"GENETIC RELATIONSHIPS BETWEEN POPULATIONS OF ANDALUSIAN BOVINE LOCAL BREEDS IN DANGER OF EXTINCTION FROM THE POLYMORPHISM OF MICROSATELLITES OF ADN"

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SUMMARY

Within a wider program of characterisation and evaluation of the Black and Red Berrenda, the Pajuna and Cárdena breeds aimed at their conservation and improvement, a genetic study using 31 microsatellite DNA markers was included.

191 animals of the Black Berrenda breed belonging to 25 farms and 170 animals of the Red Berrenda, belonging to 23 farms as well as 51 animals of the Pajuna breed from 3 farms and 14 animals of the Cárdena breed were genotyped.

These are breeds which are presently endangered, according to our census; they are raised on open range systems in Andalusia, Extremadura, Madrid, Castilla-La Mancha and Castilla-Leon. They are used chiefly in meat production as well as in bullfighting and as draught animals in religious processions.

All the loci were polymorphic in all breeds and even though some othem were studied with a small sample, drift did not seem to play an important role. The differences among the heterocygosities seem to demonstrate that the Wahlund effect was not intense. When the genetic distances were estimated among the four breeds, its was seen that the Berrenda and the Pajuna breeds were near each other and far from the Cárdena breed.

The genetic differences among the four breeds are very small and clearly less than the differences within breeds. This cannot be understood as a consequence of the result of cross-breeding of individuals with outside breeds since the farms participating in this study have closed reproduction structures.

KEY WORDS: genetic variability, cattle breeds genetic markers

INTRODUCTION

Rodero et al., (1992) and (1994) assigned an index of priorities to Andalusian cattle breeds which were considered endangered in order to guarantee their conservation. In this paper, four cattle breeds having the highest index of priority were chosen: the Andalusian Cárdena, the Pajuna, the Black and the Red Berrenda. Their imporatnce as ranch breeds raised on open ranges in mountain areas was made clear in papers presented by Azor et al. (2003) and by Molina et al., (2003) at the International Sympòsium of Animal Production and Natural Resources in Mediterranean Mountain Areas held recently in Ioannina (Greece). This same importance is also clear for cultural reasons due to the fact that these breeds are used in popular bullfighting, both in the bull ring as well as in the country. They are also used as draught animals for pulling the carriages in religious pilgrimages which play an important role in traditional Andalusian religious celebrations (Rodero et al., 1989).

FAO (1998) points out, in their second document on standards for the elaboration of national management plans for animal genetic resources, that: "At least during the first years of the establishment of a conservation program, it may be useful to associate some research activities to this program in order to widen the knowledge about the breed; that is to say, in order to improve the characterisation of its phenotype and its genotype........."

For Cardellino (2002) the special studies that should complement the World Strategy for Zoo-genetic Resources should include those suggested by MODAD in order to analyze the molecular diversity of domestic animals and to establish genetic distances, markers and micro-satellites.

To do all this, one of the first actions in a breed conservation program consists in the evolution of its genetic variability and the distribution of this variability among the populations.

Among the parameters that have most been used in estimating the genetic variability of small populations has traditionally been the expected heterocygosity (He) and the number of alleles per locus (Na). According to Petit et al.(1998), a wealth of alleles is preferible, 'but Chikhi et al. (s.d.) bet again for the He as it is less affected by the unequal effect occuring in sample size. Likewise Caballero and Toro (2002) agree, and they chose the He proposed by Nei (1973) as the means of genetic diversity. However they estimate that the measurement of allele diversity is also useful in endangered populations as it is more sensitive to the bottle-neck effect originating in the past through fluctuations in population size.

Furthermore, the Wright F statistics (Fit, Fst and Fis) allow analyzing the variability both within and among the samples and allow detecting the excess or deficit of heterocygotes depending on the effects of the non-aleatory reproductive systems. These same parameters are estimated by Weir and Cockerham (1984) through the Capf, Theta and Smallf pondering the allele frequencies for sample size while part of the supposed size equality in the different samples

When some of the Andalusian cattle breeds were studied from this point of view, (Rodero et al.,2003) it was done individually, not taking into account interactions. In this paper, we are attempting to deal with information from each of the breeds in an overall way and to establish also the relationships among them through the corresponding parameters that evaluate the genetic differentiation.

