

# Genetic relationships of the Portuguese Lidia bovine populations

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## Summary

To clarify the genetic relationships among the Lidia breed lineages and two main Portuguese Lidia bovine populations, Casta Portuguesa and Brava dos Açores, 24 autosomal microsatellites were analyzed in 120 samples. Brava dos Açores showed the highest observed and expected heterozygosity (0.73 and 0.70, respectively) while Casta Portuguesa showed the lowest observed and expected heterozygosity (0.51 and 0.50, respectively). The results of this study were compared with the previous microsatellites data from the main Lidia bovine lineages. Casta Portuguesa was the most genetically isolated Lidia bovine population as revealed by the average  $F_{ST}$  genetic distance value with respect to the other lineages (32%). All the populations of Portuguese Lidia had negative  $F_{IS}$  values. The Neighbour-joining dendrogram grouped Casta Portuguesa in the same branch with Miura, which was supported by the STRUCTURE software. The results evidenced low levels of genetic diversity and high levels of genetic differentiation in Casta Portuguesa and high levels of genetic diversity in Brava dos Açores populations, probably due to the crossbreeding of different bovine lineages at origin, and genetic flow among herds.

**Key words:** Portuguese Lidia, Genetic diversity, Microsatellites, Casta Portuguesa, Brava dos Açores

## Introduction

Nowadays, Lidia bovine breed is mainly distributed in the west and the southwest of Spain, Portugal, the south of France and in South America. The uniqueness of the selection objective of the Lidia bovine breed, based on their aggressiveness, has prompted their reproductive isolation from other cattle breeds. Currently, Lidia bovine is a single breed with different morphological and behavioural phenotypes, divided into lineages (also called “encastes”) (Boletín Oficial del Estado, 2001) according to the different spectacles (bullfights) demand. During the 19th century, crossbreeding between different Lidia lineages from Spain and aggressive peninsular Portuguese native cattle originated in a small lineage Lidia population called Casta Portuguesa. The arrival of bovine animals in the Azores Islands was begun in the 15th century with the colonization of the Islands, mainly by Portuguese people. However, only at the beginning of the 20th century did different Lidia lineages, including Casta Portuguesa, start to be selected for a particular social event called “Tourada à Corda” in the Azores (Bruges, 1915; Siva, 2011). The wide diffusion of this social event, particularly in Terceira Island, favoured breeding specialization and selection of animals for this purpose, currently recognized as Brava dos Açores population. The Lidia bovine breed has been widely studied using DNA markers (Cañón *et al.*, 2008; Cortés *et al.*, 2008,

2011), revealing a high genetic differentiation between the lineages. This fact can be explained by the division of the breed in reproductive isolated lineages in which each of them kept a source of genetic variation (Wang and Caballero, 1999). However the genetic relationships between Lidia bovine breed and Brava dos Açores population and a small Lidia population located in continental Portugal, called Casta Portuguesa, is scarce in spite of the great amount of genetic analysis published about the Lidia bovine breed (Cañón *et al.*, 2008; Cortés *et al.*, 2008, 2011).

The objective of this study was to investigate genetic relationships between the Lidia breed and the two major Lidia bovine Portuguese populations: Casta Portuguesa and the autochthonous lineage from the Azores Islands, called Brava dos Açores.

## Materials and Methods

A total of 120 peripheral blood samples of animals belonging to the two major Portuguese Lidia bovine populations were collected: Irmãos Dias (ID) (n=30) belonging to Casta Portuguesa and José Albino Fernandes (JAF; n=30), Rego Botelho (RB; n=30) and Eliseu Gomes (EG; n=30) belonging to Brava dos Açores. The same 24 microsatellites previously analyzed in the Lidia bovine breed (Cañón *et al.*, 2008) were genotyped. PCR reactions were performed as described by Cañón *et al.* (2008). Table 1 shows the number of

