https://doi.org/10.22319/rmcp.v11i4.5302

Article

Genomic diversity and structure of Lidia breed cattle in Mexico

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Abstract:

First documented in the 13th Century on the Iberian Peninsula, the Lidia cattle breed has since been the preferred breed for producing bulls for social celebrations known as "bullfighting", an expression of regional cultural identity in several countries. Specialization of the breed in Mexico began in the late 19th Century when four Mexican families imported a small number of Lidia animals from Spain. Of these original imports, only the lines derived from the Llaguno and González families remain. Different breeding strategies were implemented in the Llaguno family. Antonio Llaguno crossed the recently imported Spanish animals among each other, resulting in what is currently recognized in Mexico as the "Pure" line. Julián Llaguno crossed Creole dams with Spanish sires, creating the line known as "Impure". In addition, Lidia breed lines such as Domecq, Murube and Santa Coloma were brought to Mexico between 1996 and 1997. The present study objective was to use SNP molecular markers to analyze genomic diversity, population structure, endogamy levels and genetic relationships between Lidia lines in Mexico. Five lines within the Mexican population were studied: Antonio Llaguno, Julián Llaguno, González, Domecq and Santa Coloma. All five lines were found to be genetically distinct, although the Antonio and Julián Llaguno lines are more similar than the others. Genetic isolation between the different lines of the Lidia breed in Mexico has resulted in their being unique.

Key words: Lidia breed, population genetics, genetic diversity, genetic structure.

Received: 23/02/2019

Accepted: 07/10/2019

Introduction

First documented in the 13th Century on the Iberian Peninsula, the Lidia cattle breed is distinguished by selection for behavioral characteristics that enhance aggressiveness and for its use in civil and religious events[1]. Various social and cultural phenomena involving bulls are currently included in what is known as "bullfighting"[2,3]. Several countries consider different bullfighting traditions as practices that reinforce regional cultural identity[2,3]; indeed, in Spain and Peru it has been designated an intangible cultural heritage[4]. The Lidia breed is characterized for having low genetic and ecological interchangeability[5,6].

The first documented bullfighting celebration in Mexico was held in 1523 using aggressive cattle brought mainly from the Navarra region in Spain, which is where the Casta Navarra breed originates[7]. It was not until the turn of the 20th Century, however, that specialized breeding of Lidia began in Mexico with importation of a small number of animals from Spain by four breeding families: Llaguno, González, Barbabosa and Madrazo[8,9]. Only genetic lines originating with the Llaguno and González families are still extant today[8,10].

The Llaguno family has been located largely in north-central Mexico. Under Antonio Llaguno the reproduction system was closed involving crosses only between Lidia animals directly linked to the original imported animals; in Mexican livestock terminology these are known as "Pure" animals. Julián Llaguno, brother of Antonio, followed a different breeding strategy, crossing Creole dams with Lidia sires of known Spanish origin; these are termed "Impure"[8,9]. The González family, located in south-central Mexico, crosses imported Lidia breed animals with local cattle selected for aggressiveness[8]. With the purpose of breeding bulls for bullfights, in 1996 and 1997 a group of Mexican breeders imported animals from Spanish lines such as Domecq, Murube, Santa Coloma and Saltillo, among others; this strategy ended when livestock imports were prohibited for animal health reasons[10].

The current Lidia breed population in Mexico is approximately 110,000 animals raised on a total of around 135,000 ha[10]. This breed is raised under extensive conditions, which favors conservation of endemic flora and fauna. Its central role in many local social traditions supports Mexico's livestock economy while reinforcing regional cultural identity[7,10,11].

The genetic variability of the Lidia breed population in Mexico versus the original Spanish population has been analyzed using autosomal microsatellite markers, with differentiation between Spanish Lidia lines and the Llaguno and González family lines[12]. These results were confirmed using molecular data produced with DNA chips for bi-allelic single nucleotide polymorphism (SNP) molecular markers. Clear genetic differentiation has also been reported between the Antonio Llaguno and González family lines[13,14], although these analyses did not include samples from the Julián Llaguno line or the lines imported in the late 20th Century (Domecq, Santa Coloma, etc.).