MATERIAL AND METHODS

262 animals of the Black Berrenda breed, belonging to 25 farms, 170 animals of the Red Berrenda breed, belonging to 23 farms along with 51 animals of the Pajuna breed coming from 3 groups and 14 animals of the Cárdena breed were genotyped.

31 polymorphic loci from the DNA of the microsatellites were analyzed in order to make it possible to undertake later comparative studies. These markers were chosen by following the recommendations of FAO (MODAD), ISAG and those used in the European Project For Biodiversity in Cattle (http://www,ri.bbsrc.ac.uk/cdiv_www/infor,htm). This high number of loci permitted obtaining a reliability factor higher than 90%, as estimated by Baker and Manwel (1991)

The DNA was extracted from blood samples using the Kit BLOODCLEAN from BIOTOOLS laboratories, following the recommendations of the manufacturer for blood samples.

The amplification through a polymerase chain reaction (PCR) was done in a thermocyclator PTC-100 (MJ-Research) using various Multiplex reactions. To do this, primers stained with fluorochrome were used which allowed allele characterisation with an automatic sequencer, "ABI 373 Stretch. Through the Genescan Analysis 3.1.2. program, the data collected by the automatic sequencer were analyzed and using the Genotyper 2.5.2. program, the different alleles present on each of the micro-satellites were identified.

The programs used to calculate the different parameters for measuring DNA variability and for analyzing the genetic structure of each population were FSTAT and Genetix.

RESULTS AND DISCUSSION

Genetic Variability of Breeds.

The calculation of the allele frequencies and the number of alleles found per locus in each breed (Table 1) demonstrated that all loci were polymorphic in all breeds studied, obtaining a total of 321 alleles for the 31 loci. The least polymorphic locus was ILSTS5 for which only two alleles were found but they were present in all 4 breeds. The most common allele frequency was not very high (a maximum 0.8443 for allele 100 of the INRA35 locus in the Red Berrenda) so there seems to be no tendency to fix any of the allelles found. This seems to indicate that drift did not play an important role in these endangered populations.

A recount was taken of the private alleles, referring to those that were present in only one breed and the percentage that these represented was calculated (Table 1).

The data indicated the differences among the 4 breeds as to their allele constitution. Even though this type of allele must be taken into consideration, their frequency was so low that it suggests that no close relationship occured among the individuals of these breeds. It should be pointed out that the alleles found in the Cárdena breed for the BM2113 and BM1818 loci were of a private character with relation to the other Andalusia breeds.

Table 1 also shows the average number of alleles per locus. When comparing them with the figures obtained by McHunght (2000) for a total of 20 loci from 20 cattle breeds, British (5.2 alleles per locus), European (6.3), African (6.4) and Zebuinas (7), a higher number of alleles per locus were found in the two Berrenda breeds, whereas the values in the Pajuna and Cárdena breeds were very close to those found in the European breed.

Nevertheless, the differences may have been influenced by the different sample sizes. For that reason, we also obtained allele richness (Table 2) where it can easily be seen that Pajuna had the least allele wealth for each locus and the least average value per loci in this parameter. This is especially notable since the Cárdena animals sampled all came from the same herd.

Nei estimates of Heterocygosity.

Table 3 shows the expected heterocygosity observed in each breed.

All four breeds had very similar values in these statistics although the Pajuna breed had somewhat lesser values compared to the other three breeds.

Overall values found both for the average of all loci as well as those found for each locus were high, reaching more than 0.5 and 1, with the exceptions of loci ILSTS5 and INRA35 where the values were in all cases less that 0.5.For the Pajuna breed, locus CSRM60 was also inferior. It should be kept in mind that the number of alleles affects the results of heterocygosity and, in this case, the loci showing an atypical behaviour were those with the least number of alleles.

In all breeds the average value of Hnb (H sin sesgo) only slightly surpassed Ho which seems to indicate that if there was any inbreeding or Whalund effect, it was very slight.

The Nei estimates for Heterocygosity for all breeds studied both for each locus as well as for total loci are shown in Table 4. The total proportion observed of heterocygotes (Ho) had a value equal to 0.663 which is clearly below the heterocygosity calculated according to gene diversity within a breed (hs) which value is 0.714, also below the estimate for total gene diversity (Ht=0.742. All this seems to indicate that the heterocygosity found was caused more by in-breed effect than by racial effect

When these parameters were analyzed for the different loci, the aforementioned atypical behaviour of Loci ILSTS5 and INRA35 was confirmed.