samples collected from the two Portuguese Lidia bovine populations and the Lidia bovine breed lineages previously analyzed with the same set of microsatellites (1071 samples). Lidia bovine breed samples belonging to three Portuguese herds-Louro Fernandez de Castro (UAP), Oliveira Irmãos (UGM) and Passanha (UGY), were analysed independently because they are located in Portugal. For each lineage, the observed heterozygosity,  $H_O$ , and unbiased expected heterozygosity,  $H_E$ , were estimated as proposed by Nei (1978). The Hardy-Weinberg test was done using Wright's  $F_{IS}$ . The null distribution of  $F_{IS}$  was approximated by permuting alleles 1000 times within each lineage.

Genetic relationships between lineages were inferred using two approaches. First, pairwise  $F_{ST}$  distances were computed for all pairs of the lineages using the software ARLEQUIN package v3.11 (Excoffier *et al.*, 2005). Second, a multivariate approach was considered; more specifically, a correspondence analysis (Benzécri, 1973) was performed using GENETIX 4.04 (Belkhir *et al.*, 2001) to plot the representation of lineages in a metric space. Genetic distances obtained by the first approach were also used to obtain a transformed matrix with ultrametric properties (Weitzman 1992, 1993), and a

Neighbour-joining tree for this new matrix was drawn using MEGA 2.0 software (Kumar *et al.*, 2001). In order to analyse lineages influencing the two Portuguese Lidia bovine populations, the method of Pritchard *et al.* (2000), implemented in the STRUCTURE Software was used. Different numbers of assumed populations were evaluated with the mixed ancestry model ( $K=3, 8, 12$  and  $25$ ). Low number of populations defined ( $K=3$  and  $8$ ) shown the lineages cluster where Casta Portuguesa and Brava dos Açores are grouped, and higher  $k$  values ( $k=12$  and  $25$ ) evidenced the differentiation among the Portuguese bovine populations and the remaining Lidia lineages. Animals were allowed to have mixed ancestry and burn-in and run-in lengths of 200,000 and 300,000; respectively.

## Results

Table 1 summarizes the within breed genetic diversity parameters. The results showed that Brava dos Açores had the highest  $H_O$  and  $H_E$ . On the other hand, the  $H_O$  and  $H_E$  of Casta Portuguesa matched the Lidia breed lineages, considered individually, were lower than that observed for the three Portuguese Lidia herds

**Table 1:** Sample description and genetic diversity parameters estimated

Population	Lineage/Herd	Acronym	N	$H_E$	$H_O$	$F_{IS}$
Lidia breed lineages	Arauz de Robles	ARA	52	0.54	0.53	0.012
	Atanasio Fernández	ATA	50	0.50	0.49	0.025
	Baltasar Iban	BAL	33	0.57	0.55	0.040
	Braganza	BRA	25	0.59	0.58	0.020
	Carlos Nuñez	CAR	19	0.61	0.59	0.038
	Concha y Sierra	SIE	46	0.65	0.61	0.066
	Conde de la Corte	COR	30	0.47	0.47	-0.003
	Contreras	CON	9	0.48	0.52	-0.078
	Félix Gómez	CRM	44	0.59	0.58	0.018
	Gamero Cívico	GAM	41	0.54	0.45	0.170
	Hidalgo Barquero	HID	15	0.45	0.46	-0.012
	Jacinto Ortega Casado	UGP	30	0.63	0.65	-0.028
	Jose Escobar	UJV	30	0.53	0.55	-0.021
	Juan Pedro Domecq	DOM	59	0.56	0.49	0.133
	Louro Fernandes de Castro <sup>1</sup>	UAP	30	0.58	0.58	-0.001
	Maria Montalvo	MON	7	0.51	0.51	-0.004
	Marques de Albaserrada	ALB	46	0.53	0.48	0.092
	Miura	MIU	46	0.59	0.53	0.107
	Murube	MUR	51	0.56	0.46	0.178
	Oliveira Irmãos <sup>1</sup>	UGM	29	0.59	0.63	-0.072
	Pablo Romero	PAB	50	0.57	0.54	0.054
	Passanha <sup>1</sup>	UGY	30	0.55	0.56	-0.018
	Pedradas	PED	48	0.57	0.49	0.148
	Saltillo	SAL	38	0.59	0.50	0.151
	Santa Coloma	COL	142	0.65	0.52	0.193
	Urcola	URC	19	0.62	0.65	-0.037
Marques de Villamarta	VIL	20	0.61	0.54	0.123	
Veragua	VER	32	0.60	0.58	0.041	
Average across Lidia breed lineages			1071	0.57	0.54	0.050
Casta Portuguesa	Irmãos Dias	ID	30	0.50	0.51	-0.021
	José Albino Fernandes	JAF	30	0.69	0.72	-0.048
Brava dos Açores	Rego Botelho	RB	30	0.72	0.76	-0.055
	Eliseu Gomes	EG	30	0.69	0.69	-0.008
Average across Brava dos Açores			90	0.70	0.73	-0.037

