

Phylogenetic relationships of Argentinean Creole with other Latin American Creole Cattle as revealed by a medium density Single Nucleotide Polymorphism microarray

María Agustina Raschia  Mario Andrés Poli 

Instituto Nacional de Tecnología Agropecuaria, CICVyA-CNIA, Instituto de Genética "Ewald A. Favret", Nicolás Repetto y de Los Reseros s/n, Hurlingham (B1686), Buenos Aires, Argentina

The demographic history of Creole cattle in Argentina and in Latin America dates back to the time of the Spanish colonization. This study aimed to investigate the potential use of a medium-density SNP array to describe cattle from the most representative and oldest herds of the Argentine Creole cattle breed registered at the time of the constitution of the Argentinean Creole Cattle Breeders Association and to explore the phylogenetic relationship with Creole cattle from other Latin American countries. To achieve this goal, genotypes from 51 animals on 34,008 autosomal SNP were used to generate genetic distance matrices based on the proportion of shared identical-by-state alleles among individual animals and animals clustered according to their origin, analyzed by the PLINK program. A neighbor-joining phylogenetic tree based on pairwise genetic distance was constructed using PHYLIP and was prepared for visualization using FigTree. A multidimensional scaling analysis was performed to evaluate the level of relationship in terms of genetic distance among the different animal clusters. Genetic distances between animals varied from 0.186 to 0.357 when considering all pairs of animals, and from 0.186 to 0.338 when considering Creole pairs. The dendrogram obtained showed three major clusters. Cluster 1 included Latin American Creole cattle from Colombia, Guadalupe, Paraguay, and Uruguay, and the reference groups of Holstein and Jersey cattle. Cluster 2 contained exclusively Patagonian Creole cattle, while the third cluster included the remaining Argentinean Creoles. The genetic relationship patterns obtained via multidimensional scaling showed a close relationship among four groups of Creole animals from Argentina. The closeness between clusters can be explained in part on the basis of early migration of animals that gave rise to founders herds at some Argentinean locations. The outcomes of this study contribute to a better understanding of the composition of the early founder herds of Creole cattle in Argentina and the relationship with other Latin America Creole cattle populations.

Keywords: SNP microarray, multidimensional scaling, phylogenetic tree, Argentinean Creole cattle, Latin America Creole cattle

Relaciones filogenéticas del Criollo argentino con otros bovinos Criollos de América Latina según lo revelado por un microarreglo de polimorfismos de nucleótido simple de mediana densidad

La historia demográfica del bovino Criollo en Argentina y en América Latina en general se remonta a la época de la colonización española. El objetivo de este estudio fue investigar el uso potencial de un microarreglo de SNP de mediana densidad para describir animales de los rodeos más representativos y antiguos en el tiempo en que se constituyó la Asociación Argentina de Criadores de Ganado Bovino Criollo y explorar la relación filogenética con otros bovinos criollos de Latinoamérica. Con los genotipos de 51 animales en 34 008 SNP autosómicos se generaron matrices de distancias genéticas basadas en la proporción de alelos idénticos por estado compartidos entre animales y entre animales agrupados según su origen, con el programa PLINK. Se construyó un árbol filogenético basado en distancias genéticas entre pares de animales usando PHYLIP, y se preparó para su visualización con FigTree. Se realizó un análisis de escalamiento multidimensional para evaluar la relación en términos de distancia genética entre los diferentes grupos de animales. Las distancias genéticas entre animales variaron de 0.186 a 0.357 al considerar todos los pares de animales, y de 0.186 a 0.338 al considerar pares de criollos. El dendrograma obtenido presentó tres agrupamientos. El grupo 1 incluyó ganado criollo de Colombia, Guadalupe, Paraguay y Uruguay, y los grupos de referencia Holando y Jersey. El grupo 2 comprendió exclusivamente ganado criollo patagónico, mientras que el tercer grupo incluyó los restantes criollos argentinos. Los patrones de relación genética obtenidos a través del escalamiento multidimensional demostraron una íntima relación entre criollos de cuatro orígenes de Argentina. Esto puede explicarse en parte por la migración temprana de animales que originaron los rodeos

fundadores en algunas localidades argentinas. Los resultados de este estudio contribuirán a una mejor comprensión de la formación de los rodeos fundadores de criollos en Argentina y de la relación con otras poblaciones de ganado criollo de América Latina.

Palabras clave: Microarreglo de SNP, escalamiento multidimensional, árbol filogenético, bovino Criollo Argentina, Bovinos Criollos Latinoamérica

Introduction

The current territory of Argentina received the first cattle through four routes in the 16th century: Bolivia in 1549, Chile in 1551, Paraguay in 1554, and the South of Brazil. Later, the introduction of animals from the Canary Islands to the Río de la Plata also occurred. The spread of cattle before and during that period was a consequence of the foundation of cities by Spanish colonizers (Giberti, 1970).

The geographic conditions, moderate climate, abundance of pastures, and nearly complete absence of predators in the central and eastern regions of Argentina (Pampas) resulted in a great expansion of livestock. By 1850 the first cattle breed from northern Europe were imported (Shorthorn, Hereford, Angus). In the following decades, the crossbreeding process was very extensive in order to obtain animals with a greater tendency to fatness as required by the export market. The consequence was a total absorption of the Creole cattle of the Pampas region. Creole cattle were then displaced and confined to regions where other breeds could not survive, including tropical, subtropical, arid, and Southwest Patagonia areas (Martinez et al., 2000).

