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RESEARCH ARTICLE

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Genomic analysis of inbreeding level, kinship and breed relationships in Creole cattle from South America

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Abstract

The conservation of animal genetic resources refers to measures taken to prevent the loss of genetic diversity in livestock populations, including the protection of breeds from extinction. Creole cattle populations have suffered a drastic reduction in recent decades owing to absorbent crosses or replacement with commercial breeds of European or Indian origin. Genetic characterization can serve as a source of information for conservation strategies to maintain genetic variation. The objective of this work was to evaluate the levels of inbreeding and kinship through the use of genomic information. A total of 903 DNAs from 13 cattle populations from Argentina, Bolivia and Uruguay were genotyped using an SNP panel of 48 K. Also, a dataset of 76 K SNPs from Peruvian Creole was included. Two inbreeding indices (F_{ROH} and *Fhat2*) and kinship relationships were calculated. In addition, effective population size (N_e), linkage disequilibrium, population composition and phylogenetic relationships were estimated. In Creole cattle, F_{ROH} ranged from 0.14 to 0.03, and *Fhat2* was close to zero. The inferred N_e trends exhibited a decline toward the present for all populations, whereas Creole cattle presented a lower magnitude of N_e than foreign breeds. Cluster analysis clearly differentiated the taurine and Zebu components (K2) and showed that Bolivian Creole cattle presented Zebu gene introgression. Despite the population reduction, Creole populations did not present extreme values of consanguinity and kinship and maintain high levels of genetic diversity. The information obtained in this work may be useful for planning conservation programmes for these valuable local animal genetic resources.

KEYWORDS
breed purity, cattle, consanguinity, effective population size, genetic relatedness, microarray, SNPs

INTRODUCTION

Creole cattle were brought to the American continent by Spanish conquerors in 1493. They were initially introduced to the Caribbean islands and rapidly spread across the continent. Subsequent expeditions brought additional cattle, introducing the first animals to the Viceroyalty of the Rio de la Plata in 1549 from Potosi (Bolivia). In 1555, conquerors landed in southern Brazil and advanced to Asunción (Paraguay), where the group divided into two expeditions, one of which initiated the cattle population in the Argentine pampa (Giberti, 1970; Primo, 1992). With the more recent introduction of British and zebuine breeds, these Creole

populations were relegated to marginal areas and some of them are currently endangered (Domestic Animal Diversity Information System of the Food and Agriculture Organization of the United Nations, FAO, 2021, https://www.fao.org/dad-is).

The genetic diversity of Latin American Creole cattle populations has long been investigated. The first studies were done by analyzing their blood group and protein polymorphisms (Giovambattista et al., 2010). Over the years, these technologies were replaced by the use of molecular markers, including autosomal microsatellites, maternal mitochondrial DNA (mtDNA) and Y chromosome genetic markers, which have been widely used to characterize different Creole cattle populations and their genetic relationships (Armstrong et al., 2013; Ginja et al., 2010; Lirón et al., 2002; Lirón, Bravi, et al., 2006; Lirón, Peral-García, et al., 2006; Magee et al., 2002; Martínez et al., 2012; Ocampo et al., 2021). In 2019, Ginja and coauthors published a comprehensive work that included data from all Creole cattle populations from the USA to Argentina and summarized the contribution of the three types of genetic markers. In recent years, Creole cattle studies have migrated to genomic analysis, mainly using low- and medium-density single nucleotide polymorphism (SNP) microarrays (Campos et al., 2017; Corredor et al., 2023). In this sense, Falomir-Lockhart et al. (2019) studied the origin of the polled mutation in Argentine Creole cattle, and other works described the genetic diversity and genetic structure of Argentine Creole cattle and explored their relationship with Creole cattle from other Latin American countries (McTavish et al., 2013; Michiels et al., 2023; Raschia & Poli, 2021). This genomic approach has improved the precision of studies conducted with microsatellites and uniparental markers by carrying out analysis of breed composition and gene introgression as well as breed and individual relationships. Also, this technique can include additional analyses, like level of inbreeding, kinship, footprint and whole genome association studies (Eusebi et al., 2019).

Genetic characterization can serve as a source of information for conservation strategies. Livestock populations provide a variety of products and services as a result of their genetic diversity, which is essential for evolution by natural selection and for genetic improvement programmes. The conservation of animal genetic resources refers to measures taken to prevent the loss of genetic diversity in livestock populations, including the protection of breeds from extinction (FAO, 2007). Several countries, such as Argentina, Bolivia and Brazil, have established conservation plans to preserve Creole populations (da Silva Mariante, 1990a, 1990b; Michiels et al., 2023). These plans are fundamental because endangered native populations are naturally adapted to the local environment and contribute a high proportion of the species genetic diversity. These populations are vulnerable to accelerated genetic erosion and may need controlled schemes of support to maintain the individual genome and diversity of each breed, especially because of the small herd size (Alderson, 2018; FAO, 2007). In this sense, genomic data can provide highly useful information concerning conservation programme efficiency and success, enabling a continuous monitoring of genetic diversity and an improvement of breeding programmes and mating decisions, leading to a better management of these key resources (Kristensen et al., 2015).

According to the FAO, there are two main approaches to manage the genetic diversity of small populations of livestock. One includes programmes to maintain or increase the genetic variability (by implementing a general breeding strategy to maintain the breed, planning a mating strategy to decrease inbreeding and the incorporation of embryos and semen cryoconservation in the *in situ* programme). The second strategy is the optimization of selection response and genetic variability within the population (this approach focuses on adopting a general breeding strategy to maintain the breed and the design of a breeding programme that generates genetic improvement while maintaining genetic variability; FAO, 2013).

