

GENETIC VARIABILITY AND PHYLOGENETIC RELATIONSHIPS BETWEEN TEN NATIVE CATTLE BREEDS FROM GALICIA AND THE NORTH OF PORTUGAL

VARIABILIDAD GENÉTICA Y RELACIONES FILOGENÉTICAS ENTRE DIEZ RAZAS BOVINAS AUTÓCTONAS DE GALICIA Y REGIÓN NORTE DE PORTUGAL

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ADDITIONAL KEYWORDS

Native cattle. Biochemical polymorphism. Heterozygosity. Genetic relationships.

PALABRAS CLAVE ADICIONALES

Bovinos autóctonos. Polimorfismos bioquímicos. Heterozigosidad. Relaciones genéticas.

SUMMARY

A total of 450 animals belonging to ten different native cattle breeds from Galicia and the north of Portugal were examined. They were distributed as follows: Galega (60), Maronesa (58), Barrosã (56), Arouquesa (63), Mirandesa (70), Cachena (27), Caldelana (33), Limiana (26), Frieiresa (26) y Vianesa (31). Breeds with a small number of samples belong to populations with limited census and endangered. Eleven blood proteins that show polymorphism were chosen to develop the present study. Allelic frequencies for each system and breed were calculated. The estimated values of the average heterozygosity ranged from 0.193 (Mirandesa) to 0.311 (Maronesa). From the matrix of Nei's genetic distances obtained, cluster analysis (dendrogram) and ordination techniques (nonmetric multidimensional scaling analysis, MDS; principal coordinates analysis, PCA and minimum length spanning tree, MST) were used to establish the possible phylogenetic relationships among the ten cattle breeds investigated. Three different clusters have been revealed: Cluster I (it consists of Galega, Barrosã and Cachena

breeds, it's related to the prehistoric form *Bos desertorum*), Cluster II (Arouquesa, Frieiresa, Caldelana, Limiana, Mirandesa and Vianesa breeds were in this group, connected with the mutation form *Bos primigenius estrepserus*) and Cluster III (it consists of Maronesa breed which seems to be related to the prehistoric form *Bos brachycerus*).

RESUMEN

Se han estudiado un total de 450 animales pertenecientes a diez razas bovinas autóctonas de Galicia y Región Norte de Portugal que se distribuyen de la siguiente forma: Galega (60), Maronesa (58), Barrosã (56), Arouquesa (63), Mirandesa (70), Cachena (27), Caldelana (33), Limiana (26), Frieiresa (26) y Vianesa (31). Las razas con reducido número muestral pertenecen a poblaciones con escaso censo y en peligro de extinción. Para el desarrollo del presente estudio se eligieron once proteínas sanguíneas que

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muestran polimorfismo. Se han estimado las frecuencias alélicas para cada sistema genético y raza. La heterocigosis media estimada ha presentado un rango de valores comprendido entre 0,193 (Mirandesa) y 0,311 (Maronesa). A partir de la matriz de distancias genéticas de Nei obtenidas se han utilizado análisis de agrupamientos (dendrograma) y técnicas geométricas (escalograma multidimensional no-métrico, MDS; análisis de coordenadas principales, PCA y árbol de longitud mínima expandida, MST) para establecer las posibles relaciones filogenéticas entre las diez razas bovinas investigadas. Se han puesto de manifiesto tres grupos de poblaciones: Tronco I (constituido por las razas Galega, Barrosã y Cachena, relacionado con el tronco prehistórico *Bos desertorum*), Tronco II (formado por Arouquesa, Frieiresa, Caldelana, Limiana, Mirandesa y Vianesa, adscribiéndose a la forma mutante *Bos primigenius estrepiscerus*) y Tronco III (integrado por la raza Maronesa que derivaría del *Bos brachycerus*).

INTRODUCTION

The present dynamic of animal production has led to the reduction and even the extinction of many breeds, which means the consequent loss of their genes before their productivity and their response to genetic improvement have been known (Sánchez *et al.*, 1992).