The parameters of gene diversiy within breeds (dst and D'st) showed an average of 0.028 and 0.032 for an uncorrected and corrected variable depending on the sample size, respectively. That is, a very small value for variability existing in each breed. The same thing occurred in each locus although the greatest value was in the loci BM1818 (0.106 and 0.142) and INRA37 (0.089 and 0.118) in accordance with those that showed the greatest proportion of private alleles (Table 2).

This was also confirmed by the calculations made for Gst and G'st, these also being the estimators of Fst or of the differences and distances among the breeds

The overall result of the Gis (Fis) =0.070 indicates an average level of inbreeding in all the breeds.

Wright, Weir and Cockerham F values

The reults previously obtained were completed or made clear by the data from the Wright F statistics.

In first place, the Fis values for each and every loci each breed were calculated (Table 5). The lowest values were shown by the Pajuna breed (0.041) and the highest by the Black Berrenda (0.096). Nevertheless, as far as the respective size for each of these breeds goes (Rodero et al., 2002), the increase of expected inbreeding in Andalusian cattle breeds should be calculated from 3000 animals and, following this criterion, the Pajuna appeared to be the breed with the highest risk.

Once again the INRA35 locus wass atypical, giving extraordinarily high figures for Fis that range from 0.342 in the Black Berrenda to 0.626 in the Cárdena, the latter also showing an excess of heterocygotes on a greater number of loci in function of a negative sign of the values found since 10 of the 31 loci analyzed had a negative Fis value. This fact is especially remarkable, taking into account that a very small sample size was taken belonging to one single herd existing of this breed that only has 15 studs. It is the smallest of all Andalusian cattle breeds.

Table 6 shows the values of the Weir and Cockerham (1984) parameters for all loci. These parameter values corresponded to the Wright F values. The result of Theta (Fst) for the total prove that the differences among the breeds are very slight (2.3%). The Sig show that the main source of variation in the analysis of variance was within individuals and the smallest was among the breeds. For this reason, the average relationships of individuals within samples or breeds with the total (Relat) was also estimated, rendering a value of 0.042 which, when corrected for in-breeding effect, gave a value of Relat= -0.108. Thus, it seems that in-breeding did not substantially alter the relationships.

Taking into account the scarce number of breeds that were studied in this paper, the standard errors were estimated for these parameters by Jackknifing (Table 7) and the statistical significance through the Bootstrapping method with the following Intervals of reliability for 95% :

Capf: 0.081- 0.129

Theta: 0.017- 0.030

Smallf: 0.063- 0.106

Relat: 0.031- 0.053

Nei Distances among breeds

The Nei distances (1972) shown in Table 8 and graphically in the cluster in Figure 1 confirm what waa already detectable from the parameters of differentiation among previously discussed populations (DstGst, and Fst): the greatest proximity was between the two Berrenda breeds and the greatest degree of distance was between the Cárdena and the other breeds studied, especially the Pajuna. The relationship of genetic differences corresponded to the relationship with the geographical spatial distances in the occupying areas of the breed in question. The isolation mechanism (Dobzansky, 1951) that coincided in the differentiation of these breeds might initially have been of a spatial or geographical nature and later could have been strengthened by the consequential reproductive isolation resulting from the situation.

CONCLUSIONS

1. Although breeds having a low census were studied in this work, it is not believed that their endangered nature is due to effects such as Wahlund, drift, or to in-breeding but rather to the reproductive structure of these animals.
2. It should be pointed out that in the Cárdena breed all the alleles of the two loci were of a private character with relation to the other breeds. This is in accord with the results of the genetic distribution meaning that this breed is clearly different from the other three breeds and this is further justified by its different origin.
3. The genetic differences among the four breeds are very small and clearly less than the differences within breeds. This cannot be understood as a consequence of the result of cross-breeding of individuals with outside breeds since the farms participating in this study have closed reproduction structures.

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TABLES AND FIGURES.

Table 1 Number of alleles (Na) found in each locus and in the total and percentage of private alleles (np) for each of the endangered Andalusian cattle breeds and for the total population studied.