In 1959, the Instituto Nacional de Tecnología Agropecuaria (INTA) established the first experimental Creole cattle herd (consisting of 35 cows and 2 bulls from the NW region) in Leales in Tucumán province. During 1959-1970 Creole animals were used as a control "local" breed for the crosses of European breeds with Zebu cattle. Later (1971-1988), the objectives were to intensify Creole characterization and to develop a select nucleus of Creole cattle with emphasis on the diffusion and insertion of this breed into the national cattle herds (Holgado and Ortega,

2019). In 1985 and by the joint initiative of private breeders and INTA, the current Argentinean Creole Cattle Breeders Association was established.

Creole cattle in Argentina have been studied by other institutions and their productive and reproductive behavior has been characterized in different environments, both in purebred and crossbred states (Corva et al., 1995; Holgado and Rabasa, 2001; Martinez et al., 2003; Rabasa and Holgado, 2000; Rabasa et al., 2005; Sal Paz et al., 1976). Early studies involved characterization by blood groups and other biochemical polymorphisms (Poli, 1986; Poli and Antonini, 1991). Later, studies of genetic diversity using molecular markers at the DNA level, including microsatellites and mitochondrial DNA, were carried out (Giovambattista et al., 1996, 2001; Lirón et al., 2006; Martinez et al., 2003). Since the advancement in high-throughput genotyping techniques such as SNP (single nucleotide polymorphism) microarrays, several studies using a relatively large number of SNPs to explore the genetic diversity, demographic history, and relatedness between different cattle breeds have been published (Browett et al., 2018; Mastrangelo et al., 2018; Sermyagin et al., 2018; Frantz et al., 2020; Meseret et al., 2020; Upadhyay et al., 2019). The purpose of this study was to investigate the potential use of a medium-density SNP array to describe cattle from the most representative and oldest herds of the Argentine Creole cattle breed registered at the time of the constitution of the Argentinean Creole Cattle Breeders Association and to explore the phylogenetic relationship with Creole cattle from other Latin American countries.

Material and Methods

Animals

A total of 42 samples of Creole cattle were used in this study from the DNA repository of Creole cattle that INTA has at the Institute of Genetics. Animals were sampled from 13 herds from 5 countries whose geographic distribution is shown in Figure 1. Table 1 shows the name of the farm where samples were collected, the acronyms used in this work, the number of samples genotyped, the country, and the author and year of collection. All samples from Argentina were

taken during the phenotypic inspection and registration process of the first animals in the breeder association record book. After the Breeders Association was established and in the following years a breed standard was drawn up, and training and dissemination courses on productive and phenotypic traits of this breed were held. The genotyped samples were from unrelated animals selected based on the structure of the herd to which they belonged, their origins, and the availability of historical, phenotypic, and pedigree information. The samples from Paraguay



and Uruguay were taken in the framework of the PROCISUR PTR-Genomics applied to beef production (2003-2006) project and those from Colombia and Guadalupe were sent by German Martínez Correal

and Michel Naves, respectively. Furthermore, five Holstein and five Jersey bulls samples from the central area of Argentina were included as outgroups.

