Genetic diversity of the Mexican Lidia bovine breed and its divergence from the Spanish population

P.G. Eusebi | O. Cortés | S. Dunner | J. Cañón

1Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Barcelona, Spain
2Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain

Correspondence
P.G. Eusebi, Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain.
Email: pau.g_e@hotmail.com

Funding information
the Consejo Nacional de los Recursos Genéticos Pecuarios; the Genetics Laboratory of the Animal Production Department at the Universidad Complutense of Madrid

Summary
Lidia bovine breed exists since the XIV century in the Iberian Peninsula. These animals were initially produced for meat but some, showing an aggressive behaviour which diffculted their management, were used to participate in popular traditional and social events. A specialization of the breed giving rise to the original Lidia population is documented in Spain since mid-XVIII century. Following the same tradition than in the Spanish population, Mexico used aggressive animals at the beginning of the XX century until two families of breeders started importing Lidia breed bovines from Spain with the aim of specializing their production. Each family (Llaguno and González) followed different breeding managements, and currently, most of the Lidia Mexican population derives from the Llaguno line. Although genetic structure and diversity of the Spanish population have been studied (using autosomal microsatellite markers, Y chromosome DNA markers and mitochondrial DNA sequences), the Mexican population is not analysed. The aim of the study was to assess both the genetic structure and diversity of the Mexican Lidia breed and its relationship with the original Spanish population using the same molecular tools. A total of 306 animals belonging to 20 breeders issued from both existing Mexican families were genotyped, and the genetic information was compared to the previously existing Spanish information. Slightly higher levels of genetic diversity in Mexican population were found when comparing to the Spanish population, and the variability among populations accounted for differences within them showing mean values of 0.18 and 0.12, respectively. Animals from the Mexican breeders, belonging to each of the two families, clustered together, and there was little evidence of admixture with the Spanish population. The analysis of Y chromosome diversity showed a high frequency of the H6 haplotype in the Mexican population, whereas this haplotype is rare in the Spanish, which is only found in the Miura (100%) and Casta Navarra (38%) lineages. Mitochondrial DNA revealed similar haplotypic pattern in both Spanish and Mexican populations, which is in accordance with most of the Mediterranean bovine breeds. In conclusion, as the Mexican Lidia population had initially a small number of founders and its current population has been reared isolated from their Spanish ancestors since a long time, these bottleneck effects and a combination of mixed cattle origin are the factors that might erase any trace of the Spanish origin of this population.

KEYWORDS
D-loop, Lidia cattle, microsatellite, Y Chromosome
1 | INTRODUCTION

Cattle did not exist in America at the time of its discovery as the first bovines arrived to the continent with the second trip of Columbus in 1493, and cattle expansion was favoured by the colonization of the American continent (Ginja et al., 2010). Spanish colonizers brought also to the new lands their traditions and social events which often involved cattle shows.

The participation of bovines is well documented during the first commemorative celebrations of the conquest and of the Mexico foundation in 1523 (Scherrer, 1983), specifying that cattle from Navarrean territories was used. Later and for festivity purposes, the most aggressive animals among those mainly intended for meat production were selected (Domecq, 2009). Towards the mid-eighteenth century, these celebrations acquired such popularity in Mexico that breeders began intensive breeding among the most aggressive animals available in the country. Meanwhile, the same phenomenon was happening in Spain, where the use of specific breeding management and a clear reproductive isolation gave rise to the starting Lidia population division into a small number of differentiated lineages or strains. (Mateus, Penedo, Alves, Ramos & Rangel-Figueiredo, 2004; Prieto-Garrido, 2012).

The growing demand of the Mexican breeders for cattle with this particular behavioural performance to be destined to festivities favoured the arrival of an important number of Spanish Lidia breed individuals from different lineages to Mexico, and thus, the non-specialized animals used during the past two centuries were discarded by most of the breeders. Cattle from Navarrean territories long used in shows became obsolete due to its unwanted behaviour in both countries, and in Mexico, those animals were then relocated to defend mines and monasteries from bandit attacks (Domecq, 2009; Scherrer, 1983).