Locus	**BC**		**BN**		**Cárdena**		**Pajuna**		**Total**
	na	**np**	**na**	**np**	**na**	**np**	**na**	**np**	
N	**168**		**262**		**14**		**51**		**386**
BM1824	4	0.00	6	33.33	5	0.00	5	0.00	**7**
BM2113	12	8.30	12	8.30	6	100.00	10	0.00	**19**
ETH10	7	0.00	8	12.50	6	0.00	7	0.00	**8**
ETH31	9	0.00	10	10.00	5	0.00	6	0.00	**10**
HAUT27	9	0.00	11	18.18	7	0.00	8	0.00	**11**
HEL5	9	0.00	10	0.00	7	28.57	8	0.00	**11**
ILSTS5	2	0.00	2	0.00	2	0.00	2	0.00	**2**
TGLA12	17	5.88	18	0.00	8	0.00	8	0.00	**19**
TGLA22	12	8.30	12	0.00	9	0.00	2	0.00	**13**
BM1314	9	11.11	8	0.00	5	0.00	7	0.00	**9**
BM1818	6	0.00	8	25.00	6	100.00	6	0.00	**14**
ETH152	9	11.11	8	0.00	6	0.00	6	0.00	**9**
ETH225	6	16.66	6	0.00	5	0.00	6	0.00	**8**
HAUT24	6	0.00	6	0.00	5	0.00	6	0.00	**6**
ILSTS6	10	10.00	9	0.00	7	14.28	6	0.00	**11**
INRA5	5	20.00	4	0.00	3	0.00	3	0.00	**5**
INRA63	6	0.00	7	14.28	4	0.00	5	0.00	**7**
SPS115	6	0.00	7	0.00	7	0.00	6	0.00	**7**
TGLA12	9	22.22	7	0.00	4	0.00	7	0.00	**9**
CSRM60	8	12.50	7	0.00	6	0.00	2	0.00	**8**
CSSM66	12	16.66	12	18.33	8	0.00	11	9.00	**15**
HEL13	5	0.00	6	16.66	4	0.00	5	0.00	**6**
HEL9	11	18.18	11	9.09	5	0.00	9	0.00	**13**

INRA23	12	25.00	10	0.00	6	0.00	9	0.00	**13**
INRA37	11	0.00	12	0.00	5	0.00	8	0.00	**13**
TGLA53	17	11.76	15	0.00	9	0.00	11	0.00	**17**
ETH185	8	0.00	9	0.00	8	12.50	10	0.00	**13**
HEL1	8	25.00	7	28.57	5	20.00	4	0.00	**11**
INRA32	7	0.00	7	0.00	6	0.00	6	0.00	**7**
INRA35	6	0.00	8	25.00	3	0.00	6	0.00	**9**
MM12	11	0.00	10	0.00	9	0.00	10	0.00	**11**
Na/np total	**269**	**22**	**273**	**17**	**181**	**17**	**205**	**3**	**321**
na medio por locus / % np total	**8.67**	**6.98**	**8.80**	**6.22**	**5.83**	**9.39**	**6.61**	**1.46**	**10.35**

Na: número de alelos; np: alelos privados

Table 2. Allelic Richness on each locus and average allele wealth in the total loci for each of the endangered Andalusian cattle breeds and for the total population studied .

Locus	**BC**	**BN**	**Cárdena**	**Pajuna**	**Total**
BM1824	2.695	2.728	2.759	2.715	2.731
BM2113	3.299	3.062	3.036	3.169	3.258
ETH10	2.705	2.915	2.871	2.911	2.874
ETH31	2.917	2.882	2.176	2.432	2.846
HAUT27	2.431	2.575	2.869	2.583	2.566
HEL5	2.818	2.683	3.011	2.738	2.790
ILSTS5	1.788	1.730	1.868	1.882	1.779
TGLA12	3.091	3.136	3.347	3.081	3.183
TGLA22	3.324	3.277	3.289	2.000	3.310
BM1314	2.811	2.835	2.647	2.673	2.822
BM1818	2.520	2.760	2.825	2.580	2.759
ETH152	2.722	2.774	2.718	2.789	2.770
ETH225	2.822	2.661	2.882	2.547	2.774
HAUT24	2.893	2.822	2.171	2.816	2.872
ILSTS6	2.958	3.033	2.855	2.844	3.049
INRA5	2.376	2.357	2.362	2.100	2.396
INRA63	2.377	2.227	2.110	2.383	2.299
SPS115	2.126	2.318	2.897	2.078	2.250
TGLA12	2.792	2.751	2.553	2.695	2.755
CSRM60	2.834	2.787	2.659	1.372	2.773
CSSM66	3.251	3.260	3.222	3.131	3.288
HEL13	2.159	2.398	2.099	2.475	2.331
HEL9	3.015	2.781	2.663	2.898	2.920
INRA23	3.014	2.935	2.812	2.883	2.964
INRA37	2.837	2.748	2.331	2.332	2.856
TGLA53	3.362	3.256	3.299	3.221	3.321
ETH185	2.868	2.641	3.189	2.976	2.798
HEL1	2.546	2.587	2.754	2.463	2.617
INRA32	2.533	2.469	2.993	2.628	2.529
INRA35	1.567	1.880	1.764	1.822	1.770
MM12	3.032	2.889	3.309	2.986	2.967
ALL	2.725	2.715	2.721	2.587	2.749