<sup>1</sup> Portuguese Lidia herds belonging to Spanish lineages independently analyzed

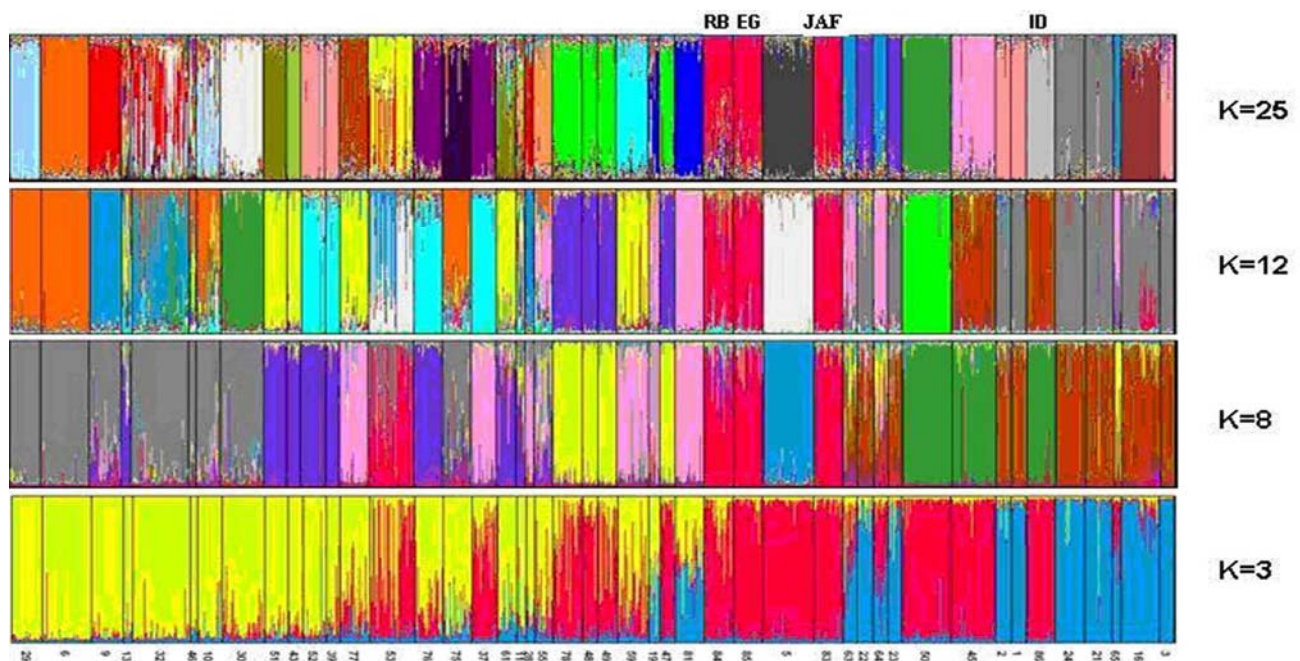
**Table 2:** Average  $F_{ST}$  values for each lineage/herd with respect to the others. JAF: José Albino Fernandes, RB: Rego Botelho, EG: Eliseu Gomes, and ID: Irmãos Dias

