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Evaluation of runs of homozygosity and genomic endogamy in the Creole breeds Guaymi and Guabala in Panama

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The inbreeding coefficient measures the likelihood of identical alleles at a locus in a population due to descent from a common ancestor, highlighting potential negative impacts on health and fitness in both natural and domesticated populations. This study focuses on homozygous segments continuous genomic regions of homozygosity resulting from the inheritance of identical haplotypes from both parents and their role in assessing genomic inbreeding and understanding genetic history and relationships within populations. Such analysis can reveal recessive disease risks. Specifically, the research assessed homozygous segments and the genomic inbreeding coefficient in Creole cattle breeds Guaymi and Guabala in Panama using 10,000 single nucleotide polymorphisms (SNP) markers. Findings showed significant differences in homozygosity between breeds, with Guabala exhibiting higher inbreeding levels, suggesting varied breeding histories or intense selection. The study also detected homozygosity patterns indicating genetic links or shared ancestors between breeds, underscoring the impact of environmental factors and human intervention on genetic diversity. Geographic isolation and artificial selection were key influences on the genetic structures of Guaymi and Guabala breeds, respectively. This underscores the balance between maintaining genetic diversity for adaptability and selecting for desirable traits, emphasizing the importance of managing genetic health and biodiversity for sustainable population viability.

Key words: Bioinformatics, biotechnology, genomics, creole, livestock, conservancy, Guaymi, Guabala, Panama.

INTRODUCTION

The inbreeding coefficient (F) is defined as the probability that two alleles at a randomly sampled locus in a population are identical by descent (IBD) relative to a base population in which all alleles are independent (Wright, 1922). Inbreeding is the result of mating between closely related individuals, and the resulting detrimental

effects on progeny performance and fitness have been widely documented in both natural and domesticated animal populations (Bjelland et al., 2013).

Runs of homozygosity (ROH) are contiguous regions of the genome in which an individual is homozygous at all sites (Gibson et al., 2006). ROHs arise when two copies of an ancestral haplotype come together in an individual. Consequently, this haplotype is autozygous, that is, homozygous by descent (Ceballos et al., 2018).

ROH in cattle have been used to analyze the history of a population after a recent selection event (Purfield et al., 2012), to estimate the coefficients of consanguinity (Ferencakovic et al., 2011, 2013), to study the detrimental effects of inbreeding on the characteristics that affect the profitability of a farm (Bjelland et al., 2013) and to control the increase in inbreeding in cross designs assisted by genome analysis (Pryce et al., 2012).

A fundamental aspect in the study of genomic homozygosity is islands of ROH. Unlike homozygous segments, which include any genomic area where the alleles of homologous chromosomes are identical, a situation that can arise both by inbreeding and randomly, especially in short regions of the genome (Purfield et al., 2012), ROH islands specifically refer to homozygous segments that are remarkably extensive and/or frequent in a population. These islands are of special relevance in population genetics since they can indicate histories of inbreeding or natural selection processes or even be linked to certain diseases or phenotypic characteristics (Toro-Ospina et al., 2022).

The study of genomic inbreeding based on ROH can provide important information about the genetic history and kinship relationships within a population. It may also have health implications, as homozygous regions may contain rare genetic variants associated with recessive diseases. Furthermore, genomic inbreeding can affect the viability and general health of a population through the loss of genetic diversity and the appearance of deleterious recessive traits (Ferencakovic et al., 2013; Forutan et al., 2018).

The coefficient of inbreeding F_{ROH} is a specific parameter that is related to ROH and is used to quantify genomic inbreeding based on ROH in an individual or a population (Scienscki et al., 2019). The F_{ROH} coefficient is calculated by dividing the sum of the lengths of all the ROH segments in an individual's genome by the total length of the autosomal genome (Peripolli et al., 2018a).

Principal Component Analysis (PCA) is a widely used statistical technique in population genetics to identify structures in the distribution of genetic variation across geographical locations and ethnic origins. It has been applied in livestock to analyze single nucleotide polymorphisms (SNPs) data to detect population structures and potential outliers, as well as to identify small panels of genetic markers that can be used to trace the origin of unknown livestock samples. For instance, Abraham and Inouye (2014) developed flashpca, a highly efficient PCA implementation based on randomized algorithms, capable of performing PCA on large SNP datasets much faster than existing tools, without losing accuracy in extracting the principal components (Abraham and Inouye, 2014).

