in a BTA18 CSS cluster region for all three breeds. In addition, *CCM2* which regulates heart and
355 blood vessel formation and integrity, is located in a Limonero BTA4 CSS cluster region and was also
356 highlighted by Ben-Jemaa and colleagues in North African cattle [76]. This may represent an
357 adaptation that increases heat transfer from the interior to the skin, which is also supported by the
358 enhanced vascularisation of the dermis that has been documented in Limonero cattle [69]. Another
359 gene, *SESN2*, located in a BTA2 CSS cluster detected in the Hartón del Valle and the Blanco
360 Orejinegro breeds may also be associated with adaptation to tropical environments. This gene was
361 observed to be downregulated in a functional genomics study of heat-stress responses in dermal
362 fibroblast cells from indicine and indicine × taurine crossbred cattle [77].

363 The BTA11 Limonero CSS cluster region contains *FBXO4* that encodes a protein, which
364 regulates body temperature through interactions with the heath shock protein HSPB6 (previously
365 HSP20) [72, 78]. We also observed that *DNAJA2* was in a Blanco Orejinegro BTA18 CSS cluster
366 region. This gene encodes a member of the DNAJ/HSP40 family of proteins and shows increased
367 expression in peripheral blood from Holstein calves exposed to heat stress [79]. Similarly, *HIGD1A*
368 (BTA22 CSS cluster) and *CBFA2T3* (a BTA18 CSS cluster distinct from that containing *DNAJA2*),

369 encode proteins associated with cellular responses to hypoxia [80, 81], which could be indicative of
370 adaptation to oxidative stress caused by high temperatures [82].

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

372 Compared to taurine breeds from temperate zones, Criollo cattle are renowned for high fertility
373 and excellent reproductive performance [21, 83] and using the CSS method we detected several genes
374 associated with these traits. For example, in the Blanco Orejinegro breed a BTA7 CSS cluster region
375 contains the *CATSPER3* gene, which encodes a sperm-specific ion channel protein directly linked to
376 sperm motility and male fertility in many species, including cattle [84]. Another CSS cluster region
377 in the Blanco Orejinegro on BTA20 contained *SPEF2*, which is involved in the formation and
378 functionality of sperm flagella [85]. Additionally, the *CREM* gene, implicated in spermatogenesis,
379 was located within a Blanco Orejinegro CSS cluster region on BTA13 [86]. Other genes related to
380 reproductive physiology were located within CSS cluster regions for the Hartón del Valle breed
381 including *HKDC1* on BTA28 that exhibits high expression in the testes [87], and *MISP3* on BTA7,
382 which is implicated in spermatogenesis [88]. For the Limonero breed, CSS cluster regions included
383 one on BTA10 that contains *FSIP1* a gene crucial for normal spermiogenesis and flagella
384 development [89]. In addition, the *PTGES* gene within a Limonero CSS cluster region on BTA11
385 encodes an enzyme involved in the synthesis of prostaglandin E2 (*PGE2*), which is a signalling
386 molecule with a crucial role in reproductive processes [90]. In parallel to this, the QTL analysis
387 (electronic supplementary material, table S8) for the Blanco Orejinegro breed identified QTLs
388 predominantly associated with reproduction, notably calving ease, and lactation. These findings
389 corroborate previous results obtained using SNP array data for Blanco Orejinegro cattle [20, 91]. The
390 results of the QTL analysis in the CSS cluster regions for the Limonero breed were similar to those
391 for the Blanco Orejinegro breed.

392 **Genomic adaptations to infectious diseases in Criollo cattle**

393 Comparable to reproductive and fertility traits, desirable disease resistance and tolerance traits
394 have been highlighted as important features of Criollo cattle populations. However, empirical

395 evidence for the genetic basis of these traits is often lacking; instead, much of the evidence base is
396 anecdotal and derived from practical knowledge and experiences of veterinarians, farmers and
397 breeders [70]. In the present study, however, we have begun to address this knowledge gap through
398 identification of several immune genes in CSS cluster regions for the Blanco Orejinegro, Hartón del
399 Valle, and Limonero breeds. For example, the *CBFA2T3* gene located in the BTA18 CSS cluster
400 region detected for all three breeds and discussed in the context of hypoxia, has a role in the bovine
401 immune response to mammary gland inflammation [92]. Similarly, *CXCL14*, located in a BTA7 CSS
402 cluster region for the Blanco Orejinegro and Limonero breeds has also been implicated in the immune
403 response to bovine mastitis caused by *Staphylococcus aureus* [93]. In addition, for the Limonero, a
404 BTA11 CSS cluster region contains the *LRRC8A* gene, which is essential for development and
405 function of T lymphocytes [94].

406 In tropical and subtropical regions, there is a significant prevalence of vector-borne
407 haemoparasitic infections [95] that induce anaemia, fever, wasting, and reproductive dysfunction
408 [96]. These diseases are primarily caused by eukaryotic parasites within the *Babesia* [97] and
409 *Trypanosoma* [98] genera, and bacterial pathogens from the *Anaplasma* genus [99]. It is noteworthy,
410 therefore, that the *EPB41* gene located within a Blanco Orejinegro and Hartón del Valle BTA2 CSS
411 cluster region is associated with resistance to anaemia and trypanotolerance in African cattle [100].
412 Also, for the Blanco Orejinegro and Limonero, *RPS27* and *RPS20* are located in CSS cluster regions
413 on BTA3 and BTA14, respectively and mutations in these genes can cause Diamond-Blackfan
414 anaemia [101, 102]. Furthermore, in the Blanco Orejinegro breed, the *ZFPM1* gene within a BTA18
415 CSS cluster region, has been shown to play a role in cardiac and hematopoietic development. In a
416 murine *Trypanosoma congolense* infection model, mice exhibiting decreased *ZFPM1* expression
417 recovered more effectively from anaemia [103].

418 Gene GO term analysis in the Criollo CSS cluster regions revealed a marked overrepresentation
419 of immunobiological processes for the Blanco Orejinegro and Limonero breeds, many of which
420 overlapped between the two breeds (figure 4*a* and 4*c* and electronic supplementary material, tables

421 S5 and S7). The statistically significant overrepresented biological process GO terms in the Blanco
422 Orejinegro and Limonero included several terms related to intracellular pathogens, including
423 *phagocytosis* (GO:0006909), *phagocytosis, engulfment* (GO:0006911), and *phagocytosis,*
424 *recognition* (GO:0006910). It is therefore noteworthy that Blanco Orejinegro cattle have a
425 documented resistance to brucellosis [104], a disease characterized by abortion and retention of the
426 placenta, which is principally caused by *Brucella abortus* an intracellular bacterium that subverts
427 phagocytic pathways and processes to enter and manipulate host cells [105].

428 CONCLUSIONS

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

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