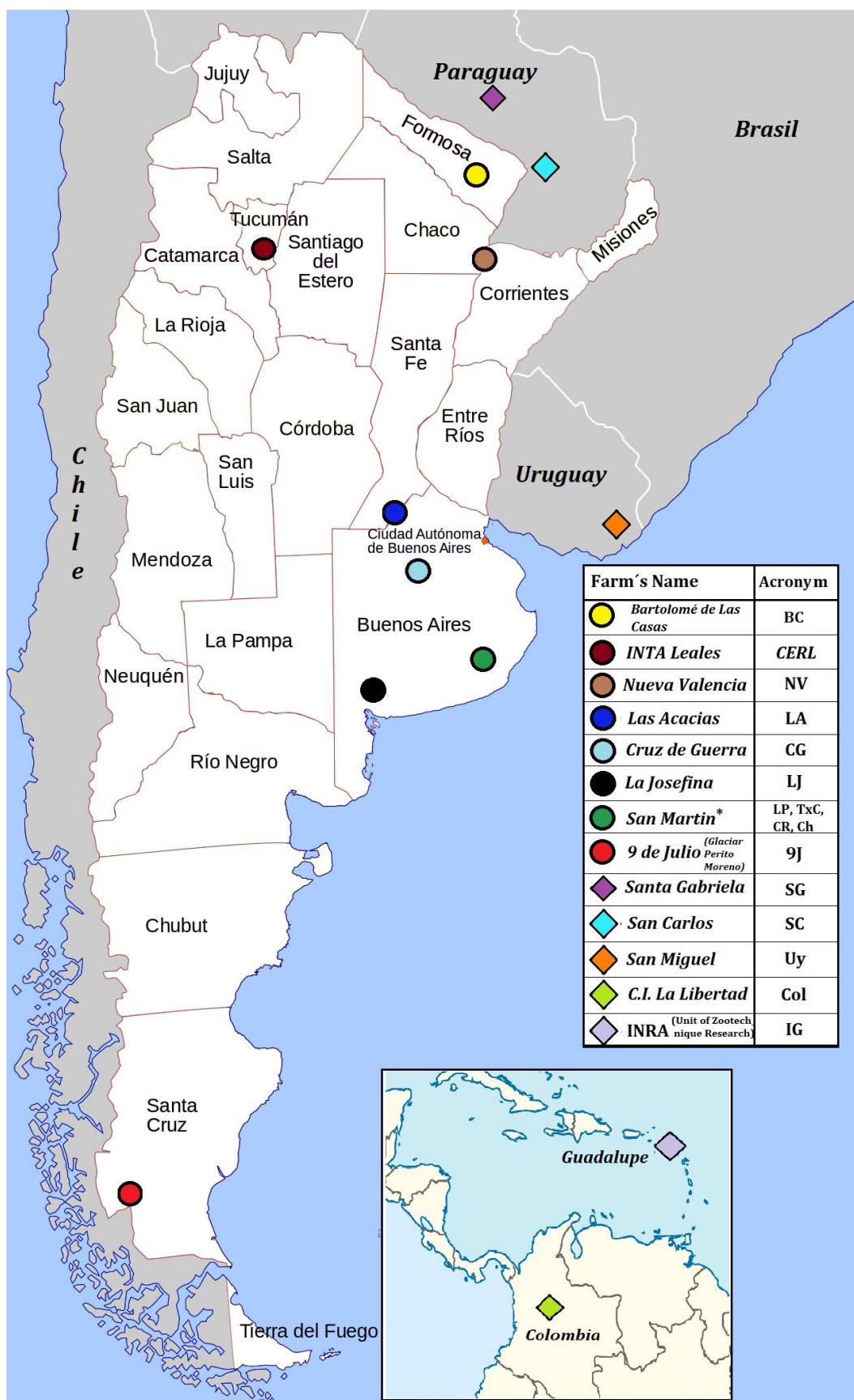


Figure 1. Geographical location of the farms from which samples referenced in Table 1 were taken.

Table 1. Farm name, acronym used, number of samples genotyped, country and name of the Creole breed and year of sample collection.

Farm's Name	Acronym	N	Country (Criollo's name)	Sample collection
9 de Julio	9J	3	Argentina (Criollo Argentino Patagónico)	1991
Cruz de Guerra	CG	5	Argentina (Criollo Argentino)	1992
INTA Leales	CERL	4	Argentina (Criollo Argentino)	1992
La Josefina	LJ	2	Argentina (Criollo Argentino)	1992
San Martín ¹	LP, TxC, CR, Ch	8	Argentina (Criollo Argentino)	1993
Nueva Valencia	NV	1	Argentina (Criollo Argentino)	1993
Las Acacias	LA	4	Argentina (Criollo Argentino)	1995
Bartolomé de Las Casas	BC	2	Argentina (Criollo Argentino)	1995
San Carlos	SC	2	Paraguay (Criollo Pilcomayo)	2007
Santa Gabriela	SG	2	Paraguay (Criollo Pampa Chaqueño)	2007
Centro de Investigación la Libertad de CORPOICA Col		3	Colombia (Sanmartinero)	2000
Reserva Parque Nacional				
San Miguel	Uy	3	Uruguay (Criollo Uruguayo)	2007
INRA Unit of Zootechnique Research	IG	3	Guadalupe (Criollo)	2000

¹San Martín farm had identified animals from different origins as: LP-Los Planteles, TxC-Tandil x Cerrillada, CR-Carlos Romero, and Ch-Chasquivil.

²Samples from Argentina, Paraguay, Colombia, Uruguay, and Guadalupe were taken by Poli, M., Ferreira, N., Martínez Correal, G., Postiglioni, A. and Naves, M., respectively.

Sample collection and DNA extraction

Fresh blood was obtained from the jugular vein of animals using EDTA as anticoagulant. The procedure for blood sample collection was approved by the Institutional Committee for Care and Use of Experimental Animals of the National Institute of Agricultural Technology (CICUAE-INTA) and followed the guidelines described in the institutional manual. Genomic DNA was extracted using a commercial kit (AxyPrep Blood Genomic DNA Miniprep Kit, Axygen Biosciences, Union City, CA) according to the protocol supplied by the manufacturer. Genomic DNA from some bulls was obtained from semen straws using a phenol-chloroform extraction method. DNA quantity and quality were determined by measuring its UV absorption at 260 and 280 nm and the 260/280 and 260/230 absorbance ratios, using a NanoDropTM 1000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA).

Genotyping

Creole animals were genotyped with the GeneSeek® Genome Profiler (GGP) Bovine 150K v2 microarray, while the reference groups of Holstein and Jersey bulls were genotyped using the BovineSNP50 v2 BeadChip (Illumina Inc., San Diego, CA, USA). Genotyping was performed

on all animals by GeneSeek (Neogen Corporation Company, Lincoln, NE, USA).

The GGP 150K v2 microarray evaluates 138 892 SNP distributed over the 29 bovine autosomes, sex chromosomes, and mitochondrial DNA, spaced on average 18,868 bp apart throughout autosomes and 38 523 bp apart throughout the genome. The BovineSNP50 v2 BeadChip evaluates 54 609 SNP distributed over the 29 bovine autosomes and sex chromosomes, spaced on average 48 102 bp apart throughout the genome. SNP locations were mapped to the bovine assembly ARS-UCD1.2 (Rosen et al., 2020) for computations.

The quality control of genotype data was performed using PLINK program (Purcell et al., 2007) and consisted in the exclusion of SNP with unknown position on the genome, located on sex chromosomes, with a call rate lower than 0.90, a minor allele frequency lower than 0.03 or a p-value for the exact test to detect deviations from the Hardy-Weinberg equilibrium lower than 1.10-4. Animals with a call rate lower than 0.80 were also excluded.

Phylogenetic analysis

A matrix of the genetic distances for all pairs of animals, estimated as 1 – IBS, being IBS the proportion of alleles identical by state, was obtained with PLINK



program. This file was adapted to the input format required by the Neighbor program in PHYLogeny Inference Package (PHYLIP; Felsenstein, 1989) for inferring phylogenies. Briefly, the neighbor-joining method, a distance matrix method producing an unrooted tree, implemented in Neighbor was used. The output of this program, a tree file in nested-parenthesis notation, was used as input for the Consense program of PHYLIP, which computed the consensus tree by the majority rule consensus tree method. The consensus tree was prepared for visualization using FigTree v1.4.3 program (Rambaut, 2009).