However, whereas relationships among Creole cattle and their origin have been extensively studied, data on inbreeding, kinship and effective population size in these native populations are still scarce. For this reason, the objective of this study was to evaluate the levels of inbreeding and kinship within and between populations, the effective population size and the genetic diversity of Creole cattle from Argentina, Bolivia, Peru and Uruguay using medium-density SNP microarrays.

MATERIALS AND METHODS

Animal populations

A total of 903 blood samples were collected from adult cattle from 13 breeds/populations, including: Argentine Creole (CrAr), Bolivian Creole from Oruro department (CrAl), Bolivian Creole from Cochabamba department (CrCoch), Bolivian Creole from La Paz department (CrHua), Bolivia Creole from the Centro de Ecología Aplicada Simón I. Patiño (CrPat), Saavedreño Creole (CrSaa), Yacumeño Creole (CrYa) and Uruguayan Creole (CrUr); two European taurine breeds – Angus (AA) and Holstein (Ho); two zebuine – Brahman (Bh) and Nelore (Ne); and one composite breed (Brangus, Br). A previously published dataset from 12 Peruvian Arequipa Fighting Bulls (AFB) was also added to the present study (Corredor et al., 2023). It should be noted that population is a comprehensive term that includes well-defined breeds as well as groups of animals that have not yet been defined as a breed (Ajmone-Marsan et al., 2023). In this study, both terms were used as synonyms and the acronym Ze was used when both zebuine breeds (Brahman and Nellore) were analyzed

or visualized together. Table 1, Table S1 and Figure 1 summarize the main characteristics of the sampled populations.

Genotyping and quality control

Genomic DNAs were isolated from white blood cells using the commercial kit Wizard® Genomic DNA Purification (Promega, WI, USA) according to the protocol supplied by the manufacturer. They were genotyped in a GeneTitan™ platform (Applied Biosystems™, CA, USA) using the microarrays Axiom™ Bos 1 Genotyping Array r3 (Applied Biosystems™) containing 648 855 SNPs, and the custom array ArBos 1 containing 58 088 SNPs. Raw data were processed using Axiom™ Analysis Suite software 4.0 (Applied Biosystems™), setting sample and SNP call rates at ≥97%. Datasets were exported in .PED and .MAP file format for further analyses. Furthermore, the following filters were applied using the command implemented in PLINK 1.9 software (Purcell et al., 2007): genotype call rate (-mind 0.05), minor allele frequency (-maf 0.05) and Hardy–Weinberg equilibrium (-hwe >0.001). The SNP position was assigned according to the bovine genome reference UMD 3.1. A single genotype matrix was constructed using the merge function in PLINK v1.9, resulting in a final database of 48 360 common SNPs. In addition, a second dataset was constructed, comprising 4964 common SNPs between the microarrays used in the present work and the 76 524 SNPs used by Corredor et al. (2023; Illumina Bovine HD Genotyping BeadChip and Illumina GGP Bovine 100 K BeadChip) for AFB.

Genetic diversity, inbreeding coefficients and kinship

The mean genetic diversity within each population was quantified via the observed heterozygosity (H_O) and expected heterozygosity (H_E), using allele frequency with the -het function in PLINK v1.9. H_O and H_E were calculated using the 48 K database, with the exception of AFB that was estimated using the 76 K microarray.

Two parameters were selected to evaluate inbreeding levels within populations using genomic data, as described below:

Runs of homozygosity

The minimum number of SNPs to compose a run of homozygosity ROH (L) was calculated according to the formula described by Purfield et al. (2012):

$$L = \frac{\left(\frac{\alpha}{n_s \times n_i}\right)}{\left(1 - h_{et}\right)}$$

where n_s is the number of SNPs per individual, n_i is the number of individuals, α is the percentage of false positive ROH (set to 0.05) and h_{et} is the average SNP heterozygosity across all SNPs. The ROH segments were divided into groups according to their length in 1–2, 2–4, 4–8, 8–16 and >16 Mb (Goszczynski et al., 2018). To establish the number of missing genotypes (n_M) and allowed heterozygotes (n_H) in each ROH group, the following formula was used:

$$n_M = \frac{m_L}{d_S} \times m_G$$

$$n_H = \frac{m_L}{d_S} \times e_G$$

where m_L is the ROH minimum length, d_S is the average distance between SNPs in the chromosome, e_G is the genotyping error rate (0.25% according to Applied Biosystems™ standard procedures) and m_G is the average missing genotype rate in the chromosome (Purfield et al., 2012). The ROH were estimated using PLINK v1.9 specifying the parameters: -homozyg, -homozyg-density, -homozyg-kb, -homozyg-snp, -homozyg-window-het, and -homozyg-window-missing. Finally, the inbreeding coefficient (F) of RHO (F_{ROH}) was calculated for each animal by dividing the length of the genome covered by ROH by the total genotyped genome. The parameter values of the PLINK commands mentioned above and the SNP database used to estimate each ROH segment in the analyzed populations are detailed in Table S2.

Inbreeding coefficient

Fhat2, similar to the fixation index (F_{IS}), was calculated using the 48 K database applying the function --ibc in PLINK 1.9 software. This index depends on reliable minor allele frequency estimations, for this reason it was only calculated for breeds/populations with at least 20 animals.

Pairwise kinship coefficients between individuals within and among populations were estimated using the command --make-king-table in PLINK 2 (Chang et al., 2015). This parameter was calculated using the 48 K database, with the exception of AFB that was estimated using the 76 K microarray. The inbreeding and kinship results were visualized in a violin plot using the GGPLOT R package (https://cran.r-project.org/).

