Pergamon

PII: S0305-1978(96)00054-3

Biochemical Systematics and Ecology, Vol. 24, No. 6, pp. 485–498, 1996

0305–1978/96 $15.00+0.00

The *"Bruna dels Pirineus"* (Pyrenean brown breed): a Genetic Study of a Rare Cattle Breed in Catalonia (Spain)

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Key Word Index—Animal genetic resources; rare cattle breed; genetic distance; F-Statistics; biochemical polymorphism; gene flow.

Abstract—According to the "Food and Agriculture Organization of the United Nations" (FAO), animal genetic resources available throughout the world are in a dramatic state of decline. This results in the disappearance of a substantial number of local animal populations, and the consequent loss of their ability to adapt genetically to their local environments.

A basic aim of conservation biology is to maintain genetic variability. This study characterizes and analyzes the genetic structure of a limited rare population, a local beef cattle breed from the Catalonian Pyrenees (Spain), *Bruna dels Pirineus*.

Five hundred and forty-three individuals were analyzed for five polymorphic genetic *loci*. Animals were sorted according to two criteria: Region level (7 subpopulations) and Nucleus of Origin level (9 subpopulations). The genetic structures and relationships among these subpopulations are analyzed and principal migratory paths and rough estimates of inbreeding among the subpopulations are discussed. The migratory trend from the northwest (Pyrenean mountain areas) to the southeast (pre-Pyrenean areas) is confirmed.

This breed exhibits genetic uniformity. The average genetic differentiation among subpopulations (Nucleus of Origin) was 1.6% ($F_{ST}=0.016$; $P<0.001$). The effective number of individuals exchanged between subpopulations (Nuclei) per generation (gene flow) is estimated to be 12.1.

We propose the animals from Pyrenean mountain areas to be the nucleus of conservation and maintenance of genetic variability for this breed. This information could be integrated into the FAO Global Data Bank on Domestic Animal Diversity.

Introduction

The *Bruna dels Pirineus* is a local cattle breed of the Catalonian Pyrenees whose probable origin is a fusion between local ecotypes of the "Pirenaica" breed and the "Brown Swiss" breed, introduced via France through the Aran Valley. This population was subsequently upgraded with the old "Brown Swiss" and later empirically selected for meat production (Parés and Vilaró, 1994).

The analysis of 29 morphological traits allowed us to place this population within the *Bos taurus turdetanus* lineage that includes breeds such as "Palmera", "Rubia Gallega" and "Pirenaica" (Jordana *et al.*, 1991). The "Turdetanus lineage" comes from the ancestral *Bos brachyceros* (Sánchez Belda, 1984), which originated in Mesopotamia and Asia Minor and which could have been introduced into Africa and Europe via Egypt and the Strait of Gibraltar.

The number of cows maintaining a high degree of racial purity is about 9,000–10,000 (Martell, unpublished observations). These figures correspond to the Rare Breed category proposed by the FAO Expert Consultation (Anonymous, 1992) or

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(*Received* 19 *October* 1995; *accepted* 29 *May* 1996)

to the category of Insecure Status (5,000–10,000 females) according to the Red Data Book System, implying the breed may become endangered in the near future (Bodó, 1992).

Our objective was to characterize and analyze the genetic structure of a relatively small population, the *Bruna dels Pirineus* breed, which appears to be in imminent danger. An analysis of the intra-racial genetic variability was carried out at two levels: REGION (or place of animal production), and NUCLEUS OF ORIGIN (place of birth or origin of the animals).

The results from analysis of the F-statistics of Wright (Nei, 1977; Wright, 1978) may provide some insight into the reproductive history of the population as well as on the principal migratory routes. These analyses also will allow us to estimate the inbreeding coefficients of the subpopulations and of the whole population (Weir and Cockerham, 1984; Weir, 1990).

Materials and Methods

Animals, breed subdivision and genetic systems. Blood samples were collected from 543 individuals belonging to the *Bruna dels Pirineus* cattle breed. Blood samples were taken with EDTA 2Na (1 mg per ml of blood), separated into plasma and red blood cells, and stored at −20°C.