Multidimensional scaling

A multidimensional scaling (MDS) analysis on the mean identical by state (IBS) pairwise genetic distances between animals clustered according to their geographical origin was performed with PLINK program. Then, a reduced representation of the data in two dimensions was generated. Each point in this plot represents a group of animals clustered according to their origin.

Results and Discussion

After genomic data quality control, genotypes from 51 animals on 34 008 autosome SNP shared by both microarrays used were available for subsequent analyses. Genotypes on those SNP were used to calculate the genetic distance for all pairs of animals. Genetic distances between animals varied from 0.186 to 0.357 when considering all pairs of animals. The genetic distance among all Argentinean creole varied from 0.186 to 0.338. While the highest genetic distance (0.357) was found between two bulls, a Holstein and a Jersey, from the outgroups, among Creole animals the

highest distance (0.338) was observed between an animal from Guadalupe and another from 9 de Julio.

The dendrogram in Figure 2 shows that the 51 animals were grouped into three major clusters. Cluster 1 included Creole cattle from Colombia, Guadalupe, Paraguay, Uruguay, and the two outgroups Holstein and Jersey. Cluster 2 contained only and exclusively animals from Patagonia (9 de Julio farm) and cluster 3 all the remaining Argentinean Creole.

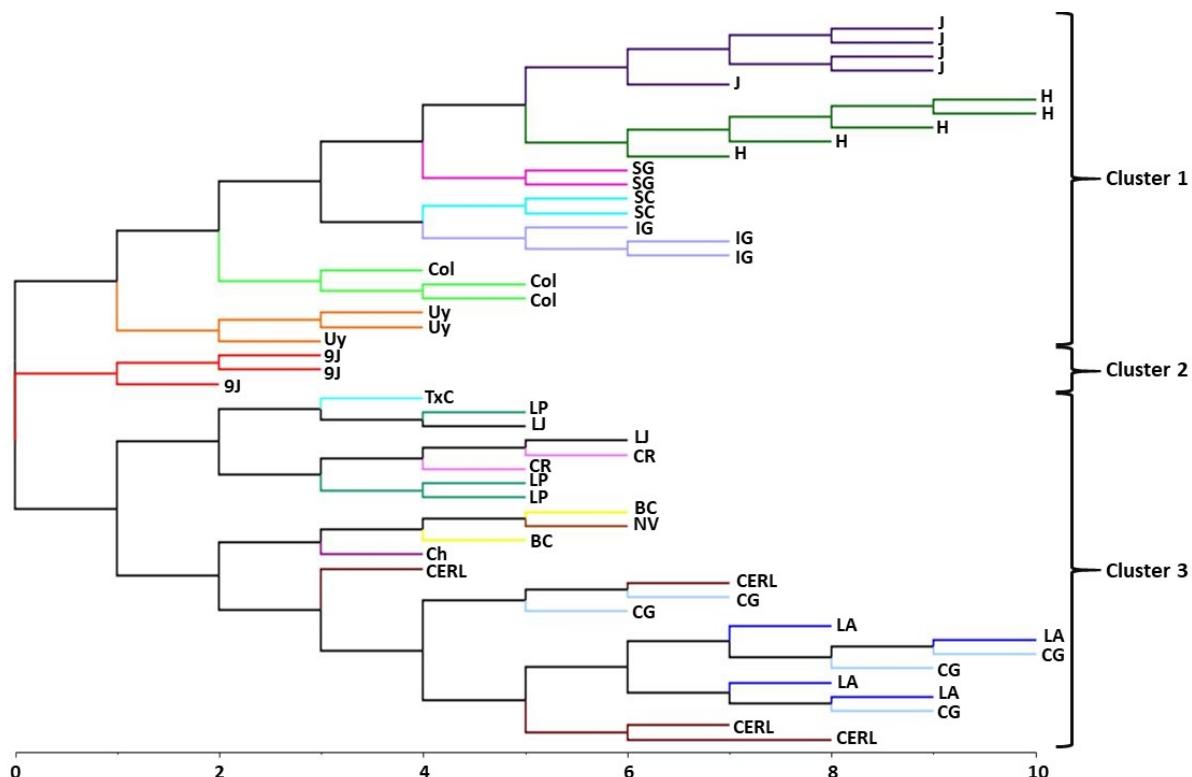


Figure 2. Dendrogram built based on genetic distances between animals estimated according to the proportion of shared IBS alleles J, Jersey; H, Holstein; SG (Santa Gabriela - Paraguay); IG, (Isla Guadalupe); SC (San Carlos – Paraguay); Col (Colombia); Uy (Reserva Parque Nacional San Miguel, Uruguay); and Argentinean Creole cattle: 9J (9 de Julio); LJ (La Josefina); LP (Los Planteles); TxC (Tandil x Cerrillada); CR (Carlos Romero); NV (Nueva Valencia); BC (Bartolomé de las Casas); Ch (Chasquivil); CERL (INTA Leales); CG (Cruz de Guerra); LA (Las Acacias).



Cluster 1 presented five subgroups of Creole cattle from four Latin American countries and both breeds used as outgroups (Holstein and Jersey), all clearly differentiated. The phylogenetically closest subgroup to the Argentinean Creole cattle was that from Uruguay. Creole animals from these two countries are phenotypically very similar on coat color diversity and body and horn shape (Personal inspection, October 2019). Genetic distances among the Argentinean and Uruguayan Creole animals genotyped varied between 0.294 and 0.32, with a mean of 0.305. Uruguayan Creole Cattle samples came from a single population of approximately 600 pure individuals restricted to San Miguel National Park, in the Northeast of Uruguay. The foundation herd consisted of 35 Creole cows, bulls, and calves brought from different locations around 70 years ago (Armstrong, et al., 2013).

