Journal of
Animal Breeding and Genetics

J. Anim. Breed. Genet. ISSN 0931-2668

ORIGINAL ARTICLE

Y chromosome genetic diversity in the Lidia bovine breed: a highly fragmented population

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Keywords
Bovine; fighting bull; genetic diversity; microsatellite; Y chromosome.

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Received: 1 February 2011;
accepted: 22 July 2011

Summary

To assess the paternal gene pool in the Lidia bovine breed (or fighting bull), a total of 603 animals belonging to 81 herds classified in 33 lineages were genotyped for six Y chromosome microsatellites, one single nucleotide polymorphism and one indel. A total of 10 haplotypes were determined with a high level of frequency variation between them, ranging from 0.2 to 74%. All the haplotypes identified belong to two previously defined major haplogroups (Y1 and Y2). Two major paternal influences were identified, corresponding to the two most common haplotypes (H1Y1 and H3Y2) with frequencies of 74 and 18%, respectively. The detection of the INRA189-104 allele evidenced an African influence in the Lidia bovine breed. Low levels of haplotype diversity have been achieved and only eight lineages showed more than one haplotype. Analysis of molecular variance showed a high level of interlineage variance (F_{ST} = 86%). Network results evidenced two main clusters made for those haplotypes belonging to Y1 and Y2 haplogroups, respectively. The findings support a high level of genetic structure together with a low level of genetic diversity in the Lidia bovine breed.

Introduction

The development of the Lidia bovine breed (otherwise known as the fighting bull) has been well documented since the 18th century (http://www.toroslidia.com). Nevertheless, the first reference to a Lidia herd is found in the 14th century literature of the kingdom of Navarre (Larrea *et al.* 2005). From the 18th century, the most aggressive animals from the traditional herds, which mainly produce meat, were selected for social events. Subsequently, the different social spectacles demanding different bull behaviour characteristics prompted the population fragmentation into small lineages, traditionally called 'encastes' (Boletín Oficial del Estado, 2001), consisting of a variable number of herds with a high level of reproductive isolation among lineages. The selection objective of the Lidia bovine breed, focused on aggressiveness, favoured its reproductive isolation from the rest of the domestic bovine populations, where this characteristic was not desirable. Today, the Lidia breed is one of the Spanish domestic breeds most widespread round the world: it has spread to other European countries such as France and Portugal and to many Central and South American countries (http://www.toroslidia.com; Cañón *et al.* 2008).

The genetic variability of the Lidia breed has been widely analysed using nuclear autosomal microsatellite markers (Cañón *et al.* 2008). The results revealed that an important part of the genetic variability remains between lineages, and consequently, a high genetic differentiation among lineages is found. The division of the breed in reproductive isolated lineages can explain the high level of genetic variability of the Lidia breed, where each lineage kept a source of genetic variation because the different lineages fixed different alleles (Fernández *et al.* 2008).

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Recent mitochondrial DNA analysis in the Lidia breed (Cortés *et al.* 2008) observed two principal maternal influences, one from Europe (haplotype T3) and the other from Africa (haplotype T1). Nevertheless, the Lidia breed showed a high level of maternal genetic diversity, closer to that found in the Middle Eastern bovine breeds than to that of European populations (Cortés *et al.* 2008).

Little is known about the paternal genetic influences during the Lidia breed development. Recent bovine Y chromosome polymorphisms described have been used in the analysis of domesticated bovine breeds, showing new perspectives in the paternal origin and the development of a breed (Edwards *et al.* 2000; Gotherstrom *et al.* 2005; Li *et al.* 2007; Kantanen *et al.* 2009; Edwards *et al.* 2011).The aim of the present study is to use a representative sampling of the Lidia breed to analyse the extant paternal gene pool and the influences during the breed development using paternal markers located in the non-recombinant Y chromosome region.

