Diversity and population structure of Criollo Lechero Tropical and Romosinuano cattle breeds in Mexico

Diversidad y estructura poblacional de las razas bovinas Criollo Lechero Tropical y Romosinuano en México

Diversidade e estrutura populacional das raças de gado Criollo Lechero Tropical e Romosinuano no México

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Abstract

Background: The assessment of genetic diversity, population structure, as well as within and across breed relationships using DNA markers is essential to develop conservation and genetic improvement programs. **Objective:** To assess the genetic diversity and population structure of Criollo Lechero Tropical (CLT) and Romosinuano (ROMO) cattle breeds in Mexico and compare them with other Criollo and Spanish breeds. **Methods:** The study included CLT and ROMO genotypes from the 65k Axiom©BovMDv3 array. Genomic data of Criollo and Spanish breeds were retrieved from a repository. Genetic diversity across breeds was evaluated by pairwise Wright's F_{ST}, principal components, and structure analysis. **Results:** Observed and expected heterozygosity, inbreeding coefficient, and effective population size (*Ne*) were 0.31, 0.30, -0.018 and 56 in CLT, and 0.32, 0.31, -0.023 and 99 in ROMO, respectively. Wright's F_{ST} indicated that both breeds are related to American Criollo and some Spanish cattle. ROMO cattle showed more significant Spanish ancestry than CLT. **Conclusions:** This study presents novel evidence indicating that, although the *Ne* of these breeds is small, they are still diverse and can be used as genetic reservoirs in conservation programs.

Keywords: *cattle; Criollo Lechero Tropical; genetic diversity; DNA markers; effective population size; inbreeding; population structure; Romosinuano.*

Resumen

Antecedentes: La evaluación de la diversidad genética, de la estructura de la población y de las relaciones dentro y entre razas utilizando marcadores de ADN es esencial para el desarrollo de programas de conservación y mejora genética. **Objetivo:** Evaluar la diversidad genética y la estructura poblacional de las razas bovinas Criollo Lechero Tropical (CLT) y Romosinuano (ROMO) de México y compararlas con otras razas criollas y españolas. **Métodos:** El estudio incluyó genotipos CLT y ROMO de la matriz Axiom©BovMDv3 de 65k. Los datos genómicos de razas criollas y españolas fueron obtenidos de un repositorio. La diversidad genética entre razas se evaluó mediante el F_{ST} de Wright para pares de razas, componentes principales, y análisis de estructura. **Resultados:** La heterocigosidad observada y esperada, el coeficiente de endogamia y el tamaño efectivo de la población (*Ne*) fueron 0,31, 0,30, -0,018 y 56 en CLT, y 0,32, 0,31, -0,023 y 99 en ROMO, respectivamente. El F_{ST} de Wright indicó que ambas razas están relacionadas con el criollo americano y algunos bovinos españoles. El ganado ROMO muestra una ascendencia española más significativa que el CLT. **Conclusiones:** El estudio presenta evidencia novedosa que indica que aunque el *Ne* de estas razas es pequeño, todavía son diversas y pueden ser utilizadas como reservorios genéticos en programas de conservación.

Palabras clave: *bovinos; Criollo Lechero Tropical; diversidad genética; endogamia; estructura de la población; marcadores de ADN; Romosinuano; tamaño efectivo de la población.*

Resumo

Antecedentes: A avaliação da diversidade genética, da estrutura populacional e das relações dentro e de todas as raças usando marcadores de DNA é essencial para o desenvolvimento de programas de conservação e melhoramento genético. **Objetivo:** Avaliar a diversidade genética e a estrutura populacional das raças Criollo Lechero Tropical (CLT) e Romosinuano (ROMO) do México e compará-las com outras raças crioulas e espanholas. **Métodos:** O estudo incluiu genótipos CLT e ROMO da matriz Axiom©BovMDv3 de 65k. Dados genômicos de raças crioulas e espanholas foram recuperados de um repositório. A diversidade genética entre as raças foi avaliada pelo F_{ST} de Wright, principais componentes e análise de estrutura. **Resultados:** Heterozisia observada e esperada, coeficiente de endogamia e tamanho populacional efetivo (*Ne*) foram 0,31, 0,30, -0,018 e 56 na CLT, e 0,32, 0,31, -0,023 e 99 em ROMO, respectivamente. A F_{ST} de Wright indicou que ambas as raças estão relacionadas com o crioulo americano e alguns bovinos espanhóis. O gado ROMO mostra ascendência espanhola mais significativa do que a CLT. **Conclusões:** O estudo apresenta novas evidências indicando que, embora *Ne* dessas raças seja pequeno, elas ainda são diversas e podem ser utilizadas como reservatórios genéticos em programas de conservação.