On the other hand there has been lately an increasing interest in the preservation of local hardy breeds. The reasons for this are mainly two, firstly they may possess valuable gene combinations that might otherwise be irreversibly lost, and secondly, they represent part of cultural and historic heritage.

The study of protein polymorphisms, quantified by electrophoresis,

identifies the amount of between and within-breed genetic variability of a population and this is useful in elucidating the evolutionary relationship of organisms. Although the basic genetic change occurs in DNA, it is important to know the evolutionary change of proteins, since they are essential molecules for building morphological characters and carrying out physiological functions (Nei, 1987). Other methods, such as microsatellite analysis, are also currently being used in our laboratory.

The aim of the present study was to examine the population genetic structure of ten different native cattle breeds from the north-west of Iberian Peninsula using genetic markers, and to clarify their evolutionary history or phylogenetic relationships.

MATERIALS AND METHODS

BREEDS STUDIED

Cattle from ten different local breeds (450 animals) were included in the study. They were distributed as follows: Galega (60), Maronesa (58), Barrosã (56), Arouquesa (63), Mirandesa (70), Cachena (27), Caldelana (33), Limiana (26), Frieiresa (26) and Vianesa (31). Breeds with a small number of samples (less than 50) correspond to populations with limited census and endangered. The geographical distribution of the original breeds is shown in **figure 1**. Few studies concerning the origin of native cattle breeds from the northwest of Iberian Peninsula exist. Most hypotheses are based on those from the classic writers.

RELATIONSHIPS BETWEEN GALICIA-NORTH PORTUGAL NATIVE CATTLE



Figure 1. Geographical location of the ten native cattle breeds. (Localización geográfica de las diez razas bovinas autóctonas).

Cachena

This breed probably originated from *Bos desertorum* (Aparicio, 1960). Jordana *et al.* (1991) have included it in the Cantabrian group in accordance with qualitative and quantitative analyses.

Caldelana

Sánchez Belda (1984) includes this breed in the Iberian group as descendant of the *Bos taurus ibericus*.

Frieirisa, Limiana and Vianesa

According to some authors (Sánchez Belda, 1984; Jordana *et al.*, 1991) these breeds are included in the Cantabrian group.

Galega

Several authors (Bernardo de Lima, 1873; Tierno, 1908) consider the origin of this breed in the convex red cattle breeds, which might have descended from the crossing between *Bos primigenius estrepsicerus* or *Bos taurus ibericus* and *Bos primigenius hahni* (Bernardo de Lima, 1873; Miranda do Vale, 1907).

Maronesa

Certain hypotheses (Bernardo de Lima, 1873; Miranda do Vale, 1907) suppose that Maronesa cattle descends from the Barrosã breed, while Miranda do Vale (1949) supports the idea that the Maronesa was originated from matings between Barrosã and Mirandesa.

Barrosã

Miranda do Vale (1907) place the origin of the Barrosã in the Mauritanian group (*Bos primigenius mauritanicus*) which would have been brought to Iberian Peninsula during the Muslim invasion. Tierno (1908), however, considers that the origin of this breed may be intracontinental and a direct descendant of the Castilian group.

Arouquesa

This breed probably originated from matings between Atlantican and Aquitanian groups (Miranda do Vale, 1907). Machado and Pereira (1981) agree that this breed seems to be the result of a crossing in which characters of the Mauritanian, Iberian and Aquitanian groups are dominant.

Mirandesa

For Tierno (1904) the Mirandesa

breed descends from the Iberian group (*Bos taurus primigenius*). Nevertheless, Pereira (1978) supports the idea that the origin of this breed could be the result of crosses between *Bos taurus brachycerus* and *Bos taurus primigenius*.