Creole cattle are significant genetic assets because of their exceptional adaptation to local environments and their resistance to diseases. A thorough genetic characterization, encompassing Runs of Homozygosity (ROH) analysis, islands of ROH, and F_{ROH}, can provide valuable insights for the conservation of these breeds (Gaspar et al., 2023). This process is instrumental in identifying unique genetic markers and devising strategies for the sustainable preservation and utilization of these valuable genetic resources. Conversely, inbreeding poses a risk by potentially increasing the prevalence of harmful alleles, which can diminish individual fitness and the overall viability of the population (Dixit et al., 2020). By quantifying F_{ROH}, we can gauge the levels of inbreeding and assess its possible effects on the health, productivity, and genetic robustness of these breeds. This evaluation is especially vital for conservation and breeding programs aimed at sustaining healthy populations while safeguarding their distinctive genetic characteristics (Liu et al., 2022). The objective of this work was to evaluate the ROH and quantify the genomic inbreeding coefficient derived from the ROH (F_{ROH}) of the Creole cattle breeds Guaymi and Guabala in Panama.

MATERIALS AND METHODS

Thirty-four samples from the Creole cattle breeds Guabala (15) and Guaymi (19) selected from an array of 10,000 SNP markers were analyzed using a DNA sequencer from the company Affymetrix as part of the Innovative Management of Animal Genetic Resources (IMAGE) project sponsored by the FAO. The objective of this project is to develop and provide free, publicly accessible multi-species single nucleotide polymorphism (SNP) arrays tailored for major farm animal species. These arrays are designed to genotype genetic collections at an affordable cost, aiming to keep expenses under $20 per sample https://www.imageh2020.eu/conteudo.php?idm=18&lang=en. Five milliliters of venous blood were taken from the jugular area of each animal. The samples were collected in tubes with EDTA and kept in a container with ice until their arrival at the laboratory, where they were immediately processed. DNA extraction was carried out using a commercial DNeasy Blood and Tissue Kit from Qiagen (Germany), for which the average concentration was 45 ng/ml and the volume was 50 μL per sample, for a total amount of 2.5 μg of DNA. The extracted DNA was sent to the company Affymetrix in the Netherlands for analysis. This work complied with the Nagoya Protocol for Access and Benefit Sharing of Genetic Resources (FAO, 2019) through a material transfer agreement (MTA) between the Institute of Agricultural Innovation and Wagenigen University and transfer permit # SEX/A-1-2021 from the Ministry of the Environment of the Republic of Panama.

Of the 10,000 SNPs selected, 8,416 met the company's quality control criteria. All the SNPs were aligned with the reference genome (*Bos taurus* UMD 3.1.1/bos Tau8). Once the data were obtained, quality control was carried out to eliminate the SNPs that did not meet the established criteria (filtered SNPs with a high failure rate, SNPs with high variability and SNPs in linkage
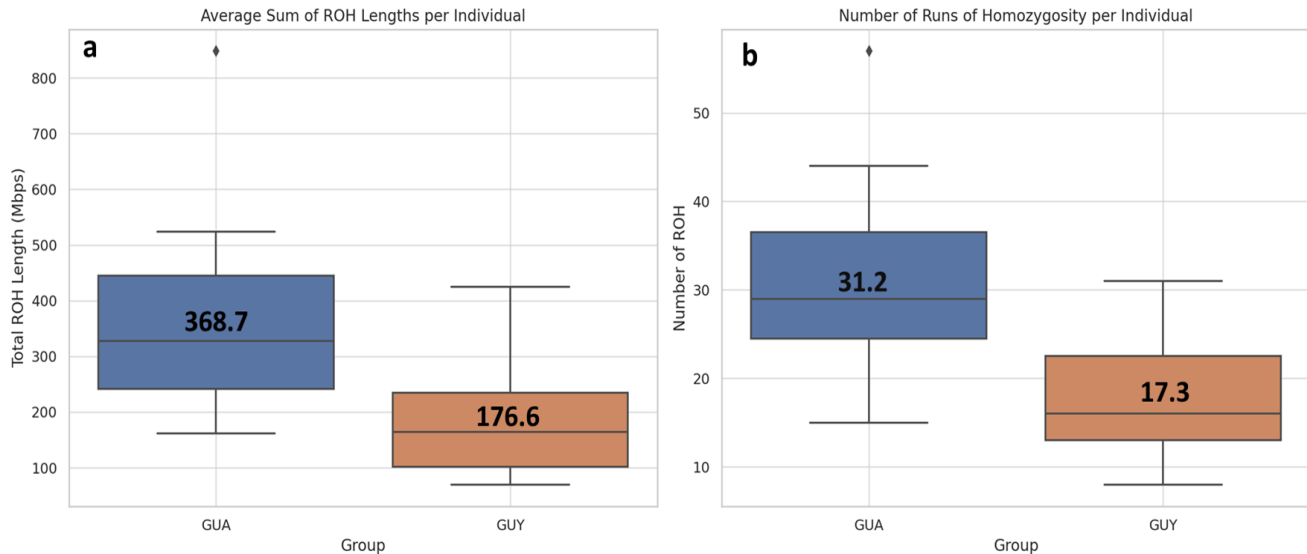
Figure 1. (a) Average length of ROH by population; (b) average number of ROH per population.

