Universitat Autònoma de Barcelona

Genetic Characterization of the Mexican Bovine Lidia Breed

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Thesis
Submitted in fulfillment of the requirements for the degree of doctor
under the supervision of:

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UNIVERSITAT AUTÒNOMA DE BARCELONA
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We should always put a little art into what we do.

It's better that way

–Jules Verne

El **Dr. Javier Cañón Ferreras**, Catedrático del Departamento de Producción Animal

y

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CERTIFICAN:

Que **Paulina García Eusebi** ha realizado bajo su dirección el trabajo de investigación titulado

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Summary

The cattle of the Lidia breed have been selected during centuries for behavioral related traits, a peculiarity that distinguishes it from the rest of the bovine breeds, selected mostly for characteristics of productive interest, such as meat and milk. In Spain, the original Lidia population has been studied through genomic data, allowing to know that the genetic richness of the breed is owed to the contribution of each of the multiple lineages or encastes in which it is subdivided. In Mexico, the Lidia breed represents an important historical and cultural legacy and currently, its population has not been genetically characterized.

In this thesis we analyze the genetic diversity and structure of the Mexican population and compared it with data from the original Spanish population by using genomic information derived from different types of molecular markers.

First, we analyzed parameters of genetic diversity in both populations using Microsatellite and Single Nucleotide Polymorphisms autosomal markers, finding similar values of expected heterozygosities with both types of molecular markers. We found also high values in terms of F_{IS} in both populations. Both, the high values of F_{IS} in the lineages and the behavior of the Runs of Homocigosity are a consequence of the lineages´ low census, contributing hence to increase the inbreeding rate. Furthermore, we detected high genetic differentiation between populations with both types of molecular markers: microsatellite and SNP, and the partition of the total genetic variability analyzed with SNPs showed that 19% of the variation is explained by the genetic differences among lineages within populations. Curiously, the genetic structure of the Mexican population revealed that it shares few common genetic origins with the original Spanish population, placing both populations in different groups.

The Y chromosome analysis evidenced the paternal footprint that Casta Navarra has left in the Mexican population through a high frequency of the H6 Haplotype, exclusive of this

lineage. Mitochondrial DNA analyzes, on the other hand, revealed similar haplotype patterns in both populations.

Finally, considering the peculiarity of the selection performed in this breed, we carried out an analysis to detect signatures of selection that could affect agonistic behavioral related traits, using as a reference two tamed Spanish breeds. Using two methods based on Bayesian inferences, we jointly identified two selected genomic regions. Also, the direction and intensity in the frequency of the allele selected of the Lidia breed is opposite to that of the tame breeds. In these regions were detected genes associated to metabolic pathways such as serotonin and dopamine, as well as genes expressed in the brain cortex, which have been related to patterns of aggressive behavior in humans and laboratory animals.

Resumen

El ganado de la raza de Lidia ha sido seleccionado durante siglos por caracteres relacionados al comportamiento, una peculiaridad que la distingue del resto de las razas vacunas, principalmente seleccionadas por características de interés productivo, como carne y leche. En España, la población de Lidia originaria ha sido estudiada por medio de información genómica, permitiendo conocer que la riqueza genética de ésta raza se debe al aporte proporcionado por cada uno de los múltiples encastes o linajes en los que se subdivide. En México la raza de Lidia representa un legado histórico y cultural importante y actualmente, su población no ha sido caracterizada genéticamente.

En esta tesis analizamos la diversidad y estructura genética de la población Mexicana y la comparamos con información proveniente de la población originaria Española utilizando información genómica mediante diferentes tipos de marcadores moleculares.

Primero analizamos los parámetros de diversidad genética en ambas poblaciones con marcadores autosómicos de tipo Microsatélite y Polimorfismos de nucleótido único, encontrando valores similares de heterocigosis esperada con ambos tipos de marcadores moleculares. Encontramos también valores elevados en términos de F_{IS} en ambas poblaciones. Tanto los valores elevados de F_{IS} en los encastes así como el comportamiento que presentan las Carreras de Homocigosis son consecuencia del bajo censo de los encastes, contribuyendo por ende a incrementar la tasa de endogamia. También encontramos una alta diferenciación genética entre poblaciones con ambos tipos marcadores moleculares; microsatélites y SNPs. La partición de la variabilidad genética total analizada con SNPs mostró que el 19% de la variación se explica por las diferencias genéticas entre linajes. Curiosamente, la estructura genética de la población mexicana reveló que comparte escasos orígenes genéticos en común con la población originaria española, ubicando a ambas poblaciones en grupos diferentes.

Summary

El análisis de cromosoma Y mostró que la Casta Navarra ha dejado huella paterna en la población mexicana mediante una frecuencia elevada en el haplotipo H6, exclusivo de ésta casta así como del encaste de Miura. Los análisis de ADN mitocondrial, por otro lado, revelaron patrones de haplotipos similares en ambas poblaciones.

Por último, considerando la peculiaridad en la selección de esta raza, realizamos un análisis para detectar huellas de selección que pudieran afectar caracteres asociados a comportamiento de tipo agonista, utilizando dos razas mansas españolas como referencia. Utilizando dos métodos que se basan en inferencias bayesianas, identificamos en común dos regiones genómicas seleccionadas. A demás, la dirección e intensidad en la frecuencia del alelo seleccionado en la raza de Lidia es opuesto a los de las razas mansas. En éstas regiones detectamos genes asociados a rutas metabólicas como las de la serotonina y la dopamina, así como genes expresados en corteza cerebral, los cuáles han sido relacionados con patrones de comportamiento agresivo en humanos y animales de laboratorio.

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Abbreviations

B.P.	Before Present
bp	Base pair
CHR	Chromosome
Ha	Hectares
Ho	Observed heterozygosity
He	Expected heterozygosity
HWE	Hardy- Weinberg equilibrium
LD	Linkage Disequilibrium
Mb	Mega base pair
mtDNA	Mitocondrial DNA
PcoA	Principal Coordinates Analysis
ROH	Runs of Homozygosity
SNP	Single Nucleotide Polymorphism

GENERAL INTRODUCTION

1. NATURAL HISTORY OF THE TAURINE CATTLE

1.1 Domestication, migration and origin of cattle breeds

The bovine cattle have been associated socially, culturally and economically to the development of the human kind, and is one of the most important livestock species (Maudet, 2010). The domestication of the taurine bovines (*Bos taurus taurus*) occurred between 10,300–10,800 years ago in the Fertile Crescent, placed at the west of the Turkish-Syrian border (Helmer et al., 2005; Vigne et al., 2011).

The taurine cattle descend from the extinct aurochs (*Bos primigenius primigenius*); a savage type of bovines that ranged over most of the Eurasian continent. Molecular estimations of the divergence time of the aurochs subspecies are of 147,000 years BP and, thus of taurine (335,000 BP) and zebu cattle (350,000 BP) is estimated to happen long before. The most recent divergence time of the aurochs is believed to happen given the difficulty of their management and breeding (Felius et al., 2014). This huge and reputedly fierce species is extinct since 1627, dying the last animal in Poland (Edwards et al., 2007).

From the center of domestication, the lack of pasture during the winters and the harsh climatic conditions gave rise to the transhumance, and thus, a large proportion of the domesticated cattle began to migrate and expand during the Neolithic transition (Felius et al., 2014). First, with a westward expansion of the agricultural societies approximately 10,000 to 8,500 years BP to Europe (Conolly et al., 2012) and then a second eastward migration to China, between 5,000 and 4,000 BP (Payne et al., 1997). The expansion of cattle to Europe followed two routes (**Figure 1**): The Mediterranean, where the first bovine settlements were placed in the south of Italy, north of Africa, the Tyrhenian Islands, south of France and in the Iberian Peninsula, and the Danube route, which followed a north route bordering the Balkan

rivers, establishing then the first bovine populations in the Centre and North of the European Continent (Shceu et al., 2015). Also, an influence of North African cattle is well documented on Iberian breeds, attributed mainly to the Moorish occupation and to cattle exchanges via the Straits of Gibraltar during the Bronze Age (Cymbron et al., 2005; Beja-Pereira et al., 2006).

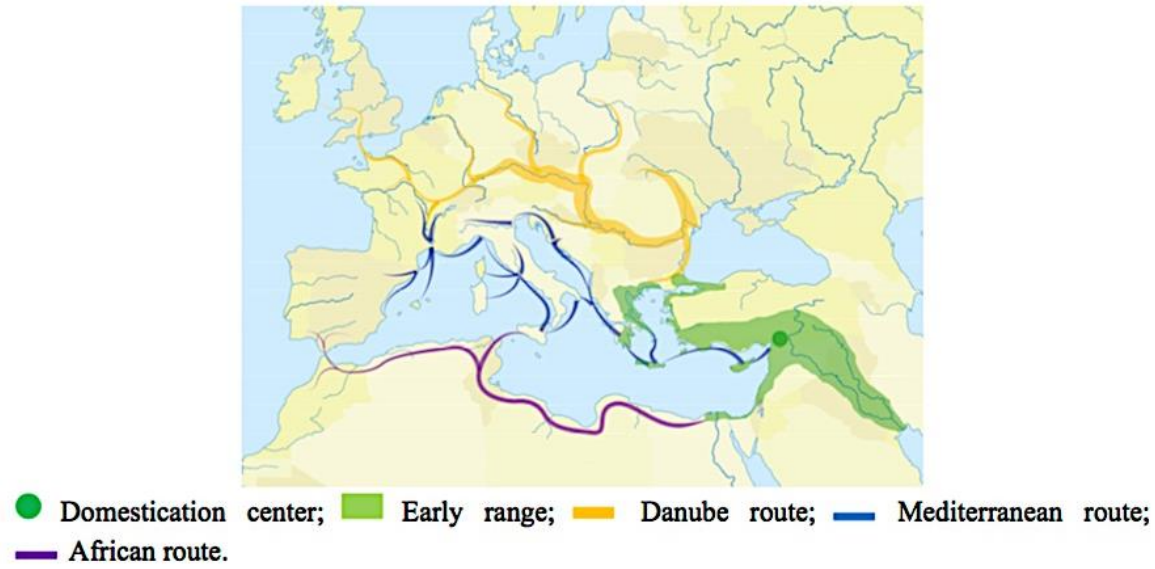

Figure 1 Migration routes of the taurine cattle in Europe (Felius et al., 2014).

The demographic events that took place after the domestication have been described by means of archeological evidence along with molecular tools using autosomal DNA, mitochondrial DNA (Ajmone-Marsan et al., 2010), and Y-chromosomal DNA (Pérez-Pardal et al., 2010). The phylogenetic analyses of mitochondrial DNA sequence variations of the modern *Bos Taurus* allowed identifying four maternal clusters of haplogroups designated as T1, T2 and T3, which coalesce to a central haplogroup T (Troy et al., 2001). The geographic haplogroup distribution shown several spatial clusters such as: the high haplgroup diversity in Southwest Asia with the presence of the four major mitochondrial haplogroups, the prevalence of the T3 haplogroup in Europe and the almost exclusive occurrence of T1 in Africa (Lenstra et al., 2014). Furthermore the frequencies of the T1 haplogroup in Spain (15%) and Portugal (11%), and also in Italian and Greek bovine breeds, depict the influence of the migration of African cattle into Europe across the sea straits of the Mediterranean

route. This is believed to happen at the early Bronze Age or during the Muslim occupation (Anderung et al., 2005; Beja-Pereira et al., 2006).

The paternal genetic origins based on Y chromosome data that help to depict the migration pattern of the cattle into Europe allowed identifying two haplotypes (Y1 and Y2) preceding the contemporary cattle breeds (Götherström et al., 2005). The geographic distribution of these two haplotypes follows a clear geographic structure; the Y2 haplotype has high frequencies in the south of Europe (the Iberian Peninsula, France Switzerland and Italy), while the haplotype Y1 in the north European breeds (Götherström et al., 2005). Some of the north-south interpretations of the Y1 and Y2 haplotype distributions mention that these distributions are result of two different migration events coming from the Near-east through the Danubian and Mediterranean routes, and it also can be due to adaptative differences along geographical areas that shift allele frequencies as a response to selection (Beja Pereira et al., 2006). There is also the hypothesis that the haplotype Y2 colonized Europe earlier, with a first European cattle arrival, followed by a local introgression with auroch bulls that may contributed to create the Y1 haplotype (Götherström et al., 2005).

From the Hellenic period to the Middle Ages, cattle were not differentiated in breeds as we know them now. Livestock at that period of time was raised in order to meet the population´s needs, which varied over the regions. For example, in the Netherlands at the middle of the XVI century the cattle were already recognized in the region as specialized in milk production, leading place to migrations of cattle throughout Europe (Bieleman, 2000). Over the years, at the beginning of the industrial revolution, a great diversity of cattle populations were already classified under a breed´s name and livestock husbandry became organized following breeding systems as a consequence of the farmers concern to increase the productivity of their animals (Felius et al., 2011). Animal breeding then, became a social concept of the upper bourgeoisie through breeding societies that created the *herd books*

making emphasis on the livestock selection towards the “racial purity” of their animals, and aiming to get an attribute of prestige for the animals with pedigree records (Felius et al., 2011).

At the XVIII century almost all the sires of the current main productive bovine breeds were selected following a breeding criterion, some of their pedigrees were registered in the herd books and begin to spread all over Europe and then to America and Australia (Felius et al., 2015).

The creation of breeds changed the distribution of the diversity in a way that the groups of herds that constituted a breed acquired uniformity, emphasizing their differentiation. From the XVIII century, popular breeds spread widely outside their region of origin and by the XX century, two centuries later, most of the more popular breeds got differentiated because of their productivity in meat or milk production, like the Holstein-Freisians, where the most numerous cattle worldwide (Felius et al., 2015). Conversely, the dispersal of the popular breeds favored the disappearance of local breeds, less productive but adapted to their geographical environment.

1.2 Cattle arrival to America

Considered one of the most important events in the history of the human kind, the discovery of America produced great social and economic changes at both sides of the Atlantic Ocean (Martínez et al., 2012). With the arrival of the Spanish colonizers in 1492 a whole new world was discovered for cattle, non-existant in America, where the pre-Columbian civilizations bred mainly dogs, turkeys, guinea pigs, and three species of Andean camelids. In this sense, Columbus's trips had a great impact on the exchange of animal and plant genetic resources between continents that revolutionized the population's lifestyle in terms of food types and nutritional habits (Crosby, 2003).

The bovines were firstly introduced to America by Christopher Columbus on his second trip which departed from Cadiz in 1493 to the Caribbean island of "La Española" (**Figure 2**) transporting mainly horses, a few calves, goats and pigs. It was until his third trip in 1498 when more bovines were brought to the island (Payne et al., 1997). In “La Española”, livestock breeding was a challenge, presenting some complications in the first years after their introduction because those animals had to overcome and adapt to the new meteorological and forage conditions, along to the natural difficulties of the tropical ecosystems. For example, in 1505 a hurricane devastated almost all the bovine population and, due to these meteorological disasters a law was crreated, avoiding the arrival of cattle to the island. Thus the breeding of the extant bovines became an exclusive practice and a privilege for a reduced amount of farmers (Payne et al., 1997). For the next fifty years, each ship departing to America could legally transport just five or six bovines, and from them, just two or three were expected to survive the journey.

Figure 2 Columbus route during his second trip (1493). Image obtained from http://www.crossingtheoceansea.com

As consequence of such restrictions, the bovine population census at the Caribbean colonies in 1524 was around 1,000 bovines, and from these islands some were distributed to the Spanish colonies in the continental America. Most of the bovines that were transported belonged to populations of the northwestern African coast and the Canary Islands that were

part of the Spanish occupation since 1479. This was the last port where the ships stop before crossing the Atlantic Ocean (de Alba, 1987; Martínez et al., 2012).

As mentioned above, it is complicated to define the cattle breeds that were brought to America since, the concept of breed was not defined at this period of time. However, it is documented that, besides the Canarian and African bovines, the cattle that populated America between 1493 and 1512 became also from the Iberian Peninsula, one in four animals became from the marshlands of the Guadalquivir river, so those animals could be considered today as belonging to the Retinta Andaluza and the Marismeña breeds (Rodero et al., 1992). A second group of bovines arose from the North-westlands of Spain in the provinces of Galicia and Asturias, where bovines from the breeds Asturianas and Gallegas are identified (de Alba, 1987). And finally the Palmera breed of the Canary Islands is considered also a basis of the formation of the American creole breeds, as it was the last port of landing before embarking to America (Rodero et al., 1992).

1.3 Cattle arrival to Mexico

The first 50 bovines that landed in the Mexican territories where introduced by Gregorio Villalobos in 1521. Later in 1524, Rodrigo de Bastidas carried from the island “la Española” 200 bovines to Mexico. Those animals constituted the first bovine population in the continental land (Suárez-Domínguez & López-Tirado, 1996). These animals were distributed through different regions along the coast of the Gulf of Mexico, and by 1540 husbandry practices were already spread into the central Mexican steppe.

After, during 1565 cattle of Iberian origin was brought to the Pacific coast of Mexico, getting to the Peninsula of Baja California in 1670 (Ulloa-Arvizu et al., 2008).

The importations of cattle from Spain to Mexico and, in general, to all of the new colonies, were highly controlled by the “House of Contract”, an institution that was responsible to dealt

with the illegal commerce of livestock at the moment of crossing through the Atlantic Ocean. This institution lasted 200 years and became very popular in the Canary Islands, where all the cattle transported to America was registered (Rodero et al., 1992). It is difficult to know the census of the Mexican cattle populations during the first two centuries after their introduction, the only information that allows knowing an approximation of the census are the marketing records of taxes from the 18th century onwards that provide the records of the movements of cattle to the big cities (Celaya-Nández, 2003).

The bovine cattle imported until the end of the XIX century were taurine cattle (*Bos t*. *taurus* or humpless*)* and at the beginning of the XX century the first indicine bovines (*Bos t*. *indicus* or humped) were imported, because humped cattle is better adapted to the meteorological conditions in tropical regions located at the south-east region of Mexico (Guevara & Lira-Noriega, 2011).

2 THE LIDIA BREED

2.1 It's Origin, the concept of *Tauromaquia* and its representations

As a need to represent the strength of the nature in a cultural way, the man has used the bovines as a symbol to represent it and, in a certain way representing a defeat of this natural world (Viard, 2014). Those animals that most probably were the fierce aurochs are the iconography represented in the paintings at caves representing the prehistoric hunts at the Paleolithic and Neolithic ages all over the Iberian Peninsula (Viard, 2014; Felius et al., 2015). Later, the Classic Civilizations used bulls also in games and festivities always with a religious nuance, such examples are embodied in the murals at the Cnosos palace in Creta, the *taurokhatapsies* in Thessaly and also in the evidences derived from the Mitriac rituals and the *venations* of the Roman Empire (Viard, 2014).