BIOCHEMICAL POLYMORPHISMS

Eleven genetic systems were investigated: haemoglobin (Hb), NADH diaphorase (Dia), catalase (Cat), purine nucleoside phosphorylase (NP), carbonic anhydrase (CA), amilase I (AmI), ceruloplasmin (Cp), albumin (Alb), transferrin (Tf), post-transferrin (Ptf-2) and post-albumin (Pa) using starch and polyacrylamide gel electrophoresis. Biochemical polymorphisms were determined by means of these specific procedures: Hb (Braend, 1963), Dia and Cat (Cepica and Statil, 1978), NP and CA (Tucker et al., 1967), AmI (Trowbridge and Hines, 1979), Cp (Kristjansson, 1963), Alb (Krishnamurthy *et al.*, 1974) and Tf, Ptf-2 and Pa (Gahne *et al.*, 1977).

MEASUREMENT OF GENETIC VARIATION *Gene frequencies*

The allelic frequencies were calculated by direct counting, since the genotypes were known. Most protein polymorphisms detectable by electrophoresis are controlled by codominant alleles. In NP *locus* dominance relationships among alleles exist.

Genetic diversity

The genetic diversity of the populations examined was investigated using the heterozygosity per *locus* criterion

(Nei, 1978) which is defined as:

$$h_k = (1 - \sum_{i=1}^{l} p_i^2) 2N / 2N - 1$$

where p_i is the frequency of the i^{th} allele at a k^{th} *locus*, l is the number of alleles and N is the analyzed sample size.

The average heterozygosity (H) is estimated as the average of h_k over all *loci*. If r *loci* are examined, average heterozygosity is given by:

$$H = \sum_{k=1}^{k=r} h_k / r$$

Standard error (SE) may be obtained by:

$$SE = [\sum (h_k - H)^2 / r(r - 1)]^{1/2}$$

Genetic distances

Genetic distance (D) and identity (I) were calculated according to Nei (1972). It was tried to measure the number of aminoacid substitutions per locus (electrophoretically detected) occurred after divergence of the two populations under consideration.

$$D = - \log_e I$$

Graphical representations

Two graphical procedures were used to depict the genetic relationships among populations. A dendrogram was constructed from a matrix of genetic distances with the unweighted pair group method of arithmetic averages: UPGMA (Sneath and Sokal, 1973).

Two ordination techniques were used as an alternative method: non-metric multidimensional scaling

RELATIONSHIPS BETWEEN GALICIA-NORTH PORTUGAL NATIVE CATTLE

Table I. Polymorphic loci, alleles and allele frequencies in ten native cattle breeds from Galicia and the North of Portugal. (Loci polimórficos, alelos y frecuencias alélicas en diez razas bovinas autóctonas de Galicia y Norte de Portugal).

Locus	Allele	Breed									
		GA	MA	BA	AR	MI	CC	CA	LI	FR	VI
Hb	A	0.867	0.974	0.991	0.905	0.979	1.000	0.985	1.000	0.980	0.984
	B	0.133	0.026	0.009	0.095	0.021	0.000	0.015	0.000	0.020	0.016
Dia	F	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	S	1.000	0.957	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CA	F	0.267	0.328	0.215	0.183	0.014	0.259	0.121	0.058	0.173	0.113
	S	0.733	0.672	0.786	0.817	0.986	0.741	0.879	0.942	0.827	0.887
NP	H	0.173	0.318	0.187	0.174	0.000	0.161	0.063	0.060	0.101	0.016
	L	0.827	0.682	0.813	0.826	1.000	0.839	0.937	0.940	0.899	0.984
Aml	B	0.775	0.698	0.750	0.595	0.371	0.815	0.576	0.481	0.654	0.532
	C	0.225	0.302	0.250	0.405	0.629	0.185	0.424	0.519	0.346	0.468
CP	A	0.833	0.914	0.920	0.690	0.679	0.907	0.879	0.827	0.692	0.790
	C	0.167	0.086	0.080	0.310	0.321	0.093	0.121	0.173	0.308	0.210
Alb	F	0.975	0.948	0.982	1.000	0.993	0.982	0.985	0.885	0.942	0.952
	S	0.025	0.052	0.018	0.000	0.007	0.018	0.015	0.115	0.058	0.048
Tf	A	0.475	0.586	0.687	0.452	0.607	0.351	0.424	0.538	0.519	0.678
	D ₁	0.092	0.017	0.009	0.087	0.000	0.130	0.015	0.039	0.096	0.032
	D ₂	0.433	0.345	0.232	0.318	0.393	0.463	0.561	0.423	0.385	0.290
	E	0.000	0.052	0.072	0.143	0.000	0.056	0.000	0.000	0.000	0.000
Ptf-2	F	0.775	0.759	0.795	0.897	0.893	0.796	0.818	0.885	0.981	0.855
	S	0.225	0.241	0.205	0.103	0.107	0.204	0.182	0.115	0.019	0.145
Pa	F	0.075	0.526	0.143	0.150	0.157	0.223	0.303	0.250	0.250	0.113
	S	0.925	0.474	0.857	0.850	0.843	0.777	0.697	0.750	0.750	0.887