disequilibrium) using the PLINK 1.9 program in the R and RStudio platforms. The following criteria were applied: loss by SNP, --gene (0.1), losses per individual, --mind (0.1), minor allele frequency, --maf (0.05), and deviations from Hardy equilibrium -Weinberg --hwe (0.001). After quality control, there remained 7,282 SNP variants that fulfilled the quality control criteria.

The ROH were estimated for each individual separately and subsequently classified into five categories according to length (0-2, 2-4, 4-8, 8-16, and > 16 Mb) following the classification methods used in similar studies (Kirin et al., 2010; Marras et al., 2015). For each ROH category in both breeds, the total number of ROH per breed (nROH), percentage of ROH per breed (%ROH), and mean number of ROH per breed (M_{ROH}) were calculated.

To identify islands of homozygosity, a comparative analysis of ROH between individuals was performed. The runs were grouped by their genomic location, considering as islands those regions of homozygosity that appeared in a significant percentage of the population. An initial threshold of 50 to 20% was established until the presence of at least one island of ROH was determined. ROH that overlapped or were close to each other in at least 20 to 50% of the individuals were considered part of an island of ROH.

To carry out the genomic inbreeding analysis, two parameters were used. First, the F_{ROH} coefficient was calculated by determining the ratio of the length of the genome found in ROH to the total length of the genome covered by the SNPs using PLINK 1.9 software (Purcell et al., 2007). The second parameter was the inbreeding coefficient, focusing especially on the F_{HOM} coefficient, which is based on the difference between the observed and expected number of homozygous genotypes; this analysis was also performed using PLINK 1.9 (Purcell et al., 2007). To complement the genetic study of the Guaymi and Guabala cattle populations, a Principal Component Analysis (PCA) was carried out using the PLINK 1.9 software (Purcell et al., 2007), utilizing the dataset of 7,200 SNP data that passed quality control. The analysis allowed for summarizing the genetic variation in a few dimensions representing the largest sources of variability among the samples. The PCA provides an additional perspective on the genetic structure of the studied populations, complementing the ROH and genomic inbreeding analyses for a better understanding of diversity and inbreeding in these Creole breeds.

RESULTS AND DISCUSSION

One of the critical considerations of this study is the sample size analyzed. The populations of the Guaymi and Guabala breeds examined come exclusively from conservation centers, where their genotype is known, and their genetic purity is guaranteed. This methodological selection was intentionally designed to ensure that the analyses accurately reflect the inherent genetic structure of these breeds, free from external genetic contamination that could distort interpretations of inbreeding and genetic diversity. However, it is important to acknowledge that, although this strategy ensures the authenticity of the samples, it also limits the generalization of our findings to all populations of these breeds. The representativeness of our samples is affected by the fact that, even within conservation centers, the populations of Guaymi and Guabala are relatively small. This implies that any extrapolation of our results to the general populations of these breeds must be done with caution.

The evaluation of the ROH in the Guabala and Guaymi cattle populations revealed notable differences. In the Guabala cattle, 14 ROH were identified, with an average length of approximately 1468.45 kb. On the other hand, in the Guaymi cattle, 11 ROH were found, with an average length of 1004.48 kb. These findings suggest a greater extent and number of ROH in Guabala cattle, indicating differentiated levels of inbreeding or different breeding histories between the two populations.

Similarly, Figure 1a shows that the total length of the ROH in the Guabala cattle, with 368.70 Mega base pairs (Mbps), was greater than that reported in other breeds, such as Hereford, with 378 Mbps (Szmatola et al., 2019), and significantly greater than that in *Bos indicus* breeds,

Table 1. Descriptive statistics of homozygous individuals, total number of ROHs by race (nROH), percentage of ROHs by race (% ROH) and mean number of ROHs by race (M ROH).

