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## Genetic diversity measures of the bovine *Alberes* breed using microsatellites: variability among herds and types of coat colour\*

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### Summary

The *Alberes* population is a native bovine breed of Catalonia with an unclear origin, which historically some authors have assumed as being composed of two different colour varieties (black and fawn). Sixteen microsatellite loci were analysed, all of them included in the AIRE2066 European Concerted Action list. Overall expected and observed heterozygosities reached values of 0.649 and 0.662, respectively. Genetic differences among black and fawn varieties were not significant ( $F_{ST} = 0.007$ ), and therefore the population is a single variety with a great colour gradation. On the contrary, we detected significant genetic differences among herds ( $F_{ST} = 0.026$ ;  $p < 0.001$ ), showing a genetic heterogeneity over short geographical distances. The number of migrants per generation among pairs of herds oscillates between 1.46 (*Roig* and *Freixe* herds) and 5.62 (*Castanyers* and *Roig* herds). Moreover, inbreeding and bottleneck situations can be rejected. The *Alberes* breed has been grouped within the Cantabrian trunk, closely related to the *Asturiana de la Montaña* and *Alistana* breeds, although some other breeds may also have influenced the population along its history.

### Zusammenfassung

Erfassung der genetischen Diversität der Rinderrasse *Alberes* mit Hilfe von Mikrosatelliten: Variabilität zwischen Herden und Typen der Fellfarbe

Die Rasse *Alberes* ist eine einheimische Rinderrasse Kataloniens mit unklarer Herkunft, von der historisch einige Autoren vermuten, dass sie eine Zusammensetzung aus zwei verschiedenen Farbvarianten ist (schwarz und hellbraun). Es wurden sechzehn Mikrosatelliten, die alle aus der „AIRE2066 European Concerted Action list“ stammen, analysiert. Der gesamte erwartete und beobachtete Heterozygotiegrad erreichte Werte von 0,649 beziehungsweise 0,662. Die genetischen Unterschiede zwischen den schwarzen und hellbraunen Varianten waren nicht signifikant ( $F_{ST} = 0,007$ ), so dass die Rasse als eine einzige Variante mit einer großen Farbabstufung gilt, die wir erhalten müssen. Im Gegensatz dazu entdeckten wir signifikante genetische Unterschiede innerhalb der Herden ( $F_{ST} = 0,026$ ,  $p < 0,001$ ), die eine genetische Heterogenität innerhalb kurzer geografischer Distanzen aufzeigten. Die Anzahl an Migranten pro Generation innerhalb der Herdpaaire schwankt zwischen 1,46 (*Roig*- und *Freixe*-Herden) und 5,62 (*Castanyers*- und *Roig*-Herden). Darüberhinaus kann Inzucht und eine Flaschenhalssituation zurückgewiesen werden. Die Rasse *Alberes* wurde innerhalb eines Cantabrischen Astes gruppiert, in enger Verwandtschaft zu den Rassen *Asturiana de la Montaña* und *Alistana*, obwohl im Laufe der Geschichte auch einige andere Rassen die Population beeinflusst haben könnten.

### Introduction

The *Alberes* breed is a semi-feral bovine breed of Catalonia that lives endemically in the eastern extreme of the Pyrenees, located in the Natural Park of the *Alberes* Massif, at a

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height of 200–1000 m a.s.l. Historically, the population has been distributed between the *Alt Empordà* region (Spain) and the *Vallespir* region (France), only separated by the frontier. Individuals, which live free all the year, graze shrubs and branches of Mediterranean woods in cold seasons, and ascend to pastures of the mountains during spring and summer. Supplementary feed is provided in winter and adverse periods. Animals are adapted to the geo-climatic and management conditions of the *Alberes* Massif, and they become essential for the control of vegetation and the reduction of fire risk. In winter, the animals live grouped in three herds, each of them grazing near an old farmhouse (*Castanyers*, *Roig* and *Freixe*, respectively), where animals receive supplementary feed. When the temperature increases, animals leave the herd and small groups of cows and heifers stroll among trees and alpine fields. Generally, individuals remain in the herd where they were born, but the bulls can mate with cows of neighbouring herds, when individuals of different herds graze the same zones, mainly in the summer.

