Journal of Heredity 2003:94(3):243–250
DOI: 10.1093/jhered/esg055

Genetic Characterization of Southwestern European Bovine Breeds: A Historical and Biogeographical Reassessment With a Set of 16 Microsatellites

A. BEJA-PEREIRA, P. ALEXANDRINO, I. BESSA, Y. CARRETERO, S. DUNNER, N. FERRAND, J. JORDANA, D. LALOE, K. MOAZAMI-GOUDARZI, A. SANCHEZ, AND J. CAÑON

From the Centro de Investigação em Biodiverdidade e Recursos Geneticos CIBIO-UP, Universidade do Porto, Campus Agrário de Vairão, Rua Monte-Crasto, Pt-4485-661 Vairão, Portugal (Alexandrino, Beja-Pereira, Bessa, and Ferrand); Departamento de Zoologia/Antropologia da Faculdade de Ciências, Praça Gomes Teixeira, 4099-002 Porto, Portugal (Alexandrino, Beja-Pereira, Bessa, and Ferrand); Laboratorio de Genética, Facultad de Veterinària, Universidad Complutense de Madrid, E-28040 Madrid, Spain (Carretero, Dunner, and Cañon); Unitat de Genetica i Millora Animal, Departament de Ciencia Animal i dels Aliments, Facultat de Veterinaria, UAB, 8193-Bellaterra, Barcelona, Spain (Jordana and Sanchez); Station de Génétique Quantitative et Appliquée—Institut National de la Recherche Agronomique, F-78352 Jouy-en-Josas Cedex, France (Laloe); and Laboratoire de Génétique Biochimique et de Cytogénétique, Institut National de la Recherche Agronomique, F-78352 Jouy-en-Josas Cedex, France (Moazami-Goudarzi).

Address correspondence to Albano Beja-Pereira at LECA-Génomique des Populations et Biodiversité UMR CNRS 5553, Université Joseph Fourier B.P. 53, F-38041 Grenoble Cedex 9, France, or e-mail: albano.beja-pereira@ujf-grenoble.fr.

Abstract

The origin of Iberian cattle has been suggested by some authors to be the product of European and north African cattle entrances during the last few thousands of years. However, these hypotheses were mainly based on morphological similarities. This study analyzed 889 unrelated individuals from 15 representative Iberian breeds and 3 French breeds for 16 microsatellite loci. Statistical tests were used to calculate interpopulation genetic distances (D_A) and principal components analysis (PCA). To visualize the geographical distribution of the genetic differentiation between Iberian cattle breeds, data from the PCA analysis were used to construct synthetic maps. Genetic similarity among neighboring Iberian breeds is mainly caused by gene flow. However, recent demographic fluctuations and reproductive isolation in Alistana, Mirandesa, and Tudanca has increased genetic drift, which may be the main cause for the relatively high differentiation of these populations. The synthetic maps constructed with the first and second PCs revealed (1) a large differentiation between Northern Iberian breeds rather than between more geographically distant breeds, and (2) a clear east-west gradient that may be related with the model of demic diffusion of agriculture. Finally, we detected no strong evidence for an African genetic influence in the Iberian cattle breeds analyzed in this study.

The origin of present-day Iberian cattle breeds is controversial, but the analysis of morphological evidence indicates that all entrances of cattle were from the *Bos taurus* (Taurine) lineage (Sanchez-Belda 1984; Valle 1941). As human population movements played a major role in domestic species dispersal, it is accepted that the first arrival of cattle during the late Neolithic, as well as subsequent arrivals, were mainly from two distinct places: Central Europe and north Africa (Nogueira 1929; Payne 1978; Ralo and Guerreiro 1981). A significant number of animals were probably brought, first by the Proto-Indo-European peoples, into northern Iberia (Zilhão 1993) during the fifth millennium B.P., and second by the Roman invasions ca. 2300 B.P. A more recent and sporadic introduction of cattle from north Africa may have occurred during the Moorish occupation (Payne 1978). All these historical facts highlight the importance of the

Table 1. Population names, codes, country of origin, and PC values

Population name	*N*	CODE	Country of origin	PC1	PC2
Alentejana	50	ALE	Portugal	−0.750	0.574
Barrosã	50	BAR	Portugal	−0.019	2.469
Maronesa	49	MAR	Portugal	−0.210	1.636
Mertolenga	50	MER	Portugal	−0.821	0.804
Mirandesa	50	MIR	Portugal	−2.658	−0.532
Alistana	50	ALI	Portugal	−1.087	−0.793
Asturiana Montaña	50	ASM	Spain	0.146	0.499
Asturiana Valles	50	ASV	Spain	0.219	−0.552
Avileña	50	AVI	Spain	0.175	−1.250
Bruna dels Pirineus	50	BRU	Spain	0.605	−1.468
Morucha	50	MOR	Spain	0.416	−0.047
Pirenaica	50	PIR	Spain	−0.105	−1.290
Retinta	50	RET	Spain	0.573	−0.226
Sayaguesa	50	SAY	Spain	1.557	−0.068
Tudanca	50	TUD	Spain	1.960	0.245
Aubrac	50	AUB	France	Not included	Not included
Gasconne	50	GAS	France	Not included	Not included
Salers	50	SAL	France	Not included	Not included

geographical position of the Iberian peninsula, which has played a key role in human movements between Europe and Africa (Arnaiz-Villena et al. 1999; McEvedy and Jones 1978). This importance was increased after the sixteenth century, when the Portuguese and Spanish explorers started to colonize the American continent and the Caribbean Islands and took with them Iberian cattle (Payne 1978; Rouse 1977; Wilkins 1984).