Palavras-chave: *Criollo Lechero Tropical; diversidade genética; endogamia; estrutura populacional; gado; marcadores de DNA; Romosinuano; tamanho populacional efetivo.*

Introduction

The assessment of genetic diversity, population structure, and within and across breeds relationships is essential for the development of conservation and genetic improvement programs of cattle populations (Martínez *et al.*, 2012). The genomic data of the populations, export, divergence, and admixture in domestic cattle can be precisely established (Decker *et al.*, 2014a). Genetic markers have been used worldwide to assess genetic variation between cattle breeds in regard to their geographical origin. The SNP markers have been used to develop Chip SNP, which is a highly informative and density-based microarray technology (Matukumalli *et al.*, 2009). The SNPs have been advantageous for assessing genetic diversity and estimate phylogenetic relationships (Decker *et al.*, 2014a).

Criollo cattle breeds from America have their ancestral sources in Spanish breeds from the New World colonization. It is not known whether these breeds originated from one or more cattle populations (Martínez *et al.*, 2012). The Criollo Lechero Tropical (CLT) cattle breed was developed in Nicaragua under geographic isolation of the original Spanish cattle that underwent later selection for milk production (De Alba, 2011). In Mexico, a CLT nucleus herd was established in the mid-20th century. Purebred CLT imported from Nicaragua and some bulls from Centro Agronómico Tropical de Investigación y Enseñanza (CATIE, Turrialba, Costa Rica) conformed the Mexican nucleus (Rosendo-Ponce and Becerril-Pérez, 2015). The CLT is currently widespread in tropical Mexico given it is a tropically adapted *Bos taurus* dairy breed. Due to its small population (less than 1000 cows), CLT is currently considered an endangered breed (FAO, 2013; Rosendo *et al.*, 2018).

Romosinuano (ROMO) cattle is a Colombian Criollo breed introduced to Mexico by an absorption process of individuals and semen of ROMO bulls from Florida, USA, and Turrialba, Costa Rica (De Alba, 2011). ROMO cattle stands for its easy handling, longevity (Carroll *et al.*, 2011; 2012), fertility (Riley *et al.*, 2007), and tolerance to high temperature and relative humidity (Scharf *et al.*, 2010).

Criollo breeds evolved under different climatic conditions compared with their European ancestry, so they have probably fixed or changed allele frequencies, differing from those of their ancestors due to adaptation and artificial selection for improved performance under these conditions. In Mexico, genetic diversity of CLT and ROMO has been evaluated through pedigree analysis (Rosendo *et al.*, 2018; Núñez-Domínguez *et al.*, 2020). However, within and across breed genetic diversity and structure of CLT and ROMO have not been evaluated using genomic approaches. Currently, the use of variable density SNP microarrays has been implemented to evaluate genetic diversity and differentiation between breeds to provide an efficient genome-wide perspective of genetic flow or isolation in specific populations (Mastrangelo *et al.*, 2014; Savaranan *et al.*, 2021).

Therefore, this study was aimed to assess genetic diversity and structure of CLT and ROMO cattle breeds in Mexico and compare them with other Criollo ancestor breed populations using a medium-density SNP array.