Tabla 3. Heterocygosity observed (Ho) and expected (Hex) in each locus and in each of endangered Andalusian cattle breeds. Hnb Heterocygosity non biased for size N

	BC			**BN**			**C**			**P**		
	Hex	**Hnb**	**Ho**	**Hex**	**Hnb**	**Ho**	**Hex**	**Hnb**	**Ho**	**Hex**	**Hnb**	**Ho**
BM1824	0.7362	0.7384	0.7066	0.7457	0.7471	0.7577	0.7245	0.7513	0.7857	0.7352	0.7430	0.7500
BM2113	0.8676	0.8701	0.7440	0.8108	0.8123	0.7050	0.7883	0.8175	0.9286	0.8354	0.8437	0.8235
ETH10	0.7353	0.7375	0.7246	0.7843	0.7858	0.7287	0.7500	0.7778	0.5714	0.7776	0.7853	0.7255
ETH31	0.7789	0.7812	0.7636	0.7746	0.7761	0.7088	0.5459	0.5661	0.5714	0.6405	0.6469	0.6667
HAUT-27	0.6199	0.6217	0.5389	0.6614	0.6627	0.4883	0.7398	0.7672	0.7143	0.6740	0.6822	0.4762
HEL-5	0.7528	0.7550	0.7083	0.7195	0.7209	0.6947	0.7781	0.8069	0.7143	0.7305	0.7377	0.5686
ILSTS5	0.4418	0.4431	0.4192	0.4053	0.4061	0.3740	0.4770	0.4947	0.3571	0.4998	0.5048	0.5000
TGLA12	0.8204	0.8229	0.8204	0.8306	0.8322	0.7529	0.8520	0.8836	0.7143	0.8162	0.8243	0.7451
TGLA22	0.8738	0.8764	0.8024	0.8643	0.8660	0.8154	0.8367	0.8677	0.8571	0.3750	0.5000	0.5000
BM1314	0.7517	0.7540	0.6607	0.7636	0.7650	0.7023	0.6888	0.7143	0.6429	0.7203	0.7274	0.6667
BM1818	0.6753	0.6773	0.6190	0.7510	0.7525	0.6409	0.7372	0.7646	0.7143	0.6920	0.6989	0.7647
ETH152	0.7263	0.7286	0.6728	0.7465	0.7482	0.6637	0.7143	0.7407	0.7857	0.7514	0.7589	0.7647
ETH225	0.7614	0.7636	0.7440	0.7247	0.7261	0.6395	0.7577	0.7857	0.8571	0.6724	0.6791	0.6863
HAUT-24	0.7775	0.7798	0.7365	0.7641	0.7656	0.6346	0.5102	0.5291	0.5714	0.7507	0.7586	0.7708
ILSTS6	0.7949	0.7972	0.7679	0.8093	0.8109	0.7814	0.7398	0.7672	0.7857	0.7593	0.7668	0.6471
INRA5	0.6484	0.6504	0.6646	0.6448	0.6461	0.5212	0.6301	0.6534	0.5714	0.5321	0.5379	0.5106
INRA63	0.6387	0.6406	0.5238	0.5985	0.5997	0.5326	0.5179	0.5370	0.3571	0.6466	0.6531	0.5600
SPS115	0.5104	0.5119	0.5179	0.5802	0.5813	0.5517	0.7449	0.7725	0.7143	0.4898	0.4947	0.4706
TGLA12	0.7554	0.7576	0.7066	0.7461	0.7476	0.7280	0.6811	0.7063	0.6429	0.7276	0.7349	0.8000
CSRM60	0.7610	0.7633	0.7246	0.7501	0.7515	0.7088	0.6811	0.7063	0.5714	0.1884	0.1935	0.2105
CSSM66	0.8587	0.8614	0.8519	0.8604	0.8620	0.8314	0.8265	0.8571	0.8571	0.8254	0.8337	0.7000
HEL13	0.5394	0.5410	0.4606	0.6394	0.6407	0.5615	0.4974	0.5159	0.6429	0.6515	0.6586	0.6304
HEL9	0.8056	0.8080	0.6707	0.7397	0.7412	0.6808	0.7066	0.7328	0.6429	0.7689	0.7765	0.7451
INRA23	0.8068	0.8092	0.7622	0.7843	0.7858	0.7782	0.7372	0.7646	0.8571	0.7628	0.7711	0.8043
INRA37	0.7643	0.7668	0.5548	0.7386	0.7401	0.5346	0.6020	0.6243	0.5000	0.5858	0.5918	0.5102
TGLA50	0.8807	0.8836	0.8000	0.8564	0.8581	0.7647	0.8418	0.8730	0.8571	0.8444	0.8539	0.8667
ETH185	0.7751	0.7774	0.7844	0.7122	0.7136	0.6996	0.8050	0.8474	0.8000	0.7874	0.7954	0.7800
HEL1	0.6892	0.6913	0.6871	0.7067	0.7081	0.6641	0.7219	0.7487	0.6429	0.6645	0.6714	0.7959
INRA32	0.6737	0.6758	0.6296	0.6600	0.6613	0.6423	0.7781	0.8069	0.6429	0.7049	0.7122	0.7755
INRA35	0.2771	0.2779	0.1677	0.4126	0.4134	0.2720	0.3597	0.3730	0.1429	0.3845	0.3883	0.2157
MM12	0.8064	0.8088	0.7784	0.7682	0.7696	0.6973	0.8418	0.8730	1.0000	0.7918	0.7997	0.8431
Media todos loci	0.7195	0.7217	0.6682	0.7211	0.7225	0.6534	0.6972	0.7234	0.6779	0.6705	0.6814	0.6540