GA= Galega; MA= Maronesa; BA= Barrosá; AR= Arouquesa; MI= Mirandesa; CC= Cachena; CA= Caldelana; LI= Limiana; FR= Frieiresa; VI= Vianesa

analysis (MDS) and principal coordinates analysis (PCA). In practice the results of MDS and PCA are rather similar. However, the smaller inter-point distances tend to be preserved more faithfully in MDS than in PCA.

Minimum length spanning tree (MST) is useful for superimposition on ordination analysis to help detect local distortions, and for showing the nearest neighbors.

Computations for 3-Dimensional analysis were made using NTSYS package (Rohlf, 1992).

RESULTS

Twenty three alleles were found in the present investigation (table I). It is interesting to note that the allele Dia^S is present only in the Maronesa breed.

Table II. Estimated values for heterozygosity per locus, average heterozygosity and standard errors among ten native cattle breeds from Galicia and the North of Portugal. (Estimaciones de la heterozigosis por *locus*, heterozigosidad media racial y errores standard en diez razas bovinas autóctonas de Galicia y Norte de Portugal).

Locus	Breed									
	GA	MA	BA	AR	MI	CC	CA	LI	FR	VI
<i>Hh</i>	0.233	0.050	0.017	0.173	0.042	0.000	0.030	0.000	0.038	0.032
<i>Dia</i>	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>CA</i>	0.394	0.444	0.339	0.300	0.028	0.391	0.216	0.110	0.291	0.203
<i>NP</i>	0.289	0.437	0.307	0.289	0.000	0.275	0.119	0.114	0.185	0.032
<i>Anil</i>	0.351	0.425	0.378	0.485	0.470	0.307	0.496	0.509	0.461	0.506
<i>CP</i>	0.280	0.158	0.149	0.430	0.439	0.171	0.213	0.291	0.434	0.336
<i>Alb</i>	0.049	0.098	0.035	0.000	0.014	0.037	0.030	0.208	0.110	0.093
<i>Tf</i>	0.583	0.539	0.472	0.671	0.480	0.654	0.513	0.531	0.584	0.463
<i>Pif-2</i>	0.351	0.369	0.329	0.186	0.192	0.330	0.302	0.208	0.038	0.252
<i>Pa</i>	0.139	0.503	0.247	0.258	0.266	0.352	0.428	0.382	0.382	0.203
<i>H</i>	0.267	0.311	0.227	0.279	0.193	0.251	0.235	0.235	0.252	0.212
<i>SE</i>	0.054	0.060	0.074	0.066	0.065	0.065	0.061	0.060	0.065	0.056

GA= Galega; MA= Maronesa; BA= Barrosã; AR= Arouquesa; MI= Mirandesa; CC= Cachena; CA= Caldelana; LI= Limiana; FR= Frieiresa; VI= Vianesa

Of the 11 *loci* examined, 6 were polymorphic (using the 0,99 common allele frequency criterion) in all the studied populations. The other 5 *loci* were monomorphic for all the breeds (Cat) or for some of them (Hb, Dia, NP, and Alb).