Materials and Methods

Source of data

All CLT and ROMO animals included in this study belong to herds affiliated to *Asociación Mexicana de Criadores de Ganado Romosinuano y Lechero Tropical*, and are being used in a genetic improvement program by the breeders. Seventy-five animals were randomly sampled from each breed. Hair follicles were collected from each animal. Samples were genotyped using the Axiom© BovMDv3 array consisting of 63,791 SNPs (Affymetrix, Santa Clara, CA, USA). Monomorphic SNPs were excluded, resulting in 55,760 SNPs for CLT and 55,579 for ROMO. Genotypic data from an Illumina BovineSNP50 BeadChip array with 1,539 animals of 127 breeds were retrieved from a public repository (Decker *et al.*, 2014b). The SNPs had been filtered by the authors based on a calling rate of 0.9, and

monomorphic SNPs were excluded, remaining 43,043 SNPs available. To properly compare genotypic data from the present study with those of the public repository, we used a similar SNP edition to the one used by Decker *et al.* (2014b), which ignores the use of linkage disequilibrium. Only data from Holstein, Jersey, Angus, and Brown Swiss animals were included as European cattle breeds. Eleven native Spanish breeds (Berrenda Negro, Berrenda Colorado, Negra Andaluza, Cárdena Andaluza, Toro de Lidia, Pirenaica, Mostrenca, Terrana, Menorquina, Morucha, Retinta), five Criollo breeds (Texas Longhorn, Colombian Romosinuano, Corriente, Senepol, Florida Cracker), and three breeds of Asian indicine cattle (Guzerat, Sahiwal, Gyr) were selected for further analysis. Genotypic data were merged by SNP´s name and location with CLT and ROMO data using PLINK v1.9 software (Purcell *et al.*, 2007). Finally, a total of 18,192 SNPs were shared between the panels.

Within-breed genetic diversity

Observed (*Ho*) and expected (*He*) heterozygosity and inbreeding coefficient (*F_{IS}*) were calculated for the CLT and ROMO samples using the PLINK software (Purcell *et al.*, 2007). Trends of the effective population size (*Ne*) were calculated from linkage disequilibrium throughout the CLT and ROMO genomes using the SNeP V1.1 software (Barbato *et al.*, 2015).

Across-breed genetic diversity

Pairwise *F_{ST}* between CLT, ROMO, and Criollo, Spanish, and indicine breeds were calculated using the PLINK software (Purcell *et al.*, 2007). Principal components (PC) were calculated using the merged SNP dataset considering only autosomal SNPs. The genomic relationship matrix was calculated using the GCTA software (Yang *et al.*, 2011). The R package OmicKriging (Im *et al.*, 2016) was used to access the matrix information and subsequently estimate PC using the RSpectra package of R (Qiu *et al.*, 2019), and the top two PC were plotted against each other considering CLT, ROMO, and the five Criollo, eleven Spanish, and three indicine breeds.

Using the SRUCTURE v2.3.4 software (Pritchard *et al.*, 2000), population structure analyses were performed with an initial Burn-in of 25,000, and 25,000 additional Markov Chain Monte Carlo (MCMC) runs. The dataset was processed for five iterative cycles, each with constant Burn-in and MCMC. The four European, eleven native Spanish, five Criollo, and three breeds of Asian indicine cattle were included to consider the possible subpopulations of ancestry in CLT and ROMO. K-values from 2 to 13 were tested with the STRUCTURE harvester software (Earl and vonHoldt, 2012) to infer the best fit Delta K-value between populations using the Evanno's method (Evanno *et al.*, 2005).

Results

Within-breed genetic diversity

The *Ho* and *He*, average *F_{IS}*, and *Ne* were calculated (Table 1). ROMO showed greater difference between *Ho* and *He* compared to CLT cattle. The *Ne* trend across the last 100 generations is shown in Figure 1. The *Ne* for both breeds has been decreasing over time. The generation intervals of ROMO and CLT breeds were 6.7 (Núñez-Domínguez *et al.*, 2020) and 6.9 years (Rosendo *et al.*, 2018), respectively. The first trips carrying cattle from Spain to America were reported in 1524 (Ajmone-Marsan *et al.*, 2010). In 70 generations, the *Ne* was reduced from 317 to 56 in CLT, and from 394 to 99 in ROMO. None of these breeds had a *Ne* value less than the lower limit (<50) recommended for conservation of genetic resources (FAO, 1998).