A controversy exists concerning the phylogenetic origin of this breed. Initially, SÁNCHEZ BELDA (1984) assumed that these animals came from the Black Iberian trunk (Black Orthoide trunk), but in his last publication he allocates this breed to the Turdetanus trunk (Red Convex trunk), together with Majorcan and Sardinian cattle breeds (SÁNCHEZ BELDA 2000). However, JORDANA et al. (1991), on the basis of morphological characteristics, included the *Alberes* breed within the Cantabrian trunk (Brown Concave trunk), in a similar way as GARCÍA-DORY et al. (1990). Moreover, several authors have described two coat-colour varieties: fawn *Alberes*, also called *Fagina*, and black *Alberes*, with different phylogenetic origins (MASCORT 1957; JORDANA et al. 1999; SÁNCHEZ BELDA 2000). Whereas MASCORT (1957) assumed that fawn animals were the native breed and black individuals arrived later, JORDANA et al. (1999) and SÁNCHEZ BELDA (2000) suggested that black animals belonged to the old *Alberes* breed and the fawn variety proceeded from the ancient *Rossa Empordanesa* breed (SÁNCHEZ BELDA 2000) or from crossbreeding with *Pirenaica* and *Gasconne* breeds, mainly (JORDANA et al. 1999). In addition, according to several authors, the *Alberes* breed has received influences from different breeds. In the middle of the 20th century, MASCORT (1957) reported that crossbreeding with Brown Swiss animals was important, and that some animals showed influences from the ancient *Marinera* breed (Turdetanus trunk), and bulls coming from the *Auvergne* region (French) were imported in 1925. Furthermore, the influence of *Charolaise*, *Gasconne*, *Pirenaica*, *Bruna dels Pirineus* and *Limousine* breeds has increased in recent decades (MASCORT 1957; SÁNCHEZ BELDA 2000).

In 2001–2002, we carried out a preliminary census that included most of the animals (but not all), and found 135 black, 52 fawn and 158 crossed cows of 3 years and older, following the traditional classification of *Alberes* individuals according to their coat colour. The black variety has a relatively homogeneous coat colour that fluctuates from solid black (extremely rare) to chocolate animals with degradations on the nose, fringe, ears, eyes, tail and a narrow ribbon on the vertebral column. At birth, very few animals are black, most of the calves being blond and darkening their coat colour as they grow. However, the fawn type showed a greater variation between individuals because they oscillated from blond animals to dark individuals with the trunk degraded.

Unfortunately, the low-effective population size has created a dramatic situation and starting a conservation programme has become vital. The current population size fits into the category of *Endangered Breed* proposal of the FAO (SCHERF 2000) and *Critically Endangered* according to EAAP classification (<http://www.tiho-hannover.de/einricht/zucht/eaap/factors.htm>). Currently, the conservation programme needs a previous step where the black and the fawn coat-colour varieties are studied in order to determine if the *Alberes* population is a breed with two varieties clearly differentiated or if there is a single variety with a great colour gradation which will have to be maintained. In this context, this paper is an attempt to estimate genetic diversity of *Alberes* breed using microsatellites, to study carefully the relationships between black and *Fagina* varieties, and to classify the *Alberes* breed among the South-European cattle breeds.

## Materials and methods

During the autumn and winter of 2001–2002, 82 fresh blood samples from six bulls (one fawn- and five black-coloured) and 76 cows and heifers (27 fawn- and 49 black-coloured) of the *Alberes* cattle breed were collected in a conserving buffer. All available bulls were included in this study, and cows and heifers were sampled at random within-generation and within-herd. Twenty-five individuals came from the *Castanyers* herd (eight fawn and 17 black), 23 animals were sampled in the *Roig* herd (seven fawn and 16 black), 29 samples belonged to the *Freixe* herd (11 fawn and 18 black) and five cows had an unclear origin because their herd could not be determined exactly. The 16 microsatellite loci studied were: CSSM 66, ETH 003, ETH 010, ETH 152, ETH 225, HEL 1, HEL 5, HEL 9, ILSTS 005, INRA 005, INRA 023, INRA 032, INRA 035, INRA 037, INRA 063 and TGLA 44, which are included in the AIRE2066 European Concerted Action list (FAO list) with the exception of the TGLA 44 locus. DNA was extracted using established procedures (JEANPIERRE 1987). Primers and polymerase chain reaction (PCR) conditions were the same as those described by CAÑON et al. (2001). The PCR analysis of microsatellites was carried out using a capillary electrophoresis analyser (Applied Biosystems 3100, Foster City, CA, USA).