The oldest evidence of festivities that resembles the present *tauromaquias* belong to the XII century in the south of France and north of Spain, where frequently, the cattle that crossed the main streets of the towns running in the way to the slaughterhouse and, those animals whose aggressive behavior made difficult their handling where set apart and destined to take part in the festivities (Domecq, 2009; Viard, 2014). However, these bovines were reproduced un-orderly until the middle of the XVIII century and, from then, the breeders created the herd book and with it the breeding of their animals became specialized, creating rational selection systems based on their morphological traits and behavioral characteristics (Domecq, 2009; Prieto-Garrido, 2012).

In that period of time it was economically more profitable to raise cattle of the Lidia breed as it has higher prices than those of the cattle selected for meat production and, has also more regularity in the buyers demand (Martínez, 1995).

The term *tauromaquia* is related frequently to a restrictive meaning associated to the "*Spanish corrida*" which is a total misconception. The term "*Tauromaquia*" makes reference to numerous cultural representations of the acts and festivities involving bovines (Maudet, 2010). The cultural air of the *tauromaquias* has been extended from the southwest Europe to America; where a wide spectrum of practices are found (from the great American rodeo in North America, the Charreada and Jaripeo in Mexico, the bulls collected in Colombia and Venezuela, the Rodeo montubio in Ecuador, the *vaquejada* and rodeo creole in Brazil, the Chilean rodeo, etc.), all along with the traditional festivities of Spanish origin (Saumade, 2014).

In Portugal, there is a variation of the Spanish corrida known as *Portuguese corrida*, and in France there is a tauromaquia based on popular courses or races in the Camarguesse and Landaise regions. Also, it is remarkable the great diversity of popular spectacles using Lidia breed cattle, such as the *encierros,* the *bous al carrer*, the *bou embolat*, the *encaixonats,* etc.

(Saumade, 2014). The *tauromaquias* are hence, a depository of a set of rituals in which the central axis is the bovine, constituted in all their historical, geographical and cultural contexts.

2.2 Original population and the founding "Castes"

The founding castes are the original or first populations of aggressive selected bovine cattle, whose production and reproduction became specialized at mid-XVIII century. Those bovines where classified according to their morphological and behavioral differences which coincided also with differences in geographical origin (Domecq, 2009). The regions with the largest number of Lidia cattle were located in Navarre, Castilla and Andalusia, and in lesser extent in Extremadura, Aragon and Portugal. Showing a strong relation between the castes and the principal Spanish rivers; like the Navarrese (Ebro), the Castilian (Duero, Tajo and Guadiana) and the Andalusian (Guadalquivir) river basins (**Figure 3**).

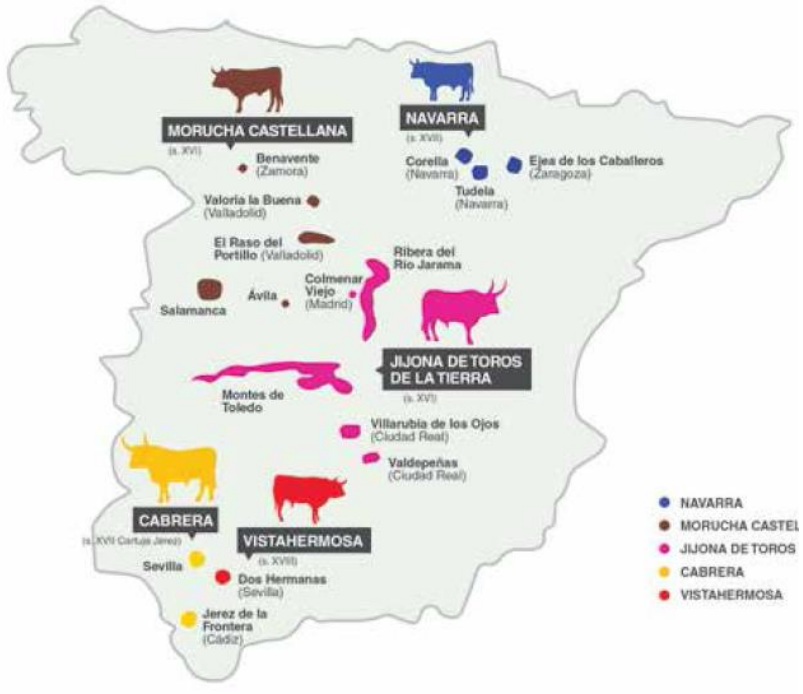

Figure 3 Geographical locations of the bovine nuclei where the original castes were born (U.C.T.L., 2018).

According to the Lidia Breeders Association (U.C.T.L., 1995) there are identified seven founding castes, which are: Morucha Castellana, Navarra, Jijona, Cabrera, Vazqueña, Vistahermosa and Gallardo (**Figure 4**). These castes are defined in the racial standards stated by the Spanish Boletin Oficial del Estado (B.O.E., 2001) in which the original diversity of the Lidia bovine breed are legally defined and, specified also the subdivision into subpopulations named "encastes" or lineages, whose behavior and morphology is different among them.

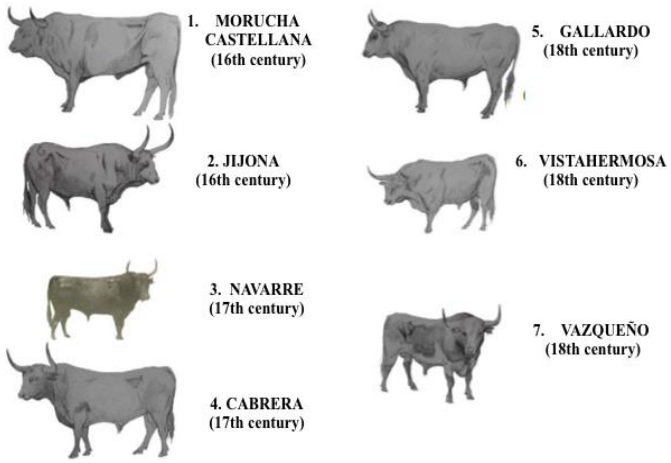

Figure 4 Lidia original founding castes and its raising century.

2.3 Current genetic diversity and structure of the Spanish population

Different types of traditional popular events demanded different types of behavior for the bulls taking part in the events, this fact favored the subdivision of the original founding castes into lineages that, at present are officially recognized (B.O.E., 2001). At present, the main representative's lineages are located mainly at the west and southwest of the Iberian Peninsula, and do not follow any particular distribution pattern across the geographical distribution areas (Cortés et al., 2008).

Numerous studies have been done to characterize genetically and depict the paternal and maternal influences of the Spanish Lidia breed (Cañón et al., 2008; Cortés et al., 2008; Cortés et al., 2011; Pelayo et al., 2015).

The genetic diversity of the Spanish lineages was described by Cañón et al. (2008) whom using autosomic microsatellite DNA genotypic information detected that: **(1)** the Lidia breed as a whole population (including all the lineages) possess high levels of genetic diversity but, those levels are low within lineages, **(2)** significant inbreeding values were identified in the lineages, mainly due to the small population sizes, and **(3)** a great level of genetic structure of the breed is a reflection of the subdivision of the Spanish population into lineages, classifying them in 29 the extant lineages genetically differentiated. As a result of both, the genetic drift and different selection objectives of the breeders, such lineages become genetically differentiated over the time.

According to Cañón et al. (2008) as a consequence of the subdivision of the breed, certain alleles are fixed within the different lineages, and kept as a source of variation as long as the lineage persists. The subdivision into lineages increases the number of homozygotes per lineage, with the risk that this entails. As inbreeding increases, it also increase the risk of disappearance of a lineage and therefore its exclusive alleles, and this process goes faster if the effective size of the population is smaller; as is the case of some extant lineages. Cañón et al. (2008) mentioned that the subdivision of the Lidia population into lineages, reproductively isolated among them, could have been a good strategy for the maintenance of the whole genetic variability of the population. To date, no action has been taken for the conservation of the genetic variability of the Spanish Lidia breed.

The haplotype diversity of the Y chromosome determined that the lineages of the Spanish Lidia breed population belong to two major haplogoups (Y1 and Y2) subdivided in 10

(Cortés et al., 2011), and 38 haplotypes (Pelayo et al., 2015). Both studies agree finding diversity from both Y1 and Y2 haplogoups. Those two major paternal influences are associated to two most common haplotypes, the H1 (Y1) and H3 (Y2) (Cortés et al., 2008). It has been also detected the presence of the microsatellite allele INRA189-104 evidencing an African paternal influence (Cortés et al., 2011).

The maternal lineages in the Lidia Spanish population revealed similar mtDNA diversity richness within the Spanish Lidia lineages than the observed in the Middle East cattle breeds and, greater than the recorded in most of the European breeds (Cortés et al., 2008). The haplogroup T3 has the highest frequency (81%) as with most of the European cattle breeds, followed by the African T1 haplogroup (17%) which has lower frequency in the European breeds (Cymbron et al., 1999; Troy et al., 2001), and then lower frequencies of the haplogroups T and T2. The high genetic variability of the Lidia breed is in part explained because of the high frequency of the African-African haplogroup T1, observed with less frequency on the rest of the European cattle breeds. Although, its distribution is widely variated, for example, in five lineages the T1 haplotype was not detected, however in the Miura lineage, its percentage is higher than 50%, varying within the lineages between 3% and 31% (Cortés et al., 2008).

At present the breeding of the Lidia breed in Spain population is organized in five herdbooks. According to the U.C.T.L.(2018) the Lidia population is extended in more than 250,000 ha of the Spanish territories, mostly in the Mediterranean forest ecosystem traditionally known as Dehesa (grassland in between Mediterranean oaks) (Cañón et al., 2008). There are 976 herds registered and by 2017 the census was estimated in 213,457 animals registered in the national genealogical record (U.C.T.L., 2018).

2.4 The Lidia breed in Mexico

In 1522 Juan Gutierrez Altamirano, cousin of the conqueror Hernán Cortés, brought to the New Spain (Mexico) ~30 bovines of Navarre origin, destined to take part in the first festivities involving cattle that commemorated the conquest of the Aztec empire. At this period of time cattle from Navarre was already being set apart for their aggressiveness, recognizing these herds among the founding castas (U.C.T.L. 1995). In Mexico for many years (1611-1679) it became popular among the breeders (that, in those years many of them where ecclesiastics) to import Navarre cattle to keep their monasteries and to keep safe the mines from the bandits' (Prieto-Garrido, 2012).

The Lidia breed cattle in Mexico were held un-orderly in the same way as it happened in Europe before the industrial revolution and the creation of herd-books. The specialization of the Lidia breed in Mexico began at the last years of the XIX century and beginning of the XX century, where four Mexican families of breeders: the Llaguno, González, Barbabosa and Madrazo started raising Lidia cattle by reproducing the aggressive selected cattle already set in Mexico, with Spanish Lidia sires imported to be sacrificed in the festivities (Scherrer, 1983). Then, between 1908 and 1912 the Llaguno´s and the González families imported a reduced number of Spanish Lidia bovines destined specifically to be breeders of their herds (Niño de Rivera, 2004).

Each family followed different breeding strategies, the Llaguno family followed a closed breeding scheme reproducing the newly imported animals among them in one "line" named "San Mateo", and in a second line "Torrecilla" crossing the imported sires with local aggressive cows (Niño de Rivera, 2004). Meanwhile, the González family crossed the new imported animals among them and also with local cows selected for aggressiveness.

The census of the Mexican Lidia population suffered dramatic losses during the post-revolution period, which lasted ten years (1910-1920). After those years, breeders recovered the Lidia population from cattle that derived either from the Llaguno or González families. At present an 80% of the Lidia Mexican breed derives from one of these two families, while the bovine population legacy from Barbabosa and Madrazo families was lost during the post-revolution period (Scherrer, 1983).

In recent years, between 1996 and 1997, a few Mexican breeders imported ≈1,000 Spanish Lidia bovines belonging from different lineages before closing borders of importations from Spain according data from the Mexican Lidia Breeders Association (A.N.C.T.L., 2017). These breeders reproduced the new imported animals among them and kept them apart, or reproduced the new imported animals with the local Lidia animals derived from Llaguno or González "lines". To date, this recent refreshment suggests a strong impact in the genetic structure of the herds belonging from the breeders that took part in those importations. But still, the major part of the Mexican Lidia population derives from the elder González and Llaguno families (Niño de Rivera, 2004).

In Mexico the *tauromaquia* is a deep-rooted tradition that has been declared as national intangible cultural heritage by the United Nations Organization for Education, Science and Culture (U.N.E.S.C.O.). Currently there are 262 breeders registered in the National Breeders Association of Lidia Cattle (Arévalo, 2015). It is estimated that each breeder has an average of 232 cows with a fertility rate of 80%, and the average land extension of 649.2 hectares per farm for breeder (C.O.T.E.C.O.C.A., 2017). It is difficult to have a precise census of the Mexican Lidia population, but the estimated data from the A.N.C.T.L. (2017) is of 109,204 animals (Arévalo, 2015).

Regarding the tauromaquia festivities, between the years 2000 and 2015 average number of animals per year that took place in the different types of festivities was around 592 per year (3,173 in 15 years). It is estimated that the value of the tauromaquia industry per year is of ≈ €270,000 (Arévalo, 2015). Despite that the main use of the Lidia cattle is to participate in festivities, at the end of the festivities all the byproducts are used. For example, there is a special dish made with the bull´s tail named "*rabo de toro*" which is highly valued by the butchers for sale in restaurants.

3. GENETIC DIVERSITY

3.1 Concept and need to preserve the genetic diversity

The concept of genetic diversity is defined by the Food and Agriculture Organization of the United Nations (F.A.O.) as the genetic variety in the diverse animal genetic resources, as it happens with the breeds within the different species (Henson, 1992). The genetic diversity therefore, can be studied at all different levels, for example: species within ecosystems, breeds within species, populations within breeds. So, "global diversity" can be defined as the combination of all those sources of variability. The diversity can be studied at a molecular level, which can be defined as the additive genetic variance within and between breeds or populations (Meuwissen, 2009). The analyses of genetic variance can provide information of the population´s genic structure.

The concern of preserving the biodiversity in domestic species is mainly due to: their biological value, the sustainability that they bring to the ecosystems and also because of their economic value for humans. At present almost two billion people depend at least partially on domestic animals as economic support, and 12% of them depend of them almost completely (F.A.O., 1999).

The genetic diversity allows the livestock to deal with several adversities such as new diseases (infectious or parasitic), to wide variations in the availability and quality of the food and water resources, among other limiting factors. The breeds adapted to local environments may be modestly less productive compared with the highly specialized breeds, but instead are very efficient in the use of the local resources, and are more sustainable in a long term. In fact, on many occasions, imported animals from widespread commercial breeds with higher productivity are not able to reproduce or survive in some regions, as are the locally adapted breeds (F.A.O., 1999).

The maintenance of the genetic diversity is, hence, a priority objective in programs for the conservation of the biodiversity (Fernández et al., 2004) in order to have sufficiently variated genetic resources that guarantee the adaptation and viability of a species or breeds to variable environments (Barker, 1999; INRA & CIRAD, 2002; Gandini & Oldenbroek, 2007).

The conservation of genetic diversity represents a safeguard against challenges from unpredictable events, such as changes in the consumer´s preferences which may modify the animal selection targets, the appearance of new diseases, new social trending's that modify the different systems of feeding or management of the animals, among others.

The loss of genetic variability, on the contrary, can lead a breed or a species to extinction. The risk of losing a breed can be taken as a criterion of prioritization to stablish strategies of conservation of the diversity, but it is complicated to provide an objective value of the real risk of extinction of a breed. This is mainly because the main international organizations, like the F.A.O., the European Federation of Animal Science (E.A.A.P.) and the European Union (E.U.), do not share common criteria to categorize the levels of extinction risk. It is challenging to gather all the elements influencing the extent of danger that a breed may have. For instance, two breeds that apparently could be in the same category according to the F.A.O., E.A.A.P. or E.U. regulation, may be subject to very different risks. To sum up,

different factors like sanitarian, social, political, economic or cultural and of course, genetic, can alter significantly the possibilities of survival of a population.

3.2 Measurements of population´s genetic diversity

Many parameters, indicators and measurements are used to estimate genetic diversity; in this chapter we make a brief review of the classic ways to evaluate it.

The information provided by genetic markers has been traditionally used to calculate parameters related to the distribution of the genetic diversity in a sub-divided populations, e.g. within and among breeds of domestic species. Indicators of diversity within breeds are, for example, the heterozygosis (Lin et al., 2010), the average number of alleles (Zenger et al., 2007), or the F_{IS} statistic (Wright, 1951). Moreover, through the assessment of genetic distances the genetic relationships of breeds among a species, or as in our case, lineages among a breed, can be assessed.

One of the parameters used traditionally as a measure of the genetic diversity is the heterozygosis that estimates the proportion of heterozygous individuals for a specific marker or, in an extended way, for an average set of markers (Nei, 1978). There are two ways of measuring the heterozygosis; one is by estimating the proportion of heterozygous individuals by counting the number of heterozygous genotypes, or by acumulating the genic frequencies of these, which is known as "observed heterozygosis (*Ho*)". The second is the "expected heterozygosis (*He*)" also known as "gene diversity" that is defined as the heterozygosis value that would be expected under conditions of Hardy-Weinberg equilibrium (Weir & Ott 1997).

A subdivided population generally shows lower observed heterozygosis levels than the expected; that reduction in the observed heterozygosis can be used to quantify the degree or extent of differentiation between subpopulations. In order to measure this inter-population

genetic diversity it is necessary to estimate the genetic distances among populations. There are different approaches to estimate genetic distance, such as the F-statisctics (Wright, 1951; Cockerham, 1969). According to Wright´s denomination, the F-statistics are denoted by the F_{IS}, F_{ST} and F_{IT}. while according to Cockerham´s definition, the statistics are denoted as f, θ and F. Both annotations are correct and widely used in current population genetics studies. To define them, the following values are considered:

- *Ho*: Medium observed heterozygosity per individual within a population
- *He*: Medium expected heterozygosity of an individual within a population
- *Ht*: Medium expected heterozygosity of the whole population (estimated from the average allele frequencies among subpopulations).

The F statistics are defined as follows:

$F_{IS} = f = \frac{H_e - H_o}{H_e}$ The F_{IS} provides a way of measuring mean heterozygosis reduction of an individual within a subpopulation (inbreeding) (Holsinger & Weir, 2009). It varies between -1 (all individuals are heterozygous) to +1 (absence of observed heterozygotes).

The F_{ST} or fixation index: $F_{ST} = \theta = \frac{H_t - H_e}{H_e}$ is the most widely used of the three statistics. It measures the average reduction of heterozygosity of a subpopulation relative to the total population due to genetic drift between subpopulations and.is, therefore, a measure of the degree of genetic differentiation between subpopulations. It represents the percentage of genetic variation that can be inferred as the differences between populations, and it is complementary. Hence, the proportion attributable to differences within populations varies between 0 (there is no differentiation) and 1 (complete differentiation).