Table 1. Observed (*Ho*) and expected (*He*) heterozygosity, inbreeding coefficient (*F_{IS}*), and effective population size (*Ne*) of Criollo Lechero Tropical (CLT) and Romosinuano cattle from Mexico.

Breed	*Ho*	*He*	*F_{IS}*	*Ne*
CLT	0.310	0.304	-0.018	56
Romosinuano	0.326	0.318	-0.023	99

Across-breed genetic diversity

Wright's *F_{ST}* values calculated between CLT, ROMO, and established cattle breeds are shown

in Table 2. The smallest Wright's F_{ST} value among ROMO with a Criollo breed (omitting the comparison with Colombian Romosinuano) was with Texas Longhorn, while the lowest values compared to Spanish breeds were with Andalusian breeds. The same results were observed with CLT. The highest F_{ST} values were with *Bos indicus* breeds for both CLT and ROMO. The F_{ST} values between CLT and ROMO with *Bos indicus* breeds were 0.25 and 0.23, respectively.

The main two PC were calculated, and animal samples were graphically represented (Figure 2). *Bos taurus* and *Bos indicus* species showed the largest difference in principal components. Criollo and Spanish cattle breeds showed a slight separation between clusters. CLT and ROMO were clustered with the Criollo breeds; however, these breeds exhibited large spread as PC points.

Genetic structure analyses are shown in Figure 3. The best fit given all the populations was determined as K=10, indicating that both CLT and ROMO show different structure compared to the other breeds in the study. However, considering the K-value of 4, ROMO and CLT shared 80 and 81%, respectively, of a common ancestral Criollo and Spanish subpopulation breeds, which shows the relationship between Criollo and Spanish cattle, grouping in the same cluster as observed in the PC analysis. The largest ancestral subpopulation that shows ROMO in Mexico is shared with Colombian Romosinuano. This subpopulation represents, on average, 75% of ROMO genes, which at the same time, this subpopulation represents on average 7% in the Spanish breeds, The other ancestral subpopulations in the ROMO population represent 10% of the subpopulation shared mainly with CLT; 2% of the subpopulation shared with Criollo cattle such as Texas Longhorn and Corriente; and 8.7% belongs mainly to subpopulations of *Bos indicus* ancestors. CLT cattle shows a subpopulation that covers 89% of its ancestry. This ancestral subpopulation is not shared with high percentages in other bovine breeds. Spanish breeds such as Cárdena Andaluza, Negra Andaluza, and Berrenda en Colorado show a slight proportion of this ancestral subpopulation (3% on average).

Table 2. Pairwise F_{ST} between Criollo Lechero Tropical (CLT) and Romosinuano breeds with other cattle breeds.