Categories (Mb)	nROH		% ROH		M_{ROH}	
	GUA	GUY	GUA	GUY	GUA	GUY
0-2	26	32	5.60	9.70	0.71	1.18
2-4	26	22	5.60	6.70	2.72	2.96
4-8	123	90	26.30	27.40	6.18	6.42
8-16	193	135	41.20	41.00	11.17	11.02
> 16	100	50	21.40	15.20	25.26	23.76

such as Gyr, 211 Mbps; Haryana, 106 Mbps; Ongole, 188 Mbps; and Kangayan, 283 Mbps (Dixit et al., 2020). The Guaymi breed, with an average of 176.65 Mbps, had a shorter total length of ROH, similar to that observed in the Blanco-Orejinegro breed (170.36 Mbps) (Caivio-Nasner et al., 2021).

Regarding the average number of ROHs per individual, that in the Guabala cattle was 31.20, while that in the Guaymi cattle was 7.32. This difference suggested variability in genetic structure between the two groups, with the Guabala cattle exhibiting more homozygous events. These values are lower than those of the Hereford breed (80.6 ROH per individual) and higher than those of several native Polish breeds (such as White-Backed (23.9), Polish Red (23.3), and Polish Red-and-White (21.8)); lower than those of Indica breeds, such as Kangayan (63.6), Gyr (45.3), and Ongole (44.9); higher than those of the Tharpakar (24.6), Hariana (26.3), and Sahiwal (24.6) breeds; and higher than White Orejinegro breed, which has a low value of 18.35 (Szmatola et al., 2019; Dixit et al., 2020; Caivio-Nasner et al., 2021).

The five length categories of the ROH (homozygous segments): 0-2, 2-4, 4-8, 8-16, and > 16 Mb (Table 1). In the short-length categories (0-2 and 2-4 Mb), both breeds exhibited similar numbers of ROH, suggesting comparable homozygous patterns. On the other hand, in the intermediate and long length categories, the Guabala breed had a greater number of ROH, which may be indicative of greater consanguinity in these segments. These findings are consistent with previous studies on inbreeding and genetic diversity in these populations (Purfield et al., 2012; Scienski et al., 2021).

Regarding the percentage of the genome covered by ROH (%ROH), the short segments of both populations suggest that there is still a level of genetic diversity, although the reported values are contrary to those observed in Holstein cattle in China by Liu et al. (2021), who reported higher percentages of these short genome segments. Comparable results were reported by Peripolli et al. (2018a) in Nellore cattle from Brazil. Short ROH are often associated with older shared ancestry and less immediate inbreeding (Sumreddee et al., 2020). On the other hand, the high percentage of ROH in the medium and long segments in both populations indicates a significant level of inbreeding (Peripolli et al., 2018b).

This information is consistent with the way in which the Guabala breed developed within the Castrellón family, who described the formation of this breed as being driven by the selection of the color red among other traits.

The analysis of the mean number of ROH by breed (M_{ROH}) revealed notable differences between the Guaymi and Guabala breeds. The Guaymi breed originated in the mountainous areas of the Gnobe-Buglé region, such as Tolé and Cerro Plata, and the Guabala breed was developed by the Castrellón family in the Guabala area through selection for color and other characteristics of interest (Villalobos et al., 2010). The data revealed intriguing patterns of homozygosity in both populations. In the shorter segments (0-2 and 2-4 Mb), the Guaymi population consistently presented higher mean numbers of ROH than did the Guabala. This finding suggested greater homozygosity, which could be indicative of a smaller effective population size or a lower genetic flow with other populations, possibly due to the geographic isolation inherent to its location and later confinement to conservation centers (Addo et al., 2021). These factors could have contributed to greater fixation of homozygous alleles in the short and middle segments of the genome. On the other hand, in the longest segments (especially> 16 Mb), an inverse pattern was observed with the Guabala breed showing a greater mean number of ROH. This observation is consistent with the effects of artificial selection practiced by the Castrejon family that developed this breed in lowland areas in the eastern region of the Chiriquí province (Villalobos et al., 2010). Selection directed toward specific characteristics, such as color, could have led to the fixation of certain alleles, resulting in long stretches of homozygous DNA. This pattern is indicative of an intensive selection process, which in turn reduces genetic diversity in specific regions of the genome (Liu et al., 2022).