The Mexican revolution began in 1910, and as a consequence, the lands destined for agriculture and livestock suffered many losses during the following 10 years. Lidia census was dramatically reduced, and two main families imported a reduced number of Spanish Lidia animals between 1908 and 1912 (Niño de Rivera, 2004). The Llaguno family, located in the north-central region of Mexico, maintained the population since then in a closed breeding management system. Meanwhile, González family, located in the south-central region and which also imported Lidia individuals, followed different breeding strategies, matching the new imported bovines with the local ones selected for aggressiveness (Niño de Rivera, 2004).

The current Mexican Lidia breed has derived from animals of both families—80% of breeders arising from Llaguno line, 10% from González family, and the remaining 10% arise from a few lineages imported during 1996 and 1997 before Mexico closed borders to Spanish bovine importations (according to the data provided by the Mexican Lidia Breeders Association’s Herd Book—ANCTL). Currently, Mexican Lidia population comprises around 110,000 animals (ANCTL) distributed in an area of 135,000 hectares and held under traditional free-range conditions, which add a strong impact on landscape conservation. Lidia breed social events play a key role in the Mexican economy and are also part of social traditions that reinforce the identity of local communities (Niño de Rivera, 2004; Scherrer, 1983).

Molecular markers allow detecting breed relationships and geographic patterns of diversity studies as indicators of migrations, admixture and genetic bottlenecks (Groeneveld et al., 2010). Genetic variability of the Spanish Lidia breed has been previously analysed with autosomal microsatellite markers, revealing high genetic differentiation among lineages (Cañón et al., 2008). Also, genetic analysis showed two major maternal and paternal lineages: T3 and T1 for the former, and Y1 and Y2 for the latter (Cortés, Tupac-Yupanqui, Dunner, Fernández & Cañón, 2011; Cortés et al., 2008). Although there is a trend to switch to SNP markers for use in genetic diversity studies, there is an important amount of genetic data based on microsatellite markers proposed for the FAO (2015) which were used for the measurement of animal genetic diversity in several breeds such as bovine Lidia and Creole breeds (Cañón et al., 2008; Delgado et al., 2012; Martinez et al., 2012). Moreover, SNP genetic information in those populations is either not available or scarce. As the genetic structure and genetic diversity of the Mexican Lidia breed and its relationship with the original Spanish population have never been explicitly studied before, the aim of this study was to investigate these aspects using three molecular sources of information: autosomal microsatellite markers, Y chromosome DNA markers and mitochondrial DNA (D-loop) sequences.

2 | MATERIAL AND METHODS

2.1 | Mexican population sampling

A total of 306 bovine samples were collected from randomly chosen animals belonging to 20 different Mexican breeders [three breeders raising animals whose origin is the González family (G) and seventeen breeders belonging to Llaguno family (L)] as defined in Table S1 according to the standards set by the ANCTL.

Samples were collected in Magic Buffer® tubes (Biogen Diagnostica, Spain), and these were maintained at 15°C until use, guaranteeing DNA integrity (Dunner & Cañón, 2006).
2.2 | Spanish population

In accordance with the aim of the study, genotypic information derived from 24 autosomal microsatellites previously used by Cañón et al. (2008) was used to determine genetic variation of the Spanish lineages. Relying on historical information of the importations made to Mexico from bovines of selected lineages since the early XXth century, and to track those lineages, 854 Spanish genotypes belonging to 14 lineages provided by the Genetics Laboratory of the Animal Production Department of the Universidad Complutense of Madrid as shown in Table S1 were selected. In addition, mtDNA (D-loop) sequences and Y chromosome markers derived from previous analysis (Cortés et al., 2008, 2011) were used.

2.3 | Microsatellite genotyping and Sequencing alignment

Genomic DNA for the 306 samples was obtained using a standard phenol/chloroform method (Sambrook, Fritsch & Maniatis, 1989). Twenty-four microsatellite loci were used according to the FAO-recommended microsatellites list (Cañón et al., 2008) to allow accordance with Spanish lineages genotypes. PCR products were marked with fluorochromes according to the fragment to amplify, and capillary electrophoresis was performed in an automatic sequencer ABI Prism® 3500 Genetic Analyzer (Applied Biosystem, USA).