To carry out the intra-racial analysis of this population, the randomly sampled animals were assigned to different subpopulations grouped under two different criteria:

1. REGION Level: A total of 543 animals were assigned to several groups according to the place of animal production (Fig. 1). There are seven subpopulations with an average sample size of 78 individuals: (AR) Alta Ribagorça (83), (PS) Pallars Sobirà (170), (PJ) Pallars Jussà (52), (CE) Cerdanya (23), (RI) Ripollès (56), (OB) Osona-Berguedà (102) and (SO) Solsonès (57).
2. NUCLEUS OF ORIGIN Level: The 525 individuals also were assigned to several subpopulations according to their place of birth or origin. Nine subpopulations were distinguished, with an average sample size of 60 individuals: (VA) Vall d'Aran (44), (AR) Alta Ribagorça (85), (PJ) Pallars Jussà (42), (CE) Cerdanya (32) and (RI) Ripollès (41); the other four nuclei correspond to geographical subareas of the Pallars Sobirà (PS) Region. This last subdivision was noted due to the fact that (PS) is the area with a greater number of individuals and is the principal exporter of breeding animals. The

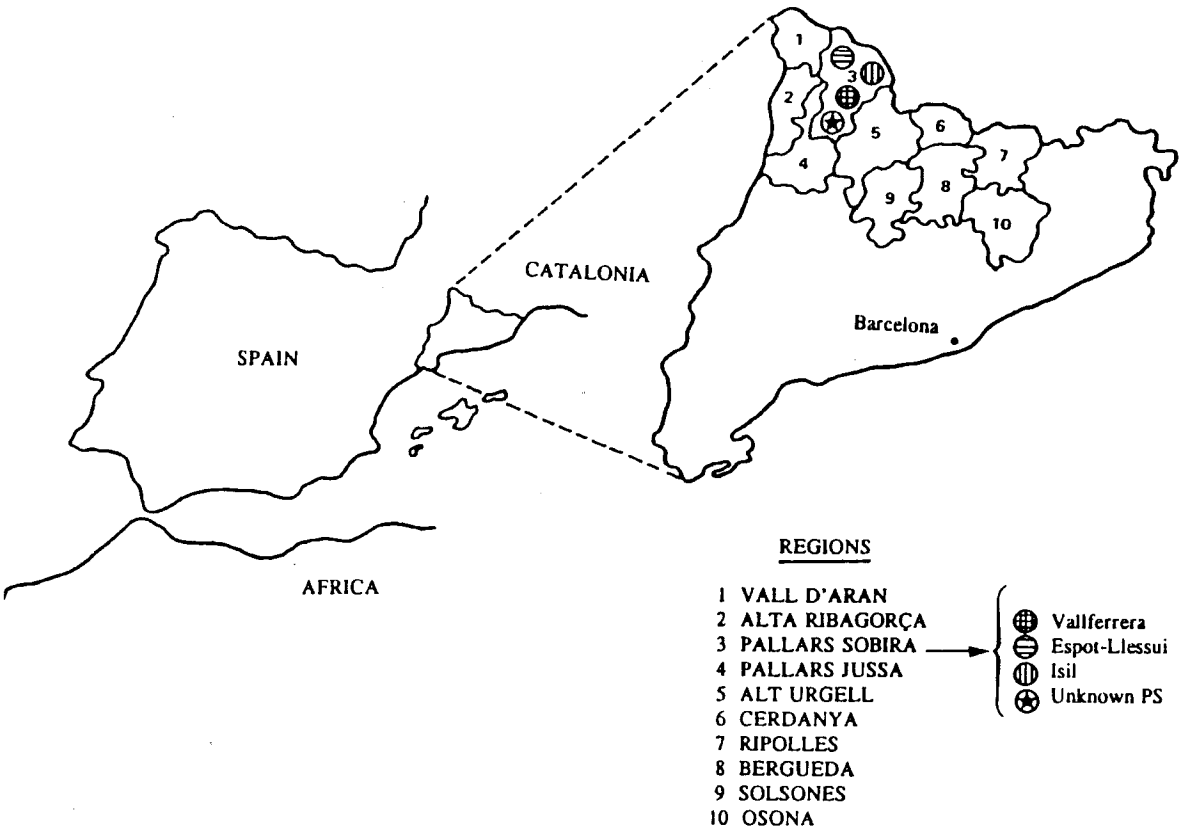

FIG. 1. GEOGRAPHICAL LOCATION OF SUBPOPULATIONS OF THE *BRUNA DELS PIRINEUS* BREED.

areas are: (VF) Vallferrera (42), (EL) Espot-Llessui (83), (IS) Isil (69) and (DS) Unknown Pallars Sobirà (87). The DS individuals had unknown local origin, but all come from the Pallars Sobirà (PS) region. Not included in this analysis were 18 individuals of unknown origin.