Linkage disequilibrium and effective population size

Linkage disequilibrium (LD) was estimated within each population using the command --r2 --ld-window 1000 --ld-window-kb 1000 --ld-window-r2 0 implemented in PLINK 1.9 and visualized with the ggplot R package. In the case of effective population size (N_e),

TABLE 1 Main characteristics of the analyzed populations. Samples sizes were taken from the Domestic Animal Diversity Information System (https://www.fao.org/dad-is).

Breed/population	Population size	Sample size and sex	Breed/population origin	Sample site	Production purpose	Environment
Arequipa Fighting Bull (AFB)	4000000[a]	12 (unknown)	Perú	Arequipa, Perú	Dairy	Highland plain
Argentine (CrAr)	15000	192 (15 males and 179 females)	Argentina	Tucumán, Argentina	Beef	Subtropical to temperate
Altiplano (CrAl)	125000[b]	52 (25 males and 27 females)	Bolivia	Oruro, Bolivia	Beef	Highland plain
Cochabamba (CrCoch)		14 (14 males)	Bolivia	Cochabamba, Bolivia	Beef	Highland plain
Huarino (CrHua)		9 (1 male and 8 females)	Bolivia	La Paz, Bolivia	Beef	Highland plain
Patiño (CrPat)	82	29 (4 males and 25 females)	Bolivia	Santa Cruz, Bolivia	Beef and dairy	Subtropical plain
Saavedreño (CrSaa)	1000	10 (5 males and 5 females)	Bolivia	Santa Cruz, Bolivia	Beef and dairy	Subtropical plain
Yacumeño (CrYa)	1000	17 (4 males and 13 females)	Bolivia	Santa Cruz, Bolivia	Beef	Flood plain
Uruguayan (CrUr)	600	14 (14 males)	Uruguay	Rocha, Uruguay	Beef	Temperate plain
Angus (AA)	Millions worldwide[c]	94 (94 males)	Great Britain	Argentina	Beef	Temperate plain
Holstein (Ho)	Millions worldwide[c]	88 (11 males – 79 females)	Netherlands	Argentina	Dairy	Temperate plain
Brahman (Bh)	Millions worldwide[c]	45 (28 males and 18 females)	United States	Argentina	Beef	Subtropical plain
Nellore (Ne)	Millions worldwide[c]	4 (3 males and 1 female)	Brazil	Santa Cruz, Bolivia	Beef	Subtropical plain
Brangus (Br)	Millions worldwide[c]	335 (301 males and 58 females)	United States	Argentina	Beef	Subtropical plain

[a]Includes the entire Peruvian Creole cattle population that comprises different local types.
[b]The population of Creole cattle on the highland plains (Departments of Oruro, La Paz, Cochabamba) is approximately 125000.
[c]These breeds are spread over several countries comprising millions of heads.

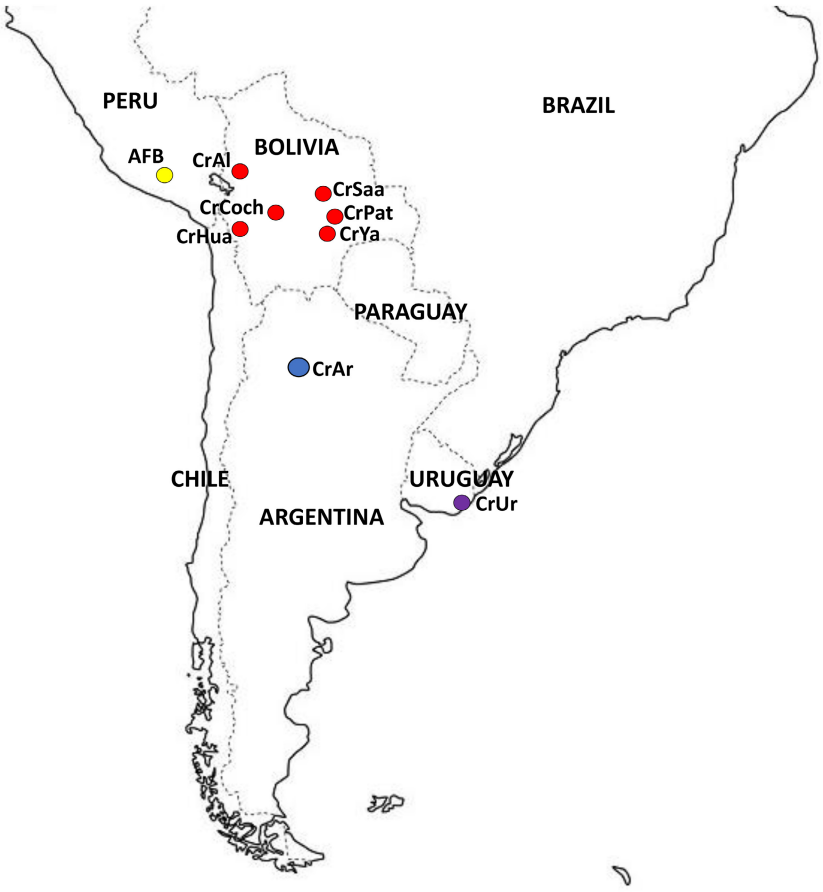

FIGURE 1 Sampling sites of Creole cattle: Argentine Creole (CrAr; blue); Bolivian Creole (red) from Oruro (CrAl), La Paz (CrHua), Cochabamba (CrCoch), Centro de Ecología Aplicada Simón I. Patiño (CrPat), Saavedreño (CrSaa), Yacumeño (CrYa), Peruvian Creole (AFB; yellow) and Uruguayan Creole (CrUr; purple).

which represents the number of reproducing individuals, it was calculated based on LD according to the method implemented in the SNEP software (Barbato et al., 2015) and for the current generation was based on LD using N_EESTIMATOR v2 (Do et al., 2013). The LD and N_e were estimated using the 48 K database, with the exception of AFB that was calculated using the 76 K microarray.