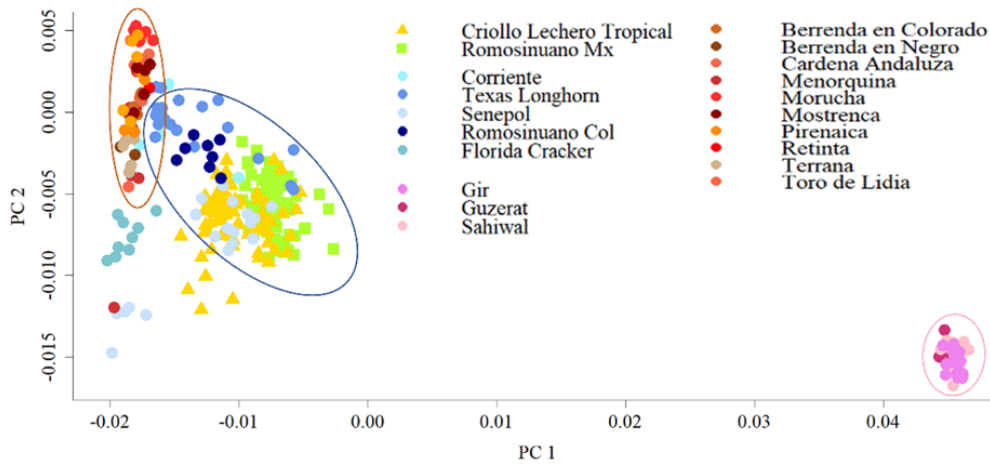| Breed	Romosinuano	CLT
Texas Longhorn	0.0760	0.0791
Colombian Romosinuano	0.0769	0.1006
Corriente	0.0795	0.0825
Senepol	0.0822	0.0854
Florida Cracker	0.1191	0.1188
Berrenda Negro	0.0698	0.0685
Berrenda Colorado	0.0757	0.0768
Negra Andaluza	0.0800	0.0802
Cárdena Andaluza	0.0821	0.0828
Toro de Lidia	0.0854	0.0850
Pirenaica	0.0860	0.0860
Mostrenca	0.0882	0.0876
Terrana	0.0971	0.0960
Menorquina	0.1027	0.1023
Morucha	0.1512	0.1513
Retinta	0.1612	0.1611
Angus	0.1204	0.1203
Brown Swiss	0.1343	0.1369
Holstein	0.1082	0.1035
Jersey	0.1513	0.1447
Guzerat	0.2083	0.2275
Sahiwal	0.2394	0.2584
Gir	0.2417	0.2603