Presently, the Iberian breeds are classified into three main groups—the Iberian, the Blond-Brown Cantábrico, and the Turdetano—defined by their geographical position, related origin, and morphological characteristics, from which coat color, horn, and head shape are the most important (Jordana et al. 1991; Sanchez-Belda 1984; Valle 1941). These three major groups encompass most Iberian cattle breeds, with the exception of the Portuguese Barrosã, which has a very peculiar hyperconcave head and lyre-shaped horns that many authors often associate with an older north African origin (Epstein 1971; Valle 1941).

Cattle, like other livestock species, are recognized as important components of world biodiversity, because the genes and gene combinations they carry may be useful to agriculture in the future (Hall and Bradley 1995). Local cattle breeds are considered, for many reasons, precious genetic resources that tend to disappear due to new market and agricultural demands. Nevertheless, there is a serious risk that most of these breeds will disappear before they have been fully identified (FAO 2000), and this applies particularly to the Iberian autochthonous races that have suffered a dramatic loss of economic importance and subsequent population size reduction.

The relatively ease of microsatellite typing has led to the buildup of large sets of data, which permit fine-scale analysis of genetic structure and the precise measurement of population genetic variation (Moazami-Goudarzi et al. 1997). Microsatellites have also been extensively used in the genetic characterization of cattle breeds (Cañon et al. 2001; Edwards et al. 2000; Loftus et al. 1999; MacHugh et al. 1997).

During the last two decades, Cavalli-Sforza and his colleagues introduced the methodology of synthetic map construction, which are geographic maps of isoplets (lines of equal values) of principal components analysis values (PCA). Synthetic maps have been widely used to study the patterns of evolution of human populations (Menozzi et al. 1978; Piazza et al. 1981). This methodology uses the principal components values to construct a map of isoplets, generally designated as synthetic genetic maps. The isoplet map that each PC generates is independent of all other maps and explains an estimated fraction of the total genetic variation (Cavalli-Sforza et al. 1993). In spite of a few small studies made on immunogenetics (Kidd et al. 1980), protein polymorphism (Arranz et al. 1996; Fernández et al. 1998), and more recently, molecular genetics (Martin-Burriel et al. 1999), relatively little is known about the history and genetic variation of Iberian cattle. The aim of this study is to combine genetic data with geographical positioning, using geostatistical models, as a new approach to study genetic relationships among Iberian breeds and to reassess the putative influence of African cattle in their genetic diversity, as recently suggested by Cymbron et al. (1999). For the first time, the 15 most important Iberian (Portuguese and Spanish), and three French, cattle breeds were characterized, under similar sampling procedures, for an identical set of 16 microsatellites.

Materials and Methods

Allelic frequencies and distributions of 889 unrelated individuals from 18 breeds (for breed names, codes, and sample origins, see Table 1) obtained with a set of 16 microsatellites—CSSM66, ETH10, ETH152, ETH225, ETH3, HEL1, HEL5, HEL9, ILSTS005, INRA023,

INRA032, INRA035, INRA037, INRA005, INRA063, and TGLA44—were computed using the results from direct gene counting. Unbiased estimates of gene diversity (He) were determined across loci and populations following Nei (1987), and mean number of alleles (MNA) was estimated to analyze the levels of variability. An exact test for deviations from Hardy–Weinberg equilibrium (HWE) was performed using the GENEPOP package, version 3.0 (Raymond and Rousset 1995).

The genetic relationships between the studied breeds were inferred from Nei's D_A (Nei et al. 1983) using DISPAN computer package (Ota 1993). The resulting matrix was used to perform a multidimensional scaling (MDS) analysis for illustration of genetic distances. Data from the literature were used to investigate the genetic relationships between Iberian and other breeds. Allele frequencies on five common microsatellite markers (ETH225, ETH152, HEL1, HEL9, HEL5) assembled from a study on African, European, and Asian cattle (MacHugh 1997) were used to estimate D_A genetic distances between the studied breeds and four European (Hereford, Friesian, Simmental, and Jersey) and two African *B. taurus* populations (N'Dama Gambia and Guinea), providing that allele nomenclature used was uniform. Standardization of the allele nomenclature (size) was done using reference samples provided by D. MacHugh (Department of Animal Science and Production and Conway Institute of Biomolecular and Biomedical Research, Faculty of Agriculture, University College, Dublin). As *Bos indicus* may also have been introduced in Iberia, data on six breeds from this lineage were also included (Butana, Kenana, Tharparker, Sahiwal, Gobra, Maure). This raised the number of breeds analyzed to 31. Distances between breeds were used for dendrogram construction according to the Neighbor-Joining (NJ) algorithm (Saitou and Nei 1987). The tree topology was constructed using the algorithm implemented in PHYLIP package (Felsenstein, 1993) and the reliability of each node was estimated by 1,000 random bootstrap resamplings of the data.