The present study objective was to use SNP molecular markers to analyze the genomic diversity, population structure, endogamy levels and genetic relationships in representative populations of the Lidia breed in Mexico.

Material and methods

A total of 306 blood samples were randomly collected from animals belonging to 32 ranches in Mexico affiliated with the Union of Lidia Bull Breeders (Unión de Criadores de Toros de Lidia). The samples were classified into five lines based on the historical origins of each: Antonio Llaguno, Julián Llaguno, González, Domecq and Santa Coloma (Table 1). The samples were collected in tubes containing Magic Buffer® preservative (Biogen Diagnostica, Spain) and kept at 15 °C until DNA extraction. Genomic DNA was extracted using a standard phenol/chloroform protocol[15], and the samples were later genotyped with the 50K medium density SNP bovine chip (http://www.illumina.com).

Table 1: Number of analyzed animals (N), genetic distance by ranch and averaged by line (F_{ST}), endogamy coefficient (F_{IS}), observed heterozygosity (Ho) and genetic diversity (He)

Line	Ranch	N	F_{ST}	F_{IS}	Ho	He
Julian Llaguno	Pozo Hondo	21	0.05	0.13	0.27	0.73
	Valparaiso	15	0.06	0.19	0.25	0.75
	El Sauz	8	0.07	0.21	0.24	0.76
	Caparica	11	0.05	0.17	0.26	0.74
	Total	55	Avg. = 0.06			
Antonio Llaguno	San Mateo	6	0.09	0.21	0.24	0.76
	Reyes Huerta	39	0.06	0.21	0.24	0.76
	Fernando de la Mora	6	0.10	0.04	0.30	0.70
	Los Cues	7	0.07	0.29	0.22	0.78
	Garfias	6	0.08	0.26	0.23	0.77
	Antigua	6	0.09	0.27	0.23	0.77
	Xajay	6	0.05	0.15	0.26	0.74
	Teófilo Gómez	6	0.07	0.19	0.25	0.75
	Celia Barbabosa	6	0.06	0.15	0.26	0.74
	Boquilla del Cármen	6	0.06	0.29	0.22	0.78
	Fermín Rivera	6	0.07	0.18	0.25	0.75
	Corlomé	6	0.13	0.00	0.31	0.69
	Arroyo Zarco	6	0.05	0.18	0.26	0.74
	Marrón	6	0.05	0.11	0.28	0.72
	La Punta	19	0.10	0.11	0.27	0.73
	Total	137	Avg. = 0.07			
González	Tenexac	8	0.13	0.29	0.22	0.78
	Yturbe	5	0.11	0.14	0.27	0.73
	De Haro	6	0.08	0.12	0.27	0.73
	Castañeda	6	0.11	0.25	0.23	0.77
	Zacatepec	12	0.12	0.03	0.30	0.70
	Rancho Seco	6	0.14	0.05	0.29	0.71
	Total	43	Avg. = 0.11			
Domecq	La Joya	17	0.10	0.15	0.26	0.74
	Santa Maria de Xalpa	17	0.08	0.06	0.29	0.71
	Jaral de Peñas	17	0.06	-0.03	0.32	0.68
	Torreon de Cañas	6	0.09	-0.02	0.32	0.68
	Jose Julian Llaguno	10	0.07	0.00	0.31	0.69
	Total	106	Avg. = 0.08			
Santa Coloma	Los Encinos	5	0.02	0.17	0.26	0.74
	San José	6	0.02	0.10	0.28	0.72
	Total	11	Avg. = 0.02			

Using the PLINK ver. 1.07 software[16], the information was refined by excluding SNPs located on sex chromosomes, those exhibiting a minor (<0.01) allele frequency (MAF), those with <20% missing genotypes and those diverging from Hardy-Weinberg equilibrium (P<0.001). A total of 41,455 SNPs remained for analysis.