The $F_{IT} = F = \frac{H_e - H_o}{H_o}$ statistic represents the mean reduction of heterozygosis of an individual regarding the whole population.

These three parameters are related to each other through the following ecuation:

$$(1-F_{IT}) = (1-F_{IS})(1-F_{ST}).$$

The statistical methodology for estimating genetic distances is well established, and the availability of new methods to estimate locus- and population- specific effects on F_{ST} (Weir & Hill, 2002; Balding, 2003; Beaumont & Balding 2004; Weir et al. 2005; Foll & Gaggiotti, 2008; Guo et al., 2009), provides a set of tools that allows identifying genomic regions or populations with unusual evolutionary histories.

A significant part of this thesis is to assess the genetic structure of the Mexican population, which theoretically, was originated from the original Spanish population.

The estimation of the allelic frequencies of sub-populations allows analyzing the extent of differentiation among them and depending of that, the possibilities of assigning the genome of individuals to one or several sub-populations. Depending of the type of information used, Pritchard et al. (2000) for microsatellite data in the STRUCTURE software and Alexander & Lange (2011) for SNPs in the ADMIXTURE software, developed non-supervised methods based on a Bayesian inference that estimates the ancestry or number of different common genetic origins assigning proportions of each animal genome to those inferred common genetic origins. Nevertheless, STRUCTURE samples the posterior distribution via Markov Chain Monte Carlo (MCMC) instead of ADMIXTURE which maximizes the likelihood. Maximum likelihood approach in ADMIXTURE can accommodate a higher number of markers and use then a further bootstrap to estimate standard error of the parameters. Both approaches are well suited estimators of ancestry parameters and *k*-cluster.

Besides, the Multidimensional Scaling (MDS) analyses are methods to visualize the level of similarity of the individuals of a dataset, in particular to display information contained in a distance matrix. A Principal Coordinates Analysis (PcoA) is a classical multidimensional scalling grapphic used to explore and visualize genetic structure of populations (Novembre & Stephens, 2008). PcoA in population genetics is widely used as it has the advantage of lacking the historical model to interpretation, because the representation depends of the input data. But the same advantage makes this technique very sensitive to the choice of the dataset in the way that unequal sampling may lead to misinterpretation of population structure (McVean, 2009).

3.3 Genetic diversity estimation using molecular markers

The molecular markers are a good alternative to estimate genetic diversity more easily than the traditional pedigree data sources of information (Schlötterer, 2004). An overview of the most common markers that have been used for genetic diversity is shown in **Table 1**.

Table 1 Examples of molecular markers used in genetic diversity studies.

Marker	Typical example	Number of Marker	alleles per marker
Blood groups	Buys, 1990	1	11
Allozymes	Taggart et al., 1981	12	2-5
AFLP	Ajmone-Marsan et al., 2001	219	2
RAPD	Kantanen et al., 1995	3-7	2
Microsatellite	MacHug et al., 1997	20	8,4 (on average)
SNP	Decker et al., 2013	Depending the density	2
Sequence	Frischknecht et al.,-2018	>1,000,000	1-2

Based on the inheritance of the red cell antigens, the blood groups were the first molecular markers in cattle in the late 80´s and 90´s decade and were widely used. However, their low

number of markers show biases on the estimation of the genetic diversity of animal breeds (Larsen & Hansen, 1986). The gene´s coding protein polymorphisms or allozymes were widely used markers in livestock in the past decades, but they had a limitation on the number of loci and the low polymorphism level (Toro et al., 2009). With the development of new DNA technologies these markers were replaced by markers at the DNA chain level.

The AFLPs (amplified fragment length polymorphism) and RAPDs (randomly amplified polymorphic DNAs) are genetic markers that were widely used in the decade of the 1990´s. The AFLPs and RAPDs are dominant bi-allelic markers widely used to analyze genome-wide variation and population genetic structure, but their dominant mode of inheritance and difficulty to reproduce are pitfalls that reduces the power to analyze within breed diversity (Toro et al., 2009). In recent years, microsatellites have been the most popular markers of choice to study genetic variation. They are based upon sites in which the same short sequence of nucleotides is many times repeated, presenting a high mutation rate and codominant nature, which makes them appropriate for the study of both within- and between-breed genetic diversity (Schlötterer, 2004).

The recent development of the genome-wide SNP (single nucleotide polymorphisms) allows estimating genetic diversity and genetic structure at higher level of resolution, hard to reach with other types of markers. An SNP marker is a single base change in a DNA sequence, with two possible nucleotides at a given position (Vignal et al., 2002).

A great advantage of the SNPs with respect to other markers is their possibility to make high throughput analyses at a relatively low-cost and, as they are uniformly distributed over the whole genome, the estimation of genetic diversity across the genome is expected to be more informative of the processes involved. In livestock, SNP markers have been widely used to analyze genetic histories of bovine populations (Gibbs et al., 2009; Gautier et al., 2010). The

SNP markers have also been used for the analysis of genetic diversity and genetic structure, as well as for QTL analysis and genomic selection (Bovine HapMap Consortium, 2009).

In recent years, new generation of sequencing technologies usually referred to as “second generation” or “next generation” sequencing technologies, from Illumina/Solexa, ABI/SOLiD, Roche's 454 and Helicos offer represents a promise for marker discovery due to their ability to generate large amounts of sequence data (Morozova & Marra, 2008). These instruments have been extensively used for genome sequencing, re-sequencing and SNP discovery (Morozova & Marra, 2008). The most effective way to genotype large numbers of SNPs is trough desining high-density assay that includes tens of thousands of SNPs distributed throughout the genome. These SNP “chips” are a valuable resource for genetic studies in livestock species (Meuweissen et al., 2001; Matukumalli et al., 2009). To date, high-density SNP chips are available for bovine cattle (http://www.illumina.com; http://www.affymetrix.com)

The availability of the SNPs markers allows tracking genomic regions that, as consequence of domestication and artificial selection of the animals for their economic or morphological characteristics, have left as a variety of imprints (Purfield et al., 2017). Recent studies are focused on searching for contiguous lengths of homozygous genotypes that are present in an individual due to parents transmitting identical haplotypes to their offspring, named Runs of homozygosity (ROH) (McQuillan et al., 2008; Purfield et al., 2012).

The extent and frequency across the genome of the ROHs allows depicting patterns of ancestry of an animal and hence of a population. The presence of long ROHs may inform a recent inbreeding within a pedigree, while the distribution of shorter ROH segments may also inform on the presence of more ancient relatedness (Purfield et al., 2012). In this sense, as selection is characterized for reductions in haplotype diversity, the analysis of distribution of

the ROH patterns across the genome may provide insights of the patterns of recent or ancient selective pressure in a population (Pryce et al., 2014; Purfield et al., 2017).

Apart from the identification of ROH, the SNP markers allow detecting a large amount of polymorphism data that can be used to estimate how happened the selective adaptation processes, bottlenecks, genetic drifts and migrations affected the variation in different regions of the genome, this is known as selective sweep or signature of selection (Pritchard et al., 2010).

Several methodologies have been developed to detect signatures of selection in cattle when, under pressure of selection a novel genetic variant can be detected at a genomic level by means of different tests like measuring allele frequencies, an excess in homozygotes, high frequency of long haplotypes, or by detecting higher genetic differentiation among populations (Qanbari & Simianer, 2014; Randhawa et al., 2016).

OBJECTIVES

Objectives

The main objective of this thesis is to analyze the genetic diversity and genetic structure of the Mexican Lidia population, and their relative genetic position with respect to the Spanish Lidia breed, based on four molecular sources of information: autosomal microsatellite markers, SNP information over the whole genome, Y-chromosome (Microsatellites and SNPs) and mitochondrial DNA sequences. Besides we wanted to determine whether there are differences in diversity and structure between the Mexican and the Spanish Lidia populations and finally to track possible signatures of selection associated to behavioral characteristics in the Lidia breed.

To achieve this objective we propose the following research design:

- First, we used autosomal microsatellite markers to study the genetic diversity and structure of the Mexican Lidia population and its relationship with the original Spanish population. We also used Y chromosome DNA markers and mitochondrial DNA sequences to explore the maternal and paternal influences of the Mexican population.
- Then we used autosomal SNPs selected from the 50K Beadchip to perform two studies. In the first, we selected a panel of 573 SNPs to explore the genomic diversity and structure of the Mexican population. In the second study we used a panel of 37,148 SNPs to analyze the same parameters, comparing them with Spanish autochthonous and American native cattle breeds. Besides, we explored the distribution of the ROHs in these populations.
- Finally, we used information provided by the SNPs to locate genomic regions associated with aggressive related traits in the Lidia breed, using two Spanish tamed breeds as a reference. We also identified putative candidate genes mapping within these regions in order to understand the evolutionary mechanisms of the Lidia breed.

PUBLICATIONS

Genetic Diversity of the Mexican Lidia Bovine Breed and its divergence from the Spanish Population

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

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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 difficulted 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 bovines 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 (Canón 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 & Canón, 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 (Canón et al., 2008; Delgado et al., 2012; Martínez 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[a] tubes (Biogen Diagnostica, Spain), and these were maintained at 15°C until use, guaranteeing DNA integrity (Dunner & Canón, 2006).

2.2 | Spanish population

In accordance with the aim of the study, genotypic information derived from 24 autosomal microsatellites previously used by Canón 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 (Canón 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 (H_o) and expected (H_e) 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 G6therstr6m 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 F_{ST} values (bootstrapped p-value <.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 F_{ST} 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 Canón 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 Castaneda 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 Castaneda 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, Gamero 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 Gonzalez 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 Gonzalez and Llaguno breeders except San José (JOS), Torreón de Canas (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 Canas (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, Koolmees, 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

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) ($p < 0.01$)

Pop	Lineage	Acronym	He	Ho	MNA	NE	AR	F_{IS}	DHWE
Spain	**Albaserrada**	**ALB**	**0.54**	**0.56**	**3.1**	**2.2**	**2.8**	**0.036**	**1**
	Anastasio Martín	**ANA**	**0.64**	**0.67**	**4.8**	**2.8**	**3.7**	**0.054***	**0**
	Atanasio Fernández	**ATA**	**0.50**	**0.49**	**3.8**	**2.0**	**2.9**	**0.025**	**4**
	Casta Navarra	**NAV**	**0.73**	**0.67**	**7.4**	**3.7**	**4.9**	**0.086***	**5**
	Conde de Santa Coloma	**COL**	**0.66**	**0.53**	**6.9**	**3.0**	**4.0**	**0.203***	**19**
	Conde la Corte	**COR**	**0.47**	**0.47**	**3.5**	**1.9**	**2.7**	**−0.003**	**2**
	Juan Pedro Domecq	**DOM**	**0.56**	**0.49**	**4.8**	**2.3**	**3.4**	**0.134***	**6**
	Garnero Cívico	**GAM**	**0.55**	**0.43**	**4.7**	**2.2**	**3.3**	**0.214***	**11**
	Miura	**MIU**	**0.59**	**0.53**	**4.7**	**2.4**	**3.4**	**0.108***	**6**
	Murube	**MUR**	**0.58**	**0.51**	**5.8**	**2.4**	**3.5**	**0.120***	**6**
	Pablo Romero	**PAB**	**0.57**	**0.54**	**4.4**	**2.3**	**3.3**	**0.055***	**7**
	Saltillo	**SAL**	**0.59**	**0.50**	**7.7**	**2.4**	**3.6**	**0.153***	**6**
	Concha y Sierra	**SIE**	**0.65**	**0.61**	**5.1**	**2.8**	**3.9**	**0.057***	**9**
	Veragua	**VER**	**0.67**	**0.61**	**6.0**	**3.1**	**4.1**	**0.094***	**9**
Mexico									
González	**Rancho Seco**	**SEC**	**0.70**	**0.57**	**5.1**	**3.3**	**4.3**	**0.183***	**5**
	Carlos Castaneda	**CAS**	**0.51**	**0.47**	**3.8**	**2.1**	**3.2**	**0.095***	**2**
	de Haro	**HAR**	**0.54**	**0.56**	**4.2**	**2.2**	**3.4**	**−0.042**	**1**
Llaguno	San José	JOS	0.65	0.58	5.0	2.8	4.0	0.110*	4
	Montecristo	MON	0.59	0.53	4.1	2.4	3.5	0.111*	2
	Torreón de Canas	TOR	0.68	0.59	4.8	3.1	4.1	0.130*	2
	Reyes Huerta	REY	0.64	0.58	5.0	2.8	4.0	0.090*	2
	Fernando de la Mora	FER	0.66	0.67	4.4	2.9	4.1	−0.019	0
	Garfias	GAR	0.54	0.51	3.4	2.2	3.2	0.053	1
	Xajay	XAJ	0.61	0.59	4.5	2.6	3.8	0.033	1
	Teófilo Gómez	TEO	0.66	0.64	5.4	3.0	4.2	0.033	1
	Los Encinos	ENC	0.62	0.60	4.5	2.6	3.8	0.040	4
	La Antigua	IGU	0.60	0.57	3.9	2.5	3.5	0.050	4
	Celia Barbabosa	BAR	0.62	0.61	4.7	2.7	3.9	0.029	0
	Boquilla del Carmen	BOQ	0.59	0.56	4.2	2.4	3.5	0.049	1
	Fermín Rivera	RIV	0.62	0.62	4.3	2.6	3.6	0.005	2
	Corlomé	CRL	0.57	0.61	3.5	2.3	3.4	−0.077	1
	Arroyo Zarco	ZAR	0.63	0.60	4.7	2.7	3.9	0.043	3
	Marrón	MAR	0.65	0.67	4.3	2.9	3.9	0.036	2
	San Mateo	MAT	0.59	0.63	4.2	2.4	3.5	−0.059	3
Average Spain			**0.61**	**0.54**		**2.56**	**3.5**	**0.12**	**7**
Average Mexico			0.62	0.59		2.63	3.8	0.05	2

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 Castaneda 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 2 Population genetic structure of Mexican and Spanish Lidia breed cattle groups inferred by using STRUCTURE (Pritchard et al., 2000). Table on the left shows clustering when K = 2 and K = 3 for both Mexican (Gonzalez (G) and Llaguno (L) breeders) and Spanish populations and table on the right shows clustering when K = 2, 4 of the Mexican breeders

		K = 2		K = 3					K = 2		K = 4			
Country	Lineage/Breeder	1	2	1	2	3	Family	Breeder	1	2	1	2	3	4
Spain	**Albaserrada**	**0.75**	**0.25**	**0.04**	**0.53**	**0.43**	**González**	**Rancho Seco**	**0.16**	**0.84**	**0.07**	**0.46**	**0.36**	**0.11**
	Santa Coloma	**0.43**	**0.57**	**0.04**	**0.14**	**0.82**		**de Haro**	**0.08**	**0.92**	**0.88**	**0.04**	**0.01**	**0.06**
	Casta Navarra	**0.92**	**0.08**	**0.10**	**0.84**	**0.06**		**Carlos Castaneda**	**0.08**	**0.92**	**0.90**	**0.01**	**0.03**	**0.06**
	Miura	**0.98**	**0.02**	**0.02**	**0.96**	**0.02**	**Llaguno**	**San José**	**0.24**	**0.77**	**0.03**	**0.77**	**0.07**	**0.13**
	Concha y Sierra	**0.98**	**0.02**	**0.02**	**0.95**	**0.03**		**Montecristo**	**0.96**	**0.04**	**0.01**	**0.01**	**0.01**	**0.97**
	Saltillo	**0.78**	**0.22**	**0.09**	**0.53**	**0.38**		**Torreón de Canas**	**0.37**	**0.63**	**0.02**	**0.02**	**0.61**	**0.35**
México	(G) Rancho Seco	0.09	0.91	0.38	0.29	0.34		Reyes Huerta	0.93	0.07	0.02	0.04	0.01	0.93
	(G) de Haro	0.08	0.92	0.76	0.04	0.21		Fernando de la Mora	0.50	0.50	0.06	0.35	0.10	0.49
	(G)Carlos Castaneda	0.01	0.99	0.72	0.07	0.21		Garfias	0.98	0.03	0.01	0.01	0.01	0.97
	(L)San José	0.01	0.99	0.41	0.06	0.53		Xajay	0.83	0.17	0.01	0.19	0.01	0.79
	(L)Montecristo	0.41	0.59	0.98	0.01	0.01		Teófilo Gómez	0.83	0.17	0.01	0.06	0.12	0.82
	(L)Torreón de Canas	0.01	0.99	0.57	0.39	0.04		Los Encinos	0.64	0.36	0.01	0.47	0.01	0.51
	(L)Reyes Huerta	0.05	0.95	0.97	0.01	0.02		La Antigua	0.92	0.08	0.03	0.03	0.02	0.92
	(L)Fernando de la Mora	0.02	0.98	0.80	0.05	0.16		Celia Barbabosa	0.90	0.10	0.02	0.03	0.04	0.92
	(L)Garfias	0.10	0.90	0.98	0.01	0.01		Boquilla del Carmen	0.92	0.08	0.05	0.03	0.01	0.91
	(L)Xajay	0.02	0.98	0.89	0.02	0.09		Fermín Rivera	0.88	0.12	0.03	0.03	0.04	0.91
	(L)Teófilo Gómez	0.03	0.97	0.88	0.10	0.03		Corlomé	0.66	0.34	0.02	0.34	0.03	0.60
	(L)Los Encinos	0.03	0.98	0.79	0.01	0.20		Arroyo Zarco	0.90	0.10	0.02	0.07	0.02	0.89
	(L)La Antigua	0.01	0.99	0.96	0.03	0.02		Marrón	0.80	0.20	0.03	0.19	0.01	0.78
	(L)Celia Barbabosa	0.02	0.98	0.96	0.02	0.02		San Mateo	0.94	0.06	0.04	0.01	0.01	0.95
	(L)Boquilla del Carmen	0.08	0.92	0.97	0.01	0.02								
	(L)Fermín Rivera	0.02	0.98	0.95	0.03	0.03								
	(L)Corlomé	0.02	0.98	0.84	0.08	0.08								
	(L)Arroyo Zarco	0.02	0.98	0.95	0.02	0.03								
	(L)Marrón	0.31	0.69	0.87	0.02	0.12								
	(L)San Mateo	0.04	0.96	0.98	0.01	0.01								

Numbers in bold highlight the major contribution for each Lineage/Breeder.

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: Avilena, 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.