These results highlight how environmental factors and human action can significantly influence the genetic makeup of populations (Beishova et al., 2022). In the Guaymi breed (originated in the mountainous regions of the Ngäbe-Buglé comarca), natural factors such as geographic isolation seem to play a crucial role in the development of homozygosity, while the genetic makeup of the Guabala breed has been shaped by human interventions through artificial selection. These findings
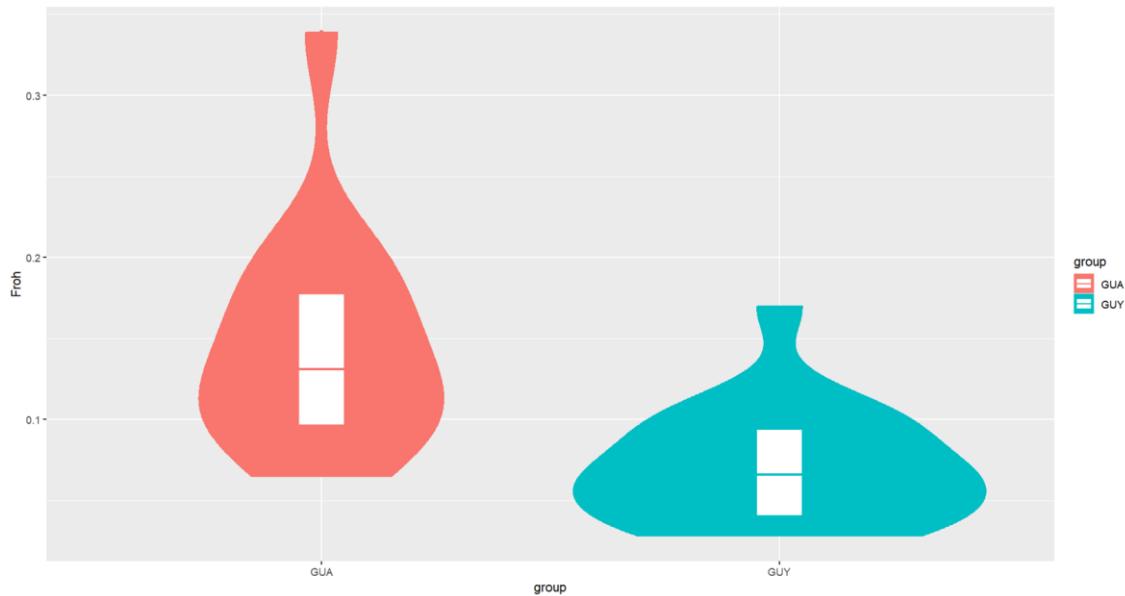
Figure 2. Genomic inbreeding coefficient of the Guaymi and Guabala breeds

provide a deeper understanding of how selection practices and environmental factors can direct the genetic evolution of populations.

In the study of population genetics, the identification of ROH is crucial for understanding genetic diversity and evolutionary history. After adjusting the threshold to 20%, significant islands of ROH were identified in the populations studied, standing out on three specific chromosomes:

1. Chromosome 15: A segment from 51,528,617 to 59,333,016 common to approximately 20.59% of individuals.
2. Chromosome 16: A segment from 25,623,468 to 26,455,382, shared by approximately 35.29% of individuals.
3. Chromosome 18: A segment between 63,878,550 and 65,978,584, shared by approximately 23.53% of individuals.

These ROH segments are shared by individuals of the Guabala and Guaymi breeds, which suggest a history of genetic exchange between these groups or a common ancestor. This observation indicates shared genetic diversity, possibly reflecting past evolutionary or migratory events (Gaspar et al., 2023; Mulim et al., 2022). The presence of these genes with important functions in both groups could indicate natural selection in a shared environment (Szmatoła et al., 2019).

The intraracial homogeneity in the Guabala breed, evidenced by the shared segments with ROH, could be derived from historical selection practices in animals. For example, the Castrellón family has maintained certain

characteristics, such as red coloration and traits of Spanish origin, while avoiding crosses with *Bos indicus* breeds (Villalobos et al., 2010; Martinez et al., 2012). This trend is not unusual in populations with histories of relative isolation and may be a result of inbreeding and inheritance of identical alleles from common ancestors (Gorssen et al., 2021).

The uniqueness of the Guabala breed reflects a specific subpopulation characterized by marked genetic homogeneity. In the context of significant artificial selection, such homogeneity is expected and often intentional, with ROH acting as markers of this genetic uniformity (Scienski et al., 2019).