The BIOSYS-1 package (SWOFFORD and SELANDER 1989) was used to compute unbiased estimates of the number of alleles, allele frequencies, and observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity, as well as the Hardy–Weinberg equilibrium test for each locus, herd or colour variety. Wright F-statistics (WRIGHT 1965; NEI 1977) were estimated in accordance with the procedures described by WEIR and COCKERHAM (1984), using the FSTAT 2.9.3. software (GOUDET 1995). The F-statistics were estimated in two ways, grouping the animals according to the coat colour and the herd of origin, respectively.  $F_{ST}$  between herds and between colour types within herd were also calculated. The statistical significance of the  $F_{ST}$  statistics was tested by the chi-square test shown in equation (1) with  $(k - 1)(s - 1)$  degrees of freedom, where N is the total sample size, k is the number of alleles, and s is the number of subpopulations (CHESSER 1983).

$$\chi^2 = 2NF_{ST}(k - 1) \quad (1)$$

The deviations of  $F_{IT}$  or  $F_{IS}$  (F) from zero were tested by the chi-square test showed in equation (2) (LI and HORVITZ 1953; NEI 1987), with  $k(k - 1)/2$  degrees of freedom.

$$\chi^2 = NF^2(k - 1) \quad (2)$$

In order to quantify the gene flow among *Alberes* subpopulations (herds or colour types), the rates of migration in balance with genetic drift ( $N_m$ ) were calculated using the WRIGHT (1965) approximation, modified by TAKAHATA (1983), to take into account the number of populations (n) (equation 3).

$$N_m = \frac{1 - F_{ST}}{4F_{ST}\left(\frac{n}{n-1}\right)^2} \quad (3)$$

In order to quantify the percentage of molecular variance due to differences among herds and differences between coat-colour varieties, a hierarchical analysis of variance of group colour within-herd using the AMOVA programme included in the ARLEQUIN package (SCHNEIDER et al. 1997) was carried out (hierarchical AMOVA with haplotypic data and several groups of populations). Variance components were used to compute fixation indices (WRIGHT 1965) and the significance of these estimates was tested using a non-parametric permutation approach described by EXCOFFIER et al. (1992), permuting haplotypes among colour subpopulations among herds ( $F_{ST}$ ) or within herds ( $F_{SC}$ ) and permuting colour subpopulations among herds ( $F_{CT}$ ).  $F_{SC}$  and  $F_{ST}$  are measures of the differences between

individuals in different subpopulations (groups of coat colour within-herd), as a proportion of the total genetic variance ( $F_{ST}$ ) or as a proportion of the herd variance ( $F_{SC}$ ). Conversely, parameter  $F_{CT}$  is a measure of the degree of resemblance between individuals of a herd, expressed as a proportion of the total variance (SCHNEIDER et al. 1997).

Populations that have recently suffered a severe reduction in size are especially important to identify for conservation. They are most likely to suffer an increased risk of extinction (CORNUET and LUIKART 1996) because bottlenecks reduce adaptive potential, increasing the rates of inbreeding, the loss of genetic variation and the fixation of mildly deleterious alleles. The BOTTLENECK software identifies populations that have experienced a severe reduction in effective population size ( $N_e$ ) along the last  $2N_e-4N_e$  generations (PIRY et al. 1999). This programme assumes two basic mutational models, the Infinite Allele Model (IAM; KIMURA and CROW 1964) and the Stepwise Mutation Model (SMM; OHTA and KIMURA 1973), which represent two extreme models of mutation along a continuum of possible models (CHAKRABORTY and JIN 1992). The IAM is recommended for allozyme data (PIRY et al. 1999), whereas the SMM is generally more appropriate when testing microsatellite loci (CORNUET and LUIKART 1996), although contradictory results have been reported (MACHUGH 1996). Because few microsatellite loci follow the strict (one-step) SMM (DI RIENZO et al. 1994), the Two-Phased Model of mutation (TPM; LI 1976; DI RIENZO et al. 1994) has recently been implemented in BOTTLENECK package. Analyses were carried out under SMM and TPM models, and bottleneck situations have been tested within-herd and for the overall population. For TPM the values recommended by PIRY et al. (1999), 95% of single-step mutations and a variance among multiple steps of 12, have been used.