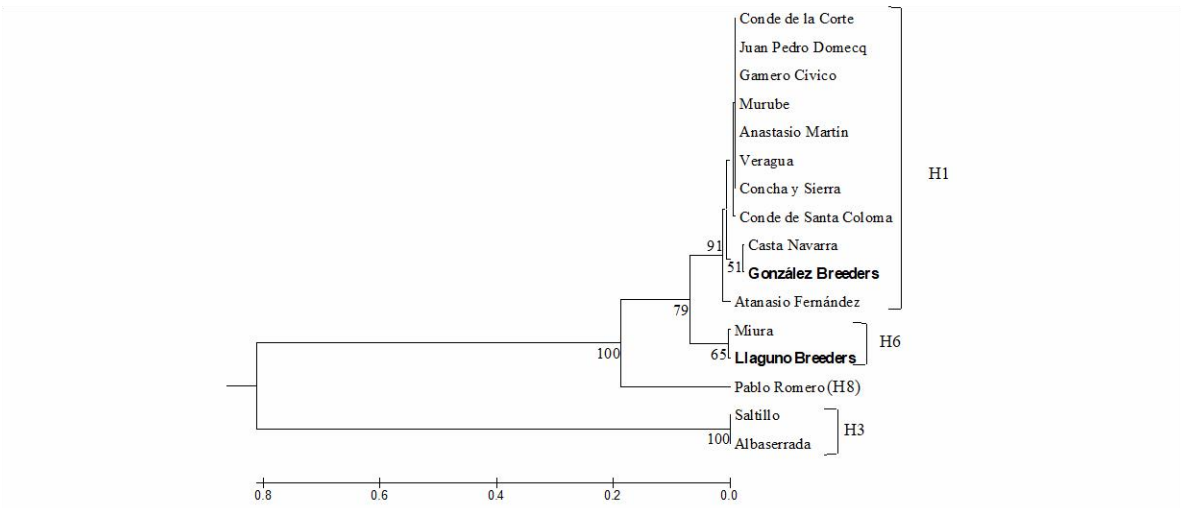

FIGURE 1 Neighbour-joining tree constructed from F_{ST} distances derived from Y chromosome microsatellite data of Mexican breeders and Spanish lineages. In bold Mexican families (González and Llaguno) grouped as defined in Table 1. Bootstrapping values higher than 50 are reflected at the left side of the branches. Brackets at the right indicate the majoritarian haplotypic group

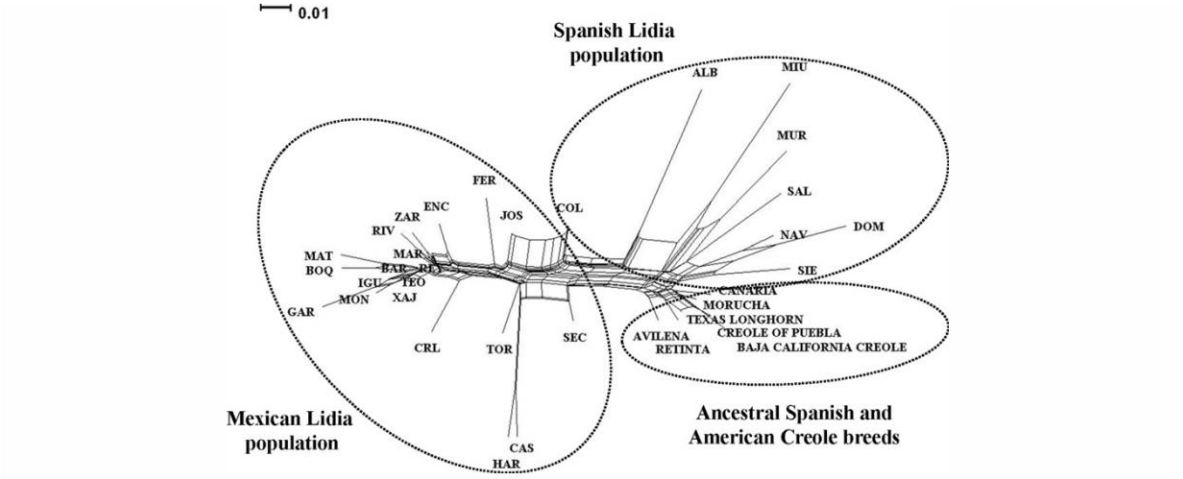

FIGURE 2 Neighbor-Network from the FST distances between Mexican breeders, Spanish Lidia lineages, Spanish ancestral and American Creole bovine breeds. Each population is grouped in circles placing Spanish ancestral and American creole together with complete name of the breeds. Spanish and Mexican Lidia populations' lineages and breeders names are as defined in Table S1

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 "Murciélago" 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 by the influence of Casta Navarra lineage (Nino 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 haplotypic 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 Saltillo lineage has been historically proven in Mexico, no traces of this ancestor were detected in this work.

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SUPPORTING INFORMATION

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

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Genetic Diversity Analysis of the Mexican Lidia Bovine Breed Population and its relation with the Spanish Population by using a subset of SNPs under low gametic disequilibrium

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Article

Genetic diversity analysis of the Mexican Lidia bovine breed population and its relation with the Spanish population by using a subset of SNPs under low gametic disequilibrium

Análisis de diversidad genética en la población de la Raza de Lidia Mexicana y su relación con la población española por medio de un panel de SNPs con bajo desequilibrio gamético

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Abstract:

Retaining features of the auroch (*Bos taurus primigenius)*, the Lidia bovine is a primitive breed originated ~250 yr ago in the Iberian Peninsula, where is still distributed, along with France and several American countries. Selected upon a behavior, which enhances their aggressiveness; these bovines were raised to participate in popular festivities that nowadays reinforce the identity of regional cultures. Different festivities demanded diverse behavior patterns, prompting a fragmentation of the breed into small lineages. In Mexico, where these bovines reached high popularity, mainly two families of breeders imported Lidia bovines from Spain in the early XX century specializing their production either reproducing the new arrivals among them or realizing systematic crosses with local populations. Genetic diversity and structure of the Mexican and Spanish Lidia populations has been assessed with microsatellite data, but nowadays SNP molecular markers allows higher resolution level. Genetic diversity of the Mexican and Spanish Lidia populations and their relationship were

assessed by using 573 SNPs with a low gametic disequilibrium (r^2<0.01) from the 50K BeadChip on 468 individuals from both populations. In both populations, similar gene diversity values were observed. Significant F_{IS} values in both populations means strong subdivision, higher F_{ST} genetic distances were observed in the Spanish than in the Mexican population. Genetic structure analysis showed similarity of three Spanish lineages with González family and some Llaguno breeders, but most Llaguno family clustered separated: genetic differentiation along with high gene diversity suggest an introgression of creole cattle in the constitution of the Mexican population.

● **Key words:** Lidia breed, Behavior selection, Population differentiation, Genetic structure, SNP.

● **Resumen:**

El bovino de Lidia pertenece a una raza primitiva originada ≈250 años en la Península Ibérica, lugar donde aún se distribuye junto con diversos países de América. Seleccionados por un comportamiento que potencia la agresividad, estos bovinos fueron criados para participar en festividades populares que en la actualidad refuerzan la identidad de las culturas regionales. Diferentes festividades han demandado la selección de diferentes comportamientos, desencadenando una fragmentación de la raza en linajes. En México donde este ganado alcanzó gran popularidad, principalmente dos familias de criadores importaron de España bovinos a comienzos del siglo XX, especializando la producción. La diversidad genética y estructura de las poblaciones mexicanas y españolas han sido evaluadas con microsatélites, pero hoy en día los marcadores de tipo SNP permiten una mayor resolución. En este sentido se analizó la diversidad genética de la población mexicana de Lidia y se evaluó su relación con la española utilizando 573 SNPs con bajo desequilibrio de ligamiento (r^2<0.01). En ambas poblaciones se observaron similares valores de diversidad genética. Valores significativos de F_{IS} en ambas poblaciones significan una subdivisión de linajes, también se observaron mayores distancias genéticas F_{ST} en la población española que en la mexicana. El análisis de estructura genética mostró similitud de tres linajes españoles con la familia González y algunos criadores de la familia Llaguno; pero la mayor parte de la familia Llaguno se agrupó separada: esta diferenciación así como la alta diversidad genética sugieren una introgresión de ganado criollo en la constitución de la población mexicana.

● **Palabras clave:** Raza de Lidia, Selección por comportamiento, Diferenciación poblaciones, Estructura genética, SNP.

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Introduction

Possessing multiple ancient features of their earliest forms originated from the auroch (*Bos taurus primigenius)*, and distinguished by its extensive management[1], the Lidia bovine is a primitive breed whose roots can be traced back to approximately 250 yr ago in the Iberian Peninsula in order to satisfy a demand of cattle destined to participate in popular spectacles. At present, shows involving cattle are found in geographical areas comprising mainly the southwest region of Europe (Italy, France, Spain and Portugal) and along the American continent involving approximately 14 countries[2]. These kinds of spectacles have their origins in the early Mediterranean civilizations, where bovines of untamed behavior, lacking of docile temperament, participated in ceremonies and rituals as an assigned symbol of the nature´s strength[3]. After, in the 13th Century those practices evolved into social events called tauromachies or "*tauromaquias*", a term that makes reference of a cultural and subjective representation of all types of games involving cattle and not as a single term for identifying one single practice (since sometimes the term is associated exclusively with the Spanish bullfight or "*corrida*"). To date, tauromaquias assemble a social and semantic construction, are an important livestock economic source and reinforce local and regional identities of the countries where are still found[2,4]. Diversity in orography and climate along with historical factors and traditions, led place to the development of different variants of bovine populations. There all were selected based upon behavioral performance of aggressiveness: the Andalusian and Navarro-Aragonese that in Spain gave rise to the original Lidia breed population, in Portugal the Lidia Portuguese breed and in France the Landaise and Camargue's cattle populations[5].

The specialization and intensification of animal husbandry did not take place until ~250 yr ago with the emergence of many specialized breeds during the industrial revolution. In Spain, to become breeder of this type of cattle provide more status to the members of aristocracy and gentry, who in search of improving the behavioral skills of their "aggressive" bovines developed a documented breeding system, giving rise to the original Lidia breed population[4,6]. Moreover, these breeders concerned about raising bovines that could be distinguished for performing different type of behavior (sometimes demanded for the different type of festivities) established closed family trees that prompted to a fragmentation of the racial group into small lineages[7].

In America, specifically in Mexico, bovines with these behavioral characteristics were imported during the colonial period (after the conquest of the Aztec empire in 1521) to take part in the festivities that were inherited as traditions of the Spanish colonizers[2]. The Lidia breed specialization began between 1908 and 1912 when mainly two families of breeders (Llaguno and González) imported a reduced number of Spanish Lidia bovines. Each family

kept different breeding strategies, the Llaguno family followed a closed breeding scheme reproducing the new imported animals among them, and the González family reproduced the imported animals with local Mexican bovines selected for aggressiveness[8].

Mexican Lidia census suffered dramatic losses during the post-revolution period, which lasted ten years (1910-1920). After those years, breeders recovered their Lidia production opting for raise cattle that derived either from the Llaguno or González families. In recent years, during 1996 and 1997, some Mexican breeders imported close to 1,000 Spanish Lidia bovines before closing borders of importations from Spain[9]. To date, this recent refreshment suggests a strong impact in the genetic structure of the herds belonging from the breeders that took part in those importations. But still, the major part of the Mexican Lidia population derives from the elder Llaguno and González families[8]. Despite both Mexican and Spanish Lidia populations are demographically well stablished, their low effective population size places them at risk of extinction[7].

Previous studies on the Spanish Lidia population found a genetic uniqueness in the breed, which is given by a high genetic differentiation between lineages[6]. Moreover, Eusebi *et al*[10] studied the genetic diversity of the Mexican Lidia population and its divergence from the Spanish Lidia population and found high genetic differentiation among them. However, both studies have been conducted by using neutral autosomal microsatellites, and recently, the availability of SNP panels allow the investigation of livestock genetic diversity and genetic structure at higher level of resolution, hard to reach with other types of markers.

In this study, a subset of 573 SNPs with low gametic disequilibrium were selected from the 50K medium density genotyping array (Illumina Inc., San Diego, CA) to assess the genetic diversity and structure of the Mexican and Spanish Lidia populations and thereafter analyze the relationships among these two populations, in order to explore the degree of admixture among them.

Material and methods

Blood samples of 468 Lidia bovines were collected: 119 belonging to the Mexican population and 349 to the Spanish population. Classification of the Spanish lineages was given according to Cañón *et al*[6] and, for the Mexican Lidia population the samples arise from 20 breeders studied independently but classified into the family that they belong to (González or

Llaguno), according to standards set by the by the Mexican Lidia Breeders Association[9]. More information is available in Table1.

Table 1. Description of the Mexican and Spanish populations (Pop) analyzed by SNP markers, providing names of breeders, their acronyms, number of breeders (NB) and (N) number of samples analyzed

Pop	Family	Name	Acronym	NB	N
MEXICO	Llaguno	Celia Barbabosa	BAR	1	6
		Boquilla del Carmen	BOQ	1	6
		Corlomé	CRL	1	6
		Los Encinos	ENC	1	5
		Fernando de la Mora	FER	1	6
		Garfias	GAR	1	6
		La Antigua	IGU	1	6
		San José	JOS	1	6
		Marrón	MAR	1	6
		San Mateo	MAT	1	6
		Montecristo	MON	1	6
		Reyes Huerta	REY	1	6
		Fermín Rivera	RIV	1	6
		Teófilo Gómez	TEO	1	6
		Torreón de Cañas	TOR	1	6
		Xajay	XAJ	1	6
		Arroyo Zarco	ZAR	1	6
	González	Carlos Castañeda	CAS	1	6
		De Haro	HAR	1	6
		Rancho Seco	SEC	1	6

Pop	Name	Acronym	NB	N
SPAIN	Albaserrada	ALB	3	14
	Anastasio Martín	ANA	1	6
	Antonio Pérez	ANT	1	9
	Araúz de Robles	ARA	1	10
	Atanasio Fernández	ATA	3	14
	Baltasar Iban	BAL	2	12
	Carlos Núñez	CAR	4	9
	Santa Coloma	COL	8	36
	Contreras	CON	3	10
	Conde de la Corte	COR	1	10
	José Marzal	CRM	1	9
	Cuadri	CUA	1	7
	Domecq	DOM	5	29
	Félix Gómez	FEL	1	9
	Gamero Cívico	GAM	3	16
	Hidalgo Barquero	HID	3	15
	Manuel Arranz	MAN	1	9
	Conde de la Maza	MAZ	1	3
	Miura	MIU	1	9
	Murube	MUR	4	16
	Pablo Romero	PAB	1	9
	Pedrajas	PED	2	10
	Saltillo	SAL	3	15
	Concha y Sierra	SIE	1	10
	Urcola	URC	1	7
	Veragua	VER	2	16
	Vega Villar	VEG	4	17
	Marqués de Villamarta	VIL	2	13

Animals were randomly chosen according to their origin, and qualified veterinarians collected the samples during routine practices in the framework of official programs aimed at applying preventive medicine. Blood samples were maintained in Magic Buffer® DNA

solution[11] until DNA extraction by standard phenol/chloroform methods[12]. Genotypes were obtained with the Illumina 50k BeadChip (Illumina Inc., San Diego, CA) and SNP quality was analyzed with the Genome Studio software (Illumina). Thereupon, by using the PLINK software[13] the dataset of SNPs was filtered according to the following excluding criteria: SNPs located on sexual chromosomes; individuals with >20% missing genotypes; SNPs with a minimum allele frequency <0.01; markers that did not match Hardy-Weinberg equilibrium expectations ($P<10^{-6}$); and a restricted linkage disequilibrium criterion of $r^2<0.01$; thus assuring low gametic disequilibrium rate among markers. Finally, the information derived from 573 SNPs spanning across all the bovine autosomal chromosomes, were selected.

Statistical estimates of genetic diversity were performed followed by a multifactorial correspondence analysis estimated to quantify genetic diversity; these analyses were carried out with the GENETIX v.4.0.5 software[14]. The proportion of mixed ancestries among populations was inferred with STRUCTURE v.2.1. software[15] which uses a hierarchical Bayesian model to infer a population structure from multilocus genotypes and assign each individual into that supposed population, assuming that each individual may have mixed ancestry from different underlying populations. The figurative number of populations or genetic clusters (*K*) ranged from 2 to 4 with six replicate chains for each value of *K*. The runs sharing maximum likelihood pattern were selected to be displayed in a graphic constructed with the DISTRUCT v.1.1. software[16].

Results

Genetic diversity

Indicators of genetic diversity estimated per population (Mexican and Spanish) and inbreeding F_{IS} estimates are shown in Table 2. In the analysis of the Mexican population, observed *(Ho)* and expected heterozygosities *(He)* ranged from 0.35 (Carlos Castañeda) to 0.48 (Teófilo Gómez) and from 0.35 (Marrón and de Haro) to 0.42 (San José, Fermín Rivera and Teófilo Gómez) respectively. Genetic diversity values from the completely Mexican population were 0.46 (*He*), 0.43 (*Ho*). Regarding F_{IS} estimates, most of the breeders presented negative values, with estimates that fluctuated from -0.17 (Corlomé) to 0.09 (Boquilla del Carmen) and a F_{IS} of 0.06 was obtained when the whole Mexican population was considered. Moreover, genetic diversity indicators in the Spanish population revealed a wider range of values compared to the Mexican population. With *He* estimates that goes from 0.26 (Cuadri) to 0.44 (Santa Coloma) and *Ho* ranging from 0.33 (Gamero Cívico) to 0.46 (Anastasio Martín and José Marzal). Genetic diversity values for the whole Spanish

population were 0.48 for *He* and 0.38 of *Ho*, and F_{IS} values going from -0.13 (Manuel Arranz) to 0.19 (Santa Coloma), thus evidencing a clear lineage subdivision.