Again using PLINK[16], analyses were done of three genetic diversity parameters: observed heterozygosity (Ho), expected heterozygosity (He) and the endogamy coefficient (F_{IS}), estimated as 1-*Ho/He*. The F_{ST} coefficients were calculated using the ARLEQUIN ver. 3.0 software[17]. For each individual, the proportion of genetic origins identifiable using the Bayesian grouping algorithm was calculated with the ADMIXTURE software[18,19]. Graphs were generated with the POPHELPER ver. 1.0.10 software[20].

A molecular analysis of variance (AMOVA) was run using a linear model to evaluate genetic variation between and within lines[17]. The analysis was done in hierarchical mode with three levels (between lines, between ranches in the same line and within ranches). The same software was used to calculate mean distance of the lines in terms of F_{ST}.

Individual runs of homozygosity (ROHs) were identified per individual[21]. This was done using PLINK with 30 SNP windows, allowing for <100 kb between two consecutive homozygous SNPs, less than two missing genotypes, one heterozygous and a 500 kbp minimum length. The average value per ranch and per line was then calculated.

Results and discussion

Genetic diversity

Average endogamy (F_{IS}) values per ranch ranged from -0.03 (Jaral de Peñas) to 0.29 (Los Cues, Boquilla del Cármen and Tenexac) (Table 1). The excess heterozygotes present at the Jaral de Peñas and Torreon de Cañas ranches, both in the Domecq line, explains the negative F_{IS} values as a consequence of the Wallhund effect[22]. The average F_{ST} distances estimated per line were similar among them (0.06 - 0.11), while average F_{ST} distances estimated per ranch ranged from 0.02 (Los Encinos and San José) to 0.14 (Rancho Seco). Ranchers in Mexico are known to exchange sires and dams, a practice more common among ranchers belonging to the same livestock groups and/or working with the same breed. Exchange frequency and the quantity of animals involved undoubtedly depends on

rancher criteria, but this could explain the minimal genetic distances between ranches in the same line.

Of total genetic variability, 10.8% was due to interline differences and 6.9% to differences between ranches in the same line (Table 2). It is to be expected that the lack of interline exchanges generates greater differences between lines than within them, where exchanges occur more regularly. The average interline F_{ST} value observed here (0.18) was similar to the average F_{ST} value reported for the Lidia population in Spain (0.15) but higher than found in other cattle breeds (values near 0.07)[6]. High F_{ST} values result from the characteristic structure of the Lidia breed, in which subdivision into subpopulations or lines produces small effective group sizes.

Table 2: Analysis of molecular variance (AMOVA) between lines, between ranches within lines and between ranches overall

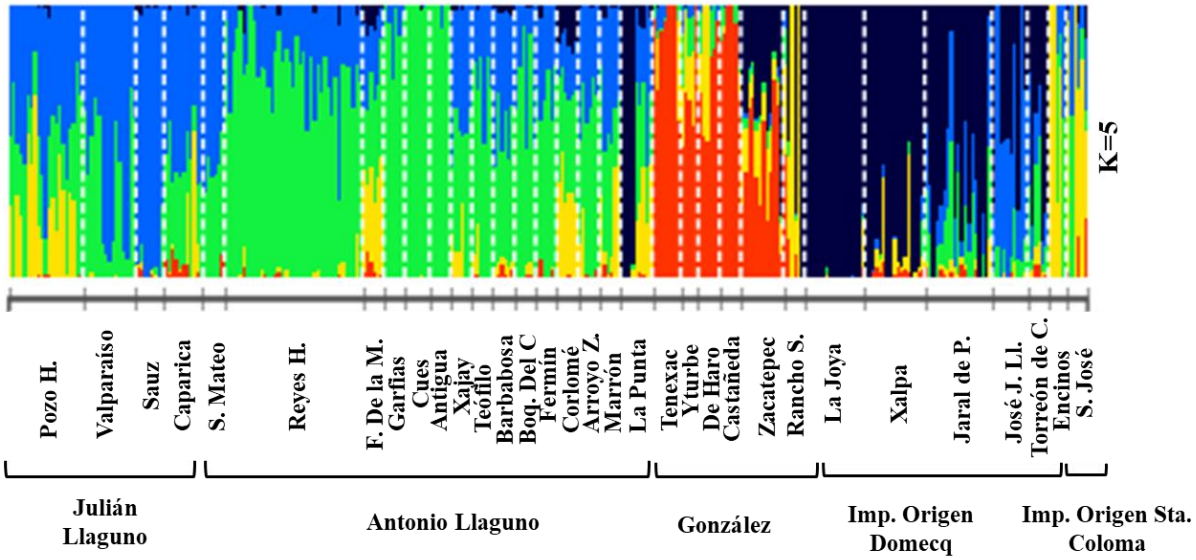
Level	Variance component	Variation (%)
Between lines	686.54	10.76
Between ranches within lines	437.74	6.86
Between ranches	5259.13	82.39
Residual	6383.41	