Y chromosome analysis was performed following the recommendations described by Cortés et al. (2011) to analyse Spanish and Mexican Lidia animals. A total of 29 samples belonging to González (5) and Llaguno (24) families (Table S1) were genotyped. Likewise, DNA material information was analysed based on the protocol described by Cortés et al. (2008). Finally, 30 samples belonging to González (4) and Llaguno (26) (L), respectively, were chosen to obtain a 521-bp fragment of mtDNA that was sequenced encompassing positions 16019–160201 (Anderson et al., 1982). Fragments were amplified using PCR and then purified with the Concert Rapid PCR Purification System (Life Technologies) according to the manufacturer’s instructions. Sequencing was performed in an ABI Prism® 3500 Genetic Analyzer (Applied Biosystem).

2.4 | Population genetics analyses

Genetic diversity parameters such as allele frequencies, total number of alleles per locus (NA), observed (Ho) and expected (He) heterozygosities, and mean number of alleles (MNA) per population were obtained using GENEPOP v.1.2 (Raymond & Rousset, 1995). Wright F-statistics were obtained with GENETIX v.4.05 software (Belkhir, Borsa, Chikhi, Raufaste & Bonhomme, 1996), and allelic richness estimation and F-statistics differences between countries were carried out with FSTAT v.2.9.3 (Goudet, 2002) program. Deviations from the Hardy–Weinberg equilibrium were tested using the chi-squared test with GENALEX v.6.5 package (Peakall & Smouse, 2012).

The proportion of mixed ancestry for Mexican and Spanish populations was analysed with the Bayesian clustering algorithm implemented in STRUCTURE software (Pritchard, Stephens & Donnelly, 2000) which uses multilocus genotypes and a Monte Carlo Markov chain simulation to infer population structure and assign individuals to a supposed population, assuming the fact that an individual may have mixed ancestry from different underlying populations. The figurative number of clusters (K) considered ranged from 2 to 6 with six replications for each value of K. We considered those runs sharing a maximum-likelihood pattern and therefore selected one of them to display the graphic with DISTRUCT v.1.1 software (Rosenberg, 2004).

Y chromosome haplotype analysis was performed with the Y-specific microsatellite markers located in the non-recombinant fragment of the Y chromosome. Genotypes were classified into their corresponding haplogroup according to Gøtherström et al. (2005), and the following analyses were performed in accordance with Cortés et al. (2011). A neighbour-joining tree was produced from the pairwise FST values (bootstrapped p-value <0.05) using the POPTREEW (Takezaki, Nei & Tamura, 2014) software.

Mitochondrial DNA sequences were restricted using the region of overlap between positions 16042 and 16280 to classify their corresponding haplotypes as defined by Anderson et al. (1982), and the following analyses were performed in accordance with the previous work performed by Cortés et al. (2008).

3 | RESULTS

3.1 | Microsatellite markers

The information obtained from the 24 microsatellite markers revealed a total of 169 alleles detected in the Mexican individuals and 233 alleles in the Spanish ones (Table S2). The number of alleles per locus ranged from 5 to 11 in the Mexican population and 6 to 20 alleles per locus in the Spanish lineages. Regarding observed heterozygosities, the means across loci were 0.59 in the Mexican samples versus 0.54 in the Spanish samples and expected heterozygosities were 0.62 and 0.59 from the Mexican and Spanish samples, respectively. The proportion of genetic variability accounted by differences among breeders or lineages within Mexico and Spain and estimated by FST had a mean value of 0.10 and 0.18, respectively (Table S2).
3.2 Genetic diversity

Genetic diversity parameters are shown in Table 1. Mexican and Spanish populations evidenced similar average number of alleles, mean number of alleles and allelic richness. Mexican Garfias and Corlomé breeders showed the lowest values for these parameters, which are similar to those previously reported by Cañón et al. (2008) for Albaserrada and Conde de la Corte Spanish lineages. Average values of expected heterozygosities were 0.61 and 0.62 for Mexican and Spanish population, respectively, and observed heterozygosities were 0.59 in the Mexican breeders and 0.54 in Spanish population, with the lowest value found for the Mexican Carlos Castañeda breeder.

The average $F_{IS}$ in Mexican population was 0.041, twice less than that in Spanish lineages (0.083). The highest $F_{IS}$ value was found in Rancho Seco breeder (0.183) derived from González family. The number of loci deviated from Hardy–Weinberg equilibrium was higher for Spanish Lidia population, with an average of seven loci per breeder comparing to the average of two loci per lineage in the Mexican population (Table 1).