Population composition

The population admixture was inferred using a Bayesian clustering algorithm in FASTSTRUCTURE v1.0 (Raj et al., 2014). To avoid the effect of uneven sample size among populations described by Raj et al. (2014), this cluster analysis was run with a similar number of individuals from each population selected using a random number generator algorithm. This analysis was performed using the 4964 SNPs dataset to include the AFB population. The ChooseK algorithm showed that K12 was the model complexity that maximized marginal likelihood and K2 was the model component used to explain structure in data. The graphical representation of the results was performed using DISTRUCT v.2.3 (Chhatre, 2018). In addition, an alternative cluster analysis was performed using the 48 K dataset, which excluded AFB. SNPs were filtered using the function --indep implemented in PLINK 1.9 with the parameters 50 5 2, resulting in a panel of 25 957 unlinked genetic markers.

Population relationships

A principal component analysis (PCA) was performed to evaluate the relationship between populations. The PCA was calculated with the 4 K and 48 K SNP dataset, with and without the AFB population, respectively, using the --pca function in PLINK v1.9 and the results were visualized using the GGPLOT2 R package. In addition, both PCA plots were also constructed excluding the Br, Bh and Ne populations to observe the relative position of Creole cattle. To avoid the effect of uneven sample size, PCAs were run with 20 individuals selected from each population using a random number generator algorithm. Populations with <20 individuals were also included with all the available samples.

A cluster-stratified gene frequency matrix was constructed using the --freq --family function in PLINK v1.9 for both 4 K and 48 K datasets. Nei's standard genetic distance (Nei, 1972) was calculated using the Gendist programme in the PHYLOGENY INFERENCE PACKAGE (PHYLIP) v3.6 (Felsenstein, 2005). The NEIGHBOR programme of PHYLIP v3.6 was used to build neighbor-joining (NJ) and UPGMA phylogenetic trees. The trees were visualized using FIGTREE v1.4.4. (Rambaut, 2018).

RESULTS

The genetic diversity analyses of Creole populations showed that H_O ranged from 0.367 in CrUr to 0.462 in AFB, whereas H_E varied from 0.352 in CrUr to 0.412 in AFB. These values were similar to those estimated for the taurine and zebuine breeds included in this work. Also, the values of H_O and H_E were similar in all cases (Table 2). The results of the inbreeding levels estimated using two coefficients (F_{ROH}, *Fhat2*) are shown in Table 2 and Figure S1a,b. The F_{ROH} showed that the proportion of the genome covered by the total ROH varied within and among populations, indicating different degrees of inbreeding. In Creole cattle, these values ranged from 0.14 in CrUr to 0.03 in CrCoch and CrPat. The F_{ROH} values were calculated according to their lengths in five categories (1–2, 2–4, 4–8, 8–16 and >16 Mb). Table S3 and Figure 2 show the contribution of each segment where the larger segments, mainly >16 Mb, accounted for the greatest fraction of the total F_{ROH}, especially in CrUr and CrAr. To assess the degree of relationship between a pair of individuals, the kinship coefficient (ϕ) was determined within and among populations. As shown in Figure S2a, the average ϕ values for Creole individuals within populations corresponded to unrelated animals (~0). However, some animals exhibited positive ϕ values compatible with third-degree, second-degree or parent–offspring/full sibling relationships. CrPat presented the highest mean ϕ (0.052 ± 0.08). Commercial breeds that included individuals from several farms and larger population sizes exhibited a similar ϕ distribution. The pairwise kinship analysis between populations had positive values for CrPat–CrSaa and CrCoch–CrAl, evidencing some degree of relatedness (Figure S2b).

Another useful parameter to manage genetic resources and conserve populations is N_e, which is also related to LD levels. Figure S3a,b shows the inferred N_e trends since 121 generations ago and the LD curves for each population, respectively. All breeds/populations exhibited a declining trend in N_e values toward the present, some of which overlapped. However, while the shapes of the curves were almost identical, Creole cattle presented a lower magnitude of N_e values than foreign breeds, with the exception of CrAl. Values of N_e were especially low in CrPat, CrAr, CrCoch and CrUr (Table 3, Table S4). This estimation evidenced that the current N_e of the analyzed Creole cattle population varied from a few individuals to dozens of animals.

The cluster analysis results are summarized in Figure 3 and Figure S4 for 4 K and 25 K SNP panels respectively. K2 clearly differentiated the taurine and Zebu components and showed that Bolivian Creole cattle populations presented Zebu gene introgression. This was mainly observed in CrSaa and CrPat (8.92 and 11.3%, respectively). K3 allowed discrimination of the two taurine components and differentiation of the AA and CrAr populations, whereas K4 added a new taurine component that distinguished Ho. This last component is also present in Creole cattle populations, with the exception of CrAr, and clearly remains in CrAl and AFB even in K13. From K5 to K13, the cluster analysis showed the complexity of Creole cattle from Uruguay, Bolivia and Peru, and evidenced the presence of four Creole groups: CrSaa–CrPat, CrAl–CrCoch–AFB, CrHua–CrYa and CrUr (see K5 and K6 in Figure 3 and Figure S4). The same general pattern was obtained when cluster analysis was performed using the 4 K and 25 K SNP datasets. The PCA results

TABLE 2 Genetic diversity, inbreeding coefficients and kinship values in Angus (AA), Brahman (Bh), Brangus (Br), Argentine Creole (CrAr), Bolivian Creole from Oruro (CrAl), Cochabamba (CrCoch), La Paz (CrHua), Centro de Ecología Aplicada Simón I. Patiño (CrPat), Saavedreño (CrSaa), Yacumeño (CrYa), Peruvian Creole (AFB), Uruguayan Creole (CrUr) and Holstein (Ho).