Genetic structure and population differentiation

The cross-validation error (CV) used in ADMIXTURE calculates values that decrease as the number of hypothetical ancestral populations (K) increases. When the CV value begins to increase it indicates the most probable hypothetical population prediction. Using the present data the most accurate prediction was identified at K = 5[18,19].

The average proportions of individuals in the ranches coincided with their assignment to each of the five ancestral populations (Figure 1). Each of the five lines largely corresponded to one of the five defined ancestral populations. Discrimination between the Julián Llaguno and Antonio Llaguno lines was less evident between some ranches in these lines, while it was greater between others (e.g., El Sauz and Valparaíso in Julián Llaguno, and Garfias, Los Cués and La Antigua in Antonio Llaguno). This analysis does not explain these differentiations between the Llaguno lines. Perhaps they result from variation in original genetic material since the Julián Llaguno line includes crosses between Creole dams and Spanish Lidia sires. Nonetheless, it is clear that both line (Antonio Llaguno and Julián Llaguno) mostly share common genetic origins. In contrast, the Gonzáles, Domecq and Santa Coloma lines are clearly genetically distinct.

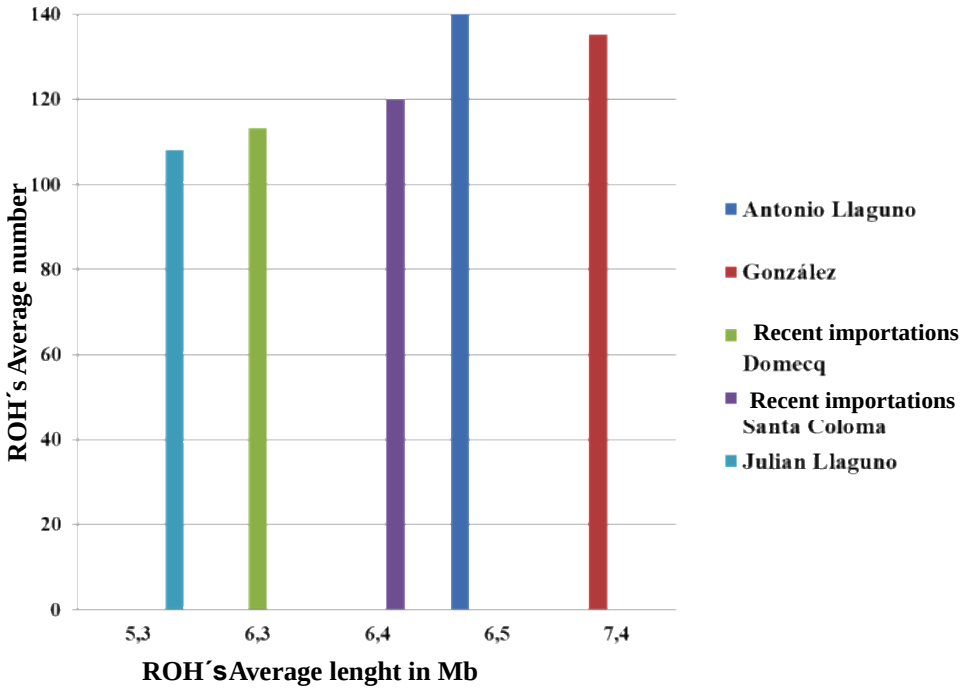
Figure 1: Cross-validation error analysis of hypothetical ancestral populations (K) using ADMIXTURE



Each vertical line represents an individual animal's total genome. The proportion of each color (genetic group, K) in the vertical lines is the proportion of each of the five ancestral populations in an individual's genome (K).