Genetic relationships within Iberian cattle breeds were also explored with multivariate statistical analysis, of which the factor analytic models (such as the PCA) are frequently applied to this kind of study (Loftus et al. 1999; MacHugh et al. 1997; Moazami-Goudarzi et al. 1997). Data from the first and second PC and the geographical coordinates for each breed were combined and plotted as contoured geographic maps using a geostatistical griding method. Because the geographical distributions of the breeds studied here do not cover all of the Iberian territory and there are missing data points in the distribution of data, a method of interpolation (for example, kriging) was used to fill in these points where no data exist. This method was developed by geostaticians to provide the best local estimates of the mean value of a regionalized variable. These interpolation methods are based on the assumption that the parameter being interpolated can be treated as a regionalized variable. A regionalized variable is intermediate between a truly random variable and a completely deterministic variable, in that it varies in a continuous manner from one location to the next, and therefore points that are near each other have a certain degree of spatial correlation, but points that are widely separated are statistically independent (Davis 1986).

Results

A total of 173 alleles were found across all loci, and the MNA per locus was 6.5 (ranged between 5.5 in the MIR and 7.2 in the GAS). The least polymorphic locus was ILST05, and the highest genetic diversity was found at the TGLA44 locus. The GAS was the breed with the highest number of private alleles (five alleles). Allele frequencies are available upon request. The unbiased estimates of gene diversity (expected heterozygosity, He) ranges from 0.611 (AUB) to 0.709 (MOR).

An exact test was used to evaluate deviation from HWE (Guo and Thompson 1992). In ten of the populations analyzed, namely Avileña, Gascogne, Maronesa, Mertolenga, Mirandesa, Pirenaica, Retinta, Sayaguesa, Salers, and Tudanca, significant deviations ($P < .018$) were observed for more than one microsatellite.

The highest and lowest DA values among the studied breeds are between Mirandesa and Tudanca (0.2091) and between Asturiana Montañas and Asturiana Valles (0.0929), respectively. The MDS graphic for the Iberian breeds depicts a large difference between Tudanca and Mirandesa, as well as between the most western Iberian and the French breeds (Table 2). However, when the genetic distances between the Iberian breeds and those from MacHugh (1997) are included, the highest values were displayed between the Indian zebu and the Central European taurine breeds.

For the 15 Iberian breeds, the first and the second PCs explain 16% and 12% of the total variation in the data, respectively. The range between the maximum and minimum values of the PCs has been divided into nine equal classes, which are indicated by different intensities of color. The synthetic map with the first PC separates Tudanca and Sayaguesa from all others at one extreme of the scale, and Mirandesa at the other. All other breeds take intermediate positions between the latter ones. In second PC the largest differentiation is obtained between the western Iberian breed (Barrosã) and the eastern Iberian breeds (Pirenaica and Bruna).

Discussion

The observed genetic differences across Iberian breeds may reflect the large differences of agricultural systems that exist within the Iberian peninsula. The less intensive agriculture (mainly shaped by the Mediterranean environmental constraints in the south) and the steep topology in the north of the peninsula, as well as the recent introgression of foreign breeds, probably contributed to the present scenario. An evidence of this is the large number of alleles with a frequency of less than 5%, of which some are private alleles. Additionally, the significant deviations from the HWE observed in ten breeds may be due to genetic substructure within

Table 2. Genetic distances (D_A) estimates between the 18 studied breeds

	ALI	ASM	ASV	SAY	TUD	ALE	BAR	MAR	MER	MIR	AUB	GAS	SAL	AVI	BRU	MOR	PIR
ASM	0.1100																
ASV	0.0954	0.0929															
SAY	0.1529	0.1561	0.1262														
TUD	0.1188	0.1089	0.1047	0.1496													
ALE	0.1297	0.1071	0.1041	0.1989	0.1429												
BAR	0.1199	0.0975	0.0977	0.1589	0.1096	0.0986											
MAR	0.1311	0.1186	0.1141	0.1877	0.1435	0.1166	0.0750										
MER	0.1436	0.1208	0.1173	0.1798	0.1510	0.0890	0.0854	0.1028									
MIR	0.0986	0.1467	0.1362	0.2091	0.2013	0.1253	0.1290	0.1336	0.1105								
AUB	0.1527	0.1209	0.1333	0.1718	0.1378	0.1401	0.1467	0.1597	0.1293	0.1657							
GAS	0.1642	0.1226	0.1218	0.1768	0.1518	0.1422	0.1215	0.1359	0.1440	0.1878	0.0945						
SAL	0.1511	0.1277	0.1233	0.1761	0.1476	0.1605	0.1506	0.1487	0.1408	0.1627	0.0703	0.0958					
AVI	0.1319	0.1212	0.1001	0.1537	0.1291	0.1042	0.1176	0.1223	0.1175	0.1440	0.1122	0.1256	0.1292				
BRU	0.1303	0.1137	0.0987	0.1682	0.1199	0.1445	0.1351	0.1441	0.1318	0.1716	0.1250	0.1160	0.1152	0.1100			
MOR	0.1457	0.1066	0.1041	0.1715	0.1116	0.1063	0.1137	0.1239	0.1275	0.1728	0.1359	0.1186	0.1432	0.1085	0.0853		
PIR	0.1374	0.1187	0.1060	0.1641	0.1396	0.1301	0.1321	0.1362	0.1026	0.1541	0.0948	0.0905	0.1023	0.1214	0.0884	0.1219	
RET	0.1342	0.0937	0.1021	0.1377	0.1235	0.0900	0.1037	0.1123	0.0948	0.1430	0.1392	0.1081	0.1297	0.0940	0.1044	0.1024	0.0948