Finally, a tree of relationship was constructed among the *Alberes* and nine cattle breeds for establishing the relationship of the *Alberes* breed within the South-European breeds. These breeds were chosen because of their geographical proximity with the *Alberes* region (*Pirenaica*, *Bruna dels Pirineus* and *Gasconne*, which may have introduced some of their genes into the *Alberes* breed), or because of their similarity in coat colour with the *Alberes* breed (*Alistana*, *Asturiana de la Montaña*, *Asturiana de los Valles*, *Sayaguesa* and *Tudanca*). *Pirenaica* and *Bruna dels Pirineus* breeds belong to the Turdetanus trunk and the remaining ones, with the exception of *Gasconne*, are breeds of the Cantabrian trunk. Furthermore, the *Avileña Negra-Iberica* breed was included as a representative of the Black Iberian trunk. A database with 50 individuals genotyped for each breed was obtained from the FAIR1 CT95 0702 project (see CAÑON et al. 2001). The joining of both microsatellite datasets was possible by using reference samples to compare allele sizes.

According to EDING and LAVAL (1999) and LAVAL et al. (2002), Reynolds genetic distance (REYNOLDS 1983) is preferable when the divergence time between populations is short, for example breeds within Europe. In addition, when different rates of evolution have to be assumed, the neighbour-joining procedure (TAKEZAKI and NEI 1996) becomes the preferred technique (EDING and LAVAL 1999). Therefore, in order to determine the position of the *Alberes* breed within the tree of South-European breeds, after calculating the Reynolds genetic distance matrix using the MICROSAT package (MINCH 1997), a neighbour-joining tree was drawn with the PHYLIP programme (FELSENSTEIN 1995).

## Results

All microsatellites analysed were polymorphic in the *Alberes* population, as well as within-herd or within-colour variety. The number of alleles detected per locus fluctuated between 3 (ILSTS 005 and INRA 005) and 10 (TGLA 44) and average values were higher in the *Castanyers* herd ( $5.3 \pm 0.5$ ) than in *Roig* ( $5.0 \pm 0.4$ ) or *Freixe* herds ( $4.7 \pm 0.3$ ), as well as in the black variety than in fawn animals ( $5.9 \pm 0.4$  versus  $4.9 \pm 0.3$ ). The  $H_o$  and  $H_e$  values fluctuated between 0.570 and 0.695 for  $H_o$ , and between 0.608 and 0.671 for  $H_e$  (Table 1).

Table 1. Number of animals (N), observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ) for each herd and colour type within herd, and overall animals

	N	$H_o$		$H_e$	
		Mean	SE	Mean	SE
Black animals	54	0.644	0.037	0.671	0.030
Fawn animals	28	0.661	0.044	0.644	0.032
<i>Castanys</i> herd	25	0.668	0.050	0.668	0.037
Black animals	17	0.695	0.048	0.668	0.034
Fawn animals	8	0.609	0.073	0.608	0.050
<i>Roig</i> herd	23	0.603	0.048	0.634	0.030
Black animals	16	0.570	0.052	0.636	0.031
Fawn animals	7	0.679	0.053	0.646	0.031
<i>Freixe</i> herd	29	0.657	0.036	0.648	0.027
Black animals	18	0.653	0.034	0.654	0.028
Fawn animals	11	0.665	0.044	0.643	0.030
Overall	82	0.649	0.038	0.662	0.030