Colombian Creole samples belonged to the Sanmartinero breed, characterized by uniform coat color, and to the founders' herds in the La Libertad Research Center of CORPOICA in Villavicencio. The samples analyzed from Paraguay did not constitute a narrow cluster and this is due to the conformation of both groups, Criollo Pilcomayo and Criollo Pampa Chaqueño. The Criollo Pilcomayo belongs to a single population and is phenotypically very similar to the Argentinean Creole cattle due to its body structure, coat color diversity, and horns. This herd was made up of animals collected by Mr. E. Prayones in the 70s in the area comprised by the coast of the Pilcomayo river (border with Argentina) and the Estero Patiño in the western region (Mr. Prayones personal communication to MP during San Carlos farm visit, August 2007). On the other hand, the Criollo Pampa Chaqueño is phenotypically similar to the Hereford breed regarding coat color and its body distribution and conformation. In a comparative study of Pampa Chaqueño, Criollo Pilcomayo, and Hereford cattle using microsatellite molecular markers, the genetic diversity and their profile were clearly different (Martínez-López, 2019). Our SNP analysis results located Criollo Pampa Chaqueño cattle as a very distant group from the other Creoles, including those from Guadalupe, and the closest to the two outgroups of European breeds.

Cluster 2 contained only animals from Patagonia (9 de Julio farm). These samples belonged to animals that were obtained by R. Martinez and A. Rodriguez with the advice of H. Echeverría (9 de Julio farm) from Los Glaciares National Park in 1990, in the framework of the Agreement project between the Lomas de Zamora University and the National Parks Administration. The origin of the Creole cattle of Patagonia was

described in detail by Martinez (2008) in his doctoral thesis, who concludes that these animals would have been the descendants of those that between the end of the 19th century and the beginning of the 20th century led the first settlers who colonized some of the sectors from current Los Glaciares National Park.

Although information on the movement of animals is scarce, the same author mentions: ". . . one of the highlights is the transfer by boat of a group of 80 cattle from the Estancia "La Cristina" to the Bahía Onelli sector (Tierra de Nadie) (Echeverría Horacio Sr., personal communication.). . . .". In 1937 the Los Glaciares National Park was created and their inhabitants were withdrawn from those territories, however, many of the cattle could not be removed and they became "feral" free-range cattle. The prolonged process of geographic isolation and free breeding of these animals has given rise to what today is the Patagonian Creole cattle origin. Although they have phenotypic characteristics almost indistinguishable from the other Creoles of Argentina, mostly in coat color, horn, and body shape, the mean genetic distance found in this study between these animals and the rest of the Argentinean Creole is 0.303. This result is in accordance with that found by Martinez (2008) using a panel of 27 microsatellites on 36 Patagonian cattle and 45 Creole cattle categorized as from NW Argentina.

In cluster 3 three major subgroups can be distinguished. The first one comprised animals sampled at San Martin farm (Los Planteles, Tandil x Cerrillada, and Carlos Romero herds; breeder M. Pereyra Iraola) and La Josefina farm (Breeder F Marenco). The animals sampled at San Martin farm were descendants from those brought by Mr. Martín Pereyra Iraola grandfather from the NW region of Argentina in 1938, kept until 1949 at "Estancia San Juan" (current Pereyra Iraola Park), and then transferred to Tandileoufu ranch near the city of Tandil (M. Pereyra Iraola personal communication, 1986-7). On the other hand, cattle from La Josefina have their origin in a herd that in 1920 Mr. Carlos Romero brought from the "Alto Peru" (Bolivia) to his farm in the province of Córdoba. The last animals of this herd were auctioned in the city of Bahía Blanca and acquired by Mr. F. Marenco, Mr. M. Eyerabide, and E..Martinez Reboul (F. Marenco and E. Martinez Reboul, personal communication, Dec2-4, 1991). The closeness of the animals in this cluster is due to the exchange between the herds from Pereyra Iraola and Carlos Romero, which can be inferred from the names of the animals registered in the Breeders Association phenotypic inspection and the Argentine Rural Society (SRA) books. Traditionally, pedigree record books



present a name for each animal which is formed in the first part by the name of the seed stock followed by the own name and then, depending on available information, the name of the father, mother, or place of origin. This can be verified in the name of some animals, for example a cow born in 1984, with Particular ID 40 (ID: animal identification, usually is a tattoo), named "PLANTELEO AVUTARDA" and sired by "ROMERO", or a bull with Particular ID 197, named. "PLANTELEO RUMBEADOR ROMERO 197". Several examples like these can be seen in the record book.

The intimate genetic relationship among LP, LJ, TxC, and CR is very clear. Probably the similarity of the animals in this cluster was given by several factors, such as the time that has elapsed since their origins (approximately 10 generations), the reduced size of the herds, mainly in Pereyra Iraola (35-40 females), the use of few bulls, and the exchange of animals between herds. Moreover, the genetic distance between two animals genotyped from Los Planteles was the lowest (0.186) and thus they had the closest relationship.

The second subgroup in cluster 3 has four samples, two animals sampled in Bartolomé de las Casas farm,

one in Nueva Valencia farm, and one in San Martin farm. The first three belonged to the same original herd, INTA Estación Experimental El Colorado (S. Luque, personal communication, April 4, 1993) and hence the close clustering. The fourth belongs to an animal originated in the province of Tucuman (Chasquivil).

The third subgroup comprised animals from CER Leales, Cruz de Guerra, and Las Acacias farms. Within this group, the lowest genetic distance (0.208), was detected between two bulls, one from Las Acacias and the other from Cruz de Guerra. Both farms, Las Acacias and Cruz de Guerra, established their first herds with Creoles from CERL (S. Rabasa, M. Garcíarena, and E. Andreani, personal communication). CERL was the main reservoir and point of dissemination of the Creole cattle from the NW region of Argentina and it has the oldest and most complete pedigree records since the '70s.