Breed	$H_O \pm SD$	$H_E \pm SD$	F_{ROH}	*Fhat2*	Kinship[a]
AA	0.359 ± 0.021	0.365 ± 0.0001	0.09 ± 0.04	0.017 ± 0.06	−0.041 ± 0.06
AFB	0.462 ± 0.102	0.412 ± 0.003	0.05 ± 0.09	n.d.	−0.0003 ± 0.13
Bh	0.349 ± 0.015	0.353 ± 0.0003	0.07 ± 0.03	0.016 ± 0.07	−0.036 ± 0.07
Br	0.431 ± 0.017	0.435 ± 0.0002	0.05 ± 0.03	0.013 ± 0.04	−0.021 ± 0.04
CrAl	0.378 ± 0.030	0.389 ± 0.0001	0.05 ± 0.07	0.027 ± 0.08	−0.068 ± 0.09
CrAr	0.377 ± 0.024	0.374 ± 0.0003	0.08 ± 0.05	0.010 ± 0.08	0.002 ± 0.07
CrCoch	0.394 ± 0.023	0.388 ± 0.00007	0.03 ± 0.05	n.d.	−0.042 ± 0.10
CrHua	0.392 ± 0.033	0.382 ± 0.00006	0.04 ± 0.04	n.d.	−0.065 ± 0.03
CrPat	0.413 ± 0.018	0.380 ± 0.0002	0.03 ± 0.04	−0.079 ± 0.07	0.052 ± 0.08
CrSaa	0.408 ± 0.019	0.384 ± 0.00009	0.04 ± 0.03	n.d.	−0.003 ± 0.05
CrUr	0.367 ± 0.031	0.352 ± 0.00007	0.14 ± 0.05	n.d.	−0.019 ± 0.05
CrYa	0.381 ± 0.009	0.368 ± 0.00007	0.04 ± 0.03	n.d.	−0.003 ± 0.05
Ho	0.379 ± 0.009	0.374 ± 0.00006	0.04 ± 0.02	−0.015 ± 0.03	0.0023 ± 0.03
Ne	0.421 ± 0.377	0.378 ± 0.00005	0.03 ± 0.02	n.d.	−0.048 ± 0.04

Abbreviations: F_{ROH}, inbreeding coefficient (F) based on runs of homozygosity (ROH) index; *Fhat2*, similar to the fixation index (F_{IS}); H_E, expected and observed heterozygosity; H_O, observed heterozygosity; n.d., not determined, for breeds with <20 sampled animals.

[a]Average kinship estimated within each population.

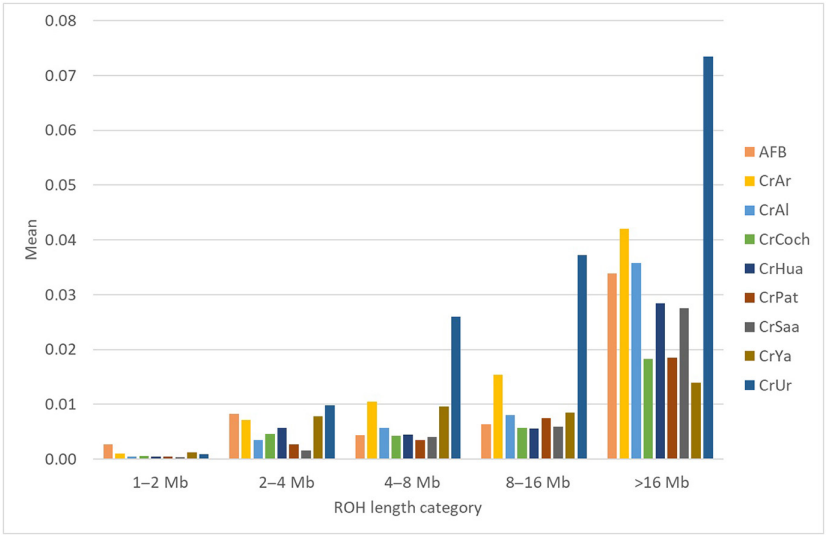

FIGURE 2 The mean sum of inbreeding coefficient (F) based on runs of homozygosity (F_{ROH}) measured in megabases (Mb) within each ROH length category for Argentine Creole (CrAr); Bolivian Creole form Oruro (CrAl), Cochabamba (CrCoch), La Paz (CrHua), Centro de Ecología Aplicada Simón I. Patiño (CrPat), Saavedreño (CrSaa), Yacumeño (CrYa), Peruvian Creole (AFB) and Uruguayan Creole (CrUr).

TABLE 3 Mean values of linkage disequilibrium (r^2[a]) and the effective population size (N_e). The interval and interval mean indicate where the r^2[a] value reaches 0.2 for each population.