Discussion

The *Ho*, *He*, and F_{IS} values have been used to reference genetic diversity of small and indigenous populations. The *Ho* values were higher than *He* in both breeds of the present study. Similarly, *Ho* values were higher than *He* in Tharparkar cattle (Saravanan *et al.*, 2020). Also, the Korea Brown Hanwoo, Brindle Hanwoo, and Jeju Black native cattle showed great genetic diversity, having *Ho* greater than *He* (Sharma *et al.*, 2016). Unfortunately, the sample of Colombian ROMO in the Decker's *et al.* (2014) paper was too small to make meaningful comparisons with the results of the present study.
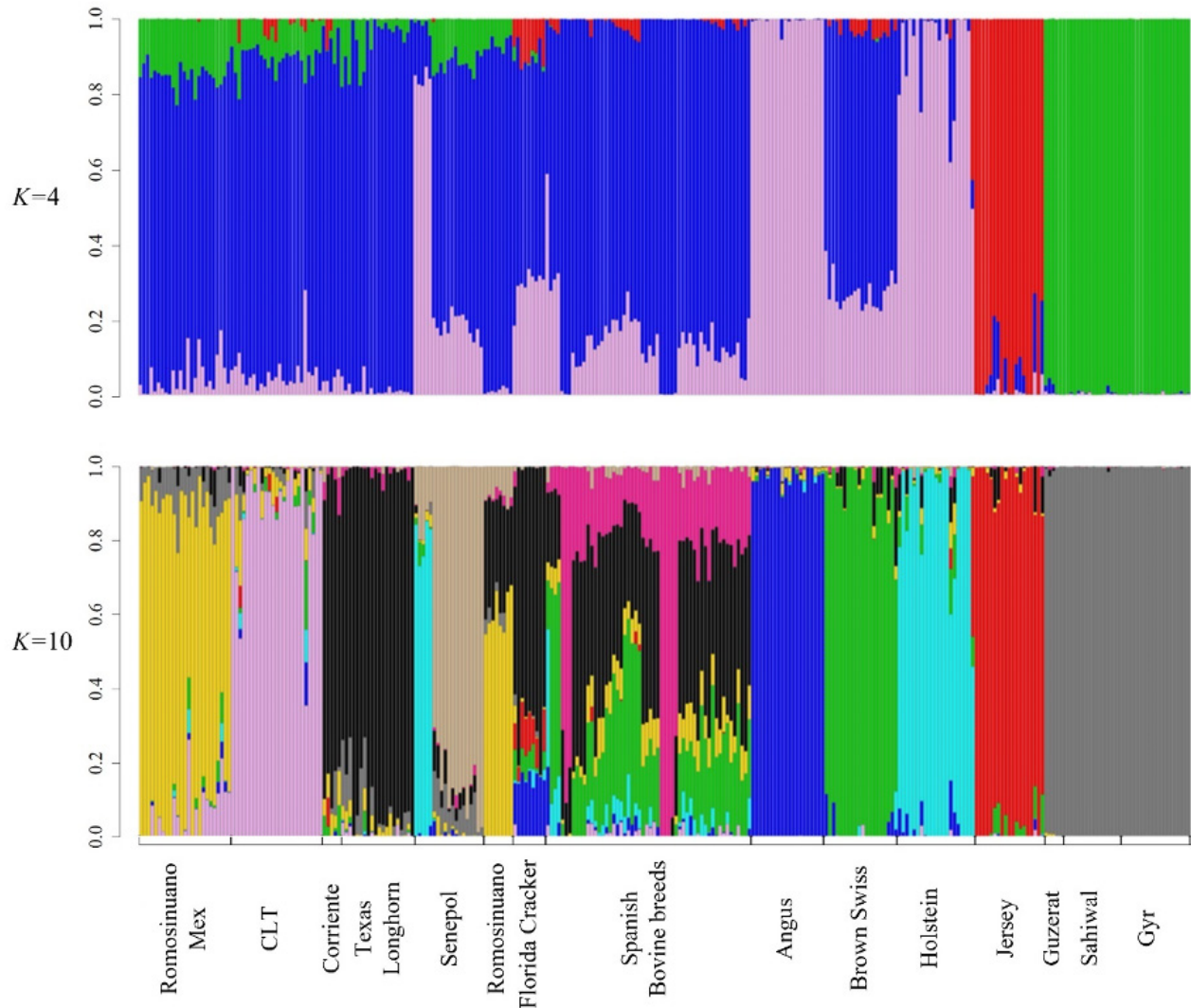
The negative F_{IS} values in the present study (-0.018 and -0.023 for CLT and ROMO, respectively) correspond to inbreeding levels
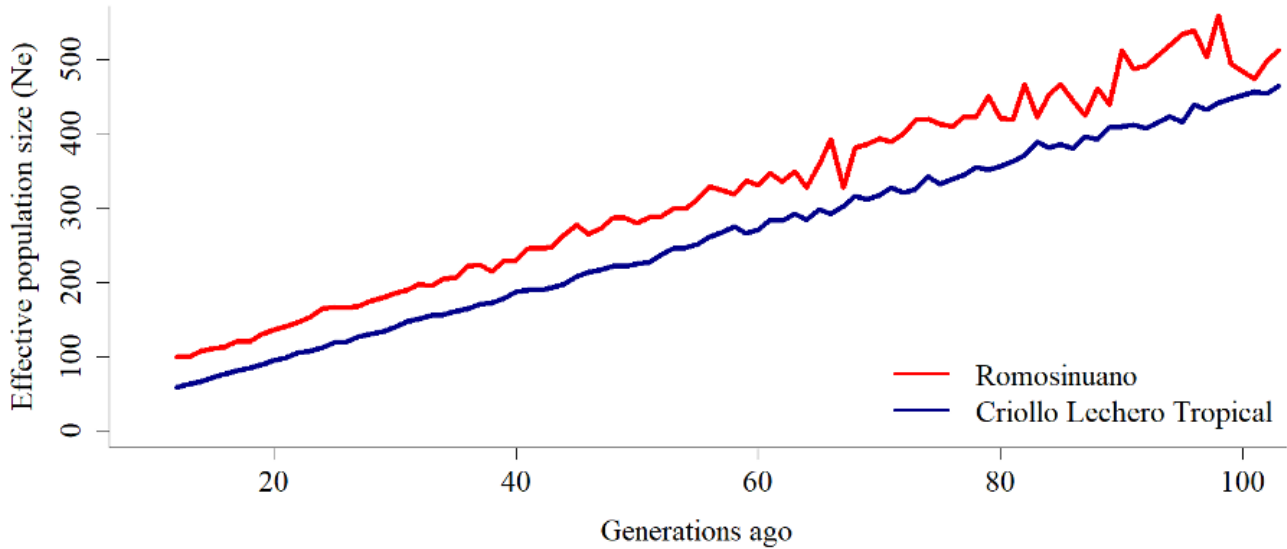
Figure 1. Estimated effective population size trend from the last 100 generations.