The variability of five genetic *loci* was assayed by different electrophoretic techniques. The haemoglobin (*Hb*) red-blood-cell system was analyzed by horizontal electrophoresis in agarose gel (Gahne and Juneja, 1985). The other four were plasma systems: albumin (*Alb*), detected by horizontal electrophoresis in starch gel (Bortolozzi, 1983); transferrin (*Tf*), group-specific component (*Gc*) and post-transferrin 2 (*Ptf-2*), typed by horizontal electrophoresis in polyacrylamide gels (Gahne *et al.*, 1977).

Statistics of genetic variability. The unbiased average expected heterozygosity (H_e; Nei, 1978), and mean number of alleles per polymorphic *locus* (n_a) have been calculated as indicators of the level of genetic variability. The values are not estimates for the breed, since only a small sample of polymorphic *loci* have been analyzed, resulting in an overestimation of H_e values (Nei, 1987; Hartl and Pucek, 1994). These estimates, however, allow us to compare the different subpopulations.

The observed genotypic frequencies were compared to those expected in Hardy-Weinberg (HWE) equilibrium by a chi-square test (χ^2). The expected genotypic frequencies have been calculated applying Levene's (1949) correction for small sample sizes. "Wright's F" (Wright, 1965) for each *locus* was calculated, giving a measurement of the HWE deviation. An ANOVA test was applied to determine if there were significant differences among all expected heterozygosity means (H_e).

Statistics of genetic differentiation. Nei's unbiased distance (a modified *D* for small sample sizes; Nei, 1978), was calculated as well as the fixation indices (F_{IS}, F_{IT} and F_{ST}) or F-statistics of Wright (1965), modified by Nei (1977) and by Wright (1978). Fixation indices seem to be more appropriate than genetic distances when studying differentiation among subpopulations of the same or different breeds.

These F-statistics provide an approximate measure of inbreeding in each subpopulation (F_{IS}) and in the entire population (F_{IT}), (Weir and Cockerham, 1984; Weir, 1990), and F_{ST} measures the degree of genetic differentiation among populations. The latter is always positive and between zero and 1, so is used as a measure of genetic distance (Gregorius and Roberds, 1986; Long, 1986; Long *et al.*, 1987; Weir, 1990). Nei's distance is appropriate for long-term evolution when populations diverge because of drift and mutation. The distance is proportional to the time since divergence in the special case of the infinite alleles mutation model and equilibrium in the ancestral population. The F_{ST} distance (or Coancestry Distance) is more appropriate for short-term evolution, for divergence due only to drift, and no assumptions need be made about the ancestral population (Reynolds *et al.*, 1983; Weir, 1990).

The statistical significance of gene frequency differences among populations was tested for each *locus* by the chi-square test. Computations were performed with the BIOSYS-1 package (Swofford and Selander, 1989). Cluster Analysis using the UPGMA algorithm (Sneath and Sokal, 1973), was applied to the F_{ST} estimated values, used as genetic distances.

Results

The allele frequencies for each electrophoretic *locus* and population analyzed are shown in Table 1, Global Population; Table 2, Regions; and Table 3, Nucleus of Origin.