breeds, resulted from different local selection strategies across herds. Indeed, these local selection strategies promote isolation between herds. Loftus et al. (1999) also found significant deviations from HWE for Anatolian and Iraqi cattle breeds, which were known to be isolated and genetically structured.

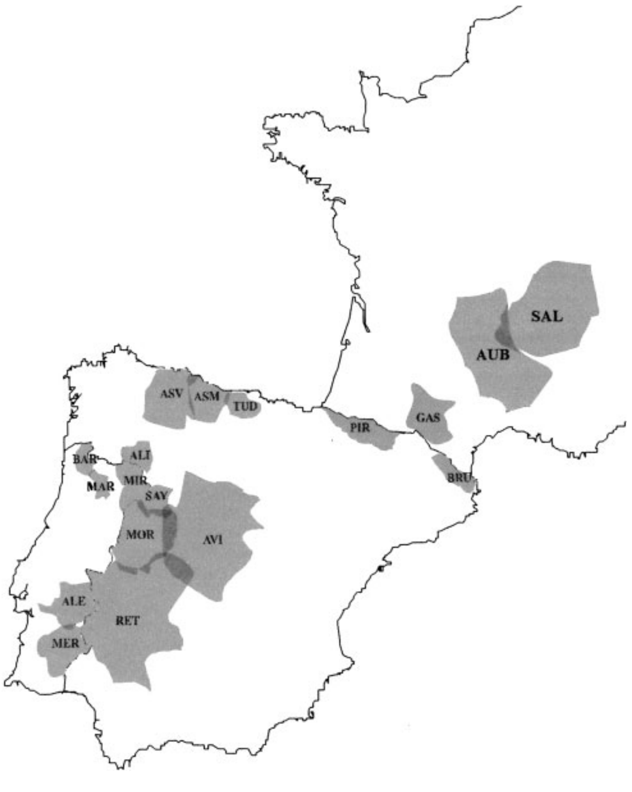

Figure 1. Geographic distribution of the 18 bovine breeds.

The visual inspection of the genetic distance matrix (Table 2) shows that the high genetic distances are more likely to be related with geographical distances rather than with the differences between classical morphological groups, to which the Iberian breeds are affiliated. Assuming that the French breeds are likely representatives of the Central European group, it is plausible to infer that the influence of the Central European gene pool in Iberian breeds decreases with geographic distance. Average genetic distances between western Iberian and French breeds (0.1258; SD ± .018) are bigger than those between eastern Iberian and French ones (0.0985; SD ± .008). In this respect, we note that Pirenaica and Bruna breeds located near to the French border are positioned near the French breeds (Figure 1). Nevertheless, we admit that factors such as geographical isolation, different selection programs, and high inbreeding levels may complicate this scenario by increasing genetic distances between breeds independently of their geographical location.

The geographical isolation argument is supported by the MDS constructed with DA distances (Figure 2). This is probably the case of the pairs formed with the northwestern Iberian Mirandesa/Alistana and Tudanca/Sayaguesa, which had dramatic decreases of effective population sizes and thus increased genetic distances relative to the other breeds. On the other hand, limited gene flow may have contributed to larger than expected differentiation between breeds that are closely related (with similar phenotypes and located in neighboring regions). The distance between the southern breeds, Alentejana and Retinta, and the close proximity of Morucha to the French and eastern Iberian breeds, are examples of this.

There are three aspects which may explain these results. The first comes from the fact that, although the two southern breeds are located in neighboring regions, the border between Portugal and Spain may have promoted reproductive isolation between them. The second is that during the last decades, these three breeds have been receiving the influence