Identification of the ROHs per line produced statistics on the average number of runs or segments and average ROH length in each of the five lines (Figure 2). The number and length of ROHs in the different lines exhibited similar patterns. Greater number of segments and length of the ROH are correlated with recent consanguinity events[23].

Figure 2: Average number of runs of homozygosity (ROHs) and their average size (Mb) in the five studied lines of the Lidia breed in Mexico



In the data for average ROH number and length by ranch (Table 3), length ranged from 5 (Jaral de Peñas) to 7.7 Mb (Boquilla del Carmen and El Sauz). This is consistent with the average F_{IS} values in which the highest values (>0.20) corresponded to Boquilla del Carmen and El Sauz while the lowest was for Jaral de Peñas (Table 1).

Table 3: Average number of runs of homozygosity (ROH) per ranch, including number of segments (NSEG) and average length (Mb)

Line	Ranch	NSEG	Average length (Mb)
Julián Llaguno	Pozo Hondo	108	5.3
	Valparaiso	134	6.2
	El Sauz	111	7.7
	Caparica	114	6.5
	Pomedio	117	6.1
Antonio Llaguno	San Mateo	141	6.5
	Reyes Huerta	124	5.9
	Fernando de la Mora	87	5.8
	Garfias	139	7.1
	Los Cués	131	7.3
	La Antigua	144	6.9
	Xajay	115	6.6
	Teófilo Gómez	135	6.0
	Celia Barbabosa	126	5.8
	Boquilla del Cármen	132	7.7
	Fermín Rivera	131	6.1
	Corlomé	79	5.3
	Arroyo Zarco	124	6.3
	Marrón	105	6.2
	La Punta	104	6.1
	Pomedio	121	6.2
González	Tenexac	135	7.4
	Gonzalo Yturbe	110	6.3
	De Haro	113	5.6
	C.Castañeda	134	6.9
	Zacatepec	83	5.7
	Rancho Seco	95	5.2
	Pomedio	109	6.2
Domecq	La Joya	113	6.3
	Sta. Maria de Xalpa	92	5.7
	Jaral de Peñas	64	5.0
	José Julián Llaguno	72	5.1
	Torréon de Cañas	73	5.2
	Pomedio	85	5.5
Santa Coloma	Los Encinos	120	6.4
	San José	105	5.8
	Pomedio	112	6.1

When animals are isolated in relatively small populations the probability is greater that they inherit identical DNA segments that account for ROH[23]. In previous studies, the high number and long length of ROHs have been associated with endogamy[23]. This coincides with the results observed here for the five studied Lidia breed lines in Mexico, which had values greater than those reported in previous analyses of ROHs in native Spanish and American Creole breeds[13]. Both the ROH and F_{IS} values in the studied Mexican Lidia population reflect subdivision into lines and its consequences: reduction of effective sizes and higher consanguinity values[24]. This subdivision is effective at preserving intrapopulation genetic variability[25],;however, as each subpopulation experiences genetic drift consanguinity will increase and genetic variability will decrease. Under these circumstances it is advisable to closely monitor degrees of endogamy.

Conclusions and implications

The genetic differentiation observed among the Mexican Lidia population, into lines and even between ranches, is due to the different genetic origins of some lines (i.e. Domecq and Santa Coloma) in conjunction with the genetic isolation maintained between the remaining lines (i.e. Antonio Llaguno, Julián Llaguno and González). Both the genetic structure and ROH analyses identified genetic isolation between the lines of the Mexican Lidia population, which has contributed to genomic variations when compared to European Lidia populations. The Mexican Lidia lines are clearly unique from their ancestral populations and quite differentiated amongst themselves.