Five loci were not in Hardy–Weinberg proportions when the overall population was tested (CSSM 66, ETH 225, ILSTS 005, INRA 032 and INRA 035). When we applied these tests to each herd or colour type separately, only the INRA 035 showed departures from the H–W proportions in all analyses. The INRA 032 locus appeared not in equilibrium in the *Castanys* herd and the black variety, HEL 1 and INRA 63 were not in equilibrium in the *Roig* herd and significant differences from the Hardy–Weinberg equilibrium for the ILSTS 5 locus in the *Freixe* herd and the ETH 225 locus in fawn animals were detected. The F-statistics estimated were not significant, with the exception of the  $F_{ST}$  value, when we tested the population subdivided by herds (Table 2).  $F_{ST}$  between pairs of herds tended to augment with the increase of the geographical distance between farmhouses, although the

Table 2. F-statistics ( $F_{IT}$ ,  $F_{ST}$  and  $F_{IS}$ ) for the 16 microsatellites analysed with two population subdivisions: herds and colour type

	Herds			Colour type		
	$F_{IT}$	$F_{ST}$	$F_{IS}$	$F_{IT}$	$F_{ST}$	$F_{IS}$
CSSM 66	-0.010	0.029***	-0.040	0.013	0.004	0.009
ETH 003	0.049	0.006	0.043	0.018	0.009	0.009
ETH 010	0.006	0.017*	-0.010	-0.029	0.003	-0.032
ETH 152	-0.049	0.002	-0.052	-0.066	0.006	-0.073
ETH 225	-0.003	0.034***	-0.038	-0.041	0.001	-0.042
HEL 1	0.047	0.017*	0.030	0.047	0.007	0.040
HEL 5	0.068	0.039***	0.031	0.025	0.004	0.022
HEL 9	0.044	0.029***	0.016	0.044	0.006	0.038
ILSTS 005	-0.088	0.003	-0.091	-0.115	0.002	-0.117
INRA 005	-0.123	0.021*	-0.147	-0.148	0.003	-0.151
INRA 023	0.074	0.075***	-0.001	0.037	0.005	0.032
INRA 032	0.004	0.054***	-0.053	-0.043	0.008	-0.052
INRA 035	0.654***	0.024**	0.643***	0.641***	0.009	0.638***
INRA 037	0.020	-0.010	0.030	0.005	0.026***	-0.022
INRA 063	-0.142	0.010	-0.154	-0.153	0.026**	-0.158
TGLA 44	0.031	0.038***	-0.007	0.012	0.004	0.003
Overall	0.035	0.026***	0.009	0.014	0.007	0.007

NS, not significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

Table 3.  $F_{ST}$ , number of migrants ( $N_m$ ) and geographical distance between pairs of herds

	$F_{ST}$	$N_m$	Geographical distance (km)
<i>Castanyers</i> – <i>Roig</i>	0.011*	5.619	0.99
<i>Roig</i> – <i>Freixe</i>	0.041***	1.462	2.10
<i>Freixe</i> – <i>Castanyers</i>	0.024***	2.542	3.03

NS, not significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

Table 4. Partitioning of genetic variability among the different sources of variation, with subpopulations grouped according to their herd

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among herds	2	20.39	0.196	4.20	$F_{SC} = 0.013^{NS}$
Among subpopulations within herd	3	15.21	0.060	1.29	$F_{ST} = 0.055^{***}$
Within subpopulations	70	308.88	4.414	94.56	$F_{CT} = 0.042^{**}$
Total	75	344.49	4.668		

NS, not significant; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

Table 5. Bottleneck analysis tested under Stepwise Mutation Model (SMM) and Two-Phased Model (TPM). Two different statistical procedures have been used, Sign Test and Wilcoxon Sign-Rank Test

	Sign Test		Wilcoxon Sign-Rank Test	
	SMM	TPM	SMM	TPM
<i>Castanyers</i> herd	NS	NS	NS	NS
<i>Roig</i> herd	NS	*	NS	NS
<i>Freixe</i> herd	NS	NS	NS	NS
Overall	NS	NS	NS	NS

NS: not significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

highest  $F_{ST}$  belonged to the intermediate geographical distance (between *Roig* and *Freixe* herds). The higher number of migrants being between *Castanyers* and *Roig* herds (Table 3).