Table 2. Genetic diversity parameters of the Mexican and Spanish Lidia breed populations: expected (*He*) and observed *(Ho*) heterozigosities, and F_{IS} inbreeding and significance *(*P*<0.01)

Pop	Family	Acronym	*He*	*Ho*	F_{IS}	Pop	Acronym	*He*	*Ho*	F_{IS}
MEXICO	Llaguno	BAR	0.39	0.46	-0.09*	SPAIN	ALB	0.33	0.34	0.03*
		BOQ	0.38	0.38	0.09*		ANA	0.38	0.46	-0.12*
		CRL	0.38	0.48	-0.17*		ANT	0.36	0.39	-0.05*
		ENC	0.39	0.41	0.07*		ARA	0.32	0.37	-0.11*
		FER	0.40	0.46	-0.07*		ATA	0.38	0.38	0.05*
		GAR	0.36	0.42	-0.04*		BAL	0.38	0.40	-0.01
		IGU	0.41	0.43	-0.04*		CAR	0.41	0.42	0.02
		JOS	0.42	0.45	0.04*		COL	0.44	0.37	0.19*
		MAR	0.35	0.40	0.02		CON	0.38	0.38	0.04*
		MAT	0.37	0.43	-0.06*		COR	0.34	0.38	-0.06*
		MON	0.39	0.45	-0.06*		CRM	0.39	0.46	-0.11*
		REY	0.38	0.44	-0.05*		CUA	0.26	0.30	-0.10*
		RIV	0.42	0.44	-0.07*		DOM	0.41	0.39	0.08*
		TEO	0.42	0.48	-0.06*		FEL	0.35	0.37	-0.01
		TOR	0.40	0.45	-0.06*		GAM	0.39	0.33	0.20*
		XAJ	0.39	0.44	-0.04*		HID	0.40	0.37	0.12*
		ZAR	0.36	0.41	-0.02		MAN	0.34	0.41	-0.13*
	González	CAS	0.30	0.35	-0.07*		MAZ	0.40	0.43	0.13*
		HAR	0.35	0.40	-0.07*		MIU	0.34	0.39	-0.07*
		SEC	0.38	0.44	0.06*		MUR	0.39	0.36	0.11*
Value of the whole population			**0.46**	**0.43**	**0.06**		PAB	0.31	0.35	-0.06*
							PED	0.37	0.35	0.11*
							SAL	0.39	0.38	0.06*
							SIE	0.37	0.41	-0.06*
							URC	0.37	0.41	-0.02
							VEG	0.39	0.34	0.15*
							VER	0.43	0.44	0.00
							VIL	0.41	0.42	0.02
						Value of the whole population		**0.48**	**0.38**	**0.21**

F_{ST} genetic distances were estimated among breeders within breeders (Mexico) and among lineages (Spain) by analyzing each population independently, followed by a second estimation of F_{ST} genetic distances including both, Mexican and Spanish populations (Table

3). The analysis of the Mexican population revealed average F_{ST} genetic distances going from 0.05 (Marrón) to 0.22 (Carlos Castañeda) when the genetic distance of each breeder to the rest of the breeders is calculated. Also F_{ST} genetic distances of each lineage to the rest of the lineages of the Spanish population ranged from 0.12 (Conde de la Maza) to 0.30 (Cuadri). Wright`s F-statistics (F_{IS} and F_{ST}) in the Mexican population were lower (Value of the whole Mexican population of F_{ST} 0.10 and F_{IS} 0.06) comparing with values obtained in the Spanish population (Value of the whole population of F_{ST} 0.18 and F_{IS} 0.21).

Table 3. F_{ST} genetic distances of the Mexican and Spanish Lidia populations with significance $P<0.05$

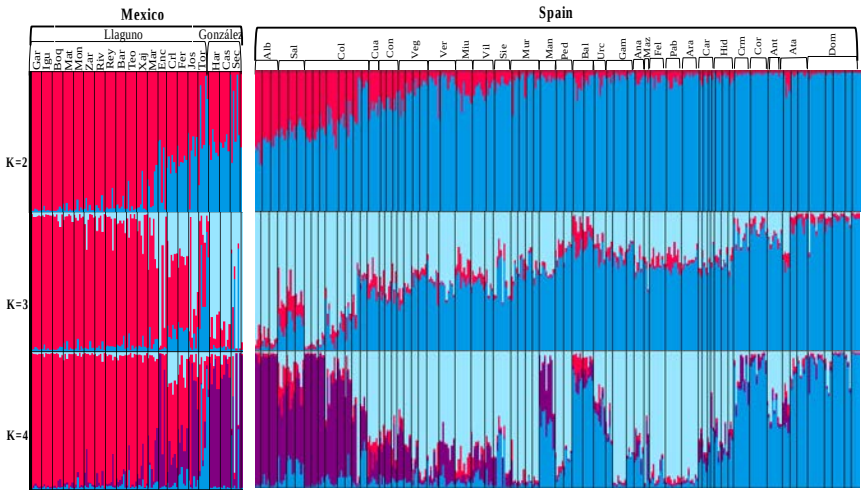
Pop	Family	Acronym	$F_{ST}^{(1)}$	$F_{ST}^{(2)}$	Pop	Acronym	$F_{ST}^{(1)}$	$F_{ST}^{(2)}$
MEXICO	Llaguno	BAR	0.08	0.14	SPAIN	ALB	0.26	0.24
		BOQ	0.07	0.15		ANA	0.17	0.17
		CRL	0.12	0.16		ANT	0.20	0.21
		ENC	0.09	0.14		ARA	0.25	0.25
		FER	0.10	0.14		ATA	0.18	0.18
		GAR	0.09	0.16		BAL	0.19	0.19
		IGU	0.11	0.18		CAR	0.15	0.15
		JOS	0.09	0.12		COL	0.13	0.12
		MAR	0.05	0.11		CON	0.20	0.19
		MAT	0.12	0.18		COR	0.22	0.23
		MON	0.09	0.16		CRM	0.17	0.17
		REY	0.07	0.14		CUA	0.30	0.30
		RIV	0.09	0.16		DOM	0.15	0.16
		TEO	0.08	0.15		FEL	0.22	0.22
		TOR	0.10	0.12		GAM	0.17	0.17
		XAJ	0.06	0.13		HID	0.16	0.16
		ZAR	0.06	0.13		MAN	0.22	0.22
	González	CAS	0.22	0.25		MAZ	0.12	0.11
		HAR	0.15	0.18		MIU	0.23	0.23
		SEC	0.10	0.12		MUR	0.18	0.18
						PAB	0.26	0.26
						PED	0.18	0.18
						SAL	0.19	0.17
						SIE	0.20	0.20
						URC	0.18	0.18
						VEG	0.18	0.18
						VER	0.14	0.14
						VIL	0.16	0.16
Value of the whole population			**0.10**		**Value of the whole population**		**0.18**	

$F_{ST}^{(1)}$ is the average F_{st} genetic distance from each lineage to the rest of the lineages from the same population. $F_{ST}^{(2)}$ is the average F_{st} genetic distance from each lineage to the rest of the lineages of both Mexican and Spanish populations.

Population relationships and clustering

The Bayesian approach implemented in STRUCTURE software[15] was used to analyse the clustering and genetic relationship among both Mexican and Spanish populations, acronyms are stated as defined in Table 1, displaying names of the breeders and their belonging family of the Mexican population, and names of the lineages of the Spanish population. The contribution of the assumed ancestral populations is graphically presented in Figure 1, with *K* populations going from 2 to 4.

Figura 1. Analysis of the genetic structure of the Mexican breeders and the Spanish lineages, the plot shows common genetic ancestors, or model based population assignments (K), for values going from from k=2 (upper) to k=4 (lower)



The acronyms are as defined in Table 1 and each acronym encloses the number of breeders belonging to each lineage.

In the Mexican population, from *k*=2 to *k*=4 a single ancestral population is observed in most of the breeders of the Llaguno family (Gar, Igu, Boq, Mat, Mon, Zar, Riv, Rey, Bar, Teo, Xaj and Mat), with a clear separation between González and Llaguno families. Mixed contributions with some of the Spanish lineages (Alb, Sal and Col) are observed in all of the

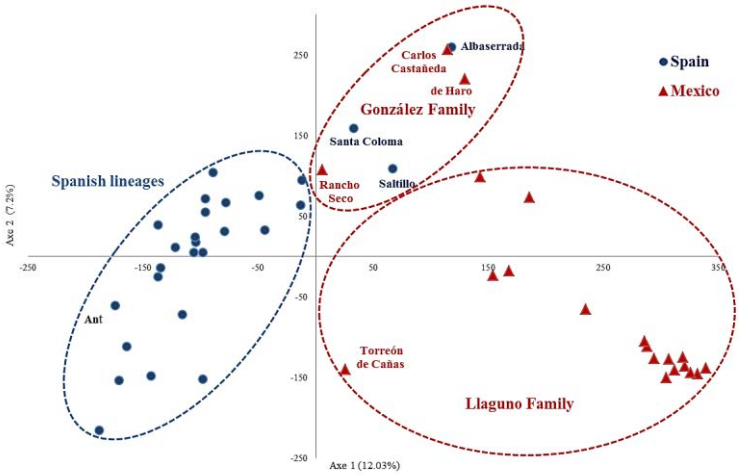
González breeders (Sec, Cas and Har) and some breeders from Llaguno family (Tor, Jos, Fer, Crl and Enc) when *k*=4.

In the Spanish population when *k*=2 most of the lineages belong to a same single ancestral population with some mixed contributions observed in Alb, Sal and Col lineages. Then when k=4 three different ancestral groups or clusters are differentiated: one conformed by Alb, Sal and Col lineages, a second cluster conformed by Cua, Con, Veg, Miu, Vil, Sie Mur, Gam, Ana, Maz, Fel, Pab and Ara and a third cluster conformed of Dom, Ata, Ant, Cor, Crm, Bal and Urc.

In general, among Spanish and Mexican populations, both showed different genetic ancestral origin with an exception of mixed contributions in the Mexican breeders of the González family and Tor, Jos, Fer, Crl, and Enc breeders from the Llaguno family with the Spanish lineages of Alb, Sal and Col.

Finally, in the correspondence analysis (Figure 2) a genetic discrimination between the Mexican and Spanish populations can be seen, with some exceptions like Sec and Tor breeders from the Mexican population who are placed closer to the Spanish Lineages than to the Mexican breeders. Furthermore, the Spanish Col, Sal and Alb: lineages are situated closer to the Mexican breeders than to the rest of the Spanish lineages.

Figura 2. Correspondence analysis of the Spanish and Mexican Lidia breed populations



Discussion

High gene diversity values were found in both the Spanish (0.48) and the Mexican populations (0.46). This value obtained in the Mexican population is remarkable, since lower gene diversity values were expected to obtain considering that, most of the current Mexican population arose from a few individuals of the Spanish Lidia population. On the contrary, similar diversity values were observed in both populations, so it is reasonable to consider certain degree of introgression with local Creole cattle populations of diverse origin during the establishment of the Mexican Lidia breed population.

Moreover, significant F_{IS} (P<0.01) values were observed in both populations which means a subdivision within each, higher (0.21) in the Spanish than in the Mexican population (0.06). This subdivision in lineages or breeders results in the preservation of more genetic variance[17], but a faster loss of genetic diversity within sub-population can be expected. Additionally, a loss of diversity due to population bottlenecks and founder effects result in increased inbreeding, resulting that the preservation of heterozygosity in the whole population is at the expense of a progressive poor genetic health within each sub-population.

Genetic diversity analysis revealed significantly higher genetic distances (P<0.05) in the Spanish population compared to the genetic distances of the Mexican population, with whole population F_{ST} values of 0.18 and 0.10 respectively (Table 3). Similar results were observed by Eusebi *et al*[10] with data obtained with microsatellite markers. In the Mexican population the lower genetic distances among breeders means higher animal exchangeability, a common practice in Mexico and less usual in Spain, where higher genetic distances between lineages were obtained, thus explained by higher genetic isolation among lineages.

Furthermore, genetic structure analysis revealed in both, Correspondence and Bayesian clustering analysis a clear separation among families (González and Llaguno) of the Mexican population and in the Spanish population three clusters are observed at k=4. The cluster with Albaserrada (Alb), Saltillo (Sal) and Santa Coloma (Col) is placed closer (correspondence analysis) and share genetic structure with the Mexican González family and some Llaguno breeders (Tor, Crl, Jos and Enc), leaving clearly differentiated the remaining Llaguno breeders. This similarity of Spanish Alb, Sal and Col lineages with the above mentioned González family and the few Llaguno breeders is not surprising, given the fact that those breeders were involved in the imports of 1996 and 1997, introducing mainly animals from Santa Coloma (Col) and in lesser extent Saltillo (Sal) and Vega Villar (Veg)[8]. But it is worth to note the proximity of Albaserrada (Alb) lineage to the Mexican population, since

Albaserrada herds have been raised under strict closed breeding schemes from 1912 onwards[18]. This genetic closeness is explained by two similar historical and genetical phenomenons' as Albaserrada lineage derive from Saltillo and Santa Coloma lineages[6] and in parallel, those similar Mexican breeders constructed their herds by mating animals from the same lineages as ancestors.

A deeper analysis of the Mexican population structure revealed that anthropogenic barriers are well documented drivers of the genetic differentiation observed among breeders (e.g., the clear genetic division observed between the González and Llaguno families). Both families where located respectively in the North and south central regions of Mexico and became much like hegemony of Lidia cattle, being in charge to supply Lidia cattle to emerging farmers in their regions. In addition, both families' bovines did not mix each other[8], confirming the different genetic origin among them.

Conclusions and implications

Isolation along with a small founder population size shaped by a classic bottleneck effect can explain the differentiation of the Llaguno Family of the Mexican population from the Spanish Lineages of which it arose. To all this, a possible introgression of Creole Cattle populations located at the north and south central regions of Mexico[19] could explain this gain of diversity. A trace-back analysis of the extant cattle populations in those regions could be footprints in the way to explain the major ancestors of the Mexican Llaguno family.

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DETECTION OF -SELECTION SIGNATURES FOR AGONISTIC BEHAVIOR IN CATTLE

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Detection of selection signatures for agonistic behaviour in cattle

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Summary
The identification of genomic regions including signatures of selection produced by domestication and its subsequent artificial selection processes allows the understanding of the evolution of bovine breeds. Although several studies describe the genomic variability among meat or milk production cattle breeds, there are limited studies orientated towards bovine behavioural features. This study is focused on mapping genomic signatures of selection which may provide insights of differentiation between neutral and selected polymorphisms. Their effects are studied in the Lidia cattle traditionally selected for agonistic behaviour compared with Spanish breeds showing tamed behaviour. Two different approaches, BayeScan and SelEstim, were applied using genotypic 50K SNP BeadChip data. Both procedures detected two genomic regions bearing genes previously related to behavioural traits. The frequencies of the selected allele in these two regions in Lidia breed were opposite to those found in the tamed breeds. In these genomic regions, several putative genes associated with enriched metabolic pathways related to the behavioural development were identified, as neurochondrin gene (NCDN) or glutamate ionotropic receptor kainate type subunit 3 (*GRIK3*) both located at BTA3 or leucine-rich repeat and Ig domain containing 2 (LINGO2) and phospholipase A2-activating protein (PLAA) at BTA8.

KEYWORDS
aggressive behaviour, Lidia breed, selective sweep, Spanish cattle

1 | INTRODUCTION

Since their domestication (~10,000 years ago), cattle populations have been subjected to natural and artificial selection processes (Ajmone-Marsan, Garcia, & Lenstra, 2010). Currently, most domesticated bovine breeds are specialized in milk and meat traits, and on a smaller scale in other economic traits of interest, such as leather or draft among others (Felius et al., 2014).

An early prerequisite of the domestication process in all the farm animals was probably to reduce their fear to humans (Belyaev, Plyusnina, & Trut, 1985). Thus, as a consequence of domestication, humans have modified the wild nature and social behaviour of bovines.

Different studies have analysed genomic changes produced by the long-term selection in most commercial bovine breeds (Pritchard, Pickrell, & Coop, 2010; Randhawa, 2016). As a consequence, several strong genomic signatures or hard sweeps belonging to traditional selected morphological traits (muscular hypertrophy, coat colour, presence/absence horns) have been reported (Druet, Pérez-Pardal, Charlier, & Gautier, 2013; González-Rodríguez et al., 2017). But so far, studies of selection signatures focused on behavioural features are limited. The Lidia bovine breed has been selected for centuries for its agonistic–aggressive behaviour by means of a series of traits registered by the breeders on a categorical scale that classifies their aggression and fighting capacity (Silva, Gonzalo, &

Cañón, 2006). Furthermore, these traits have evidenced significant heritability values, which make them suitable to genetic selection (Menéndez-Buxadera, Cortés, & Cañon, 2017; Silva et al., 2006). Unlike other bovine breeds in which aggression is an undesirable trait, it is likely that the aggressiveness selection process in the Lidia bovine breed has left detectable genomic signatures (Akey, Zhang, Zhang, Jin, & Shriver, 2002).

The detection of selective sweeps in quantitative traits still presents some limitations because many of the characters of interest, such as behavioural traits, are polygenic. In this case, the response to selection would be generated by modest allele frequency shifts at many loci, detection of which can be difficult to accomplish (Pritchard et al., 2010).

The imputation of genotypes using high-density genotyping platforms has favoured the identification of genomic selection signatures using demographic models, selections models or a combination of both (Ma et al., 2015). Under selection pressure, a new genetic variant at the genomic level may show one or more of the following features: extreme allele frequencies, excess of homozygotes, high frequency of long haplotypes and/or a higher genetic differentiation among populations (Qanbari & Simianer, 2014; Randhawa, 2016).

Several selection signature detection methods are based on allele frequencies differences among populations that may simply be identified in the extreme tails of the F_{ST} estimates distribution. Theoretically, loci under selection pressure or balancing selection are expected to evidence high and low levels of differentiation among populations, respectively. Foll (2012) extended this approach and directly estimated the probability that each locus is subject to selection using a Bayesian method that evidenced their robustness under different demographic scenarios. However, one criticism of this kind of methodologies is that they do not quantify the intensity of selection. Recently Vitalis, Gautie, Dawson, and Beaumont (2013) developed a Bayesian method which allows distinguishing between selected and nearly neutral polymorphisms and estimated the intensity of selection under a genetic model that assumes the subdivision of a population into subpopulations that may exchange migrants.

In this study, information provided by a panel of SNPs was used to analyse three groups of the Lidia bovine breed traditionally selected for agonistic-related traits, and two non-specialized tamed Spanish breeds (Asturiana de los Valles and Morenas Gallegas) as a reference to locate genomic regions associated with agonistic traits. A marginal second objective was to identify putative candidate genes mapping within these genomic regions in order to understand the evolutionary mechanisms of the Lidia breed.

2 | MATERIAL

A total of 213 (48 from Mexico and 165 from Spain) Lidia bovine breed individuals were genotyped using the Bovine 50K SNP BeadChip (http://www.illumina.com). According to Silva et al. (2006) who evidenced differences among the three main behaviour characteristics that are traditionally scored in the Lidia breed (aggressiveness, ferocity and mobility), 100 samples belonging to those Spanish lineages with higher agonist behaviour (SPA+) and 65 with the lower ones (SPA−) were selected to be genotyped. Those lineages with the higher and lower behaviour scores also evidenced the higher genetic differentiation among the Lidia breed lineages (Supplementary Table 1) (Eusebi et al., 2017; Cañón et al., 2008). In addition, animals from Asturiana de los Valles and from Morenas Gallegas bovine breeds were genotyped as reference group in which agonist behaviour is not desirable: 60 unrelated (based on genealogical information) Asturiana de los Valles breed individuals (35 genotyped with the 50k BeadChip and 25 with the 777k BeadChip) and 30 individuals from the Morenas Gallegas breed genotyped with the 50k BeadChip.