The values for average heterozygosity (**table II**) found in the examined populations ranged from 0.193 to 0.311, though they are within the values reported for other Spanish cattle breeds (Vallejo *et al.*, 1990).

From the matrix of genetic distances (**table III**) it can be observed that:

- the values found in the present

investigation, ranging between 0.0057 (Arouquesa/Frieiresa) and 0.0610 (Maronesa/Mirandesa), indicate a small genetic divergence among the breeds considered.

- according to Nei (1976) the values for genetic distance obtained are within the range of values existing among breeds.

- the highest values were found when the Maronesa was compared to the other breeds, and they ranged from 0.0610 (Maronesa/Mirandesa) and 0.0215 (Maronesa/Cachena).

Based on these values a UPGMA dendrogram was constructed (**figure 2**).

RELATIONSHIPS BETWEEN GALICIA-NORTH PORTUGAL NATIVE CATTLE

Table III. Genetic distance (above diagonal) and genetic identity (below diagonal) of ten native cattle breeds from Galicia and the North of Portugal. (Distancias e identidades genéticas obtenidas en diez razas bovinas autóctonas de Galicia y Norte de Portugal).

	GA	MA	BA	AR	MI	CC	CA	LI	FR	VI
<i>Galega</i>		0.0333	0.0092	0.0121	0.0375	0.0068	0.0182	0.0254	0.0164	0.0186
<i>Maronesa</i>	0.9673		0.0224	0.0361	0.0610	0.0215	0.0256	0.0356	0.0311	0.0431
<i>Barrosã</i>	0.9909	0.9778		0.0153	0.0347	0.0120	0.0201	0.0213	0.0174	0.0129
<i>Arouquesa</i>	0.9880	0.9646	0.9848		0.0155	0.0174	0.0152	0.0131	0.0058	0.0101
<i>Mirandesa</i>	0.9632	0.9408	0.9659	0.9846		0.0453	0.0174	0.0069	0.0159	0.0068
<i>Cachena</i>	0.9932	0.9787	0.9880	0.9827	0.9557		0.0127	0.0248	0.0164	0.0261
<i>Caldelana</i>	0.9819	0.9747	0.9801	0.9849	0.9828	0.9557		0.0057	0.0116	0.0436
<i>Limiana</i>	0.9749	0.9650	0.9789	0.9869	0.9930	0.9755	0.9943		0.0091	0.0058
<i>Frieiresa</i>	0.9837	0.9694	0.9827	0.9942	0.9842	0.9837	0.9887	0.9909		0.0100
<i>Vianesa</i>	0.9815	0.9578	0.9872	0.9899	0.9932	0.9742	0.9865	0.9942	0.9900	

DISCUSSION

The results of the present investigation indicate the existence of certain genetic divergence among the populations studied. Average hetero-

zygosity levels were, in general, larger for Galicia breeds than for Portuguese breeds. In those populations, genetic diversity has clearly been constrained by their small effective size.

In previous studies, Aparicio (1960)