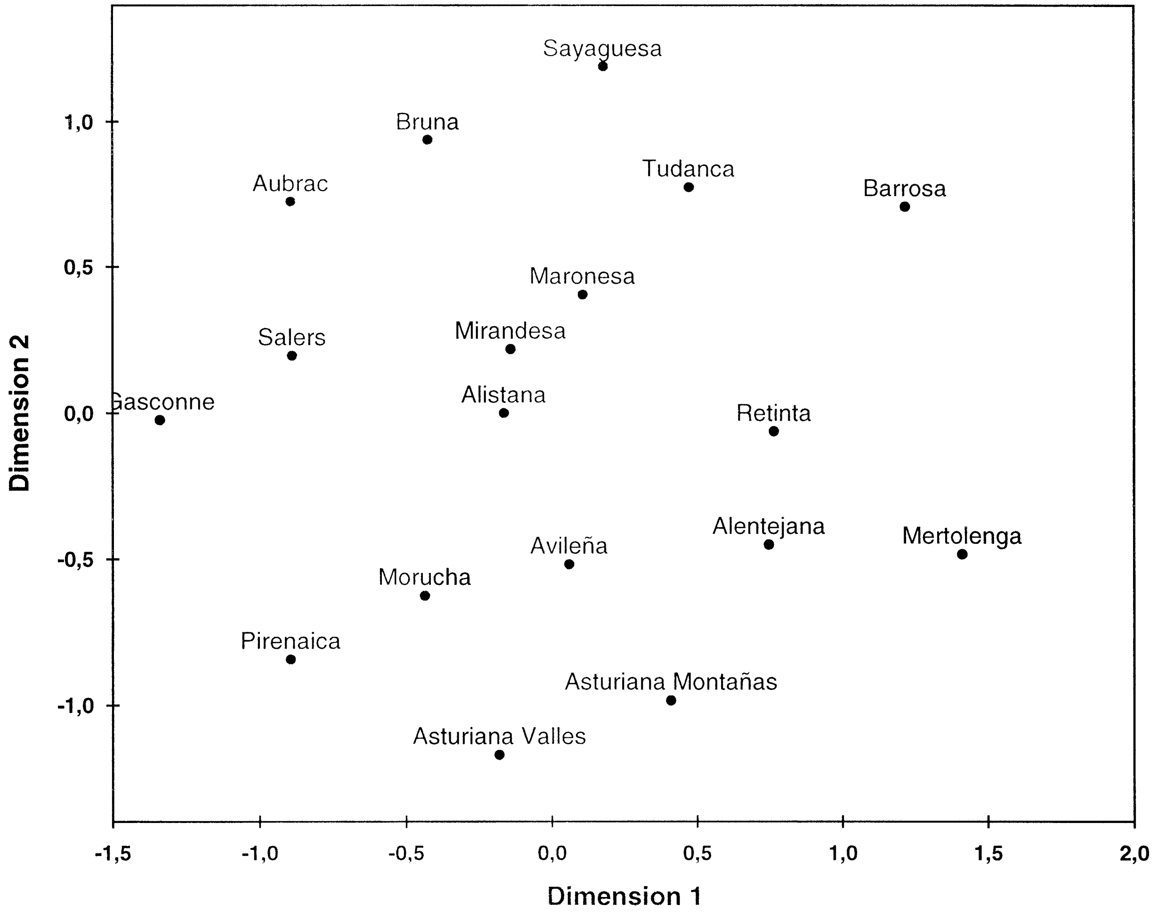

Figure 2. Two multidimensional scalling plot illustrating the DA genetic distances among 18 cattle breeds.

of many foreign ones, such as French Limousin, Salers, and the exotic Nellore (*B. indicus*) and Santa Gertrudis [(modern St. Gertrudis cattle are approximately five-eighths Shorthorn (*B. taurus*) and three-eighths Brahman (*B. indicus*)], in order to improve their beef production performances and heat resistance (Ralo and Guerreiro 1991; Sanchez-Belda 1984). Finally, in both cases, the distribution ranges are the largest among the Iberian breeds, and this may have contributed to maintaining a moderate heterogeneity within populations due to substructuring.

In Figure 3, the synthetic maps illustrate of how genetic differentiation within Iberian cattle is geographically distributed across the peninsula. The first PC shows a clear distinction of four northern Iberian breeds relative to the other Iberian breeds. Nevertheless, this pattern is more likely to be the result of different selection forces combined with genetic drift, rather than from different genetic origins. In fact, the unique agricultural system of northern Iberia is characterized by many small herds that are located in fragmented regions due to geographical and environmental barriers. These conditions, per se, promote genetic isolation and, together with the diverse local selection strategies, seem to have generated strong differentiation among closely related breeds over time (average genetic distance among northern Iberian breeds is 0.131 SD ± .011).

The fact that such remarkable differentiation could be found in only a small part of Iberia, whereas the other regions showed to be essentially homogenous, is evidence that this region may be an important reservoir of diversity. For these reasons, it is suggested that these populations should be considered important targets for conservation purposes. In contrast with this is the pattern observed for southern Iberia, where the agriculture production system corresponds to farms covering hundreds of hectares and to an extreme-open field way of raising domestic cattle. The associated small or even inexistent reproductive control may thus promote gene flow and be responsible for the small degree of genetic differentiation between populations (average genetic distance among southern Iberian breeds is 0.095 SD ± .004). The second PC shows a clear east–west gradient that may be tentatively consistent with the model of demic diffusion of agriculture suggested by Cavalli-Sforza et al. (1994). While the applicability of this model to Iberia has been challenged by Zilhão (1998), who suggests, instead, a complex mosaic pattern of different settlements on the coast of the peninsula, the fact is that the synthetic map