The multidimensional scaling analysis enabled visual identification of animal clusters and the level of relationship among them in terms of genetic distance among the animals from the different origins studied (Figure 3).

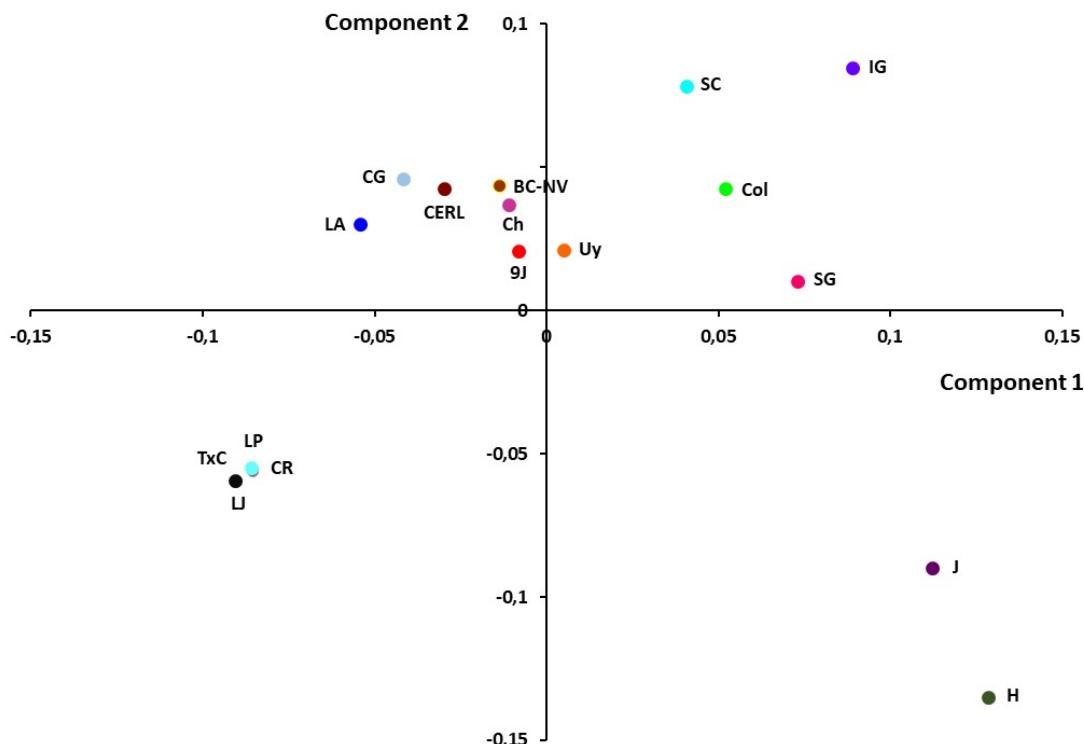


Figure 3. Multidimensional scaling plot based on the mean IBS pairwise genetic distances among animals from different geographical origins. Each dot represents a group of animals clustered according to their origin. J, Jersey; H, Holstein; SG (Santa Gabriela - Paraguay); IG, (Isla Guadalupe); SC (San Carlos – Paraguay); Col (Colombia); Uy (Reserva Parque Nacional San Miguel, Uruguay); and Argentinean Creole cattle: 9J (9 de Julio); LJ (La Josefina); LP (Los Planteles); TxC (Tandil x Cerrillada); CR (Carlos Romero); NV (Nueva Valencia); BC (Bartolomé de las Casas); Ch (Chasquivil); CERL (INTA Leales); CG (Cruz de Guerra); LA (Las Acacias).

An intimate genetic relationship among LP, LJ, TxC, and CR is suggested from the MDS analysis as well as from the phylogenetic relationship reconstruction. This can be explained, as mentioned above, by the exchange of animals between the main herds brought from the north by Iraola and Romero. Furthermore, the breeding practices and the elapsed time probably made these animals very genetically different from the other Creole cattle (mean genetic distance to other Argentinean Creoles: 0.303). Although it is very likely that the Patagonian Creoles originated from the large populations of cattle that existed in the Pampas region, the great phylogenetic differences observed in this study between them and those sampled in San Martin (Pampas region) have at least two possible explanations: 1) although Creole cattle were seen in Patagonia towards the end of the 18th century (Martinez, 2008), the sampled animals were geographically isolated since the late 19th century in Los Glaciares National Park; 2) the Creole cattle sampled in San Martin had their origins in the two herds brought from the NW between 1920 and 1938. Moreover, in the MDS the Patagonian animals seem to be more distant from those of San Martin than the rest of the Argentinean Creole and from those of Uruguay. Isolation and genetic drift can also contribute to these differences.

Animals from CG, CERL, and LA clustered into the same subgroup in the phylogenetic tree, being intermixed instead of arranged according to their origin. Moreover, the points corresponding to these three geographical origins were located very close to each other in the MDS plot. When we investigated the background of animals from Cruz de Guerra, we found that all of them came from CER Leales, confirming the results obtained in our study. The plot also clearly separates the Creoles of Colombia, Gaudalupe, and Paraguay, from the Creoles from Argentina and Uruguay.

As mentioned above, the objective of the study was to investigate the potential use of a medium-density SNP array to explore the phylogenetic relationship of Argentinean Creole cattle from founder herds with other Latin American Creoles. The results obtained from the analysis of the genetic distance matrix and showed in the dendrogram and in the MDS plot are in

In conclusion, this study demonstrates the effectiveness of using genotypes obtained through a medium-density SNP array to reconstruct the phylogenetic relationship of animals belonging to Creole founder herds of Argentina and the genetic distance among them and Creole cattle from other

agreement with the animals' origins, as well as with their demographic history and breeding information.