The pairwise matrix of $F_{ST}$ distances among lineages and breeders is shown in Table S3. It is remarkable that the highest $F_{ST}$ values between Mexican breeders (e.g., Carlos Castañeda and de Haro both belonging to González family) had a similar magnitude than the lowest value among Spanish lineages. Genetic distances among the Mexican breeders were significant ($p < .05$), with an average $F_{ST}$ value of 0.10, significantly lower ($p < .05$) than the 0.18 achieved among the Spanish lineages.

3.3 Population structure

Mexican breeders and the 14 Spanish lineages selected by historical criteria were jointly analysed using the model-based clustering method (Pritchard et al., 2000). For lower $K$ values, some Spanish lineages (Anastasio Martín, Atanasio Fernández, Conde de la Corte, Domecq, Gameiro Cívico, Murube and Veragua) and Mexican breeders were clearly separated in different clusters, and therefore, these Spanish lineages were removed in posterior analysis. Concha y Sierra, Miura, Casta Navarra, Saltillo, Albaserrada and Santa Coloma were the remaining Spanish lineages left in this analysis. Table 2 shows a certain degree of admixture of González breeders and one breeder from Llaguno (San José) with Spanish lineages Santa Coloma and in less proportion with Albaserrada and Saltillo for low $K$ values (see also Figure S1). Furthermore, the remaining Spanish lineages and Llaguno breeders were grouped in different clusters. STRUCTURE results for Mexican breeders evidenced for $K = 2$ a clear separation among González and Llaguno breeders except San José (JOS), Torreón de Cañas (TOR) and some individuals from Encinos (ENC), which were clustered with breeders from the González family. When $K = 4$, most of the genetic variability of all the Llaguno family breeders is clearly identified, with some exceptions such as Torreón de Cañas (which clustered in a second group) and to a lesser extent a third cluster composed by San José, Encinos, Corlomé, Xajay, Fernando de la Mora and Marrón (Figure S1).

3.4 Y chromosome Diversity

Three of the ten haplotypes previously identified in the Spanish population (Cortés et al., 2011) were found in the Mexican population. Mexican Y chromosome haplotype frequencies are shown in Table S4. It should be noted that haplotype H6, found at frequencies of 69% and 20% in Llaguno and González breeders, respectively, was only present in Miura (100%) and Casta Navarra (38%) lineages of the Spanish population.

The neighbour-joining dendrogram constructed from $F_{ST}$ genetic distances (Figure 1) clearly evidenced two major groups constituted by the Y1 and Y2 haplogroups; Mexican breeders grouped in their respective families were placed in different branches into the Y2 group. Llaguno family is located in the same branch with Miura as their males are carriers of H6 haplotypes, while González family, which carries H1 and H6 haplotypes, is positioned in a different branch but close to Casta Navarra.

3.5 Diversity of mtDNA

The haplotype distribution for the Mexican D-loop mitochondrial DNA sequences (Table S5) showed a typical southern European pattern according to Felius, Koolmee, Theunissen and Lenstra (2011), with T3 as the predominant haplotype (67%), T1 the less common (17%) and T at a very small frequencies (3.3%).

4 DISCUSSION

Our analysis of the Mexican Lidia population illustrates a significant differentiation from the Spanish lineages. The mean point estimate of the genetic diversity parameters estimated in the Mexican population (Table 1) is higher, although not significant, than those found in the Spanish lineages from which it hypothetically arose. So, the genetic differences among Mexican breeders are lower than the differences among Spanish lineages due to lower reproductive isolation between breeders comparing with the strict isolation among Spanish lineages.

However, the analysis of the population structure highlighted a strong clustering tendency for most of the
Mexican breeders, and both populations (Mexican and Spanish) segregated as soon as $K = 3$. Santa Coloma followed by Albaserrada and Saltillo are the lineages sharing the higher proportion of ancestry with the Mexican breeders (Table 2 and Figure S1). This supports the documented role of Santa Coloma in the development of the Mexican Lidia breed during 1996–1997 (ANCTL). Although Saltillo is considered one of the founder lineages of Mexican Lidia population, our results evidence less ancestry of this lineage in the Lidia Mexican breed than Santa Coloma. Moreover, at $K = 4$, de Haro and Carlos Castañeda breeders (both belonging to González family) show different ancestry when comparing to breeders from Llaguno family; this is the result of a strong reproductive isolation due to close-breeding strategies of these breeders in spite of the traditional conservation strategies of the Gonzalez family (Figure S1).