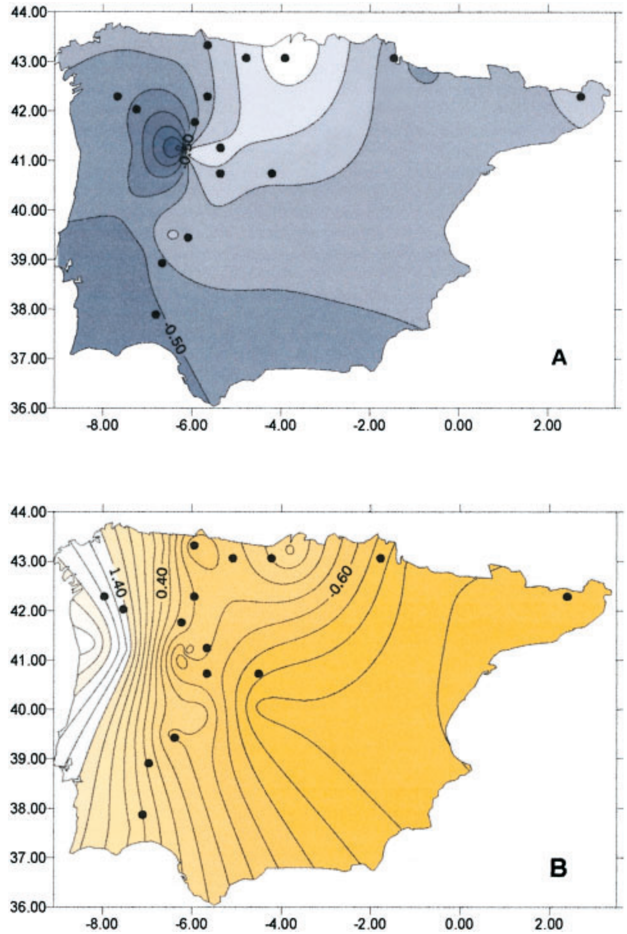

Figure 3. Synthetic genetic maps of Iberian peninsula representing 28% of the total genetic variation. **(A)** and **(B)** are the interpolated values of the first and second PCs, respectively. The PC values are grouped in isopleths and ranked according the color tonalities, dark color: high values; light color: lower values. Black dots represent the geographic coordinate used for each population.

methodology does not detect any sign of the necessarily associated differences in the origin of cattle or even of the classical morphological groups.

In a recent paper on mtDNA genetic variability of six Portuguese breeds, of which five are included in this study, Cymbron et al. (1999) detected African haplotypes in two southern Portuguese breeds, Alentejana and Mertolenga. However, screening of microsatellite locus ILST001 revealed that allele 88, typical of African taurine breeds (MacHugh et al. 1997), was only present in low frequency in the two northern Portuguese Maronesa and Barrosã breeds ($f = 0.05$ and $f = 0.02$, respectively). On the other hand, it is noteworthy that one individual from Mertolenga was found to be homozygous for allele 191 at locus ETH152, which had previously been detected only in East African Zebus (MacHugh et al. 1997). Nevertheless, results comparing Iberian breeds with the others assembled from the literature should be viewed with caution, due to the small number of loci and differing sampling strategies that may interfere with the estimation of genetic distance between those breeds. The NJ dendrogram from DA genetic distances shows that the Iberian breeds group together with other Central European ones rather than with African *B. taurus* or *B. indicus* (Figure 4). Although bootstrap values in NJ dendrogram are low (data not shown), the general topology and inferences that we draw from it are in concordance with the observation of zebu or African taurine diagnostic alleles in the Portuguese breeds (northern Iberian breeds are positioned closer to the African ones). The average genetic distances between Iberian breeds and the two African taurine breeds is 0.2443 (SD ± .039), whereas within Iberian breeds and between Iberian and other European are 0.122 (SD ± .021) and 0.1245 (SD ± .033), respectively. However, six Iberian breeds, of which four are located in northern Iberia and the other two are in southern Iberia, cluster more closely to the African. This shows that the distribution of those alleles is probably not associated with the proximity of southern Iberia to north Africa but with the large genetic diversity of Iberian cattle and recent gene flow from foreign breeds, thus implying that a clear cline-pattern of African influence in Portuguese cattle as described in Cymbron et al. (1999) is not supported by the nuclear data now investigated. An alternative explanation for these discrepant observations may be associated with the introgression of mtDNA haplotypes originated in the decades of 1960/1970, when individuals from the St. Gertrudis and Nellore breeds were often used in crosses with southern Iberian breeds (Ralo and Guerreiro 1991; Sanchez-Belda 1984).

Suggestions that north African cattle may have left a genetic signature in Iberian breeds arise from the hypothesis of human population dispersal, north African cultural influence, and historical heritage in the Iberian Peninsula. This hypothesis is in line with the origin of the most important human settlements and spread of agro-pastoral economies in the southern and western coasts of Iberia (Zilhão 1993) but is not supported by our data set of 16 microsatellites. We therefore encourage the realization of other studies using additional types of molecular markers, including north African breeds, in order to clarify this problem.

