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Session 01

Genetic diversity of the Mexican and Spanish Lidia populations by using a subset of non-linked SNPs P.G. Eusebi, O. Cortés, S. Dunner and J. Cañón

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Retaining features of the auroch (Bos taurus primigenius), the Lidia bovine is a primitive breed that has its origin ~250 years ago in the Iberian Peninsula, where is still distributed along with France and several American countries. Selected upon a behavior that enhances their aggressiveness, these bovines were raised to take part 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, mainly two families of breeders imported Lidia bovines from Spain in the early XXth 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 the 50K BeadChip on 468 individuals, who were genotyped, and after applying strict filtering criteria, 573 non-linked SNPs were selected. In both populations Similar gene diversity values were observed: remarkable for the Mexican population. Significant FIS values mean a strong subdivision within and higher FST 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.

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GENETIC DIVERSITY ANALYSIS OF THE MEXICAN AND SPANISH LIDIA POPULATIONS AND ITS RELATIONSHIPS BY USING A SUBSET OF NON-LINKED SNPs

The roots of the Lidia bovine breed can be traced back to 250 years ago in the Iberian Peninsula. It is currently found in geographical areas comprising mainly the southwest region of Europe and along the American continent involving approximately 14 countries. These kinds of shows 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 (Felius et al. 2014). 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. 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 (Saumade 1995; Maudet 2010).

The specialization and intensification of animal husbandry did not take place until ~250 years 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, 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 (Saumade 1995; Cañón et al. 2008). Moreover, different type of behavior (sometimes demanded for the different type of festivities) established closed family trees, prompting to a fragmentation of the racial group into small lineages (Cortés et al. 2014).

In Mexico, bovines with these behavioral characteristics were imported during the colonial period (after the conquest of the Aztec empire in 1521) but the Lidia breed specialization not began until 1908 when mainly two families of breeders (Llaguno and González) imported a few Spanish Lidia bovines. Each family followed different breeding strategies, either performing a closed breeding scheme reproducing the new imports among them or by reproducing the imported animals with local bovines (Niño 2004). 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 deriving either from the Llaguno or González families. In recent years (1996-1997), some breeders imported Spanish Lidia bovines before closing borders of importations from Spain. To date, this refreshment suggests an impact in the genetic structure of the herds that introduced those animals. But still, the major part of the Mexican Lidia population derives from the elder Llaguno and González families (Niño 2004). Despite both Mexican and Spanish Lidia populations are demographically well established, their low effective population size places them at risk of extinction (Cortés et al. 2014).

Previous studies on the Spanish Lidia population found a genetic uniqueness in the breed, given by a high genetic differentiation between lineages (Cañón et al. 2008). Moreover, Eusebi et al. (in press 2017) found high genetic differentiation between the Mexican and Spanish Lidia populations. However, both studies were conducted by using neutral autosomal microsatellites, and recently, the availability of SNP panels allow to investigate genetic diversity with higher level of resolution. In this study, a Bovine SNP50 medium density genotyping array (Illumina Inc., San Diego, CA) was used to select a subset of non-linked SNP markers to assess the genetic diversity and structure of the Mexican and Spanish Lidia populations and its relationship, in order to explore the degree of admixture among them.

Blood Samples of 467 Lidia bovines were collected: 119 belonging to the Mexican population and 348 to the Spanish population. Classification of the Spanish lineages was given according to Cañón et al. (2008) 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 (ANCTL 2016). Standard phenol/chloroform methods were performed for DNA extraction (Sambrook et al. 1989) and genotypes were obtained with the Illumina 50k BeadChip (Illumina Inc., San Diego, CA). Thereupon, 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 $r^2<0.01$. Finally, the information derived from 573 non-linked SNPs spanning across all the bovine autosomal chromosomes, were selected.

Statistical estimates of genetic diversity were carried out with the GENETIX v.4.0.5 software (Belkhir et al. 2004). The Proportion of mixed ancestries among populations was inferred with the hierarchical Bayesian model implemented in the STRUCTURE v.2.1. Software (Pritchard et al. 2000) 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 (Rosenberg 2004).

High gene diversity values were found in both the Spanish (0.48) and the Mexican populations (0.46) (Table 1). This value for 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. But, 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-value<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) (Table 1). This subdivision in lineages or breeders results in the preservation of more genetic variance, but a faster loss of genetic diversity within sub-population can be expected, and additionally, a loss of diversity due to population bottlenecks and founder effects result in increased inbreeding.

Genetic diversity analysis revealed significantly higher genetic distances (p-value<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. 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.

Table 1 Genetic diversity parameters of the Mexican (including family: Fam) and Spanish populations (Pop), its name and acronym (Acr) analyzed by using SNP markers: expected (He) and observed (Ho) heterozigosities, FIS inbreeding with significance (*P<0.01) and FST estimated values for each population.