Notwithstanding the migration between herds, variation among herds estimated through the analysis of molecular variance was 4.2%. On the contrary, the percentage of variation due to differences between colour types within-herd reached only 1.3%. The greatest value (excluding individual variability) belonged to variation within subpopulations (94.6%). The  $F_{SC}$  estimate did not attain statistical significance, whereas  $F_{ST}$  and  $F_{CT}$  values were 0.055 and 0.042, respectively (Table 4).

When we tested the existence of recent bottlenecks, no results were significant, with the exception of the analyses carried out within the *Roig* herd under TPM (Table 5).

According to the tree (Fig. 1), the breeds belonging to the Cantabrian trunk (*Alistana*, *Asturiana de la Montaña*, *Asturiana de los Valles*, *Sayaguesa* and *Tudanca*) were grouped, and the *Alberes* breed was included within this group, closely related to *Asturiana de la Montaña* and, to a lesser degree, to the *Alistana* breed. Unfortunately, the bootstrap values were small for all nodes, with the exception of the *Tudanca-Sayaguesa* one (81%). The node concerning to *Asturiana de la Montaña* and *Alberes* reached a moderate value (44%).

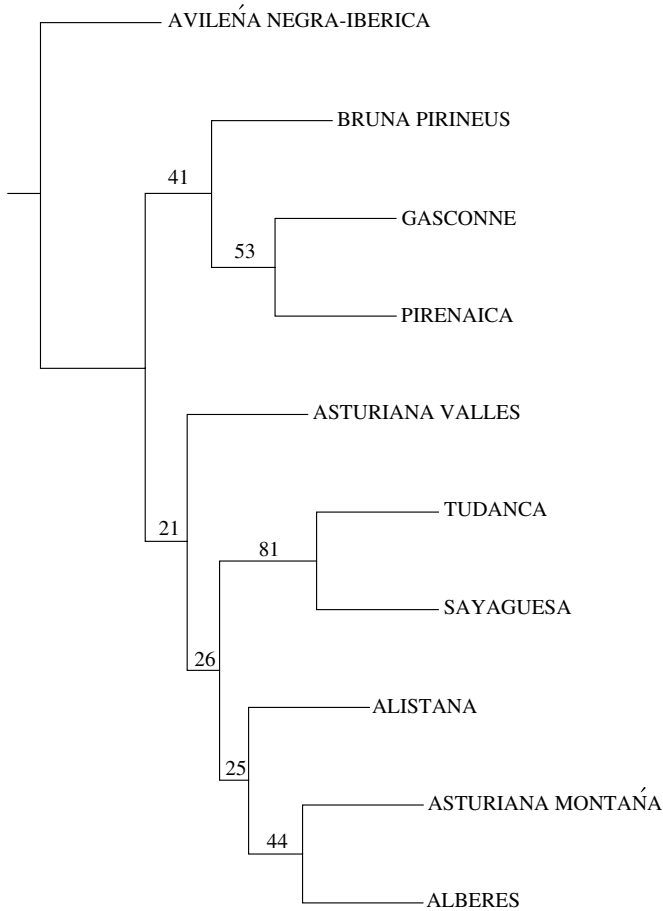


Fig. 1. Tree of relationships among breeds. Values represent the percentage of grouping confidence calculated by bootstrap analysis

*Avileña Negra-Iberica* diverged from the remaining breeds early, and *Pirenaica*, *Bruna dels Pirineus* and *Gasconne* breeds belonged to a separated cluster, however, the percentage of grouping confidence calculated by bootstrap analysis was moderate–low (41%).

### Discussion

The overall  $H_e$  ( $0.662 \pm 0.030$ ) reached intermediate values in comparison with the  $H_e$  values described by CAÑON et al. (2001), for 18 European cattle breeds, and are higher than values reported in the Majorcan breed (ARANGUREN-MÉNDEZ et al. 2001), both with the same marker sets. The average number of alleles follow a similar pattern described for  $H_e$ . Similar results for the number of alleles per locus have been described in various European cattle breeds (MOAZAMI-GOUDARZI et al. 1994; CIAMPOLINI et al. 1995; SCHMID et al. 1999).