Materials and methods

Fresh blood, collected in a conservation buffer (Dunner & Cañón 2006) until use, was taken from 603 unrelated (without common ancestors or not connected by kinship) male animals. DNA was extracted by standard phenol/chloroform methods (Sambrook *et al.* 1989). Table 1 shows the number of males sampled (603) from each herd and the number of herds (83) of each lineage (33). The primer sequences and annealing temperature of the six Y chromosome microsatellites (DYZ1, BYM1, BM861, UMN0307, INRA189 and UMN0103), the single nucleotide polymorphism (UTY19) and the indel (ZFY10) are described in Table S1.

The six microsatellite PCR fragments were separated by capillary electrophoresis on ABI3130 according to the manufacturer's recommendations. The microsatellite alleles were combined in haplotypes. The UTY19 PCR products were subjected to Single Strand Conformation Polymorphism (SSCP) analysis in a 12% (100:1) polyacrylamide gel at 300 V during 3 h. The indel ZFY19 was genotyped following an allele-specific PCR procedure. Two different PCR were carried out, one with the common primer and the specific allele primer for the CT insertion and the other with the specific allele primer for the CT deletion. Electrophoresis of the PCR products was performed separately in 10% polyacrylamide gels at 220 V during 1 h.

Y chromosome haplotypes analysis was performed with the Y-specific microsatellite markers. The ARLEQUIN package V 3.11 (Excoffier *et al.* 2005) was used to determine haplotype frequency, haplotype diversity and F_{ST} distances. The UTY19 and ZFY10 Y chromosome-specific markers were used to classify the haplotypes into it corresponding principal haplogropus previously defined (Gotherstrom *et al.* 2005). Reduced median networks were generated using the NETWORK 3.0 program (Bandelt *et al.* 1999), including the microsatellites analysed and the UTY19 and ZFY10 markers.

Results

A total of 10 haplotypes were identified using the six Y chromosome microsatellites (Table S3). Haplotypes segregating frequencies ranged from 0.2 to 74% (Table 1). The two most common haplotypes, H1 and H3, had a total frequency of 92%. Haplotype H1 had the highest frequency (74%) and was fixed in 20 lineages and is the most frequent in an additional five lineages. The second most common haplotype (H3) was fixed in four lineages and is the majority in an additional two lineages. The remaining eight haplotypes were detected with low frequencies exclusively in one lineage, which was different for each haplotype, except for the H6 and H8 haplotypes. Haplotype H6 was exclusive of the Miura lineage where it is fixed, and the Pablo Romero lineage evidenced an exclusive haplotype (H8) with a high frequency (93%). Overall, 25 lineages evidenced only one haplotype and in the remaining eight lineages, the frequency of the most frequent haplotype was >60%. All the microsatellites analysed were polymorphic except BM861 which evidenced only one allele (Table S2). All the polymorphic microsatellites had a unimodal distribution with one frequent allele with a frequency >74% (Table S2). The INRA189-104 allele, with a total frequency of 4%, previously detected in African taurine bulls (Edwards *et al.* 2000), was identified in 14 of the 15 samples belonging to the Pablo Romero lineage and in the Atanasio Fernandez lineage.

Among the 10 haplotypes found, seven were of the Y1 haplogroup (Table S3) with a total frequency of 81% and three were of the Y2 haplogroup (H3, H4 and H9) with a total frequency of 19%. The Y2 haplogroup was fixed in 25 lineages. In four lineages, the haplogroup Y1 was fixed and only four lineages showed both haplogroups. Relationships among Y chromosome haplotypes are shown in the Median-Joining network in Figure 1. The haplotypes

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Table 1 Number of samples analysed (N), haplotype frequencies (in percentage), haplogroup frequencies (in percentage) and haplotype diversity (He) for each lineage and for the entire population. Average F_{ST} distance values for each lineage compared to the rest of the lineages (F_{ST})