Table 4. Nei estimates of heterocygosity in each locus and in the total endangered Andalusian cattle breeds and for the total population studied.

LocName	**Ho**	**Hs**	**Ht**	**Dst**	**Dst'**	**Ht'**	**Gst**	**Gst'**	**Gis**
BM1824	0.750	0.745	0.757	0.012	0.016	0.761	0.016	0.021	-0.007
BM2113	0.800	0.837	0.909	0.073	0.097	0.934	0.080	0.104	0.043
ETH10	0.688	0.773	0.784	0.011	0.015	0.787	0.014	0.019	0.110
ETH31	0.678	0.694	0.707	0.013	0.017	0.711	0.018	0.024	0.023
HAUT27	0.554	0.684	0.695	0.011	0.015	0.699	0.016	0.021	0.190
HEL5	0.671	0.756	0.802	0.046	0.062	0.818	0.058	0.076	0.112
ILSTS5	0.413	0.462	0.470	0.007	0.010	0.472	0.015	0.020	0.108
TGLA12	0.758	0.841	0.880	0.039	0.052	0.893	0.044	0.058	0.099
TGLA22	0.744	0.800	0.865	0.066	0.087	0.887	0.076	0.098	0.070

BM1314	0.668	0.741	0.753	0.011	0.015	0.757	0.015	0.020	0.099
BM1818	0.685	0.724	0.830	0.106	0.142	0.865	0.128	0.164	0.054
ETH152	0.722	0.744	0.748	0.004	0.005	0.749	0.005	0.007	0.030
ETH225	0.732	0.738	0.797	0.059	0.079	0.817	0.074	0.096	0.009
HAUT24	0.678	0.710	0.745	0.035	0.047	0.757	0.047	0.062	0.045
ILSTS6	0.746	0.786	0.846	0.059	0.079	0.865	0.070	0.092	0.052
INRA5	0.567	0.623	0.690	0.067	0.089	0.712	0.097	0.125	0.089
INRA63	0.493	0.610	0.615	0.006	0.008	0.617	0.009	0.012	0.191
SPS115	0.564	0.589	0.599	0.010	0.013	0.602	0.016	0.022	0.043
TGLA12	0.719	0.737	0.737	-0.000	-0.000	0.737	-0.000	-0.001	0.024
CSRM60	0.554	0.606	0.663	0.057	0.075	0.681	0.085	0.111	0.086
CSSM66	0.810	0.854	0.866	0.012	0.016	0.870	0.014	0.018	0.052
HEL13	0.574	0.590	0.593	0.003	0.004	0.594	0.005	0.007	0.027
HEL9	0.685	0.766	0.781	0.016	0.021	0.787	0.020	0.026	0.106
INRA23	0.800	0.783	0.786	0.004	0.005	0.788	0.005	0.006	-0.023
INRA37	0.525	0.683	0.772	0.089	0.118	0.802	0.115	0.148	0.232
TGLA53	0.822	0.868	0.871	0.003	0.005	0.872	0.004	0.005	0.053
ETH185	0.766	0.783	0.791	0.008	0.010	0.793	0.010	0.013	0.022
HEL1	0.698	0.705	0.754	0.049	0.066	0.770	0.065	0.085	0.010
INRA32	0.673	0.714	0.717	0.003	0.004	0.718	0.005	0.006	0.058