The SNPs in common between the 50K and 777K chips were identified (Nicolazzi et al., 2015). Then, the data sets of the five groups were combined using PLINK v. 1.07 (Purcell et al., 2007), and the following SNP edits were applied including the removal of individuals with a call rate <80%, non-autosomal SNPs and SNPs with minor allele frequency <0.01. After edits, 38,577 SNPs on 303 individuals remained.

3 | METHODS

SelEstim procedure proposed by Vitalis et al. (2013) is a hierarchical Bayesian method whose model is a diffusion approximation for the distribution of allele frequency in a population subdivided into a number of groups that exchange migrants with a rate equal to m. This procedure provides two parameters of differentiation between groups: σ is an average effect of selection and is a hyperparameter that summarizes the strength of dispersion among groups at each specific locus, and the Kullback–Leibler divergence (KLD) which is a non-symmetric measure of difference between two probability distributions calculating the distance of the posterior distribution of σ of the centring distribution. The KLD parameter represents the neutral demographic history of the groups, and KLD values are strongly correlated with F_{ST} estimates. Also, an estimate of the migration rate among the breeds is provided, this parameter is scaled by the effective size (i.e., $M_j = 4N_j m_j$) where M_j is the scaled migration parameter in the jth

population, N is the number of diploid individuals and receives immigrants for the whole population at a rate m.

A first computation is performed on the whole data set to estimate the posterior distribution of the parameters, obtaining a "pseudo-observed" data set; in order to provide a criterion to discriminate between neutral and selected markers, the calibration computation was then performed to achieve the thresholds (0.95, 0.99, 0.995 and 0.9999) quantiles of a "centring" KLD empirical distribution computed from the pseudo-observed data set.

3.1 | Identification of genomic regions with selection signatures

Assuming that behavioural traits are polygenic, low influence of many loci is expected. Hence, a slide window of ~10 MB that contains each of the SNP with KLD higher than 99.99% was selected to identify *genomic regions with selection signatures*. Furthermore, the previously defined SNPs with KLD higher than 95% in the 10 Mb windows were counted and used to define regions of genomic selection signatures. Gene annotation was performed by exploiting the knowledge on UMD3.1 locations of genes from the NCBI (ftp://ftp.ncbi.nih.gov/genomes/Bos_taurus/mapview/seq_gene.md.gz), and as annotation of the bovine genome is still incomplete, BioMart from Ensembl Archive release 90 (www.ensembl.org/biomart) was used to determine the orthologous human gene ID for each gene detected.

BayeScan software (Foll, 2012) was also used to detect signatures of selection, with the difference that this methodology detects divergence selection from Bayesian binomial frameworks identifying loci under selection when they show F_{ST} coefficients that are significantly different to that expected under neutrality.

With BayeScan, F_{ST} coefficients are split into a population-specific component (β), common to all loci and a locus-specific component (α) shared by all the populations using a logistic regression. Allele frequencies are assumed to follow a Dirichlet distribution. Selection is detected when α is significantly different to zero; *that is*, the locus-specific component is necessary to explain the observed pattern of diversity. When $\alpha > 0$ it is assumed that directional selection is acting on the locus under analysis, while $\alpha < 0$ suggests balancing or purifying selection (Foll, 2012).

3.2 | Identification of genomic regions with selection signatures

The standard PLINK files were converted to BayeScan format with the PGDSpider v 2.0.7.3 software (Lischer & Excoffier, 2012) and used the same parameters set with SelEstim to perform the analyses. A first filter was applied to the results, setting a significance threshold of 5% false discovery rate (Randhawa et al., 2016), and then, selecting the SNPs with alpha (α) values higher than 1, as it indicates strong evidence of diversifying selection according to Jeffrey's interpretation (Foll, 2012).

4 | RESULTS

4.1 | SelEstim

A total number of 19,287 SNPs had KLD estimates over the 50% quantile: 3,857 (90%), 1,918 (95%), 386 (99%), 194 (99.5%) and 5 (99.99%). The genomic regions of positive selection containing at least one SNP in the last percentile and the remaining in the 95% are described in Table 1. The migration rates (M_j) ranged from 20.92 in the Asturiana de los Valles breed to 2.52 in the Mexican Lidia group (Table 2).

4.2 | BayeScan

A total of 249 outlier loci displayed strong signals of positive selection, $\alpha > 1$ and q value 5% (Table S2). A q-value of 5% means that it is expected that 5% of the outlier markers (those with a q-value >5%) are false positives (Foll, 2012) and therefore were discarded.

Positional coincidences with SelEstim and BayeScan were identified in chromosomes 3 and 8 (Table 3, Figure S1). Furthermore, these selective sweeps with genomic signals of positive selection were analysed more thoroughly to identify candidate genes that could have been modified by selection.

4.3 | Selection signature at BTA3

The pattern of the average values of selected alleles (κ_{ij}) shown in Figure 1 evidenced that most of the polymorphisms are positively selected in the bovine populations. However, all the groups show an outlier allele at nucleotide

TABLE 1 Putative selective sweeps identified with SelEstim

Selective sweeps	Chr	N SNP	Mb Start	Mb End	Higher KLD
1	3	9	109.49	119.08	1.92
2	8	8	14.89	27.98	1.92
3	11	11	15.07	24.92	2.55
4	13	5	26.52	31.82	1.98
5	18	12	47.83	54.86	2.22

Chr, chromosome; N SNP, number of SNPs included in the genomic region with KLD over 95% and at least one SNP over 99.99%; Mb, Mega base pairs, and the higher value of the Kullback-Leibler Divergence (KLD) of the SNPs included in the selective sweep.

TABLE 2 Estimate of the migration (M_j) parameters for the five groups, mean values and standard deviations (Std. Dev.)

Groups	Mean	Std. Dev.
Asturiana de los Valles	20.92	0.24
Morenas Gallegas	9.14	0.09
Lidia Mexico	2.52	0.02
Lidia Spain(+)	12.81	0.13
Lidia Spain(−)	4.68	0.04

TABLE 3 Genomic concordance of the selective sweeps identified with BayeScan and SelEstim approaches

	SelEstim				BayeScan	
Chr	N SNPS	Mb Start	Mb End	Higher KLD	Mb	Higher α
3	9	109.49	119.08	1.92	116.8	1.02
8	8	14.89	27.98	1.92	19.9	1.16
					22.3	1.28

Chr, chromosome; N SNP, number of markers; Mb, mega base pairs.

BTA3:110,766,510 with the highest KLD value (Table 1). At this locus, the intensity of selection (σ) estimated, which allows for the identification of the strongest selection coefficients, had the same selection direction in the Lidia breed subpopulations and the opposite in the tamed Morenas Gallegas bovine breed. This genomic region contains several genes related to different pathways, such as the serotonergic and dopaminergic signalling pathways, which contribute to the process of differentiation in a selection oriented for behavioural-related traits.

This SNP with the highest KDL value is located proximate to the Neurochondrin gene (*NCDN*) (BTA3: 110,784,499-110,793,283). This gene is highly expressed in the central nervous system (Table S3) and works as a negative regulator of the Ca_2+/calmodulin-dependent protein kinase II (*CaMKII*), a key enzyme present in the early stages of memory formation and involved also in the hippocampal synaptic plasticity (Dateki et al., 2005). This gene is highly associated with the serotonergic signalling pathway in modulating the acquisition and consolidation of memory.

The glutamate ionotropic receptor kainate type subunit 3 (*GRIK3*) gene is located close to the *NCDN* gene and has been identified previously by Qanbari and Simianer (2014) as candidate gene for signatures of selection in cattle. The *GRIK3* gene is highly expressed in the central nervous system and is included in a QTL described in the reward-related processes underlying learning and memory

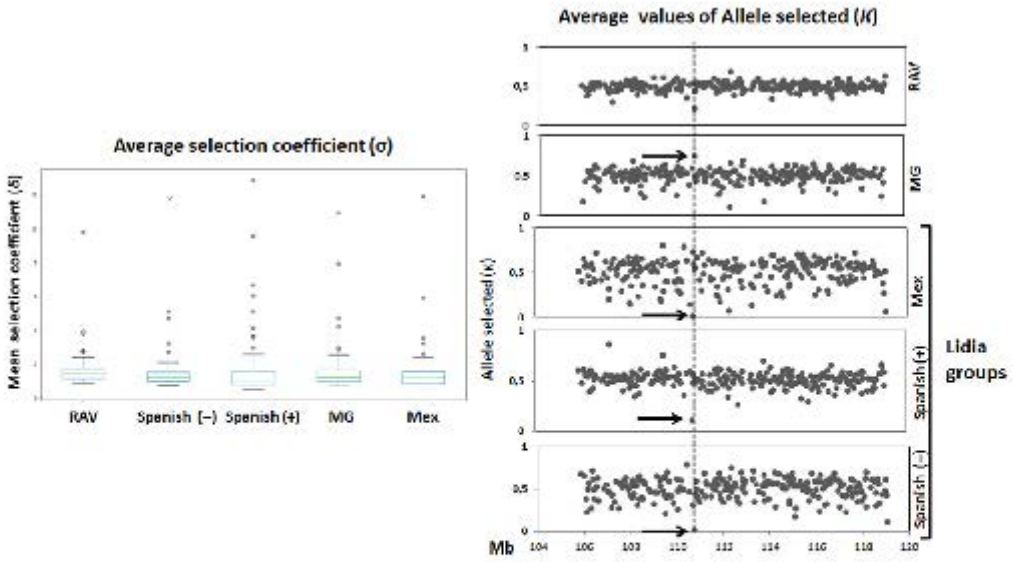

FIGURE 1 Plot of the putative selective sweep localized in BTA3 between 106 and 119 Mb. The left boxplot is the mean selection coefficient (σ) per group and in right boxplot the mean values of the allele selected (κ_{ij}) for each group, where RAV = Asturiana de los Valles, MG = Morenas Gallegas, Mex = Lidia from Mexico, Spanish(−) = Spanish Lidia less aggressive group and Spanish(+) = Spanish Lidia more aggressive group

(Minelli, Scassellati, Bonvicin, Perez, & Gennarelli, 2009). Furthermore, the disc large-associated protein 3 (*DLGAP3*) gene located within the same region is also associated with learning processes (Kähne et al., 2016).

Besides, thyroid hormone receptor-associated protein 3 (*THRAP3*) and splicing factor proline- and glutamine-rich (*SFPQ*) genes, also located within the frame of this genomic region, are linked to the circadian cycle. Other genes are associated with processes implicated in domestication-related changes like sensory perception (*GJB4*, *SAG* and *TRPM8*), brain development and neurobehavioural functioning (*POU3F1*), muscle contraction (*FHL3*) and pigmentation (*NCDN*) (Xing, Ling, Chen, & Gu, 2006) (Table S3).

4.4 | Selection signature at BTA8

The pattern of the average values of the selected alleles (κ_{ij}) revealed opposite direction of selection intensity (σ) in the Lidia breed subpopulations compared with Asturiana de los Valles and Morenas Gallegas tamed breeds (Figure 2). Also it should be noted that the SNP with the strongest intensity of selection is present in the Lidia with higher agonist behaviour (SPA+) group. Several genes are located in this genomic region; however, the leucine-rich repeat and Ig domain containing 2 (*LINGO2*) and phospholipase A2-activating protein (*PLAA*) genes are related with extreme neurobehavioural phenotypes and psychiatric disorders and probably with behaviour characteristics (Table S3).

5 | DISCUSSION

In the present study, two Bayesian approaches that are able to detect both recent and old selection events, BayeScan and SelEstim, were applied to identify genomewide signatures of selection in three bovine breeds traditionally selected for opposite behaviour characteristics.

Additionally, SelEstim procedure also estimates intensity and direction of the selection at each locus for each population and the migration rate (M_s) reflecting the relative admixture of each group with respect to all the groups. The relative genetic proximity of the Asturiana de los Valles breed respect to the rest of cattle populations analysed (Table 2) is noteworthy. A similar result for the Asturiana de los Valles breed was also obtained by González-Rodríguez et al. (2017) using seven Spanish bovine beef breeds, suggesting that this breed has been used as terminal sire line and crossbred individuals are

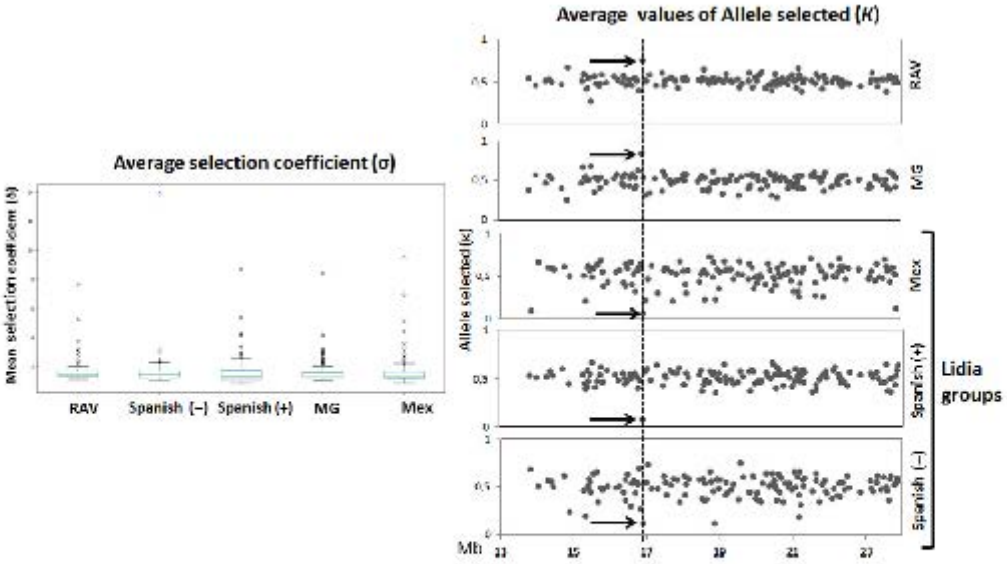

FIGURE 2 Plot of the putative selective sweep localized in BTA8 between the 13 and 24 Mb. The left boxplot is the mean selection coefficient (σ) per group and in right boxplot the mean values of the allele selected (κ_{ij}) for each group, where AST = Asturiana de los Valles, MG = Morenas Gallegas, Mex = Lidia from Mexico, Spanish(−) = Spanish Lidia less aggressive group and Spanish(+) = Spanish Lidia more aggressive group

introduced into the receptor populations. However, it is difficult to embrace this argument in our case taking into account the presence of the Lidia breed, which is extremely isolated and with low effective population sizes (Cortés, Sevane, Baro, & Cañón, 2014).

A curious appreciation is the need to decrease the threshold of KLD to 90% to identify genomic regions under selection that are known to be under positive selection, such as the one bearing MSTN or myostatin gene (Supplementary Table 4). This threshold identified 3,857 SNPs, so this large amount of polymorphisms may be related to polygenic selection or adaptation processes (Pritchard et al., 2010), involving several genes or polymorphisms with minor effects. However, when the most restrictive threshold (99.99%) was applied, the number of selected polymorphisms was reduced to only five (Table 1).

The difficulty to detect selective sweeps with statistical significance in polygenic traits, in which many loci shift their frequency moderately (Pritchard et al., 2010), could explain that only two genomic regions were shared with both methodologies. Other reasons may be the limitations of the 50K chip and the sample size of the analysis.

Furthermore, a high rate of false positives is expected due to the divergence in allelic frequencies between breeds (and groups within the Lidia breed and Morenas Gallegas) as a consequence of the genetic drift and founder effects; this is particularly important during the development of the cattle breeds (Petersen et al., 2013). These factors can bias the footprints left in the genome by selection and hamper the identification of selective sweeps.

The results of the present study suggest that the methods employed are able to detect signals of selection generated by recent selection events within populations. Furthermore, the absence of regions with strong signals of selection may be hidden considering that (i) artificial selection processes do not always leave relevant signatures of selection; (ii) the polygenic nature of the behavioural traits (Pritchard et al., 2010) and (iii) the limitations of the bovine genomic resources of the SNP BeadChip already mentioned. However, both methodologies detected genomic signatures of selection in BTA3 and BTA8 regions, where genes whose higher expression is detected mainly in the prefrontal cortex of the brain, where the reactions of violence and social aggression take place (Lotze, Veit, Anders, & Birbaumer, 2007) (Table S3).

Besides, the candidate genes *NCDN, GRIK3, DLGAP3, THRAP3* and *SFPQ* located in the selective sweep at chromosome 3 are involved in the serotonergic signalling pathway involved with the development of personality and behavioural traits (Minelli et al., 2009) and also in the development of different aggressive behaviour manifestations, such as fear-induced aggression (Popova, Naumenko, Plyusnina, & Kulikov, 2005), intermale aggression (Kulikov, Osipova, Naumenko, & Popova, 2005), predatory aggression (Nikulina & Popova, 1988) and maternal aggression (da Veiga, Miczek, Lucion, & de Almeida,2011). However, the candidate gene approach has mainly been conducted using rats, albeit with limited success. Studies involving putative behavioural genes such like those involved on serotonergic, catecholaminergic and glutamatergic pathways have failed to find variants of significance, mainly because of a small number of study subjects and a lack of functional assays (Spady & Ostrander, 2008).

In conclusion, the present study identifies two genomic regions associated with agonistic-related traits in cattle. The direction of selection of both regions differed between the aggressive Lidia breed and the tamed Asturiana de los Valles and Morenas Gallegas breeds that were used for comparative purposes. These findings corroborate that intensive targeted selection for different goal traits has left detectable imprints in the genome.

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

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COMPLEMENTARY DOCUMENTATION

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Genomic diversity and population structure of Mexican and Spanish bovine Lidia breed

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Summary

The Lidia bovine breed is distinguished for its low genetic exchangeability given its selection on aggressive behavior, its management uniqueness and its subdivided structure. In this study, we present a comprehensive genome-wide analysis of genetic diversity, population structure and admixture of 468 animals from Mexican and Spanish Lidia breed populations and 64 samples belonging to 10 Spanish native and American-creole breeds using 37 148 single nucleotide polymorphisms. We found similar average inbreeding values in the Lidia breed, with different distributions within groups; variability of inbreeding values among Spanish lineages was significant and no differences were found among the Mexican subpopulations. Together, the high F_{IS} of the lineages and the behavior of the runs of homozygosity are consequences of the lineage's small effective population sizes, contributing to their inbreeding increase. Population admixture analysis discarded any influence on the genetic structure of the Lidia populations from the Spanish native and American-creole breeds. In addition, both Lidia populations depicted different genetic origins, with the exception of some Mexican individuals whose origins traced back to recent Spanish importations.