		Haplotypes (%)										Haplogroups			
Lineage	N	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	Y1 (%)	Y2 (%)	He	Average F_{ST} distances (%)
Miura (1)[a]	15						100						100	0	0.97
Pablo Romero (1)	15	7							93				100	0.13	0.90
Diego Garrido (1)	15	100											100	0	0.25
Concha y Sierra (1)	15	100											100	0	0.25
Prieto de la Cal (1)	15	100											100	0	0.25
Jacinto Ortega Casado (1)	8	100											100	0	0.25
Braganza (1)	15	80								20		20	80	0.34	0.30
Murube (4)	29	93						7					100	0.13	0.24
Contreras (4)	15	7		80	13							93	7	0.36	0.67
Saltillo (3)	15			100								100		0	0.82
Santa Coloma (10)	50	88		12								12	88	0.22	0.24
Marques de Albaserrada (3)	15			100								100		0	0.82
Antonio Perez (1)	15	100											100	0	0.25
Samuel Flores (1)	10	100											100	0	0.25
Gamero Civico (4)	15	100											100	0	0.25
Pedrajas (2)	15	100											100	0	0.25
Conde de la Corte (1)	15	100											100	0	0.25
Juan Pedro Domecq (9)	55	96				2					2		100	0.01	0.25
Atanasio Fernandez (6)	28	64	26										100	0.48	0.36
Conde de la Maza (1)	10	100											100	0	0.25
Carlos Nuñez (6)	25	100											100	0	0.26
Urcola (1)	14	100											100	0	0.25
Dolores Rufino Martin (1)	15	100											100	0	0.25
Baltasar Iban (2)	15	100											100	0	0.25
Marques de Villamarta (3)	20	100											100	0	0.26
Cuadri (1)	15			100								100		0	0.82
Gavira	15	100											100	0	0.25
Benitez Cubero (3)	20	100											100	0	0.26
Vega Villar (4)	40	2		98								98	2	0.05	0.80
Arauz de Robles (1)	15	100											100	0	0.25
Maria Montalvo (1)	2	100											100	0	0.23
Ramon Sánchez (1)	7			100								100		0	0.81
Mariano Sanz Jimenez (1)	15	100											100	0	0.25
Lidia Breed	603	74	1.7	18.1	0.3	0.2	2.5	0.3	2.3	0.5	0.2	19	81	0.42	

[a]Between brackets: the herds sampled by lineage.

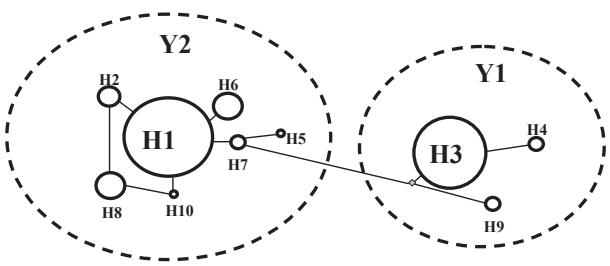

Figure 1 Network representation of the 10 haplotypes identified in the Lidia bovine breed. The sizes of the circles are proportional to their frequencies and their classification into the two aim European haplogroups (Y1 and Y2) found in *Bos taurus* (dotted circles) is shown.

belong to haplogroups Y1 and Y2 forming two clusters separated by one median vector reflecting a marked divergence between them. The median vector separated haplotype H9 and the other haplotypes belonging to the Y1 haplogroup. The two haplotypes, H2 and H8, where the African taurine allele was detected, are included in the Y2 haplogroup. Only four lineages are represented in both clusters belonging to those lineages who evidenced both haplogropus (Vega Villar, Santa Coloma, Contreras and Braganza).

Haplotype diversity was low and varied from 0.07 to 0.48 in the eight lineages where more than one haplotype were found (Table 1). The haplotype

diversity of the Lidia breed without the subdivision in lineages was 0.42.