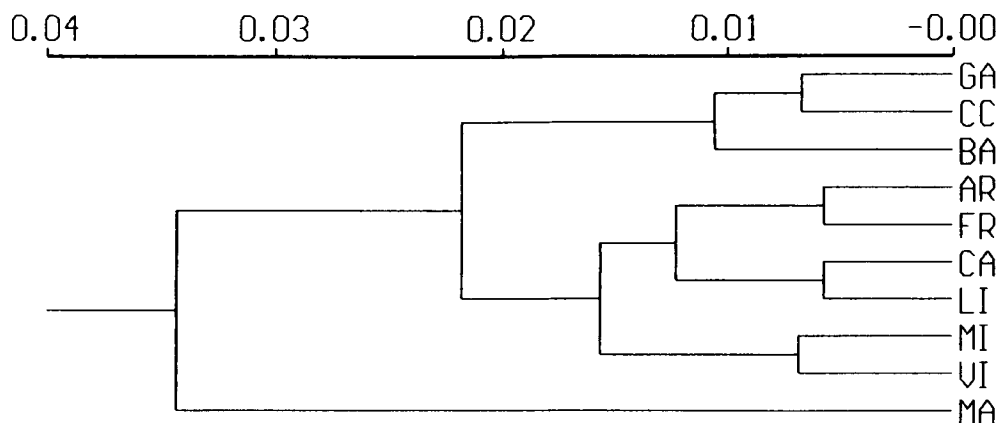
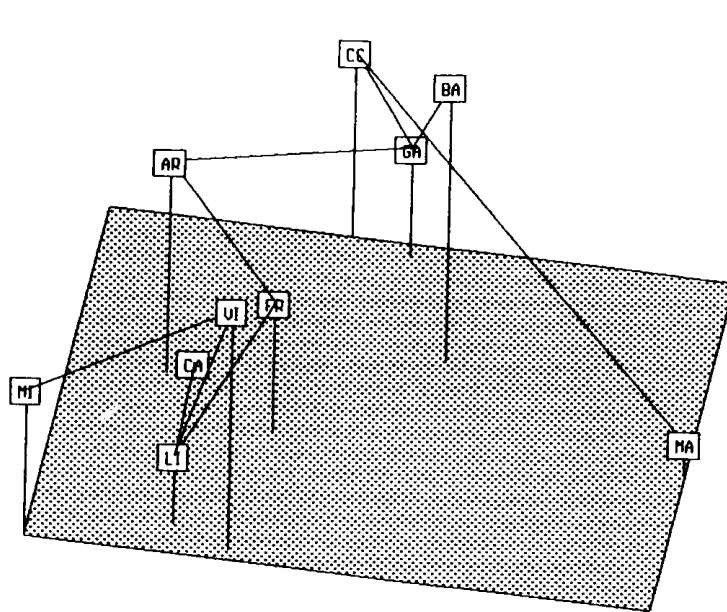


Figure 2. Dendrogram constructed by the UPGMA method and based on data of ten native cattle breeds from Galicia and North of Portugal. (Dendrograma construido según el método UPGMA y basado en datos de diez razas bovinas autóctonas de Galicia y Región Norte de Portugal). GA=Galega, MA=Maronesa, BA=Barrosã, AR=Arouquesa, MI=Mirandesa, CC=Cachena, CA=Caldelana, LI=Limiana, FR=Frieiresa y VI=Vianesa.



a=171 b= 46 r=99.0

Figure 3. Perspective view of 3-dimensional space showing the results of nonmetric multidimensional scaling analysis (MDS), with superimposition of minimum length spanning tree (MST). a= rotation angle around the Z-axis in degrees; b =tilt angle around the X-axis in degrees; r=distance from the observer to the object. (Representación tridimensional del análisis MDS con superimposición del MST. a= ángulo de rotación alrededor del eje Z en grados; b= ángulo de inclinación alrededor del eje X en grados; r= distancia entre el observador y el objeto).