Considering the evolutionary implications, natural selection and consequences for the health and biodiversity of these populations is essential. The presence of ROH suggests that certain genetic characteristics were favored throughout the evolution of the group in response to specific environmental pressures (Goszczynski et al., 2018). However, genetic homogeneity, although beneficial for the perpetuation of desirable traits, can limit the ability of a population to adapt to new environmental or health challenges, affecting its long-term viability (Medugorac et al., 2011).

The F_{ROH} coefficient is a quantitative measure of autozygosity that reflects the proportion of the genome that consists of segments of homozygosity by descent. The Guabala population had an average F_{ROH} of 0.147 ± 0.066, which is indicative of greater inbreeding than the Guaymi population, for which the average F_{ROH} was 0.071 ± 0.035.

The violin plot (Figure 2) shows not only the medians and quartiles but also the density of the distribution of the

Table 2. Values of F_{ROH} at the chromosome level (Chr) in the races Guabala (GUA) and Guaymi (GUY).

Chr	GUA	GUY
Chr1	0.199	0.129
Chr2	0.150	0.146
Chr3	0.270	0.126
Chr4	0.159	0.098
Chr5	0.187	0.111
Chr6	0.200	0.106
Chr7	0.237	0.114
Chr8	0.271	0.261
Chr9	0.280	0.151
Chr10	0.274	0.155
Chr11	0.165	0.162
Chr12	0.220	0.235
Chr13	0.161	0.151
Chr14	0.165	0.180
Chr15	0.158	0.180
Chr16	0.171	0.184
Chr17	0.430	0.179
Chr18	0.145	0.093
Chr19	0.195	0.168
Chr20	0.110	0.076
Chr21	0.145	0.107
Chr22	0.183	0.315
Chr23	0.245	0.115
Chr24	0.350	0.309
Chr25	0.256	0.377
Chr26	0.268	0.225
Chr27	0.270	0.310
Chr28	0.403	0.206
Chr29	0.292	0.182

F_{ROH} values, offering a comprehensive visualization of the intrapopulation genetic variability (Wu et al., 2021). The wide dispersion observed in the Guabala population suggests more pronounced variability in consanguinity, possibly due to mating practices between related individuals or a more subdivided population structure. In contrast, the narrower distribution in the Guaymi population points to less consanguinity and, by inference, greater genetic heterogeneity. The F_{ROH} values observed for the Guabala breed are greater than those reported by Zinovieva et al. (2020) for the native breeds of Russia, Yaroslavl (0.103) and Kholmogor (0.059). La Guaymi had an intermediate range. Determination of the coefficient of inbreeding based on ROH has several advantages compared to the classical coefficient of inbreeding calculated on the basis of pedigree data (Szmatoła et al., 2019; Toro-Ospina et al., 2022). F_{ROH} more efficiently predicts the degree of genome autozygosity and can be estimated in any animal with genotypic data, even if genealogical information is unavailable (Ferencakovic et

al., 2011; Purfield et al., 2012; Curik et al., 2014). Importantly, all regions of homozygosity have an impact on phenotypes, which suggests that genetic conservation strategies could focus on maintaining diversity in critical chromosomal regions (Peripolli et al., 2017).

This may be particularly relevant for small or isolated populations in which genetic diversity is limited and inbreeding can have more pronounced effects, such as the Guabala and Guaymi breeds (Pilon et al., 2021).

The differences between the Guabala and Guaymi breeds were evidenced by the techniques described above. Furthermore, these differences are manifested in a more complex way, not only in terms of population inbreeding levels but also at the chromosomal level, shown in Table 2.