Acknowledgments

We thank breeders' associations for their help in collecting samples, and G. Luikart and M. Branco for constructive criticisms of our data and for comments on an earlier draft of this manuscript. We also thank D. MacHugh for providing reference samples from African cattle. Our work was supported by European contract FAIR1 CT95 0702, FCT (project PRAXIS XXI 3/3.2/CA/2002/95) and CICYT (contract AGF96_1950-CE/95). A. Beja-Pereira and I. Bessa were supported by Ph.D. grants from Fundação para a Ciência e Tecnologia (BD2746/2000, PRAXISXXI, and BD/15578/98, respectively).

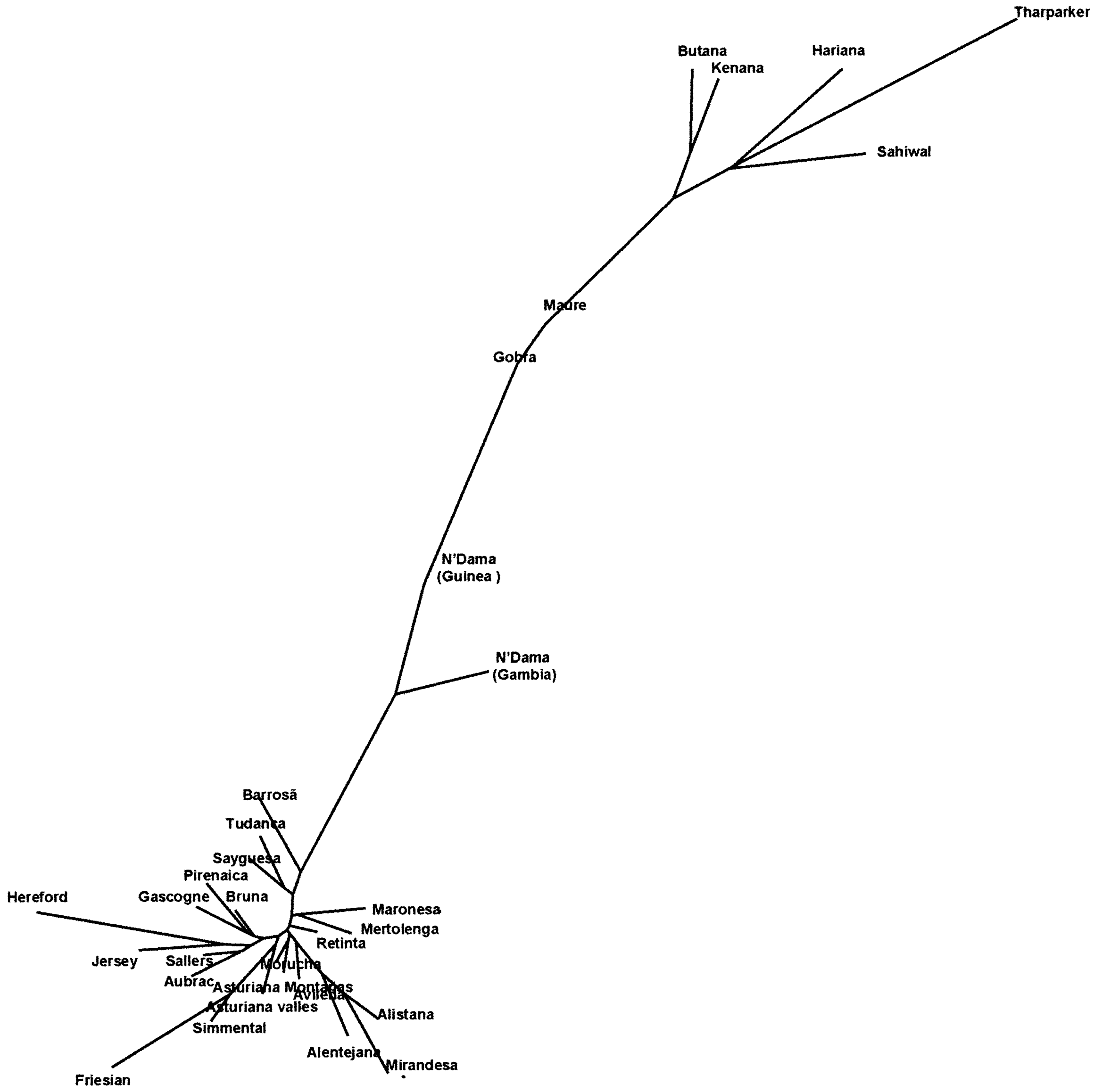

Figure 4. Unrooted NJ network derived from the DA genetic distances among 31 breeds. Data from Central European, African, and Indian cattle were assembled from MacHugh (1997).

References

Arnaiz-Villena A, Martinez-Laso J, and Alonzo-Garcia J, 1999. Iberia: population genetics, anthropology, and linguistics. Hum Biol 71:725–743.

Arranz L, Bayón Y, and San Primitivo F, 1996. Comparison of protein markers and microsatellites in differentiation of cattle populations. Anim Genet 27:415–419.

Cañon J, Alexandrino P, Bessa I, Carleos C, Carretero Y, Dunner S, Ferran N, Garcia D, Jordana J, Laloe D, et al., 2001. Genetic diversity measures of local European beef cattle breeds for conservation purposes. Genet Sel Evol 33:311–332.