An example of the usefulness of these tools can be seen with sample ID-Lab 859 (sample identification in the laboratory) which was sampled at San Martin farm (near Tandil city - Buenos Aires province) in 1993. However, in the dendrogram it was positioned in the same cluster as the animals from Bartolomé de las Casas (BC) and Nueva Valencia (NV), and the MDS plot also showed it very distant from the San Martin farm cluster. This sample was a young bull brought by Mr. Pereyra Iraola from Chasquivil (Ch), Tucumán province, in 1991, about 1300 km away to San Martin farm (M. Pereyra Iraola, personal communication, July 16, 1993). This bull is registered in the SRA book as: "Particular ID 001; Birth Date: 00/00/90; sex: M; Name: "PLANTELORO CHASQUIVIL"; Coat colors cod 2,51,96; Date of Inspection: 07/16/93".

Traditionally a breeder association is based on a phenotypic standard and genealogical records. When there are no kinship records and the animal has characteristic phenotypic traits, in general the animal is not accepted. However, some associations accept such animals as a "base" category and it takes at least three generations with full pedigree records for their progeny to be accepted as purebred.

The availability of information on single nucleotide polymorphisms in bovines has increased dramatically in the last ten years. These markers have been used to describe groups of animals and breeds based on genomic data (Gorbach, et al., 2010; Hulsegge, et al., 2013; Hulsegge, et al., 2019). However, the assignment of an animal to a specific breed by means of a genetic test requires the genotypes of a reference group of animals with defined phenotypic characteristics (breed standard) and known origin, which is called a "Reference Population". All individuals in a reference population must represent genetic diversity within the particular breed. The clusters constituted within Argentina and the Latin America Creole demonstrate that the genetic variability is very large and hence present a big challenge to build Creole reference populations and to select the minimum number of SNPs with the highest capability and reliability to differentiate between Creole breeds.

Conclusion

Latin American countries. The outcomes from this study shed light on the genetic differences of the early founder herds of Creole cattle in Argentina and on how Creole cattle populations expanded and spread throughout Latin America, in order to contribute to a better understanding of the evolution of these cattle.



Declaration of interest: The authors have no conflicts of interest to declare.

Acknowledgments

To the breeders and INTA for their vision and conviction to establish a Creole Breeders Association and also their hospitality and talks about the history of their herds and the origin of the animals during inspection and sampling in the early '90. To Germán

Martinez Correal and Michel Naves for providing samples from Sanmartinero and Isla Guadalupe, respectively and to Kathiravan Periasamy for technical assistance and guidance and Mateo Poli Tieffemberg for the edition of Figure 1.

Funding: This study was supported by Instituto Nacional de Tecnología Agropecuaria (INTA) grant PE 145; FAO-IAEA CRP D3.10.28 and PRIN2017 Program of the Italian Ministry of Education, University and Research (MIUR).

Literature Cited

Armstrong, E., A. Iriarte, A. M. Martínez, M. Feijoo, J. L. Vega-Pla, J. V. Delgado and A. Postiglioni. 2013. Genetic diversity analysis of the Uruguayan Creole cattle breed using microsatellites and mtDNA markers. *Genet. Mol. Res.* 12: 1119-1131. <http://dx.doi.org/10.4238/2013>

Browett, S., G. McHugo, I. W. Richardson, D. A. Magee, S. D. E. Park, A. G. Fahey, J. F. Kearney, C. N. Correia, I. A. S. Randhawa and D. E. MacHugh. 2018. Genomic characterization of the indigenous Irish Kerry cattle breed. *Front. Genet.* 9:51. <https://doi.org/10.3389/fgene.2018.00051>

Corva, P. M., E. L. Villarreal, C. A. Mezzadra and L. M. Merlucci. 1995. Reproductive traits of Angus, Criollo and reciprocal crossbred females in the temperate area of Argentina. *Anim. Sci.* 61: 241-249. <https://doi.org/10.1017/S135772980001376X>

Felsenstein, J. 1989. PHYLIP - Phylogeny Inference Package (Version 3.2). *Cladistics* 5: 164-166. <https://evolution.genetics.washington.edu/phylip.html>

Frantz, L.A.F., Bradley, D.G., Larson and, G., Orlando, L. 2020. Animal domestication un era of ancient genomics. *Nature reviews. Genetics.* <https://doi.org/10.1038/s41576-020-0225-0>

Giberti, H. C. E. 1970. Historia económica de la ganadería argentina. Ediciones Solar/Hachete. Buenos Aires. 217 páginas.

Giovambattista, G., C. D. Golijow, F. N. Dulout and M. M. M. Lojo. 1996. Gene frequencies of DRB3.2 locus of Argentine Creole cattle. *Anim. Genet.* 27: 55-56. <https://doi.org/10.1111/j.1365-2052.1996.tb01178.x>

Giovambattista, G., M. V. Ripoli, P. Peral-García and J. L. Bouzat. 2001. Indigenous domestic breeds as reservoirs of genetic diversity: The Argentine Creole Cattle. *Anim. Genet.* 32: 240-247. <https://doi.org/10.1046/j.1365-2052.2001.00774.x>

Gorbach, D. M., M. L. Makgahlela, J. M. Reecy, S. J. Kemp, I. Baltenweck, R. Ouma, O. Mwai, K. Marshall, B. Murdoch, S. Moore and M. F. Rothschild. 2010. Use of SNP genotyping to determine pedigree and breed composition of dairy cattle in Kenya. *J. Anim. Breed. Genet.* 127: 348-351. <https://doi.org/10.1111/j.1439-0388.2010.00864.x>