A detailed analysis of the genomic inbreeding coefficient by chromosome in the Creole cattle breeds Guabala and Guaymi, revealed notable differences. In the Guabala breed, the chromosome that stands out the most due to its high level of inbreeding is 17, with a F_{ROH}
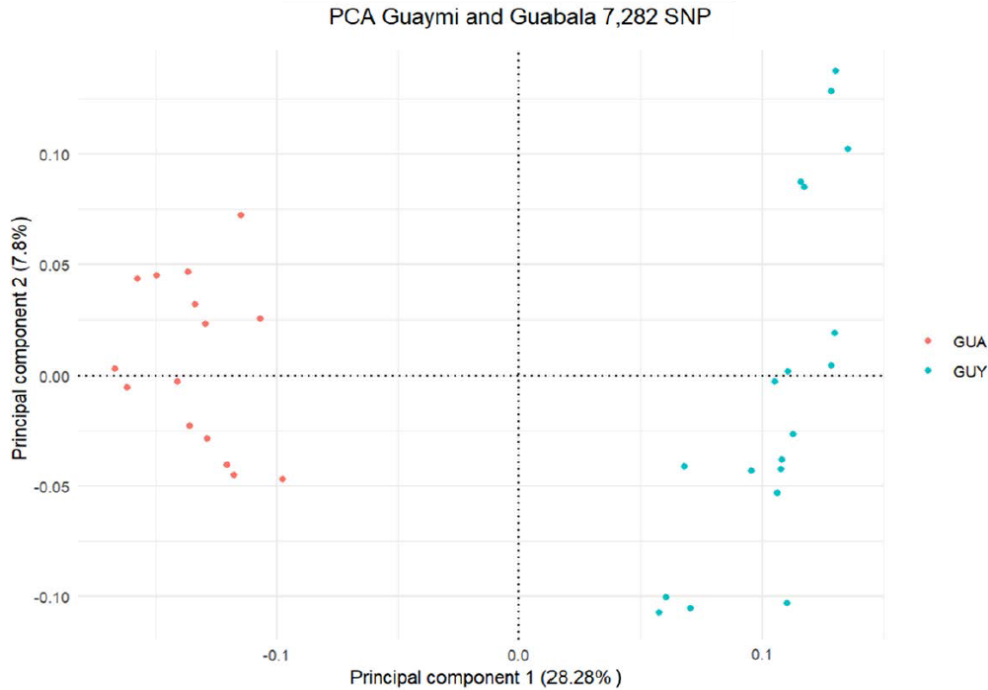
Figure 3. Principal components analysis of Guaymi and Guabala breeds.

of 0.430, indicating a significant level of inbreeding. On the other hand, chromosome chr20 showed the lowest level of inbreeding in this breed, with a F_{ROH} of only 0.109.

In the Guaymi breed, chromosome 25 was the most affected by inbreeding, with a F_{ROH} of 0.377, also indicating a high level of inbreeding. Like in the Guabala breed, chromosome 20 in the Guaymi breed showed the lowest level of inbreeding (0.076). This parallelism on chromosome 20 between the two breeds is remarkable, suggesting that this chromosome could be less susceptible to inbreeding in these bovine breeds.

The variability in the F_{ROH} values between the chromosomes of the Guabala and Guaymi breeds can have significant biological implications, particularly in the context of genetic selection and diversity (Toro-Ospina et al., 2022). These results are indicative of the degree of homozygosity in the genome. Greater homozygosity, reflected in high values, could indicate a reduction in genetic diversity, which in turn may be the result of inbreeding or a small founder population (Kim et al., 2019).

Variations in genomic inbreeding between different chromosomes can indicate differences in genetic diversity throughout the genome, which could impact the adaptive capacity of the population to environmental changes or diseases (Hohenlohe et al., 2021; Beishova et al., 2022).

Specific differences in F_{ROH} values between chromosomes could suggest the presence of selection

signatures (de Simoni et al., 2014; Gorssen et al., 2021). For example, if certain chromosomes in a breed consistently show higher homozygosity, this could be indicative of selection, either natural or artificial, for specific genetic traits. Among the chromosomes on which the Guaymi breed shows superiority to the Guabala breed, it is possible that there are genes or chromosomal regions that have been the object of selection, leading to greater homozygosity in these specific areas (Saravanan et al., 2021). High homozygosity may be associated with an increased risk of genetic diseases, particularly those involving harmful recessive genes. Understanding the distribution of homozygosity along chromosomes is crucial for the management of breeding and conservation of these breeds, allowing the development of strategies to minimize the health risks associated with inbreeding (Kardos et al., 2016).

The differences between the Guaymi and Guabala breeds, and specifically between chromosomes, could reflect their evolutionary and demographic histories, including events such as genetic bottlenecks, population expansions or migrations, which have been discussed in this work and by other authors (Murray et al., 2010; da Fonseca et al., 2019).