Lineage/Herd	Brava dos Açores				Casta Portuguesa
	JAF	RB	EG	Average	ID
Arauz de Robles	0.22	0.19	0.20	0.20	0.33
Atanasio Fernández	0.23	0.2	0.21	0.21	0.34
Baltasar Iban	0.18	0.16	0.17	0.17	0.33
Braganza	0.19	0.17	0.17	0.18	0.34
Carlos Nuñez	0.16	0.11	0.14	0.14	0.3
Concha y Sierra	0.15	0.11	0.13	0.13	0.29
Conde de la Corte	0.24	0.2	0.24	0.23	0.38
Contreras	0.25	0.23	0.25	0.24	0.39
Félix Gómez	0.18	0.16	0.18	0.17	0.33
Gamero Cívico	0.21	0.16	0.17	0.18	0.32
Hidalgo Barquero	0.23	0.2	0.23	0.22	0.36
Jacinto Ortega Casado	0.14	0.11	0.13	0.13	0.3
Jose Escobar	0.19	0.15	0.20	0.18	0.39
Juan Pedro Domecq	0.18	0.15	0.18	0.17	0.32
Louro Fernández de Castro <sup>1</sup>	0.19	0.14	0.17	0.17	0.31
Maria Montalvo	0.22	0.17	0.19	0.19	0.37
Marques de Albaserrada	0.24	0.22	0.25	0.24	0.36
Miura	0.2	0.18	0.19	0.19	0.3
Murube	0.17	0.15	0.14	0.15	0.29
Oliveira Irmãos <sup>1</sup>	0.16	0.11	0.14	0.14	0.3
Pablo Romero	0.23	0.18	0.19	0.20	0.31
Passanha <sup>1</sup>	0.18	0.14	0.15	0.16	0.32
Pedrajas	0.19	0.14	0.18	0.17	0.28
Saltillo	0.15	0.13	0.13	0.14	0.28
Santa Coloma	0.17	0.14	0.15	0.15	0.27
Urcola	0.15	0.1	0.14	0.13	0.31
Marques de Villamarta	0.18	0.13	0.16	0.16	0.29
Veragua	0.15	0.14	0.15	0.15	0.27
AVERAGE	0.19	0.16	0.18	0.18	0.32
José Albino Fernandes (JAF)		0.06	0.07		0.24
Rego Botelho (RB)			0.05		0.23
Eliseu Gomes (EG)					0.25

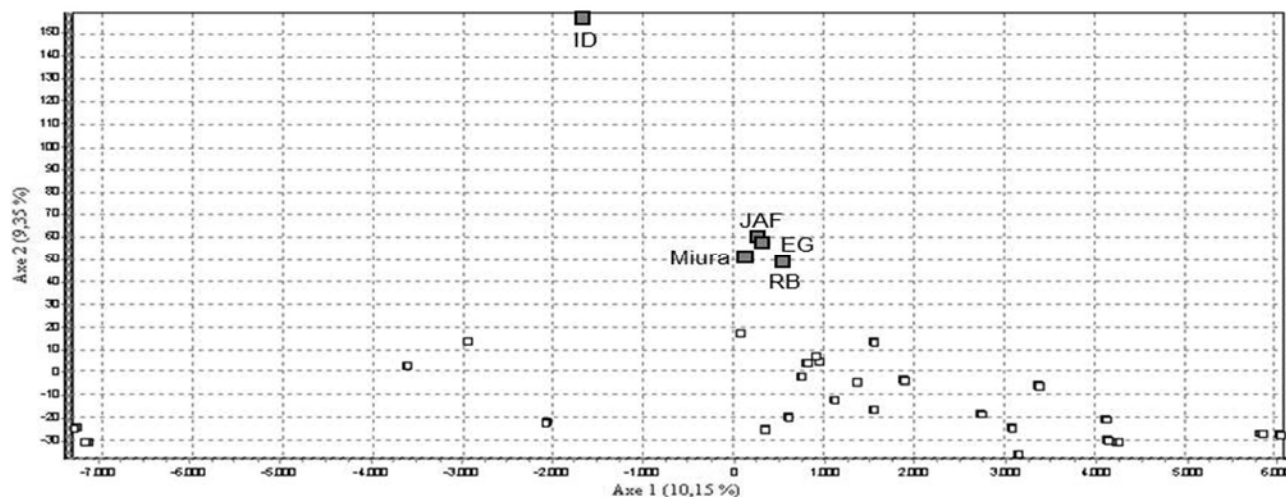
<sup>1</sup> Portuguese Lidia herds independently analyzed