Figure 2. Principal components plot of Criollo Lechero Tropical, Mexican Romosinuano, American Criollo, Spain native and indicine cattle breeds.

Figure 3. Structure analysis with K=4 and K=10 (best fit) in Criollo Lechero Tropical, Romosinuano, and cattle reference populations.

lower than average homozygosity of the populations (0.310 for CLT and 0.326 for ROMO). Different results were obtained by Eusebi *et al.* (2017) who reported average F_{IS} values of 0.06 and 0.01 for the same repository data from the Spanish and Criollo breeds used in the present study. Likewise, Rosendo *et al.* (2018) and Núñez-Domínguez *et al.* (2020), based on a pedigree information analysis, estimated 0.01 and 0.025 inbreeding coefficients for CLT and ROMO, respectively. Using SNP, the parameters calculated in the present study showed higher genetic diversity within breeds than the pedigree analysis.

Therefore, within-breed genetic diversity studies are necessary for Criollo and local breeds to be used in a productive and economic environment and to avoid inbreeding problems (Meuwissen, 2009).

In general, the differences with Spanish and Criollo breeds are categorized as 'moderate differentiation' for both breeds, while 'great differentiation' was observed with indicine breeds (Weir and Cockerham, 1984). The lowest Wright's F_{ST} values among Mexican ROMO, CLT, and the Spanish breeds agree with reports mentioning that cattle arrival to the New World occurred in the

second and later trips of Christopher Columbus. Cattle left Seville and nearby ports, so Andalusian cattle would be the most likely ancestors of Criollo breeds (Ajmone-Marsan *et al.*, 2010).

Gautier *et al.* (2010) reported F_{ST} values between European cattle and *Bos indicus* breeds between 0.29 and 0.38. This result is consistent with Decker *et al.* (2014a) and Martínez *et al.* (2012) who mention that Criollo breeds have indicine ancestry, which occurred after the arrival of Spanish cattle to the New World. Miretti *et al.* (2004) suggest that Zebu's influence in Criollo cattle may be due to imports of *Bos indicus* from Africa to improve adaptability to tropical conditions during the colonial period.

The long-term genetic variation maintained in the population is proportional to the effective population size (FAO, 1998). The *Ne* from genealogical records was calculated for CLT and ROMO in México (Rosendo *et al.*, 2018; Núñez-Domínguez *et al.*, 2020). One way to estimate *Ne* is by using pedigree; however, these estimates depend on the integrity of genealogical records (Barbato *et al.*, 2015). According to the *Ne* trends estimated in the present study the *Ne* value published by pedigree analysis (Rosendo *et al.*, 2018) was reached thirteen generations earlier, while the value has not yet been reached in ROMO (Rosendo *et al.*, 2018). Hidalgo *et al.* (2021) estimated a *Ne* of 72 in generation 1 from a genomic sample of the same ROMO population in Mexico. FAO (2013) suggested a minimum *Ne* of 50 per generation to maintain genetic diversity. The estimated *Ne* in our study was above 50.

Estimates of *F* and *Ne* values in CLT from the present study (-0.018 and 56, respectively) differ from those of Rosendo *et al.* (2018) using pedigree analyses (0.043 and 68.1). Similarly, estimates of *F* and *Ne* in ROMO from the present study (-0.023 and 99, respectively) differ from those of Núñez-Domínguez *et al.* (2020) using pedigree analyses (0.026 and 45, respectively). These differences are expected since estimates from pedigree analyses are based on probabilities of common genes among relatives, while genomic analyses rely on common SNP markers; therefore,

the latter procedure is more accurate. Conservation strategies for CLT and ROMO should consider estimates from genomic analyses to support better decision making.