INRA35	0.200	0.365	0.366	0.000	0.001	0.366	0.001	0.002	0.453
MM12	0.830	0.812	0.811	-0.001	-0.001	0.811	-0.001	-0.001	-0.022
Overall	0.663	0.714	0.742	0.028	0.038	0.751	0.038	0.050	0.070

Table 5. Fis Values in each locus and in the total for each endangered Andalusian cattle breed and for the total population studied.

Locus	**BC**	**BN**	**Cárdena**	**Pajuna**
BM1824	0.043	-0.014	-0.048	-0.010
BM2113	0.145	0.132	-0.142	0.24
ETH10	0.018	0.073	0.273	0.077
ETH31	0.023	0.087	-0.010	-0.031
HAUT27	0.134	0.264	0.071	0.304
HEL5	0.062	0.036	0.119	0.231
ILSTS5	0.054	0.079	0.286	0.010
TGLA12	0.003	0.095	0.198	0.097
TGLA22	0.085	0.059	0.013	0.000
BM1314	0.124	0.082	0.103	0.084
BM1818	0.086	0.149	0.068	-0.095
ETH152	0.077	0.113	-0.063	-0.008
ETH225	0.026	0.119	-0.095	-0.011
HAUT24	0.056	0.171	-0.083	-0.016
ILSTS6	0.037	0.037	-0.025	0.158
INRA5	-0.022	0.194	0.130	0.051
INRA63	0.183	0.112	0.343	0.144
SPS115	-0.012	0.051	0.078	0.049
TGLA12	0.068	0.026	0.093	-0.089
CSRM60	0.051	0.057	0.197	-0.091
CSSM66	0.011	0.036	0.000	0.0162
HEL13	0.149	0.124	-0.258	0.043
HEL9	0.170	0.082	0.127	0.041
INRA23	0.058	0.010	-0.126	-0.044
INRA37	0.277	0.278	0.205	0.139

TGLA53	0.095	0.109	0.019	-0.015
ETH185	-0.009	0.020	0.059	0.019
HEL1	0.006	0.062	0.146	-0.188
INRA32	0.069	0.029	0.209	-0.090
INRA35	0.397	0.342	0.626	0.447
MM12	0.038	0.094	-0.152	-0.055
Overall	0.074	0.096	0.065	0.041

Table 6. Weir and Cockerhan over all loci

Capf	Theta	Smallf	Relat	Relatc	Sig_a	Sig_b	Sig_w
0.103	0.023	0.082	0.042	-0.108	0.524	1.841	20.495

Table 7. Jackknifing over loci.

	Capf	Theta	Smallf	Relat	
total	0.103	0.023	0.082	0.042	Means
	0.013	0.003	0.011	0.006	Std. Err.

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Table 8 . Genetic Distances (Nei) between breeds.

	BC	BN	Cárdena	Pajuna
BC (168)	0.000	0.043	0.242	0.121
BN (262)	0.043	0.000	0.275	0.117
Cárdena (14)	0.242	0.275	0.000	0.294
Pajuna (51)	0.121	0.117	0.294	0.000

Figure 1. Cluster from de genetic distances between breeds