Cavalli-Sforza L, Menozzi P, and Piazza A, 1993. Demic expansions and human evolution. Science 259:639–646.

Cymbron T, Loftus R, Malheiro M, and Bradley G, 1999. Mitochondrial sequence variation suggests an African influence in Portuguese cattle. Proc R. Soc London Ser B 266:597–603.

Davis JC, 1986. Statistics and data analysis in geology. New York: Wiley.

Edwards C, Dolf G, Looft C, Loftus R, and Bradley D, 2000. Relationships between the endangered Pustertaler-Sprinzen and three related European cattle breeds as analysed with 20 microsatellite loci. Anim Genet 31:329–332.

Epstein H, 1971. The origin of domestic animals of Africa. New York: Africana.

FAO, 2000. World watch list for domestic animal diversity, 3rd ed. (Sherf B, ed). Rome: Food and Agriculture Organization.

Felsenstein J, 1993. PHYLIP (Phylogeny Inference Package) Version 3.5c. Department of Genetics, University of Washington, Seattle.

Fernández A, Viana J, Iglesias A, and Sanchez L, 1998. Genetic variability and philogenetic relationships between tem native cattle breeds from Galícia and the North of Portugal. Arch Zootenicos 47:61–71.

Guo SW and Thompson EA, 1992. Performing the exact test of Hardy–Weinberg proportions for multiple alleles. Biometrics 48:361–372.

Hall S and Bradley D, 1995. Conserving livestock breed biodiversity. Tree 10(7):267–270.

Jordana J, Pelegrin M, and Piedrafita J, 1991. Relaciones genéticas en bovinos españoles obtenidas a partir del estudio de caracteres morfológicos. ITEA 87:50–64.

Kidd K, Stone H, Crimella C, Carenzi C, Casati M, and Rognoni G, 1980. Immunogenetic and population genetic analyses of Iberian cattle. Anim Blood Groups Biochem Genet 11:21–38.

Loftus R, Ertrugrul O, Harba A, El-Barodys M, MacHugh D, Park S, and Bradley D, 1999. A microsatellite survey of cattle from a centre of origin: the Near East. Mol. Ecol 8:2015–2022.

MacHugh DE, 1997. Molecular biogeography and genetic structure of domesticated cattle (PhD dissertation). Dublin: University of Dublin.

MacHugh DE, Shriver MD, Loftus RT, Cunningham P, and Bradley DG, 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*). Genetics 146:1071–1086.

Martin-Burriel I, Garcia-Muro E, and Zaragoza P, 1999. Genetic diversity analysis of six Spanish native cattle breeds using microsatellites. Anim Genet 30:177–182.

McEvedy C and Jones R, 1978. Atlas of the world population history. London: Harmondsworth Penguin Books.

Menozzi P, Piazza A, and Cavalli-Sforza L, 1978. Synthetic maps of human gene frequencies in Europeans. Science 201:786–792.

Moazami-Goudarzi K, Laloe D, Furet JP, and Grosclaude F, 1997. Analysis of genetic relationships between 10 cattle breeds with 17 microsatellites. Anim Genet 28:338–345.

Nei M, 1987. Molecular evolutionary genetics. New York: Columbia University Press.

Nei M, Tajima F, and Tateno T, 1983. Accuracy of estimated phylogenetic trees from molecular data. J Mol. Evol 19:153–170.

Nogueira JP, 1929. Portugal: gados. Lisboa: Imprensa Nacional.

Ota T, 1993. DISPAN: Genetic distance and phylogenetic analysis. Pennsylvania State University, University Park, PA.

Payne W, 1978. Cattle production in the Tropics. London: Longman.

Piazza A, Menozzi P, and Cavalli-Sforza L, 1981. The making and testing of geographic gene-frequency maps. Biometrics 37:635–659.

Ralo A and Guerreiro A, 1981. Raças exóticas e cruzamentos. In: Bovinos em Portugal (Rodrigos A, ed). Lisboa: Direcção Geral dos Serviços Veterinários.

Raymond M and Rousset F, 1995. GENEPOP (Version 1.2), population genetics software for exact tests and ecumenicism. J Hered 86:248–249.

Rouse J, 1977. The criollo, Spanish cattle in the Americas. Norman: University of Oklahoma Press.

Saitou N and Nei M, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Sanchez-Belda A, 1984. Razas bovinas Españolas. Publicaciones de Extensión Agraria. Madrid: Mapa.

Valle J, 1941. Gado bissulco. Lisboa: Livraria Sá Costa, Colecção a Terra e o Homem.

Wilkins J, 1984. Criollo cattle of the Americas. AGRI 10:1–19.

Zilhão J, 1993. The spread of agro-pastoral economies across Mediterranean Europe: a view from the far west. J Mediterr Arch 6(1):5–63.

Zilhão J, 1998. On logical and empirical aspects of the Mesolithic-Neolithic transition in the Iberian peninsula. Curr Anthropol 39(5):690–698.

Received October 11, 2002
Accepted January 30, 2003

Corresponding Editor: James Womack