CLT and ROMO remained close to Criollo breeds in the PC plot, mainly with Texas Longhorn, Corriente, and Senepol, confirming the slight differentiation from native Spanish and wholly differentiated from *Bos indicus*-like observed in the Wright's F_{ST} comparison. Strucken *et al.* (2021) mentioned that *Bos indicus* breeds show low across-breed genetic diversity among breeds because the *Ne* of Indian indigenous breeds have been large since domestication. Contrarily, criollo breeds were developed with a small *Ne* since the arrival of the Spanish people to America; therefore, criollo breeds show wide across-breed genetic diversity.

The PC analysis considers a fraction of the variance included in the genotyped animals, which explains this variability in terms of a minimum of principal components (Figure 2). Pritchard *et al.* (2000) suggested STRUCTURE as an exploratory tool when inspecting results from a range of values of K. A delta K-value of 10 was the best at separating cattle subpopulations, being sensitive to the number of breeds and population size. A delta K-value of 4 clearly separates (Figure 3) four subpopulation groups (*Bos taurus, Bos indicus,* Criollo, and Spanish cattle), in agreement with the clusters observed in the PC analysis plot (Figure 2).

The results of the structure analysis support Wright's F_{ST} coefficients. ROMO cattle has a low percentage of indicine origin ancestry present in some Criollo populations (Decker *et al.*, 2009; Martínez *et al.*, 2012; Decker *et al.*, 2014a). CLT cattle only presented 2% of *Bos indicus* ancestors. Using only 19 microsatellite markers and calculating the contributions by the likelihood estimation of admixture proportions with the LEADMIX software, Martínez *et al.* (2012) found that Iberian breeds significantly contributed to Criollo cattle (between 70 and 80%). With the same number of microsatellite markers and a high K-level, a set of Criollo bovine breeds did

not show clear admixture evidence with any of the other breeds evaluated (Ginja *et al.*, 2019). There was a lower proportion of ancestry in other subpopulations of European breeds due to origin differences of British, continental, and Spanish cattle (Decker *et al.*, 2009). Our results strongly support the idea that Criollo breeds derived their own genetic identity and deserve to be adequately managed and conserved.

ROMO and CLT in America have originated different genetic structures between European, Spanish, and Criollo breeds. Exportation, admixture, and breed formation greatly impact variation between breeds (Decker *et al.*, 2014a). Breed diversity is helpful because it provides alternatives when commercial breeds have problems due to genetic drift or if changes in the production sector require poorly developed traits in the commercial breeds (Weir and Cockerham, 1984). For the assessed breeds, our results suggest enough diversity as a genetic reservoir considering their essential advantages in tropical environments (Hammond *et al.*, 1996; Johnson *et al.*, 2012; Becerril-Pérez *et al.*, 2020) and preserving their productivity in these conditions (Rosendo-Ponce and Becerril-Pérez, 2015; Riley *et al.*, 2015).

In conclusion, although CLT and ROMO cattle represent small populations, within-breed genetic diversity parameters confirm their degree of heterozygosity. CLT and ROMO diverge from ancestor Criollo populations suggesting that they developed their own genetic pool during adaptation to the challenging environment. Given that CLT and ROMO breeds are tropically adapted *Bos taurus*, they have become a valuable genetic resource that justifies structured and constant conservation and utilization programs to preserve their genetic diversity.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest regarding the work presented in this report.

Author contributions

All authors conceived and validated the study, contributed to methods and to writing the paper; data curation and formal analysis, Ricardo E. Martínez-Rocha, Gaspar Manuel Parra-Bracamonte and José G. García-Muñiz; writing-review and editing, Rodolfo Ramírez-Valverde, Rafael Núñez-Domínguez and José G. García-Muñiz; supervision, Rodolfo Ramírez-Valverde and Gaspar Manuel Parra-Bracamonte; management to get support from CONARGEN, Rafael Núñez-Domínguez. All authors read and agreed to the published version of the manuscript.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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