Population	Mean r^2[a]	Interval (bp)	Interval median (bp)	N_e[b] (13 gen. Ago)	N_e[2] (current)
AA	0.21	0–100000	50001	185	33.1
AFB	0.18	0–100000	50001	52	36.4
Bh	0.19	0–100000	50001	107	17.4
Br	0.16	0–100000	50001	168	21.9
CrAl	0.15	0–100000	50001	213	39.8
CrAr	0.19	200000–300000	250000	35	9.6
CrCoch	0.21	0–100000	50001	59	10.5
CrHua	0.25	0–100000	50001	35	58
CrPat	0.25	0–100000	50005	58	4.8
CrSaa	0.25	0–100000	50001	39	19
CrUr	0.20	200000–300000	250000	41	13.8
CrYa	0.24	0–100000	50001	73	22.2
Ho	0.16	0–100000	50001	184	31

Abbreviations: AA, Angus; AFB, Peruvian Creole;Bh, Brahman; Br, Brangus; CrAl, Bolivian Creole from Oruro; CrAr, Argentine Creole; CrCoch, Cochabamba; CrHua, La Paz; CrPat, Centro de Ecología Aplicada Simón I. Patiño; CrSaa, Saavedreño; CrUr, Uruguayan Creole; CrYa, Yacumeño; Ho, Holstein.

[a]Values estimated based on linkage disequilibrium (LD) using N_E ESTIMATOR v2.

[b]Values estimated based on LD using SNEP software.

including all populations are shown in Figure S5a.i. The first component accounted for 31.02% of the total variance. This component showed a visible differentiation between the taurine (negative values) and zebuine (positive values) breeds. Within the Creole populations, CrAr clustered apart, whereas the other populations appeared together in the same cloud. The second component captured 12.22% of the total variation and differentiated the taurine populations, with the CrAr located on the top side (higher positive values), the Bolivian and Uruguayan Creole in the middle (around the zero values), and the European taurine on the bottom side (higher negative values). In addition, PCA was carried out excluding the Br, Bh and Ne populations (Figure S5a.ii), showing a clearer discrimination of Creole populations in agreement with the K3 and K4 results mentioned above.

As expected, the NJ tree based on Nei's genetic distance (Figure S5b.i) clearly discriminated between the taurine and zebuine breeds. The composite Br breed was located in an intermediate position between these clusters but closer to taurine, in accordance with its breed composition (Álvarez-Cecco et al., 2022). This result was in agreement with the PCA and admixture analyses. Regarding the taurine group, Creole cattle populations were distributed in sub-clusters according to their historical and geographical distribution, and European breeds formed an additional sub-cluster. The UPGMA tree showed similar results (data not shown). Similar results were observed when the NJ

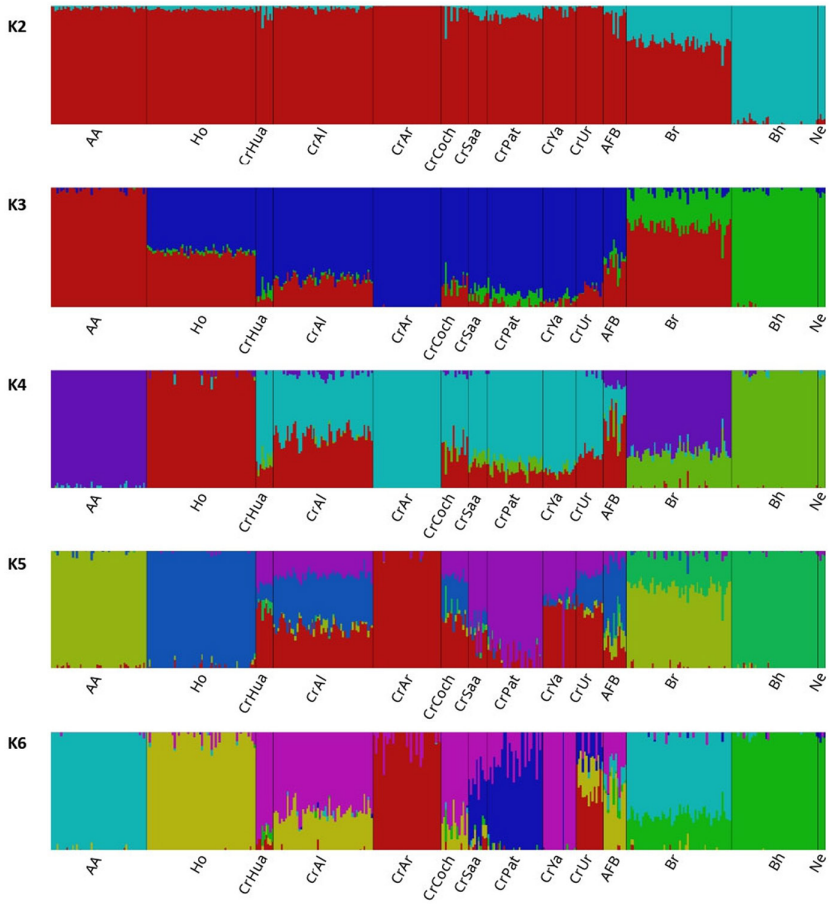

FIGURE 3 Structure analysis (K2–K6) using the 4K dataset for Angus (AA); Brahman (Bh); Brangus (Br); Argentine Creole (CrAr); Bolivian Creole form Oruro (CrAl), Cochabamba (CrCoch), La Paz (CrHua), Centro de Ecología Aplicada Simón I. Patiño (CrPat), Saavedreño (CrSaa), Yacumeño (CrYa); Peruvian Creole (AFB), Uruguayan Creole (CrUr), Holstein (Ho) and Nellore (Ne).

tree was performed with AFB using the 4K dataset (Figure S5b.ii).