Acknowledgements

The research reported here was financed by the "Macro-proyecto de Caracterización genética de la Raza de Lidia Mexicana" supported by the Consejo Nacional de los Recursos Genéticos Pecuarios (CONARGEN) and the Asociación de Criadores de Toros de Lidia. Thanks are due the Genetics Laboratory of the Animal Production Department of the Veterinary Faculty, Universidad Complutense de Madrid, and the participating Mexican Lidia ranchers.

Literature cited:

1. Domecq JP. Del toreo a la bravura. España, Alianza Editorial, 2009.

2. Maudet JB. Terres de taureaux: les jeux taurins de L´Europe à L´Amerique. 1ra ed. España, Casa de Velzaquez, 2010.

3. Prieto-Garrido JL. El toro bravo, ganaderías míticas. España Editorial Almuzara, 2012.

4. Cárdenas RVC. Situación del todo de Lidia y de la fiesta en los países de Hispano-América. Editado por García AL. Congreso Mundial Taurino de Veterinaria, Consejo General de Colegios Veterinarios de España, 2017.

5. Crandall KA, Bininda-Emonds OR, Mace GM, Wayne RK. Considering evolutionary processes in conservation biology. Trends Ecol Evol 2000;15:290-295.

6. Cañón J, Tupac-Yupanqui I, Garcia-Atance MA, Cortés O, Garcia D, Fernandez J, Dunner S. Genetic variation within the Lidia bovine breed. Anim Genet 2008;39:439-445.

7. Scherrer HL. Historia del toro bravo mexicano. México, Asociación Nacional de Criadores de Toros de Lidia (ANCTL), 1983.

8. Niño de Rivera L. Sangre de Llaguno, la razón de ser del toro bravo mexicano.1ra ed. México: Punto de Lectura; 2004.

9. Villanueva Lagar JA. San Mateo, encaste con historia. México: Aldus; 2005.

10. ANCTL. Asociación Nacional de Criadores de Toros de Lidia. México. 2019.

11. Censo Agrícola, Ganadero y Forestal 2007. INEGI. http://www.inegi.org.mx

12. Eusebi PG, Cortés O, Dunner S, Cañón J. Genetic diversity of the Mexican Lidia bovine breed and its divergence from the Spanish population. J Anim Breed Genet 2017;134(4):332-339.

13. Eusebi PG, Cortés O, Dunner S, Cañón J. Genomic diversity and population structure of Mexican and Spanish bovine Lidia breed. Anim Genet 2017;8(6):682-685.

14. Eusebi PG, Gardyn OC, Boxberger SD, Ferreras JC. 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. Rev Mex Cienc Pecu 2018;9(1):121-134.

15. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual, 2nd ed. NY, USA: Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989.

16. Purcell SM, Neale B, Todd-Brown K, Thomas L, Ferreira MAR. *et al*. PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559-575.

17. Excoffier L, Laval G, Schneider S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online 2005:1, 47.

18. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. Genome Res 2009;19:55–64.

19. Alexander DH, Lange K. Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. BMC bioinformatics 2011;12:246.

20. Francis RM. Pophelper: an R package and web app to analyze and visualize population structure. Mol Ecol Resour 2016.

21. Purfield DC, Berry DP, McParland S, Bradley DG. Runs of homozygosity and population history in cattle. BMC Genetics 2012;13:70.

22. Crow JF, Kimura M. An Introduction to Population Genetics Theory. New York. USA: Harper & Row, Publ; 1970.

23. Upadhyay MR, Chen W, Lenstra JA. *et al*. Genetic origin, admixture and population history of aurochs (*Bos primigenius*) and primitive European cattle. Heredity 2016;118:169–76.

24. Cortés O, Tupac-Yupanqui I, Dunner S, Fernández J, Cañón J. Y chromosome genetic diversity in the Lidia bovine breed: a highly fragmented population. J Anim Breed Genet 2011:491-498.

25. Wright S. The genetical structure of populations. Ann Eugenics 1951;15:323-354.