Keywords fighting bull, genetic variation, runs of heterozyosity

Retaining the aggressive features of its wild ancestors, the aurochs (*Bos taurus primigenius*), and distinguished by its extensive management, the Lidia bovine population could be considered a primitive breed which originated ~250 years ago in the Iberian Peninsula. In Mexico, most of the current Lidia population derive from a few Spanish animals imported in 1908 by two families of breeders (Llaguno and González) and a lesser proportion from more recent Spanish importations made during 1996 and 1997 (Niño de Rivera 2004).

Recently, the availability of SNP panels (Bovine HapMap Consortium 2009) has allowed a higher level of resolution when investigating livestock genetic diversity and structure. So, in this study we present for the first time a comprehensive genome-wide analysis of the genomic diversity and population structure of both Mexican and Spanish Lidia populations using the 50K Beadchip panel (Illumina Inc.). In addition, 10 local bovine breeds from Spain and North America were included in the analysis to assess possible shared genetic origins with the Lidia breed.

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A total of 468 DNA Lidia breed samples, 349 from 28 Spanish lineages classified according to Cañón *et al.* (2008) and 119 from the two Mexican lineages classified according to Eusebi *et al.* (2016), were analyzed (Table 1). Samples were genotyped for 54 609 SNPs using the Bovine 50K SNP BeadChip following standard protocols (http://www.illumina.com; data are available via the Figshare repository, https://doi.org/10.6084/m9.figshare.5394895.v3). We also included existing genotypic data kindly provided by Decker *et al.* (2014) of 64 animals from 10 breeds from the Iberian Peninsula and North America that may have possible shared genetic origins with the Lidia breed (Table S1). The animals were classified into four groups: two from the Lidia breed populations (Mexican and Spanish) divided into their corresponding lineages and two from the non-Lidia breeds (Spanish native and American-creole).

After standardizing our Lidia genotypic data with the data provided by Decker *et al.* (2014) into the UMD 3.1 assembly, we used the PLINK V 1.07 software (Purcell *et al.* 2007) to exclude individuals with more than 20% missing genotypes, SNPs located on sex chromosomes, those with a minimum allele frequency less than 0.01 and markers that did not

Table 1 Description of the groups analyzed by their origin, lineages of the Lidia groups, number of breeders (NB), Number of samples (NS) and individual inbreeding values averaged (*F*) computed from PLINK as 1 – *Ho*/*He*.

Group and lineage	Acronym	NB	NS	*F*
Lidia breed				
Mexico, F_{IS} = 0.068, F_{ST} = 0.125				
González	GON	3	16	0.29
Llaguno	LLA	17	101	0.30
Spain, F_{IS} = 0.079, F_{ST} = 0.205				
Albaserrada	ALB	3	14	0.41
Anastasio Martín	ANA	1	6	0.19
Antonio Pérez	ANT	1	9	0.32
Araúz de Robles	ARA	1	10	0.34
Atanasio Fernández	ATA	3	14	0.36
Baltasar Iban	BAL	2	12	0.28
Carlos Núñez	CAR	4	9	0.25
Santa Coloma	COL	8	36	0.35
Contreras	CON	3	10	0.32
Conde de la Corte	COR	1	10	0.39
José Marzal	CRM	1	9	0.19
Cuadri	CUA	1	7	0.44
Domecq	DOM	5	29	0.36
Félix Gómez	FEL	1	9	0.29
Gamero Cívico	GAM	3	16	0.43
Hidalgo Barquero	HID	3	15	0.31
Manuel Arranz	MAN	1	9	0.30
Conde de la Maza	MAZ	1	3	0.24
Miura	MIU	1	9	0.26
Murube	MUR	4	16	0.34
Pablo Romero	PAB	1	9	0.32
Pedrajas	PED	2	10	0.39
Saltillo	SAL	3	15	0.33
Concha y Sierra	SIE	1	10	0.22
Urcola	URC	1	7	0.26
Veragua	VER	2	16	0.19
Vega Villar	VEG	4	17	0.38
Marqués de Villamarta	VIL	2	13	0.26
Non-Lidia breeds				
Spanish native, F_{IS} = 0.065, F_{ST} = 0.093	SPA	8[1]	25	0.15
American-creole, F_{IS} = 0.010, F_{ST} = 0.024	AME	2[1]	39	0.07

[1]Non-Lidia breed groups NB correspond to the number of breeds, as defined in Table S1.

match Hardy-Weinberg equilibrium (0.001) to finally obtain 37 148 SNPs with which to perform the analyses.

Using PLINK v1.07, we calculated individual inbreeding values (*F*) and then analyzed their variability across and within groups. We also performed two analyses of molecular variance using ARLEQUIN V 3.5 software (Excoffier *et al.* 2005), adjusting a hierarchical model at three levels (groups, lineages and individuals) to assess for the different sources of genetic variation. We also computed runs of homozygosity (ROH), as described by Purfield *et al.* (2012), with a sliding window of 30 SNPs, with less than 100 kb between two consecutive homozygous SNPs, more than two missing genotypes, one possible heterozygous SNP and a minimum length of 500 kbp. ROH were classified into five length categories.

The subdivision level of the Lidia breed in terms of F_{IS} (0.076) was similar to the values found in both the Spanish (0.079) and the Mexican group (0.068) (Table 1). However, the individual inbreeding values within each group showed different distribution patterns. The country of origin of the Lidia group explained 34% of the variability of the individual inbreeding values; but, although 42% of the variability was explained by the lineages within the Spanish Lidia, differences within the Mexican group were not significant.

Previous studies associated high average number and length of ROH to practices of mating related animals (Upadhyay *et al.* 2017); this is consistent with our results, which evidenced higher number and size of ROH segments in the Spanish and Mexican Lidia than in the non-Lidia groups (Spanish native and American-creole) (Table 2, Figs S1 & S2). Both the genomic ROH achieved and high F_{IS} values in the Lidia breed are reflections of high sub-division in the lineages and its main consequences, reduced effective population sizes and high inbreeding levels (Cortés *et al.* 2014).

The genetic variability explained by the Lidia breed lineages (Mexican and Spanish) was 19% (Table S2). In the Mexican Lidia population, the lower genetic distances among breeders are the consequence of a relatively frequent exchange of sires, a common practice in Mexico but less usual in Spain. Thus, the F_{ST} value within the Spanish Lidia group was significantly higher (0.21) (Table 1), and these distances were more than twice the average values of the Spanish native (0.09) and American-creole groups (0.02).

Non-significant correlation between diversity in the origin of the Spanish and Mexican Lidia lineages and their contemporary expected diversities was found (Table S3).

We used ADMIXTURE v1.23 software (Alexander *et al.* 2009) to analyze the genetic structure and PLINK v1.07 to perform a multi-dimensional scaling analysis. We did not detect shared genomic origins between the Spanish native and American-creole groups and the Lidia breed (Fig. 1). In addition, the genetic origins of the Mexican Lidia lineages rarely coincided with that of the Spanish lineages. The spatial separation on the multi-dimensional scaling analysis of the Lidia groups (Fig. 2) confirms this differentiation.

There are arguments that explain the clear genetic differentiation between Lidia groups. In the early 20th century, a few Spanish Lidia animals were imported by both the Gonzalez and Llaguno families of breeders; each family followed different breeding strategies, mating the recent imports with the extant aggressive selected bovines or among them respectively (Niño de Rivera 2004). Additionally, we hypothesize the probability of an admixture with local cimarron genes. Cimarrons are 'run-away' individuals that escaped from their original environment and returned to its wild state; in this context, the *cimarronage* is considered a typical phenomenon of the livestock colonization in the New World (Maudet 2010).

Group	Number of ROH				Total length of genome >1 Mb			
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
Spanish Lidia	104	24.4	9	151	736.2	240.3	25.1	1630
Mexican Lidia	116	27.4	46	162	683.9	221	182.6	1264
Spanish native	43	31.1	5	125	332.2	295.9	8.3	1183.9
American-creole	22	14	6	60	160.7	109.5	14.3	438.6

Table 2 Descriptive statistics for the number of runs of heterozyosity (ROH) and total length (Mb) of genome in ROH for the four cattle groups.

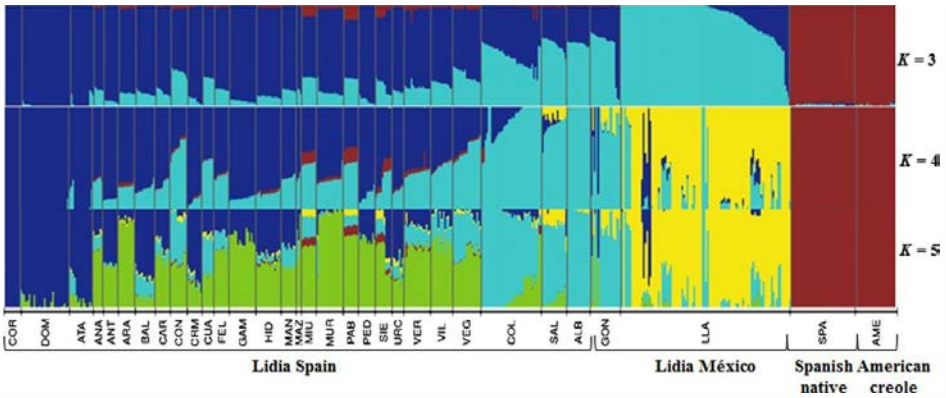

Figure 1 Graphic representation of the proportions of the sampled individual genomes belonging to 3, 4 and 5 (*K*) inferred clusters.

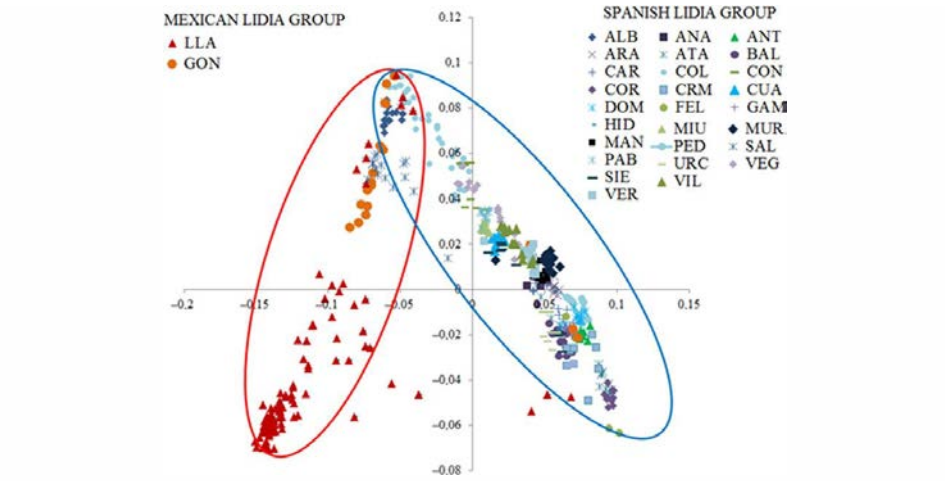

Figure 2 Multidimensional scaling plot based on the matrix of genome-wide pairwise identity-by-state distances inferred with PLINK. The graphic shows the genetic relationships between the Lidia lineages from Mexico (inside the red circle) and Spain (inside the blue circle). See Table 1 for acronym definitions.

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Supporting information

Additional supporting information may be found online in the supporting information tab for this article:

Figure S1 Relationship between the number of ROH >1 Mb and the total length (Mb) of the genome in those ROH for individuals from each group.

Figure S2 Differences between groups of the total length of genome in ROH divided into different length categories and ROH length.

Table S1 Data of the Spanish native and American-creole breeds included in the analyses according Decker *et al.* (2014).

Table S2 Analysis of molecular variance results: (a) between Mexican and Spanish Lidia groups and (b) each group analyzed separately.

Table S3 Diversity in origin (*Div*) (calculated as $1 - \Sigma(qk)$ 2, where qk is an average fraction of the breed's genetic ancestry from the K separate genetic clusters at the optimal K, identified in the ADMIXTURE analysis) and expected heterozygosity (*He*) values estimated per lineage of the Lidia groups and for the Spanish native and American-creole groups.

GENERAL DISCUSSION

Herein we provide a genomic analysis of the Mexican Lidia breed, a unique population likely founded by a few individuals of the original Spanish Lidia population. Our work conclusively shows that the Mexican population shares few common genetic origins with the hypothetical original population, which led us to propose new hypothesis about the genetic origins of the Mexican population, whose genetic resources are valuable to address the challenge of the genetic improvement of the Lidia breed.

We also studied the possible existence of genomic regions affected by selection for agonistic behavior related traits, whose is the selection target of the Lidia breed, and compared it with two tamed Spanish breeds as a reference. The results allowed identifying selected genomic regions in the Lidia breed containing genes putatively associated to behavioral features.

1. The genetic diversity of the Mexican Lidia breed

The results of the genetic diversity parameters analyzed with data derived from microsatellite and SNP molecular markers revealed, in general, similar genetic diversity values in both the Mexican and Spanish Lidia populations. The medium number of alleles (MNA) obtained with the microsatellite data in the Mexican population ranged from 3.4 to 5.4 (average of 4.4), similar to the Spanish lineages that ranged from 3.1 to 6.9 (average of 4.8). These values are lower than the observed by Cymbron et al. (2005), who worked with a pool of North European commercial cattle breeds analyzed with a panel of 20 microsatellites, and detected an average value of 7.97 MNA, and also with a pool of Mediterranean cattle breeds, finding an average value of 7.62 MNA. However, when each of these breeds was analyzed individually, the MNA ranged from 3.8 to 4.9 (Cymbron et al., 2005), placing the Lidia breed in general (including both, the Mexican and Spanish populations) within the range of these

European breeds. Curiously, the values of MNA in four Mexican creole herds, analyzed with a panel of 19 microsatellites, ranged from 6.68 to 8.3 (Ginja et al., 2013).

In terms of heterozygosity, the results were in a similar situation than the observed with the values of MNA. The mean values of expected heterozygosities were alike in both, the Mexican and the Spanish populations, using the two types of genetic markers (**Table 2**), being slightly higher the results obtained with the microsatellite data than the values obtained with SNPs. The statistical power per locus was higher in microsatellite markers than in the SNPs because their higher polymorphism, while typically SNPs have just two alleles per locus, in which case heterozygosity cannot exceed values of 0.5. Comparing the efficiency to detect genetic diversity of both types of genetic markers, microsatellite loci are more informative because of their greater extent of polymorphism, but SNPs have the advantage of being much more abundant and thus, have more power to infer differences in genetic summary statistics than the data obtained with microsatellites (Valï et al., 2008; Coates et al., 2009; Ciani et al., 2013).

Table 2 Comparison of the expected heterozygosities obtained with the different types of autosomal genetic markers used: microsatellites and SNPs.

	Gene diversity (H_{exp})		
Population	**Microsatellite data**	**573 SNP data**	**50K SNP data**
Over all Spanish lineages	0.61	0.48	0.36
Over all Mexican families	0.62	0.46	0.36

The autosomal gene diversity of the Mexican population differs from what we expected. Historical data affirms that just a few Lidia animals were imported to Mexico between 1908 and 1912 with the specific purpose of breeding (Scherrer, 1983; Niño de Rivera, 2004). Before that, the reproduction of the Lidia bovines was not held in an organized way.

The loss of genetic variability is an important aspect in the management of populations since a genetic impoverishment could ultimately lead a breed or species to extinction. Accodringly,

we made an analysis to quantify the loss of diversity in origin. We assessed the correlation between the diversity in origin per lineage and the *He* estimates and detected low correlation between these values (0.12). This result is not surprising because, in general, the Lidia breed has a reproductive system which is focused traditionally on closed breeding schemes, where the exchange of animals among breeders within lineages, although is less common in Spain (Cañón et al., 2008), than in Mexico (Eusebi et al., 2017), lead to a high genetic uniformity within the lineages and a loss of genetic diversity with respect to the diversity in origin.

Assuming simple genetic models of rapid colonization (Olivieri, 2009), we expected to find a reduction of the genetic variability in the Mexican population as a consequence of both, the founder and bottleneck effects. Conversely, we detected (with both type of molecular markers) similar patterns of genetic diversity in the Mexican population when compared to the original Spanish population.

In terms of F_{IS}, the Mexican population showed similar to lower average values (0.06 with the 573 selected SNPs and 0.05 with the Microsatellite data) than obtained in the Spanish population (0.21 with the 573 selected SNP data and 0.12 with the Microsatellite data). When we analyzed the distribution patterns of the individual inbreeding values within each population using the 50K SNP data, we found that in the Spanish population 42% of the variability is explained by the differences of the inbreeding values among lineages, and conversely, we did not detect significant differences in terms of inbreeding among the two Mexican families.

The particular racial grouping of the Spanish population must be highlighted to explain the great differences of the inbreeding variability (42%). There are different levels of gene flow within lineages; while some lineages are composed of a single herd with reduced population size e.g. Partido de Resina, Miura, Arauz de Robles or Cuadri, the remaining lineages are composed by a wide range of herds and besides, the genetic exchange between lineages is

scarce(Cañón et al., 2008). Whereas in Mexico the situation is different, where basically a few individuals from the Saltillo lineage composed the whole population and also, the exchange of reproducers is a common practice among Mexican breeders.

The partition of the total genetic variability within the Lidia breed populations (Spain and Mexico) analyzed with the 50K SNP Beadchip, showed that 19% of genetic variation is explained by the genetic differences among lineages (Eusebi et al., 2017).

Furthermore, the average genetic distances estimates in terms of F_{ST} among lineages within countries was of 0.10 in Mexico (Microsatellite and 573 SNP data) and 0.18 in Spain (**Table 3**).

Table 3 Comparison of the overall average F_{ST} genetic distances obtained with the different types of markers used: microsatellites and SNPs.

	Average F_{ST} Genetic distances		
Population	**Microsatellite data**	**573 SNP data**	**50K SNP data**
Over all Spanish lineages	0.18	0.18	0.21
Over all Mexican families	0.10	0.10	0.13

The lower genetic distances among Mexican breeders is probably a sum of different historical events: **(1)** The first importations (1908-1912) of a reduced number of animals that arised from the same lineage (Saltillo) that, in those years, was highly demanded for the festivities (Niño de Rivera, 2004), **(2)** besides, in the subsequent years after these importations, the Lidia population in Mexico experienced a drastic reduction as a consequence of the Mexican revolution and its after-effects (e.g. vandalism, poverty and hunger, land grants) (Niño de Rivera, 2004). We can suspect that individuals from both families admixed (that means, mixing of genes from populations who were previously separated), and a genetic homogenization within families explains their lower genetic distances.

Nevertheless, the average F_{ST} values of both populations (**Table 3**) are yet greater than the average pairwise FST values observed between different European bovine breeds (European Cattle Genetic Diversity Consortium, 2006).