Hulsegge, B., M. P. L. Calus, J. J. Windig, A. H. Hoving-Bolink, M. H. T. Maurice-van Eijndhoven and S. J. Hiemstra. 2013. Selection of SNP from 50 K and 777 K arrays to predict breed of origin in cattle. *J. Anim. Sci.* 91, 5128-5134. <https://doi.org/10.2527/jas.2013-6678>

Hulsegge, I., M. Schoon, J. Windig, M. Neuteboom, S. J. Hiemstra and A. Schurink. 2019. Development of a genetic tool for determining breed purity of cattle. *Livestock Science* 223: 60-67. <https://doi.org/10.1016/j.livsci.2019.03.002>

Holgado, F. D. and A. Rabasa. 2001. Herencia del carácter "sin cuernos" en el bovino Criollo Argentino. *Zootecnia Trop.* 19(2): 185-190.

Holgado, F. D. and M. F. Ortega. 2019. Caracterización productiva del bovino criollo argentino: período 2006-2016. Buenos Aires: Ediciones INTA. 26p. ISBN 978-987-521-987-8.

Lirón, J. P., P. Peral-García and G. Giovambattista. 2006. Genetic characterization of Argentine and Bolivian Creole cattle breeds assessed through microsatellites. *J. Hered.* 97(4): 331-339. <https://doi.org/10.1093/jhered/esl003>

Martínez-López, O. R., S. B. P. Barbosa, A. M. Martínez, J. V. Delgado and V. Landi. 2019. Perfil genético de dos poblaciones bovinas criollas de Paraguay. *Actas Iberoamericanas de Conservación Animal -AICA* 14: 141-153.

Martínez, R. D., E. N. Fernández, E. R. Género y F. J. L. Rumiano. 2000. El ganado bovino criollo en Argentina. *Arch. Zootec.* 49: 353-361.

Martínez, R. D. 2008. Caracterización genética y morfológica del bovino criollo argentino de origen patagónico. http://www.uco.es/conbiand/tesis/Ruen_Martinez.pdf

Martínez, R. D., G. Giovambattista, M. V. Ripoli, J. C. DeLuca and F. N. Dulout. 2003. Patagonian Argentine Creole cattle polymorphism: comparison with North-West populations of this breed. *Res. Vet. Sci.* 74: 287-290. [https://doi.org/10.1016/s0034-5288\(02\)00190-x](https://doi.org/10.1016/s0034-5288(02)00190-x)



Mastrangelo, S., E. Ciani, P. Ajmone Marsan, A. Bagnato, L. Battaglini, R. Bozzi, et al. 2018. Conservation status and historical relatedness of Italian cattle breeds. *Genet. Sel. Evol.* 50:35. <https://doi.org/10.1186/s12711-018-0406-x>

Meseret, S., Mekonnenb, Y.A., Brenigc, B., Sctz, E., Hannotte, O., Gltas, M., Schmitt, A.O. 2020. Genetic diversity and population structure of six ethiopian cattle breeds from different geographical regions using high density single nucleotide polymorphisms- *Livestock Science* 234:103979.

Poli, M. A. 1986. Asociación entre factores sanguíneos, transferrinas, genes Ps, Bs y aspectos productivos en bovinos criollos. *RIA*, XXI, N 2, 67-76.

Poli, M.A. and A. G. Antonini. 1991. Genetic structure of milk proteins in Argentinian Holstein and Argentinian Creole cattle. *Hereditas* 115: 177-182.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. R. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. W. de Bakker, M. J. Daly and P. C. Sham. 2007. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *Am. J. Hum. Genet.* 81(3): 559-575. <https://doi.org/10.1086/519795>

Rabasa, A. and F. D. Holgado. 2000. Reproductive evaluation of complete diallelic crossbreeding creole-Nelore. *Zootecnia Tropical*. 18(1): 79-90.

Rabasa, A. E., F. D. Holgado and M. A. Poli. 2005. Bovino Criollo de Argentina: diferentes aspectos en su caracterización. *Agrociencia* vol IX N° 2 y 3: 473-477.

Rambaut, A. 2009. FigTree, a graphical viewer of phylogenetic trees. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, United Kingdom.

Rosen, B. D., D. M. Bickhart, R. D. Schnabel, et al., 2020. De novo assembly of the cattle reference genome with single-molecule sequencing. *Gigascience*. 9(3):giaa021. <https://doi.org/10.1093/gigascience/giaa021>

Sal Paz A. R. de, F. Sal Paz, F. Bergmann and S. L. Sal Paz A. R. de, F. Sal Paz, F. Bergmann and S. L. Rabasa. 1976. Asociación de la fertilidad femenina con genes mendelianos mayores en bovinos Criollos. *Mendeliana* 1 (2): 91-95.

Sermyagin, A. A., A. V. Dotsev, A. A. Gladyr, A. A. Traspov, T. E. Deniskova, O. V. Kostyunina, et al. 2018. Whole-genome SNP analysis elucidates the genetic structure of Russian cattle and its relationship with Eurasian taurine breeds. *Genet. Sel. Evol.* 50:37. <https://doi.org/10.1186/s12711-018-0408-8>

Upadhyay, M., Eriksson, S., Mikko, S., Strandberg, E., St. Ihammar, H., Groenen, M.A.M., Crooijmans, R.P.M.A., Andersson, G. and Johannson, A.M. 2019. Genomic relatedness and diversity of swedish native cattle breeds. *Genet Sel Evol* 51: 56 <https://doi.org/10.1186/s12711-019-0496-0>