Frequencies of Y chromosome haplotypes were used to compute the average F_{ST} genetic distance value with respect to the rest for each lineage, with values ranging from 23 to 97% (Table 1). For those lineages where haplotype H1 was the most frequent (25 lineages), the average F_{ST} distance values ranged from 0.23 to 0.34. In another six lineages, where the most frequent haplotype was the H3, the average F_{ST} distance values varied between 0.53 and 0.80. Miura and Pablo Romero lineages showed the highest average F_{ST} distance 0.90 and 0.97, respectively, as a result of having an exclusive high-frequency haplotype. These high average F_{ST} distance values (Table S2) are in agreement with the high proportion of the total variance explained by the genetic differences among lineages obtained by the AMOVA analysis, 86%, (data not shown) which evidenced a high level of genetic subdivision.

Discussion

The average haplotype diversity found in the lineages with more than one haplotype was similar to that reported in other cattle breeds (Ginja *et al.* 2009; Pérez-Pardal *et al.* 2010). Two haplotypes (H1 and H3) among a total of 10 haplotypes identified in the 33 lineages represent 92% of the total frequency. In 24 of the 33 lineages analysed, one of those haplotypes is fixed and the frequency of the most frequent haplotype was >60% in the remaining nine lineages. The reduced levels of haplotype diversity achieved within lineages could be explained by three non-exclusive reasons: (i) the reduced Y chromosome genetic diversity of the ancestral populations of the present bovine breeds; (ii) the reproductive isolation between lineages and its low effective population size, on average around 30 (data not shown); and (iii) the traditional practice of using a small number of bulls when trying to fix desirable behaviour traits through inbreeding (Ginja *et al.* 2009).

Despite the reduced levels of haplotype diversity within lineages, the haplotype diversity found in the Lidia bovine breed (0.42) was higher than that of 0.16 and 0.30 found in northern and southern Portuguese cattle breeds, respectively, by Ginja *et al.* (2009) and in many European and African breeds (Pérez-Pardal *et al.* 2010; Edwards *et al.* 2011). The subdivision of the Lidia bovine breed in different lineages with high levels of reproductive isolation has been well documented, in some cases, since the 19th century. This reproductive management is in agreement with the traditionally postulated alternative to maintain the genetic variability of a population (Wang & Caballero 1999). However, the population subdivision can decrease the population size, generating higher levels of inbreeding (Fernández *et al.* 2008), and both side effects have been achieved in the Lidia breed (Cañón *et al.* 2008). The haplotype diversity values of those lineages with more than one haplotypes are low. Nevertheless, the haplotype diversity is higher than that in several domesticated European bovine breeds when the Lidia breed is considered as a whole. Probably, each isolated lineage kept different allelic variants and act as genetic reservoir that increase the genetic variability when the Lidia breed is considered as a whole. To decrease the negative effects of the population subdivision, an alternative postulated is the one-migrant-per-generation rule (Mills & Allendorf1996), but this methodology is against the traditional reproductive isolation management of the Lidia breeders. Traditionally, herds are herited from fathers to sons, being part of the family identity, and the reproductive isolation among lineages favoured a differentiated product from a behavioural perspective. So, designing crossbreedings to minimize the increase rate of inbreeding could be the unique available practice to avoid the increase in inbreeding levels, especially in those lineages like Miura or Pablo Romero which contribute significantly to the gene pool of the Lidia breed and are represented by a single herd.

The average F_{ST} distance of each lineage with respect to the rest of the lineages showed remarkable differences (Table 1). The average F_{ST} value ranged from 0.23 to 0.34 for those lineages where haplotype H1 was the most frequent (25 lineages), from 0.53 to 0.80 in those lineages where haplotype H3 was the most frequent and finally Miura and Pablo Romero lineages showed the highest average F_{ST} distance value, 0.90 and 0.97, respectively, as a result of having an exclusive high-frequency haplotype. These high average F_{ST} distance values (Table 1) are in agreement with the high proportion of the total variance explained by the genetic differences among lineages obtained by the AMOVA analysis, 86%, which evidenced a high level of genetic subdivision. The differences among lineages using autosomal and mitochondrial DNA markers explain 18 and a 12%, respectively, of the total genetic variation of the Lidia bovine breed (Cañón *et al.* 2008; Cortés *et al.* 2008). Also the average F_{ST} distance values using microsatellite autosomal markers ranged from 13 to 28% and from 3 to 17% using

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mitochondrial DNA (Cañón *et al.* 2008; Cortés *et al.* 2008). Otherwise, the AMOVA results and the average F_{ST} distance values of the different kind of markers reflected a high level of genetic subdivision in the Lidia bovine breed. Probably, the low male effective population size augment the genetic drift effect increasing the loss of genetic diversity and explain the higher Y-specific marker F_{ST} AMOVA results.