The fact that some diversity parameters found in the Mexican population show values as high as those found in

### TABLE 1  Genetic diversity parameters per population: population (Pop), lineage, acronym, expected heterozygosity ($H_e$), observed heterozygosity ($H_o$), mean number of alleles (MNA), effective number of alleles (NE), allelic richness per locus corrected for lineage/breeder sample size (AR), $F_{IS}$ within-lineages inbreeding coefficient and significance (*p < 0.01) and number of loci not complying with Hardy–Weinberg equilibrium (DHWE) (*p < 0.01)
the Spanish one from which it originates might suggest that native cattle breeds have probably been introgressed into the founding population of Mexican Lidia breed brought from Spain. For this reason and to test this hypothesis, we used microsatellite marker information derived from previous studies of the following breeds: Avileña, Morucha, Retinta and Canaria from Spain, due to their historical ancestry with Mexican Creole populations, and also Creole populations from Puebla and Baja California in Mexico and Texas Longhorn from the USA (Delgado et al., 2012; Martínez et al., 2012). This data set shared 16 of the 24 microsatellites originally used in the Lidia breed. After discarding those lineages not showing major relationships with either Mexican or Spanish populations, we visualized genetic $F_{ST}$ distances via NeighbourNet graphs using SPLITSTREE 4 (Huson & Bryant, 2006). Figure 2 shows the complete network of the Mexican families together with the Spanish Lidia, the ancestral Spanish and American Creole breeds. This network not only confirms previous results obtained with STRUCTURE software (Pritchard et al., 2000), but also tells us that Mexican Lidia population forms a separate cluster from the ancestral Spanish and American Creole breeds. So, the hypothesis of the genetic influence of Creole cattle in the Mexican Lidia population could not be confirmed with the samples used in this work.

Three of the ten Y chromosome haplotypes present in the Spanish Lidia breed (Cortés et al., 2011) have been found in the Mexican population. The traditional practice in this production system of using a reduced number of males, and the unjustified idea of breeders that inbreeding would fix a desirable behaviour, has led to an isolation trend between breeders and a low effective population size (Villanueva Lagar, 2005), whose effects are magnified when this type of molecular information is used.
Visualization of genetic distances in the neighbour-joining dendrogram (Figure 2) revealed the proximity of Llaguno to Miura and González to Casta Navarra lineage. These proximities are explained by the presence of Y chromosome H6 haplotype in these four groups. According to this, we propose the hypothesis that the bull “Murcielago” which belonged to Casta Navarra lineage in 1879 and was introduced into the Miura herd (López del Ramo, 1991) imprinted the H6 haplotype into this lineage. Such migration involving one individual from a subset of the Casta Navarra population would have led to a stepwise increase in genetic drift and a subsequent decrease in the genetic diversity. This founder effect could be the explanation that in Mexico, the resemblance to Miura is often considered through the influence of Casta Navarra lineage (Niño de Rivera, 2004). Despite the fact that the presence of Saltillo lineage has been historically proven, no traces of this paternal ancestor were detected in this work.
Similar genetic patterns of mtDNA haplotypes than that previously reported for the Spanish Lidia lineages (Cortés et al., 2008) and for Southern bovine European breeds (Felius et al., 2011) were observed. In addition, the T haplotype frequency was higher in the Mexican population (3.3%) than in the Spanish lineages (1.1%). The original diversity and a certain population subdivision maintain, as in the Spanish breed (Cortés et al., 2008), this haplotype richness.

The reduced population size of the Mexican Lidia breed (Villanueva Lagar, 2005) along with a reproductive isolation among breeders and a not well-defined mixed origins have erased traces of its autosomal genetic relationships with the Spanish breed and position the Mexican population separately from the Spanish lineages with some exceptions that are a result of recent introgression. Also, despite the fact that the presence of Saitillo lineage has been historically proven in Mexico, no traces of this ancestor were detected in this work.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Biobovis consortium (http://biobovis.jimdo.com/) for providing genetic information of the Spanish local and American Creole breeds, also to the breeders of the ANCTL for providing the animal material. This work has been funded by grants of the Consejo Nacional de los Recursos Genéticos Pecuarios and by the Genetics Laboratory of the Animal Production Department at the Universidad Complutense of Madrid

REFERENCES


SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.