In the present study, the genetic structure and diversity of the Guaymi and Guabala Creole breeds are examined through PCA (Figure 3). This technique has allowed a detailed visual evaluation of the genetic differentiation existing between both populations. The results obtained from the PCA highlight that the first principal component

(PC1) is responsible for 28.28% of the total observed variability, demonstrating a notable genetic distinction between the studied breeds. This degree of genetic differentiation suggests that both selective practices and environmental factors have contributed to a significant genetic divergence between the breeds, possibly reflecting adaptations to specific ecological niches or a history of selection oriented towards various phenotypic traits. On the other hand, the second principal component (PC2), which explains 7.8% of the variability, reveals the internal diversity within each breed, pointing to an underlying genetic heterogeneity. This internal variability could indicate the presence of subpopulations within the breeds or differences in inbreeding and gene flow. For example, the variability observed in the Guaymi breed may suggest a lower susceptibility to inbreeding, attributable to its origin in isolated mountainous regions. In contrast, the genetic structure of Guabala could reflect a history of more intense selection for certain phenotypic traits, such as coat color, influenced by human intervention. The assessment of the genetic structure and diversity of cattle breeds through advanced genomic analyses, such as Principal Component Analysis (PCA) and the identification of runs of homozygosity (ROH), has proven to be an essential tool for understanding genetic differentiation and the adaptation of breeds to specific ecological niches or to varied selection pressures. As observed in the study by Toro-Ospina et al. (2022), where the Criollo Caqueteño cattle breed in Colombia was analyzed, the application of these genomic methods allowed for the detection of a decrease in inbreeding frequency and the identification of genomic regions associated with economically interesting traits. This methodological approach reflects a remarkable genetic distinction and internal diversity within the studied breeds, like what is observed in our analysis of the Creole Guaymi and Guabala breeds through PCA, highlighting the importance of selective practices and environmental factors in the genetic divergence between breeds (Toro-Ospina et al., 2022). Similar to our analysis of the Creole Guaymi and Guabala breeds using Principal Component Analysis (PCA), the study by Barbato et al. (2020) provides a valuable perspective on the genetic structure and diversity in cattle populations, highlighting the importance of ancestral selection and environmental factors in the genetic differentiation among populations.

The configuration of the points in the PCA plot, and particularly the clustering of individuals from the Guabala breed, supports the hypothesis of a higher degree of inbreeding in this population, as discussed earlier. Moreover, the dispersion observed in the Guaymi breed could indicate a reduced incidence of inbreeding or a greater residual genetic diversity. These findings are of vital importance for conservation strategies and management of these breeds, providing a framework for future research on their genetic conservation.

To advance the study of the Guaymi and Guabala Creole bovine breeds, three key areas of focus are suggested. First, it is essential to conduct genomic wide association studies (GWAS) to discover genetic variants linked to desirable traits and recessive diseases. This information is necessary for the design of effective breeding programs and the conservation of these breeds. Second, given the observed inbreeding, a comprehensive analysis of the associated health risks must be performed. Identifying recessive diseases and adverse traits will help develop breeding management strategies that minimize inbreeding and maintain genetic diversity. Finally, comparing these homozygous patterns and inbreeding coefficients with those of other bovine breeds globally will provide a broader context, revealing unique evolutionary patterns and guiding conservation strategies. These steps are critical for understanding and preserving the genetic diversity and long-term health of these breeds. In this study, the lack of access to complete historical records has limited our ability to interpret some of the genetic patterns observed in the context of historical breeding practices and population dynamics. However, the recent formation of a breeders' association, along with the enactment of a law focused on promoting the conservation and study of these Creole breeds, represents a promising step towards the collection and systematization of such historical data. This initiative will not only facilitate the preservation of crucial information for future genetic analyses but also underscores the growing recognition of the importance of integrating historical and genetic knowledge for the management and conservation of Creole breeds.

Conclusions

The present study provides a deep understanding of the genetic structure and levels of consanguinity of the Guaymi and Guabala Creole bovine breeds. The results revealed significant differences in the homozygous segments and genomic inbreeding coefficients between the two breeds. A greater extension and number of ROH segments were observed in the Guabala breed than in the Guaymi breed, which indicates different levels of inbreeding or different breeding histories. Furthermore, the identification of significant islands of ROH suggests a history of genetic exchange or a common ancestor between these groups. These findings are critical for understanding the genetic diversity and evolution of these breeds and their potential health risks associated with inbreeding. The identification of selection signatures and runs of homozygosity highlights specific areas for future research, with the potential to unlock key genetic variants for desirable traits and disease resistance. Likewise, this study serves as a call to action for strengthening collaborations between academic institutions, breeder communities, and governmental bodies, thus ensuring the long-term viability and sustainability of these valuable

bovine breeds within Panama's agro-biodiversity heritage.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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