The network skeleton analyses revealed two main clusters grouping the Y1 and Y2 haplotypes in each one. The medina vector connecting both clusters evidenced the clear separation between them. Haplotype H6, exclusively found in the Miura lineage, is close to haplotype H1, only separated by one mutation event in UMN307 microsatellite: so probably few related breeding males carrying the UMN307 mutation event fixed the haplotype in the Miura lineage and its reproductive isolation prevented the spread of this genotype to other lineages.

The Y1 and Y2 haplogroups showed a clear distribution pattern between lineages (Table 1). Haplogroup Y2 was fixed in 25 lineages and haplogroup Y1 in four. The remaining four lineages evidenced both haplogroups, and the most frequent was over 80%. Furthermore, the frequency of the Y2 haplogroup (81%) reflects the fact that the present genetic constitution of the Lidia breed is closer to that found in those breeds located in the primary centre of domestication, which arrived in the Iberian Peninsula via the Mediterranean coast, than that from northern Europe, represented by the Y1 haplogroup with a total frequency of 19%. Similar results were reported for other local bovine breeds in the Iberian Peninsula. In a recent study (Pérez-Pardal *et al.* 2010) including different European and African bovine breeds, the authors did not find the Y1 haplogroup in the samples analysed belonging to the Lidia breed; however, in our results, the Y1 haplogroup recorded the important frequency of 19%. The INRA189-104 allele previously detected in African taurine bulls (Edwards *et al.* 2000) can be inferred to be the paternal African influence on the Lidia bovine breed. Two Y haplotypes showed this allele (H2 and H8) and represented 4% of the samples analysed. This result is clearly lower than the African maternal influence previously estimated at 17% (corresponding to the frequency of the T1 haplotype) in the Lidia breed (Cortés *et al.* 2008). A higher rate of cows than sires of African origin in the Lidia herds and the founder effects which are amplified on the sire genetic pathway due the common use in breeding schemes of a few selected males that produce a large number of offspring could explain the lower genetic paternal African influence in the Lidia breed.

Our results on the Y chromosome haplotype pattern support the hypothesis of two main paternal influences in the extant Lidia breed, corresponding to the two founding migrations that arrived in the Iberian Peninsula from domesticated origins (http://www.toroslidia.com; Cymbron *et al.* 1999), represented by the clearly differentiated Y1 and Y2 haplogroups. The results also evidenced a high level of genetic subdivision. The high reproductive isolation between lineages and the traditional practice of using a small number of bulls to fix desirable behavioural traits avoided the spread of both haplogroups in the lineages and explain the high genetic structure evidenced in the Lidia bovine breed using paternal inherited markers.

Acknowledgements

We wish to acknowledge the financial support of the INIA and European Regional Development Fund no. RZ2008-00005-C02-02. Thanks are also due to the breeders who provided the biological samples, to the laboratory technicians R. Parellada, E. Martin and M.I. Panadero, and to A. Holliday for providing language advice.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1 Description of the annealing temperature of the locus typed and the Y chromosome microsatellites and SNP sequence primers.

Table S2 Allele sizes and frequencies (in percentage) of the five Y chromosome microsatellites for the entire population.

Table S3 Y chromosome haplotypes defined by six Y chromosome microsatellites and their classification into the two principal European haplogroups characteristics of *B. Taurus* using UTY19 and ZFY10 Y specific chromosome markers.

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