independently analyzed (UAP, UGM and UGY). Furthermore, JAF and RB Brava dos Açores herds showed  $F_{IS}$  values significantly different from zero, caused by an excess of heterozygosity (Table 1). Genetic differentiation between Brava dos Açores and Lidia breed lineages was significantly higher with an average  $F_{ST}$  of 0.19, 0.16 and 0.18 in JAF, RB and EG, respectively. Spanish lineages, Concha y Sierra, Jacinto Ortega Casado and Urcola showed the lowest average  $F_{ST}$  distance values (0.18) to Brava dos Açores herds. On average, Casta Portuguesa was the most genetically isolated from the rest of the Lidia breed lineages and Brava dos Açores herds ( $F_{ST} = 0.32$ ) (Table 2).

The cluster analysis with the STRUCTURE software (Fig. S1) grouped Brava dos Açores populations and Concha y Sierra up to  $k=8$ . However, when a higher number of clusters was considered Brava dos Açores populations formed a single cluster. The only herd of Casta Portuguesa (ID), was grouped with Miura and Pablo Romero lineages ( $k=8$ ). When the number of defined clusters was increased, Miura and ID formed a separated one ( $k=12$ ). Finally, ID formed a single cluster when the number of defined populations was 25. The first two axes of the correspondence analysis represented 10.15% and 9.35% of the total inertia, respectively (Fig. 1). The first axis clearly separated Casta Portuguesa from other studied populations, grouping Miura lineage with RB, EG and JAF Brava dos Açores herds.