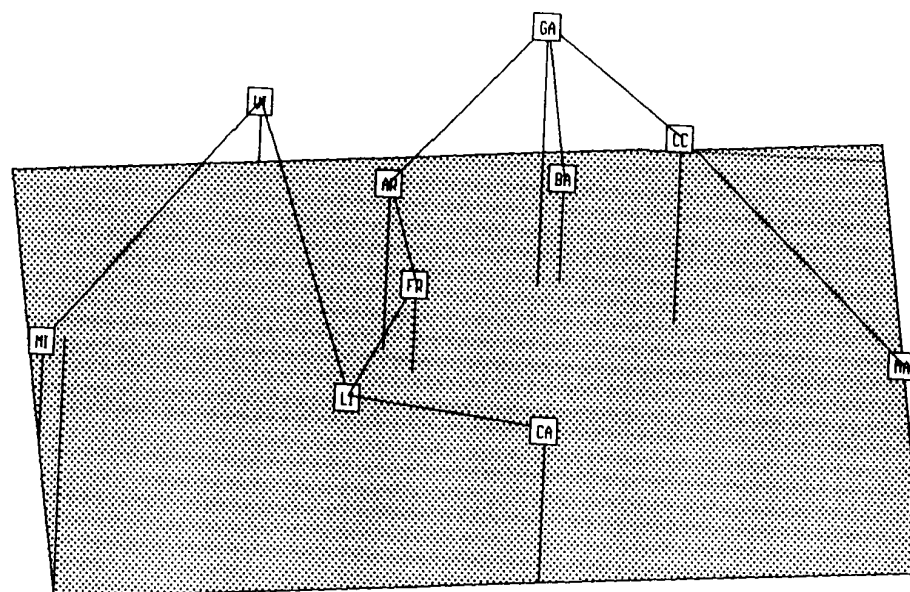
suggested that the Iberian cattle originated from two well differentiated groups: one from *Bos desertorum* and the other influenced by *Bos taurus primigenius* and *Bos taurus brachycerus*. Nevertheless, other investigations (Vallejo *et al.*, 1990) indicate the great difficulties of quantification due to the closeness of evolutionary distances studied.

The high degree of genetic similarity among cattle populations could cause distortions using cluster analysis. This can be avoided using ordination methods (PCA and MDS) optimized

with MST (**figures 3 and 4**). The results obtained show the agreement among the different graphic analyses from Nei's matrix of genetic distances. The hypothesis of phylogenetic relationships among cattle populations from the north-west of Iberian Peninsula and primitive cattle, tries to establish possible solutions. The dendrogram showed three major clusters:

Cluster I.- Galega (GA), Barrosã (BA) and Cachena (CC) breeds are included in this group. They could be connected with *Bos desertorum* whose

RELATIONSHIPS BETWEEN GALICIA-NORTH PORTUGAL NATIVE CATTLE



a= 6 b= 42 r=99,0

Figure 4. Graphical representation of principal coordinates analysis (PCO) obtained from the matrix of Nei's genetic distances, with superimposition of minimum length spanning tree (MST). (Representación gráfica del análisis PCO con superimposición del MST).

most significant characteristics were high-lyred horns and light coats (Aparicio, 1960; Sotillo and Serrano, 1985), the same described at present for Barrosã and Cachena breeds. This morphological similarity could be useful to classify breeds. In this way, Jordana *et al.* (1991) establish phylogenetic relationships of Spanish breeds using qualitative and quantitative analyses of data from morphological traits.

It is accepted the primigenic origin of the Galega breed. The results support the idea of a possible influence of the *Bos desertorum* with the contribution of the Barrosã breed in the last period. So, the origin of this Galega breed

cluster may be due to the limited area of expansion and to the closeness of Barrosã breed, which during the second half of last century showed a large expansion, due to a strong export trade to Great Britain, which meant the substitution of the Galega breed or the intensive crossing with it.

Cluster II. - It consists of Arouquesa (AR), Frieiresa (FR), Caldelana (CA), Limiana (LI), Mirandesa (MI) and Vianesa (VI) breeds.

Although in the dendrogram the Arouquesa breed (light coat) are surprisingly well differentiated from the Galega group, they are connected on the MST as nearest neighbors.

The Mirandesa samples are clearly

differentiated from the Galicia populations (Frieiresa, Caldelana, Limiana and Vianesa) but are connected to them on the MST.

The pattern of differentiation which emerged from our study is congruent with the phenotypic classification.

This cluster should originate from the mutant *Bos primigenius estrepiscerus* or *Bos taurus ibericus*.

Cluster III.- The Maronesa (MA) breed is the only one included in this group. The graphical representations indicated that separation of the Maronesa from the other breeds took place early from a hypothetical common. This breed might have been

originated from *Bos taurus brachycerus* and its divergence from the other breeds could be either the result of genetic drift or due to the isolation of the breed in a small mountain area.

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RELATIONSHIPS BETWEEN GALICIA-NORTH PORTUGAL NATIVE CATTLE

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