The non-significant  $F_{IS}$  and  $F_{IT}$  estimates in the *Alberes* breed indicate that there is no inbreeding in the population. This conclusion is in accordance with results by JORDANA

et al. (1999). Non-significant values of  $F_{IS}$  and  $F_{IT}$  parameters could also be explained by the existence of gene flow with other populations (e.g. *Bruna dels Pirineus*, *Gasconne*, *Charolaise*,...) as suggested by MASCORT (1957), JORDANA et al. (1999) and SÁNCHEZ BELDA (2000), or by the Robertson effect (ROBERTSON 1965), when there are different allele frequencies between male and female parents. This situation is particularly likely in a polygynous population because of the small number of breeding males (JORDANA et al. 1999). Although an overall genetic deficit of heterozygotes has not been detected, the INRA 035 locus showed high and significant values of  $F_{IS}$  and  $F_{IT}$ . This locus might be linked to some morphological or productive traits of selective interest, and alternatively, low numbers of sampled animals or the presence of null alleles could also break the Hardy-Weinberg equilibrium (CIAMPOLINI et al. 1995; MACHUGH 1996).

We have not detected genetic differentiation between colour types (Table 2), although the analysis within herds suffered from important limitations because of the low number of available animals. These results contradict those obtained by JORDANA et al. (1999), although non-significant values of  $F_{ST}$  between coat-colour varieties were also described by ARANGUREN-MÉNDEZ et al. (2001) in the Majorcan breed, also a chestnut breed. On the contrary, genetic differences between herds have been detected (Tables 2 and 3) showing a genetic heterogeneity over short geographical distances. This situation has been shown for domestic species like Spanish dogs (JORDANA et al. 1992) and the *Bruna dels Pirineus* beef cattle breed (JORDANA and PIEDRAFITA 1996).

Migration values can be interpreted as the upper limit of the number of migrants per generation which would allow the maintenance of the genetic differentiation observed between the herds. This value was higher between *Roig* and *Castanyers* herds (5.61) and lower between *Freixe* and *Roig* or *Castanyers* herds (1.46 and 2.45 respectively). These values are clearly smaller than the number of migrants per generation obtained between different populations of *Bruna dels Pirineus*, subdivided by geographical areas (JORDANA and PIEDRAFITA 1996).

The percentage of variation due to differences between colour varieties was minimum (1.29%), meanwhile herds had a variance of approximately 4%, in accordance with the population structure described above. The  $F_{SC}$  value was not significant and it corroborated the null genetic contribution of coat-colour differentiation.

Bottleneck analysis under the Sign Test suffers from low statistical power and the Wilcoxon Sign-Rank Test is preferable (CORNUET and LUKART 1996), but our results did not show important differences and, in accordance with the SMM and TPM models, the Spanish population of the *Alberes* breed has not suffered a bottleneck situation. Thus, we can assume that the genetic diversity of the *Alberes* breed has not been reduced significantly in recent decades. Given that the number of bulls was insufficient, the maintenance of genetic diversity may be caused by the introduction of genes from neighbouring cattle populations, apart from the preservation of the census.

According to Fig. 1, the *Alberes* breed could be classified within the Cantabrian trunk, but the percentages of grouping confidence calculated by bootstrap analysis were generally low. This situation is relatively usual when we use a reduced number of loci (MOAZAMI-GOUDARZI et al. 1997; BLOTT et al. 1998; KANTANEN et al. 1999; JORDANA et al. 2003) and the interpretation needs to be cautious. We can conclude that if the *Alberes* population has received the influence of some other breeds, the animals with their origin in the Cantabrian trunk may have contributed the most to the genepool of the current population, which would coincide with results previously reported by GARCÍA-DORY et al. (1990) and JORDANA et al. (1991).

The demographic situation of the *Alberes* breed makes starting a conservation programme vital. According to RUANE's (1999) criteria, this breed should be conserved given that it is a greatly endangered breed, has a cultural/historical value (this is one of the last native Catalonian cattle breeds), and may be potentially adapted to a specific environment (it might contribute a significantly added value in the prevention of fires).



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