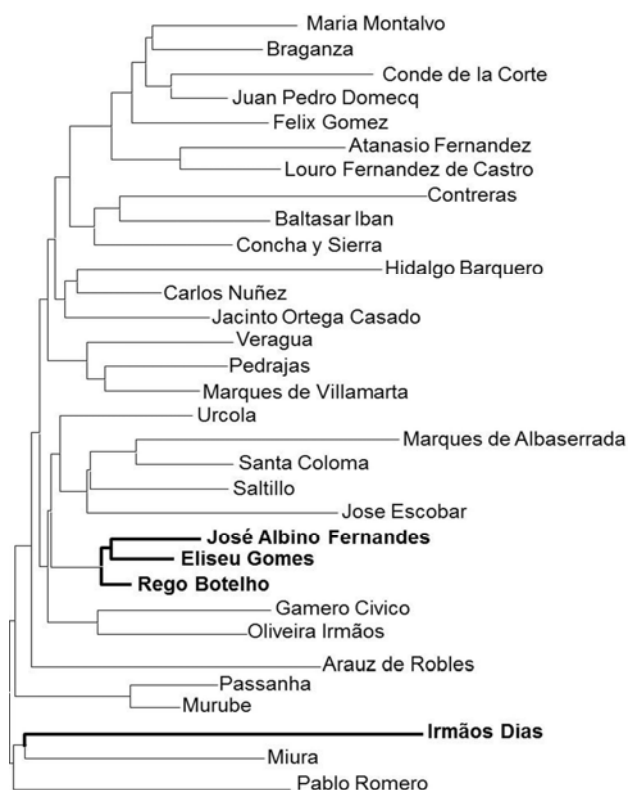


**Fig. S1:** Population structure determined using STRUCTURE with 24 autosomal microsatellites loci for  $K=3$  to  $k=25$ . Black lines separated the 46 populations analyzed. Each genotype is represented by a line divided into  $K$  colours, where  $k$  represented the number of defined clusters and the colours of each genotype represents the estimated individual membership proportion in each cluster. RB: Rego Botelho; EG: Eliseu Gomes; JAF: José Albino Fernandes; ID: Irmãos Dias; 1, 2, 3: Marques de Albaserrada; 5: Arauz de Robles; 6: Atanasio Fernández; 9: Baltasar Ibán; 10: Braganza; 11, 13: Carlos Nuñez; 16, 19, 21, 23, 24: Santa Coloma; 28: Contreras; 29: Conde de la Corte; 30: Felix Gomez; 32: Juan Pedro Domecq; 37, 39: Gamero Cívico; 43: Hidalgo Barquero; 45: Miura; 46: Maria Montalvo; 47, 48, 49: Murube; 50: Pablo Romero; 51, 52: Pedrajas; 53: Concha y Sierra; 55: Urcola; 59: Veragua; 61: Marques de Villamarta; 63, 64, 65: Saltillo; 75: Louro Fernandes de Castro; 76: Oliveira Irmãos; 77: Jacinto Ortega Casado; 78: Passanha; 81: Jose Escobar; 83: José Albino Fernandes; 84: Rego Botelho; 85: Eliseu Gomes; 86: Irmãos Dias



**Fig. 1:** Graphic of the two first axes from the factorial correspondence analysis of the Lidia breed lineages/herds and the three herds of Brava dos Açores population. JAF: José Albino Fernandes; RB: Rego Botelho; EG: Eliseu Gomes; ID: Irmãos Dias

In the Neighbour-joining dendrogram, the Brava dos Açores populations were clustered in a single branch, emphasizing the high genetic relationship among them (Fig. 2). The ID herd was grouped in a separate cluster closed to Miura and Pablo Romero lineages and highly separated from the other lineages considered.



**Fig. 2:** Neighbour-joining tree for the average  $F_{ST}$  genetic distance from one lineage/herd to the rest. Brava dos Açores herds and Casta Portuguesa are shown in bold.

## Discussion

The genetic relationship between the autochthonous Lidia bovine populations from the Azores Islands called

Brava dos Açores and Spanish and Portuguese Lidia bovine lineages was evaluated.

$H_E$  and  $H_O$  were lower than that in the majority of the Lidia bovine breeds and in the 69 bovine breeds analyzed by the European Cattle Genetic Diversity Consortium (2006) (average  $H_O=0.69$ ).

On the other hand, Brava dos Açores population showed higher  $H_E$  and  $H_O$  and was also higher than the 69 breeds analyzed in the European Cattle Genetic Diversity Consortium breeds (2006). Within herds genetic variability in Brava dos Açores evidenced an excess of heterozygosity (negative  $F_{IS}$  values) in JAF and RB herds, probably due to the crossbreeding among different Lidia breed lineages and local bovine populations during breed development. It is remarkable that the  $F_{IS}$  value in EG Brava dos Açores herd showed no significant difference from 0, probably due to the smaller size of this herd, with a number of animals around half that observed in the JAF and RB.

In previous DNA analyses in Lidia breed lineages, a high level of genetic isolation among lineages and also between herds within lineages (average  $F_{ST}=18\%$ ,  $F_{IS}=11\%$ ) was found (Cañón *et al.*, 2008). On average, Casta Portuguesa appeared as the most genetically isolated of the Portuguese Lidia populations as well as the other Lidia bovine lineages with an average  $F_{ST}$  genetic distance of 0.32. This value is also greater than that estimated in previous analyses between Spanish Lidia breed lineages (Cañón *et al.*, 2008). Also, the  $F_{ST}$  distance values among Brava dos Açores herds were in the bottom of the  $F_{ST}$  distances range (0.07-0.01) estimated in European cattle breeds (Cañón *et al.*, 2001).