Рор	Fam	Name	Acr	He	Но	F _{IS}	F_{ST}	Рор	Name	Acr	He	Но	F _{IS}	F_{ST}
MEXICO	Llaguno	Celia Barbabosa	BAR	0.39	0.4 6	- 0.09*	0.08		Albaserrada	ALB	0.33	0.34	0.03*	0.26
		Boquilla del Carmen	BOQ	0.38	0.3 8	0.09*	0.07		Anastasio Martín	ANA	0.38	0.46	-0.12*	0.17
		Corlomé	CRL	0.38	0.4 8	- 0.17*	0.12		Antonio Pérez	ANT	0.36	0.39	-0.05*	0.2
		Los Encinos	ENC	0.39	0.4 1	0.07*	0.09		Araúz de Robles	ARA	0.32	0.37	-0.11*	0.25
		Fernando de la Mora	FER	0.4	0.4 6	- 0.07*	0.1		Atanasio Fernández	ATA	0.38	0.38	0.05*	0.18
		Garfias	GAR	0.36	0.4 2	- 0.04*	0.09		Baltasar Iban	BAL	0.38	0.4	-0.01	0.19
		La Antigua	IGU	0.41	0.4 3	- 0.04*	0.11		Carlos Núñez	CAR	0.41	0.42	0.02	0.15
		San José	JOS	0.42	0.4 5	0.04*	0.09		Santa Coloma	COL	0.44	0.37	0.19*	0.13
		Marrón	MA R	0.35	0.4	0.02	0.05		Contreras	CON	0.38	0.38	0.04*	0.2
		San Mateo	MAT	0.37	0.4 3	- 0.06*	0.12	SPAIN	Conde de la Corte	COR	0.34	0.38	-0.06*	0.22
		Montecrist o	MO N	0.39	0.4 5	- 0.06*	0.09		José Marzal	CRM	0.39	0.46	-0.11*	0.17
		Reyes Huerta	REY	0.38	0.4 4	- 0.05*	0.07		Cuadri	CUA	0.26	0.3	-0.10*	0.3
		Fermín Rivera	RIV	0.42	0.4 4	- 0.07*	0.09		Domecq	DO M	0.41	0.39	0.08*	0.15
		Teófilo Gómez	TEO	0.42	0.4 8	- 0.06*	0.08		Félix Gómez	FEL	0.35	0.37	-0.01	0.22
		Torreón de Cañas	TOR	0.4	0.4 5	- 0.06*	0.1		Gamero Cívico	GA M	0.39	0.33	0.20*	0.17
		Xajay	XAJ	0.39	0.4 4	- 0.04*	0.06		Hidalgo Barquero	HID	0.4	0.37	0.12*	0.16
		Arroyo Zarco	ZAR	0.36	0.4 1	-0.02	0.06		Manuel Arranz	MA N	0.34	0.41	-0.13*	0.22
	González	Carlos Castañeda	CAS	0.3	0.3 5	- 0.07*	0.22		Conde de la Maza	MAZ	0.4	0.43	0.13*	0.12
		De Haro	HAR	0.35	0.4	- 0.07*	0.15		Miura	MIU	0.34	0.39	-0.07*	0.23
		Rancho Seco	SEC	0.38	0.4 4	0.06*	0.1		Murube	MU R	0.39	0.36	0.11*	0.18
Whole value 0.46 0.4 3 0.06 0.10							Pablo Romero	PAB	0.31	0.35	-0.06*	0.26		
								Pedrajas	PED	0.37	0.35	0.11*	0.18	
									Saltillo	SAL	0.39	0.38	0.06*	0.19
								Concha y Sierra	SIE	0.37	0.41	-0.06*	0.2	
									Urcola	URC	0.37	0.41	-0.02	0.18
									Veragua	VER	0.39	0.34	0.15*	0.18

VEG

VIL

Vega Villar

Marqués de Villamarta 0.43

0.41

0.44

0.42

0

0.02

0.14

0.16

Furthermore, genetic structure analysis in the Bayesian clustering analysis a clear separation among families (González and Llaguno) of the Mexican population and in the Spanish population (Figure 1) three clusters are observed at *k*=4. The cluster with Albaserrada (Alb), Saltillo (Sal) and Santa Coloma (Col) is placed closer 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) (Niño 2004). 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. This genetic closeness is explained by two similar historical and genetical phenomenons' as Albaserrada lineage derive from Saltillo and Santa Coloma lineages (Cañón et al. 2008) and in parallel, those similar Mexican breeders constructed their herds by mating animals from the same lineages as ancestors.

Fig.1 Genetic structure analysis plot showing model based population assignments for the values going from k=2 (upper) to k=4 (lower) of the Mexican breeders and the Spanish lineages. The acronyms are as defined in Table 1 and, for the Spanish population, each acronym enclose the number of breeders belonging to each lineage.



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 could explain this gain of diversity, although the current existence of creole populations in those regions is not reported. A trace-back analysis of the extant cattle populations could be footprints in the way to explain the mayor ancestors of the Mexican Llaguno family.

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