2. Genetic structure of the Mexican Lidia population

Currently, the use of genetic markers allows estimating ancestries or common genetic origins of individuals within populations. This is an increasingly important method applied nowadays because it helps to solve different problems such as: **(1)** the detection of population structure **(2)** defining the number of subpopulations in a sample **(3)** assigning anonymous individuals to subpopulations **(4)** defining the number of ancestral populations in admixed populations **(5)** assigning ancestral population proportions to admixed individuals.

The software STRUCTURE (Pritchard et al., 2000) uses a Bayesian clustering approach. Similarly, the ADMIXTURE (Alexander et al., 2009; Alexander et al., 2011) program uses a maximum likelihood framework, both software's aim to infer population genetic structure and assign individuals ancestry proportions to a *K* supposed population. Unlike STRUCTURE, ADMIXTURE is focused on maximum likelihood estimation (MLE) rather than sampling the posterior distribution using MCMC, and calculates the estimations via a block relaxation approach, which results in improvements in speed. This computational efficiency provides an advantage over STRUCTURE when using very large numbers of markers, for example when using dense SNP data instead of smaller microsatellite panels (Liu et al., 2013). For this reason we used the ADMIXTURE to analyze the information provided by the SNPs and STURCTURE to analyze the microsatellite data.

The analyses revealed a considerable genetic differentiation between the Mexican population and the original Spanish lineages, with the exception of some mixed contributions observed in a few individuals of the Mexican González and Llaguno families with three Spanish lineages: Santa Coloma, Saltillo and Marqués de Albaserrada. Although, when we traced back the origins of those Mexican individuals sharing common genetic origins with the

Spanish lineages and we found that these animals were admixed with Spanish individuals from recent importations made at the beginning of the 1990´s (A.N.C.T.L., 2017).

Given the clear genetic differentiation between the Mexican and the Spanish populations, we hypothesized the introduction of bovines with different genetic origins, like Spanish local or American creole breeds, all of these potential breeds that could be involved in shaping the current genetic structure of the Mexican Lidia population. To test this hypothesis we included additional microsatellite and SNPs genotypic data of these breeds and performed the respective analyses using STRUCTURE and ADMIXTURE software's. Although, we did not detect common genetic ancestry of our Lidia population and the breeds included, and thus could not provide directions of the ancestry of the Mexican population.

Initially, observing the similarity of the heterozygosity patterns between the Mexican and Spanish populations and also the different ancestry of both populations, we can argue that this differentiation can be a consequence of two concomitant and, in a certain way, related phenomena: a population bottleneck, and a founder effect. The founder effect of the Lidia Mexican population was strong because of the limited number of Spanish Lidia bovines firstly introduced at the beginning of the 20th century (Niño de Rivera, 2004).

In the view of the foregoing, we additionally hypothesize the probability of an admixture with local "Cimarron" genes, as mentioned by Eusebi et al. (2017). In this case we adopted the term cimarron that is a "run-away" individual that escape from their original environment and return to its wild state (Maudet, 2010). There are many successful examples of cimarron bovine populations in America, for example, during the first social disturbs in Texas from 1830 to 1848, about 80% of 100,000-headed cattle in the region returned to their "wild state" (Jacquin, 2015). Mexican cimarron bovines selected for their aggressiveness can be then, the source of the genetic divergence of the Mexican Lidia group. Thus, a trace-back looking for footprints of cimarron genes may provide more information of the ancestry of this lineage.

3. Analyses of the sex chromosomes

The distribution of the paternal haplogroups in both Lidia populations (Mexican and Spanish) revealed the presence of the two haplogroups (Y1 and Y2), which coincides with the geographical distribution of the majority of the northern and southern European Breeds (Götherström et al., 2005). In the Mexican Lidia breed population three of the ten haplotypes previously identified in the Spanish population (Cortés et al., 2011) were observed.

A remarkable high frequency of the haplotype H6 was detected in the Mexican population, with frequencies of 69% and 20% in Llaguno and Gonzalez breeders respectively. This haplotype was described by Cortés et al. (2008), as exclusive in the Miura lineage (frequency of 100%) and with a high frequency (38%) in Casta Navarra. The presence of this H6 haplotype in the Mexican population has a plausible explanation, which is a strong paternal influence of males from the Casta Navarra, whose introduction to Mexico after the conquest is well documented (Niño de Rivera, 2004). Similarly, the influence of this haplotype in the total paternal lineage in the Miura lineage is explained by the strong influence of a sire of Casta Navarra named "Murcielago", who was a predominant and widely used sire during the late 19th century (López del Ramo, 1991), imprinting this haplotype to the whole Miura male offspring as a classic founder effect.

The analysis of the maternal lineages revealed similar patterns of genetic haplotype diversity in the Mexican population compared to the observed in the great majority of the European bovine breeds. The lower mtDNA haplotype diversities of the Mexican population compared to the observed in the original Spanish population could be due to a combination of factors, such as **(1)** a bottleneck effect **(2)** genetic drift acting on the small population of the Mexican families. In general, the most common European haplogroup T3 is predominant

(67%) in the Mexican dataset, similar to the haplotype distribution of the southern European breeds (Felius et al., 2011). The following high frequency of T1 (17%) is also seen in the Spanish lineages, and the presence of this haplogroup might be influence of the intense migrations across the Mediterranean sea, facilitated by the proximity to northern Africa, where T1 is prevalent (Bonfiglio et al., 2012). The haplogroup T have the smallest frequencies (3.3%) but yet higher comparing with the Spanish frequency (1.1%).

Our results suggest that, the Mexican mitochondrial gene pool still preserves the genetic footprints of a different maternal origin that is observed similarly in the Spanish lineages that gave rise to this population.

4. Analysis of the Runs of Homozygosity

Both Mexican and Spanish Lidia groups displayed similar ROH patterns in terms of total length of segments, composed mostly of high number of long ROHs (**Table 4**). The results were different in the non-Lidia breeds we also analysed in order to assess for possible admixture patterns of these breeds in the formation of the Lidia breed (Eusebi et al., 2017) (Details of the Spanish native and American creole groups are defined in the **Table S1** of the annexes).

In the Spanish native breeds group the total length of ROH is composed for a very low number of large segments and the results were similar in the American creole breeds group, with some extreme animals observed who had high number of segments (125) (**Table 4**).

Table 4 Descriptive statistics of the number and total length of ROH in the genome for the four cattle groups analyzed. Mean values of the segments and its standard deviation (St.Dv.), and the size of the shortest and longest segments per group.

ROH	Group	Spanish Lidia	Mexican Lidia	Spanish native	American-creole
Number of ROH	Mean	104	116	43	22
	St. Dv.	24.4	27.4	31.1	14.0
	Min.	9	46	5	6
	Max.	151	162	125	60
Total length of genome >1Mb	Mean	736.2	683.9	332.2	160.7
	St. Dv.	240.3	221.0	295.9	109.5
	Min.	25.1	182.6	8.3	14.3
	Max.	1630.0	1264.0	1183.9	438.6

We analysed for each of the four groups the number and total length of ROHs and detected considerable variations across individuals and populations. The **Figure 5** illustrates the number of ROH greater that 1 Mb against the total ROH length obtained in the four populations.

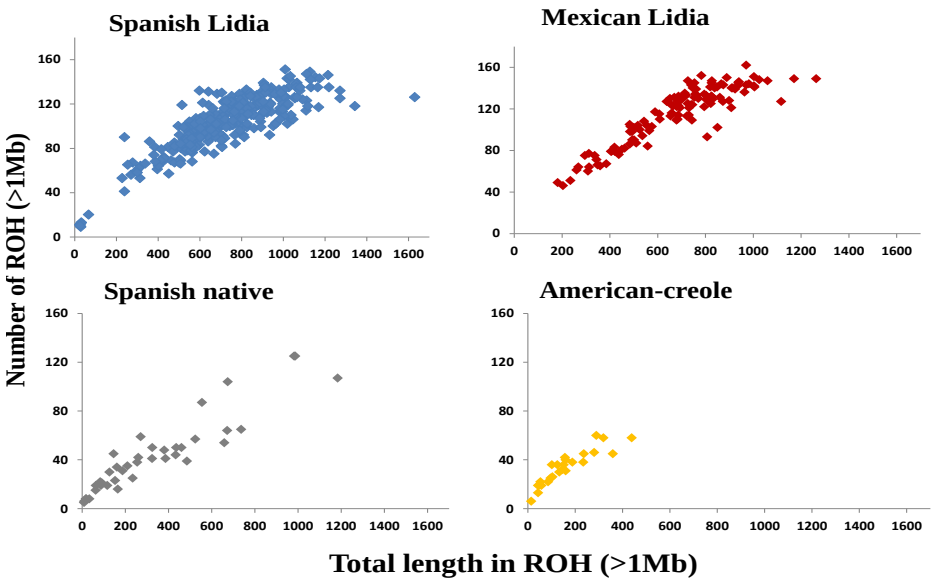

Figure 5 Relationship between the number of ROH>1 Mb and the total length (Mb) of the genome in those ROH, from each group.

When we divided ROH lengths into categories (**Figure 6**), the Mexican Lidia group showed the highest proportion (63%) of 4-6 Mb ROH length, followed by the Spanish Lidia group with 59% of 6-8 Mb ROH length. Among the four groups, the Spanish native breeds showed a greater amount of long ROH (>8 Mb).

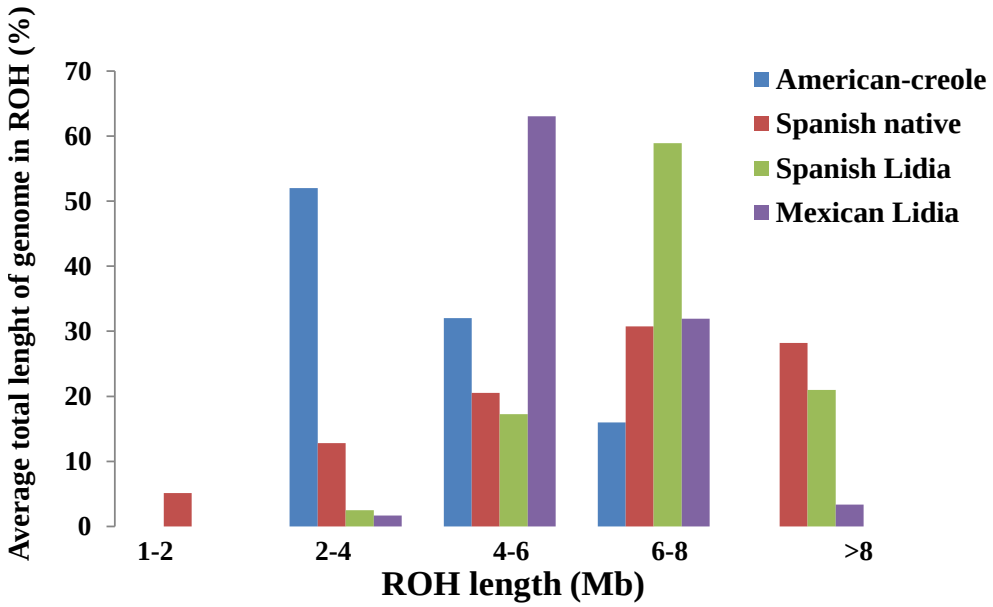

Figure 6 Differences between groups of the total length of genome in ROH divided into different length categories and ROH length.

In general, the Lidia groups have high number and large size of the ROH, the longest segments (6-8 Mb) detected in the Spanish lineages agree with the higher inbreeding values detected in terms of both, the F_{IS} and pedigree (Cortés et al., 2014) analysis and is the consequence of their historical background of the reproduction system followed by the breeders, based on a traditional closed breeding schemes (Cañón et al., 2008). Overall, the long homozygous segments throughout the genome has been reported as result of mattings of close relatives, reduction in population size and selection (Bosse et al., 2012), all of these factors match perfectly with the description of the Lidia breed populations.

5. Signatures of selection oriented to behavioral features

We analyzed data from the Mexican and Spanish Lidia populations and two other Spanish non-specialized tamed breeds: Asturiana de los Valles and Morenas Gallegas with the aim to seek selection signatures for agonistic behavioral related trait, we also aimed to identify candidate genes or metabolic processes associated with the regions involved in the selection processes that may occur during the evolution of the Lidia populations. To meet these goals we used two software's: the SelEstim program developed by Vitalis et al. (2013) based on a Bayesian approach that allows distinguishing between selected and nearly neutral polymorphisms under a genetic model that assumes the subdivision of a population into sub-populations that may exchange migrants. The second method is the Bayescan program based on a statistical methodology based on finding outlier loci based on the significantly different F_{ST}- coefficients given a model of neutrality (Foll & Gaggiotti, 2008).

An advantage of the SelEstim software is that it is able to detect a migration rate (M_i) which is equivalent to measure genetic distances and relative admixture of each group analyzed with respect to a joint pool of all of them. In this regards we detected that the tamed Asturiana de los Valles breed had the highest migration rate value, this result is similar to the finding of González-Rodriguez et al. (2017) where the explanation given for the high migration rates is that the Asturiana de los Valles has exchanged sires to other Spanish breeds. Although, this is not the same case of our study, where both Lidia and Morenas Gallegas breeds, are extremely isolated breeds (even more considering the geographical separation respect to the Mexican Lidia population), making the hypothesis of exchanging sires of the Asturiana breed not possible.

We detected two genomic regions in common with both SelEstim and Bayescan procedures. In both of genomic regions associated to selection are placed genes like the *NCDN*, *GRIK3*, *DLGAP3*, *THRAP3* and *SFPQ* which are highly expressed in the central nervous system and involved in metabolic pathways associated with processes like the development of personality, development of aggressive behavior such as fear induced behavior, intermale aggression, predatory aggression and maternal aggression (Kulikov and Popova, 1996; Kulikov et al., 2005; da Veiga et al.,2011). Although, most of these studies have been conducted mainly using laboratory animals, our results provide insights of clear signatures of selection oriented for behavioral traits left by the Lidia breed respect to other non-specialized breeds.

The low concordance of both approaches to detect regions with strong signals of selection may have different causes, (**1**) that strong selection signals may be hidden, considering that artificial selection processes do not always leave relevant signatures of selection, (**2**) we also need to consider that polygenic traits such as behavior, in which many loci are involved shifting their frequencies moderately, hampers the detection of selective sweeps with statistical significance (Pritchard et al., 2010). Besides we need to take into consideration a high rate of false positives due to the differences in the allelic frequencies between breeds, as a consequence of the genetic drift and founder effect. Despite the above mentioned, this analysis allowed identifying genomic regions with opposite selection direction in the Lidia breed compared to the tamed breeds, corroborating a target selection that may have left imprints in the genome of the Lidia breed.

CONCLUSIONS

Conclusions

1. The Mexican Lidia population is genetically differentiated from the Spanish population. We observed that the gene diversity values of the Mexican population were similar to that observed in the Spanish population, differing hence of what we expected: a gene diversity reduction in the Mexican population as a consequence of genetic drift and a bottleneck effect.

As a reflection, this clear genetic differentiation between the Mexican and the Spanish Lidia populations allows the argument that the denomination of "Lidia breed" when referring to the Mexican population actually is a rhetoric fueled result whereby the term "Lidia" has been exploited more towards the iconography that festivities represent rather to the breed itself. This may be, therefore, like a phenomenon of adaptive convergence but applied to a terminology.

2. The Spanish Lidia population is divided in lineages genetically more isolated than the families of the Mexican population.

3. The maternal lineages of the Mexican population are a skewed sample of the Spanish haplotype diversity.

4. Thanks to the exclusive haplotype of the Spanish Casta Navarra has been possible to trace the maintenance of this lineage in the current Mexican population.

5. Genomic regions with different intensity and allele frequency of selection were found in the Lidia breed than that detected in tamed cattle breeds.

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Table S1 Data of the Spanish native and American-creole breeds included in the analyses according Decker et al. (2013).

Group	Breed	Number of Samples	Continent	Geographic Origin
American creole	Corriente	5	America	Sonora, Mexico
	Texas Longhorn	20	America	Texas, United States
Spanish native	Berrenda en Negro	5	Europe	Ciudad Real, Jaen, Cordoba, Sevilla, and Huelva, Spain
	Berrenda en Colorado	5	Europe	Cordoba, Sevilla, Huelva, and Cadiz, Spain
	Cárdena Andaluza	5	Europe	Sierra Morena, Spain
	Mostrenca	5	Europe	National Park of Donana, southwestern Spain
	Morucha	5	Europe	Salamanca
	Negra Andaluza	5	Europe	Sierra Morena Mountains, Cordoba, and Sevilla Spain
	Retinta	4	Europe	Southwest of Spain and bordering Portugal
	Terreña	5	Europe	Vasconcades mountainous region of Alava, Spain

List of publications

The present Ph.D thesis is based on the list of studies bellow:

Eusebi P. G., Cortés O., Dunner S., Cañón J. 2018. Detection of -selection signatures for agonistic behavior in cattle. J.Anim.Breed.Genet. (*In press*).

Eusebi P.G., Cortés O., Dunner S., Cañón J. 2018. Genetic diversity analysis of the Mexican and Spanish Lidia populations and its relationships by using a subset of non-linked snps. Rev. Mex. Cienc. Pecu. 9:118-134.

Eusebi P.G., Cortés O., Dunner S., Cañón J. 2017. Genetic diversity of the Mexican Lidia bovine breed and its divergence from the Spanish population. J.Anim.Breed.Genet.. 134: 332-339.

Eusebi P. G., Cortés O., Dunner S., Cañón J. 2017. Genomic diversity and population structure of Mexican and Spanish bovine Lidia breed. Anim. Genet. 48: 682-685.

Eusebi P.G., Cortés O., Dunner S., Cañón J. 2017 Genetic diversity and admixture of the Mexican Lidia population inferred from medium-density genotypic data. Book of Abstracts of the International Society of Animal Genetics. p.121.

Eusebi P.G., Cortés O., Dunner S., Cañón J. 2017 Genetic analyses of the Mexican Lidia breed and its position respecting to the Spanish population. Festa Nailha Açoriana, 21: 78-81.

Eusebi P.G., Cortés O., Dunner S., Cañón J. 2017. Genomic analyses of the Mexican Lidia breed, similarities and differences with respect to the Spanish Lidia breed. Book of Abstracts of the International Congress of Veterinary of the Lidia breed. 27-31.

Eusebi, P. G., J. Cañon, and O. Cortés. 2016. P4022 Genetic diversity of Mexican cattle lidia breed and its relationships with Spanish populations through bovine SNP 50k Beadchip. J. Anim. Sci. 94:89-90. Doi:10.2134/jas2016.94supplement489x**.**

Eusebi P.G., Cortés O., Dunner S., Cañón J. 2017 Genetic diversity of the Mexican and Spanish Lidia populations by using a subset of non-linked SNPs. Book of abstracts of the EAAP-68TH Annual meeting. p.84.

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