DISCUSSION

According to the FAO, there are around 3000 cattle breeds in the world and about 800 are in danger of extinction, while the risk status of another 1500 is unknown because of a lack of population data. For this reason, the need for action to protect them is recognized in the Global Plan of Action for Animal Genetic Resources (FAO, 2007), whose Strategic Priority Area 3 is devoted to the conservation of populations, including the American Creole cattle populations. There are various methods to conserve animal genetic resources, and maintaining genetic variation remains essential. In this sense, SNP panels allow the investigation of genetic diversity between populations and individuals, population structure and inbreeding levels (FAO, 2013). The use of genomic technology in small conserved populations is very informative and highly recommended when possible (Pertoldi et al., 2014), mainly when demographic and pedigree data are incomplete or absent. Most of the previous works in Creole cattle have focused on analyzing the relationships between populations and breed composition, as well as on the detection of parental populations. The present work also evaluates parameters related to genetic erosion risk, such as inbreeding levels, N_e and kinship in Creole cattle from Argentina, Bolivia, Peru and Uruguay, using microarray data.

In the last century, Creole cattle have suffered a drastic population fragmentation and reduction in their population size (Giovambattista et al., 2001; Michiels et al., 2023) because they have been displaced and/or crossed with foreign introduced breeds. It was expected that this process could cause a significant increase of inbreeding level and kinship between purebred animals, resulting in small N_e and genetic diversity loss. Furthermore, the loss of population purity by gene introgression could affect the local fitness (outbreeding depression) of these valuable genetic resources. However, the present analysis shows that the studied populations still exhibit levels of genetic diversity (H_E and H_O ranging between 0.352 and 0.462), comparable with those reported for other cattle breeds (e.g., Cañas-Álvarez et al., 2015; Kawaguchi et al., 2022; McTavish et al., 2013; Michiels et al., 2023; Saravanan et al., 2022). In agreement with these relatively high values of genetic diversity, the Creole cattle populations showed low average inbreeding levels measured through two methods. In the currently studied Creole cattle populations, the proportion of the genome covered by ROH ranged between 3 and 7.6%, which was similar to

those reported for other cattle breeds such as Retinta (3%; Goszczynski et al., 2018), Nelore (4.7%; Zavarez et al., 2015), Tharparkar (6.4%, Saravanan et al., 2022) and Gyr cattle (7.01%, Peripolli et al., 2018). The exceptions were CrUr (14%), with a similar value to Chinese local cattle (11.5%, Xu, Zhao, et al., 2019) and a highly inbred Retinta herd (15%, Goszczynski et al., 2018). This result could be a consequence of the history of the CrUr population that was founded with only 35 animals in the 1930s and remained in genetic isolation for more than 80 years in the Santa Teresa National Park (Armstrong et al., 2013). The total proportions of ROH were distributed in groups according to their length (1–2, 2–4, 4–8, 8–16 and >16 Mb) for each population. Figure 2 shows the main contribution of long ROH, especially >16 Mb, as a result of recent breeding events (Purfield et al., 2012). Moreover, segments of ~10 Mb are traceable to inbreeding that occurred within the last five generations while inbreeding levels that correspond to events occurring in the last 50–20 generations (~1–2 Mb) in the studied Creole populations are scarce (Howrigan et al., 2011).

In accordance with the F_{ROH} results, the *Fhat2* values also showed low levels of inbreeding within Creole populations (<0.027), which were similar to those observed in other breeds like Gyr (Peripolli et al., 2018) and Chinese indigenous cattle breeds (Zhang et al., 2018), but lower than those observed by Pryce et al. (2014) and Saravanan et al. (2022).

In small cattle populations, like most of the studied Creole cattle, it is important to know the kinship between individuals to design crossbreeding that prevents inbreeding as much as possible and maximize N_e. In this study, kinship analysis showed that average values for most populations corresponded to unrelated animals, which could explain the low inbreeding levels mentioned above. However, some animals exhibited values of third-, second- and first-degree relationships between them. Kinship evaluation between populations showed that only animals from CrSaa and CrPat had positive values. This is in agreement with the origin of these populations, because CrPat was established in 2007 in the Centro de Ecología Aplicada Simón I. Patiño with CrSaa animals from the Centro de Investigación Agrícola Tropical (Pereira et al., 2015). This information could be valuable when it is necessary to introduce closely related animals to increase the size and genetic diversity of a small endangered population while maintaining the distinct characteristics of an adapted breed.