The higher genetic diversity achieved in the Brava dos Açores bovine population than in the Lidia bovine breed is a consequence of the crossbreeding of different Lidia lineages and autochthonous Azores bovine animals (Bull Breeders Association of Azorean Street Bullfighting, 2006) during the breed development. For Casta Portuguesa population it can be assumed that reproductive management was about the same as other Lidia breed lineages, characterized by a high level of

genetic isolation and low number of reproductive males used, in order to fix desirable aggressive patterns. As was expected, the genetic diversity of Casta Portuguesa was within the range achieved for the Lidia breed lineages. Furthermore, the level of differentiation between Casta Portuguesa and the remaining Lidia breed lineages was higher than that among lineages without Casta Portuguesa and also than the values obtained among European cattle breeds (MacHugh *et al.*, 1998; Cañón *et al.*, 2001). In Casta Portuguesa the effect of the genetic drift, which has been postulated as the principal source of genetic differentiation among Lidia lineages could have been greater than in the rest of the lineages for two reasons:

1) The higher reproductive isolation among Casta Portuguesa and the remaining lineages due to geographic considerations; Casta Portuguesa is located in continental Portugal while the remaining lineages are located in Spain.

2) The number of herds and animals belonging to Casta Portuguesa is lower than that in most lineages. On the other hand, the  $F_{ST}$  among Brava dos Açores populations evidenced clearly lower values than those in the Lidia breed lineages, which evidenced a higher level of genetic flow among them.

The results of cluster analysis with STRUCTURE showed different influences in the Portuguese Lidia bovine populations. At low  $k$  values, the genetic relationships of Casta Portuguesa were in agreement with the Neighbour-joining dendrogram, being grouped with Miura and Pablo Romero. When  $k$  values increased, Miura and Casta Portuguesa joined in a single cluster confirming the well documented gene flow between both lineages (Lucas, 2006). However, the results of the present study showed for the first time a genetic relationship between Casta Portuguesa and Pablo Romero. The  $F_{IS}$  value in Casta Portuguesa could be the consequence of crossbreeding with Miura lineages or recent pooling of different strains or families within ID herd, while Miura and Pablo Romero, which are represented by a single herd showed a higher deficit of heterozygotes. The gene flow among strains or families within ID is a practical reproductive strategy in order to maintain the genetic diversity and avoid inbreeding.

Brava dos Açores populations showed a different pattern of genetic structure than Casta Portuguesa, being mainly grouped with Concha y Sierra lineage up to  $k=8$ . For higher  $k$  values Brava dos Açores was grouped in a single cluster with certain mixed origin with Veragua, Murube, Gamero Cívico and Concha y Sierra. Therefore, genetic relationships observed in Brava dos Açores could explain the higher genetic diversity of this population.

Portuguese Lidia bovine populations were grouped in the same cluster only at low  $k$  values ( $k=2$ ), indicating a certain degree of Casta Portuguesa in Brava dos Açores population but less than other Lidia lineages.

Our results on the Portuguese Lidia bovine populations showed two different results involving genetic diversity:

1) Casta Portuguesa evidenced low levels of genetic

diversity and consequently high levels of genetic differentiation from the remaining Lidia breed lineages.

2) Brava dos Açores populations showed higher levels of genetic diversity than that in Casta Portuguesa, probably due to the crossbreeding at origin with different Lidia bovine lineages, and to a greater genetic flow among herds as evidenced  $F_{IS}$  values.

However, taking into account the reduced number of herds and animals of Portuguese Lidia bovine populations, a conservation program to preserve the genetic variability of both populations should be considered.

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