Maintaining the N_e across generations is crucial for the long-time survival of a particular zoogenetic resource. Demographic and pedigree data have traditionally been used to obtain N_e estimates in livestock (Flury et al., 2010). However, native populations usually have incomplete or absent demographic and pedigree data, which makes them inapplicable. These approaches remain limited to populations belonging to experimental farms, such as CrAr from Leales, considering that it contains complete pedigree information since 1959, when it was established. To overcome this limitation, we estimated the N_e based on LD using genomic SNP data. As expected, the N_e in the studied commercial and Creole populations declined over generations. The N_e estimation in Latin American Creole cattle is scarce; however, this parameter was reported for several indigenous and highly selected commercial cattle breeds, showing similar decay of N_e over generations (Biegelmeyer et al., 2016; Dlamini et al., 2022; Garcia et al., 2023; Jin et al., 2022; Sargolzaei et al., 2008; Strucken et al., 2021; Sudrajad et al., 2016; Xu, Zhu, et al., 2019). Creole populations had an accelerated decline and presented lower values, consisting of a few individuals compared with dozens of animals for worldwide distributed breeds (Sargolzaei et al., 2008; Biegelmeyer et al., 2016; Campos et al., 2022; present work). Nevertheless, these results were similar to those estimated for indigenous cattle breeds from India (Strucken et al., 2021; N_e = 13, 30 and 43), Bangladesh (Bhuiyan et al., 2021; N_e = 26.02–108.29 until five generations ago), Korea (Sudrajad et al., 2016; N_e = 53–60 11 generations ago), China and Japan (Xu, Zhu, et al., 2019; N_e = 27, 34 and 14 for South and North Chinese cattle, and Black Japanese breed, respectively) and South Africa (Dlamini et al., 2022; N_e = 56–99 for five generations ago). Other native breeds such as Dabieshan Chinese cattle (Jin et al., 2022; N_e = 86 for one generation ago) and Indian native cattle (Strucken et al., 2021; N_e = 399 for one generation ago) exhibited higher values. The significant decrease of N_e in recent generations in Creole cattle populations agrees with the high contribution of large segments of F_{ROH} to the inbreeding values. It is important to remark that values of N_e of more than 100 individuals are necessary to maintain genetic diversity and fitness over the subsequent 10 generations and would be sufficient for survival in the long term (Frankham et al., 2014; Meuwissen, 2009). Considering the estimated values of N_e for the evaluated Creole cattle, it is expected that their population will shrink at least 10% in the next 50 years. This highlights the urgent necessity of applying actions to increase the N_e.

Creole cattle populations are adapted to a wide range of environments, such as highland plateaus, temperate plains and valleys, tropical overflow plains and dry forests, and they are usually isolated from one another. During the process of migration to new environments and the further process of adaptation, these populations can diverge as a consequence of natural selection and/or stochastic evolutionary forces (genetic drift and inbreeding). In addition, recent events of admixture can contribute to genetic divergence. In this context, molecular data can not only be useful to determine the risk status as discussed above, but also to estimate the genetic distance between populations and the breed purity. High genetic similarity to not-at-risk breeds indicates low distinctiveness and thus

diminishes the conservation priority of a breed, considering that economic resources are usually limited. In this work, we addressed this issue through PCA and cluster analyses, showing that the extreme position of CrAr in PC2 agrees with data reported by Michiels et al. (2023) using low density microarrays. However, this contrasts with previous results based on microsatellite data showing that the Argentine and Bolivian Creole populations are located at an intermediate position (Lirón, Bravi, et al., 2006; Lirón, Peral-García, et al., 2006). Ginja et al. (2019) grouped the CrAr and CrUr populations in the same cluster using microsatellites, while CrYa was located with Brazilian Creole populations. In the present work, based on SNP data, Bolivian Creole cattle can be divided into three groups: populations adapted to highlands; subtropical beef lowland; and dual-purpose lowland. The analysis of population structure differentiated the taurine and Zebu components in K2, revealing the introgression of Zebu genes into the Bolivian Creole populations that are located in subtropical lowland plain, and especially in CrPat (0.114) and CrSaa (0.089). This is consistent with data presented by Giovambattista et al. (2000), indicating that Bolivian Creole populations had *Bos indicus* Y-chromosome haplotypes, which were absent in the southernmost populations (CrAr and CrUr) and highland populations (CrAl, CrHua, CrCoch and AFB). This difference could be explained by historical data of geographic distribution and adaptation to environmental conditions. In the eighteenth and nineteenth centuries, Brazil introduced zebuine animals to improve native populations in this tropical region, and these animals were exported to other Latin American countries like Bolivia and crossed with Creole cattle. On the contrary, Bolivia Creole cattle from the highland plain evidenced introgression with Ho, which could be explained by the introduction of this breed to improve dairy production during the last decade. Also, in agreement with Michiels et al. (2023), CrAr appeared as a highly differentiated genetic pool when the *K*-value increased (K3), probably because of the routes of colonization of South America (Martínez et al., 2012).

In conclusion, in the present work we show that it is possible to have a more detailed status of Creole cattle populations by combining different approaches and using genomic data. This allows a more informed management of these populations such as crossbreeding within and among private and experimental herds with similar genetic backgrounds, while avoiding high levels of inbreeding and ultimately preserving adaptive variation.

AUTHOR CONTRIBUTIONS

O. Marcuzzi: Conceptualization; data curation; formal analysis; investigation; writing – original draft; writing – review and editing. **F. Calcaterra:** Data curation; formal analysis; investigation; writing – review and editing. **A. Loza Vega:** Investigation; resources; writing – review and editing. **M. F. Ortega Masagué:** Investigation; resources; writing – review and editing. **E. Armstrong:** Investigation; resources; writing – review and editing. **J. A. Pereira Rico:** Investigation; resources; writing – review and editing. **E. Jara:** Investigation; resources; writing – review and editing. **L. H. Olivera:** Data curation; formal analysis; investigation; writing – review and editing. **P. Peral García:** Conceptualization; funding acquisition; project administration; writing – review and editing. **G. Giovambattista:** Conceptualization; data curation; formal analysis; funding acquisition; project administration; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The genomic data used in the present study are available at the Open Science Framework platform (https://osf.io/cs726; DOI 10.17605/OSF.IO/CS726).

ETHICS STATEMENT

All animal procedures were reviewed and approved by the Institutional Committee on Care and Use of Experimental Animals from the School of Veterinary Sciences of the National University of La Plata (Buenos Aires, Argentina; protocols 89-1-18T, 41.2.14T).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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