Genetic Variability and HWE Analysis

The statistics describing the amounts of genetic variation are presented in Table 4 (regionally) and Table 5 (by origin). Using Wright's F analyses, we found some regions departing from HWE: Pallars Jussà (PJ) for *Tf* ($P<0.05$), Ripollès (RI) for *Hb* ($P<0.05$) and *Gc* ($P<0.05$) systems, and Solsonès (SO) for *Hb* ($P<0.05$) and *Gc* ($P<0.05$). When examining nuclei of origin, only the population from Cerdanya (CE) had a single *locus* not in HWE (*Gc*; $P<0.01$). By considering all the individuals as members of a single population (*Bruna dels Pirineus* breed), the only *locus* that shows significant disagreement with Hardy-Weinberg proportions is the

TABLE 1. ALLELE FREQUENCIES FOR EACH ELECTROPHORETIC *LOCUS* AND POPULATION ANALYZED

Locus	Hb	Tf	Alb	Gc	Ptf2
Global Population					
	A: 0.759	A: 0.289	A: 0.952	A: 0.168	S: 0.273
	B: 0.241	D: 0.684	B: 0.048	B: 0.832	F: 0.727
		E: 0.027			

Sample size for each *locus* was 543 individuals.

TABLE 2. ALLELE FREQUENCIES FOR EACH ELECTROPHORETIC *LOCUS* AND POPULATION ANALYZED

Region (Locus)	AR* (83)**	CE (23)	OB (102)	PJ (52)	PS (170)	RI (56)	SO (57)
Regions							
Hb							
A	0.789	0.739	0.735	0.731	0.741	0.839	0.763
B	0.211	0.261	0.265	0.269	0.259	0.161	0.237
Tf							
A	0.283	0.152	0.275	0.413	0.288	0.268	0.289
D	0.693	0.826	0.691	0.577	0.685	0.696	0.684
E	0.024	0.022	0.034	0.010	0.026	0.036	0.026
Alb							
A	0.982	0.935	0.966	0.981	0.924	0.964	0.939
B	0.018	0.065	0.034	0.019	0.076	0.036	0.061
Gc							
A	0.139	0.109	0.211	0.125	0.156	0.241	0.158
B	0.861	0.891	0.789	0.875	0.844	0.759	0.842
Ptf2							
S	0.301	0.304	0.270	0.163	0.271	0.250	0.333
F	0.699	0.696	0.730	0.837	0.729	0.750	0.667

(*) See text for codes.(**) Sample size for each population is in parentheses.

TABLE 3. ALLELE FREQUENCIES FOR EACH ELECTROPHORETIC *LOCUS* AND POPULATION ANALYZED

Nucl. (Locus)	VA (44)	AR (85)	VF (42)	EL (83)	IS (69)	DS (87)	PJ (42)	CE (32)	RI (41)
Nuclei of Origin									
Hb									
A	0.841	0.800	0.810	0.723	0.761	0.701	0.655	0.734	0.793
B	0.159	0.200	0.190	0.277	0.239	0.299	0.345	0.266	0.207
Tf									
A	0.261	0.265	0.298	0.235	0.384	0.287	0.417	0.188	0.268
D	0.727	0.712	0.702	0.741	0.565	0.678	0.571	0.797	0.695
E	0.011	0.024	0.000	0.024	0.051	0.034	0.012	0.016	0.037
Alb									
A	0.955	0.982	0.905	0.970	0.891	0.954	0.976	0.922	1.000
B	0.045	0.018	0.095	0.030	0.109	0.046	0.024	0.078	0.000
Gc									
A	0.170	0.135	0.083	0.169	0.203	0.172	0.131	0.141	0.268
B	0.830	0.865	0.917	0.831	0.797	0.828	0.869	0.859	0.732
Ptf2									
S	0.273	0.300	0.333	0.271	0.246	0.259	0.202	0.297	0.268
F	0.727	0.700	0.667	0.729	0.754	0.741	0.798	0.703	0.732

Gc system ($P<0.01$), the deficit of heterozygotes (D) being -0.143. It can be concluded that each locality (regions and nuclei) and the entire population (the breed) have genotypic proportions in close agreement with HWE, with the exception of the group-specific component (*Gc*) system.

Statistics of genetic differentiation

1. Nei's unbiased genetic distance: At the region level, distances ranged from $D=0.000$ (for 8 pairs) to $D=0.018$ (for the Pallars Jussà—Cerdanya pair).

The average distance between regions was 0.003 (±0.004). At the nucleus of origin level, distances ranged from D = 0.000 (for 12 pairs) to D = 0.014 (for Pallars Jussà—Cerdanya, and Pallars Jussà—Vall d'Aran). The average distance between the nuclei was 0.004 (±0.004).

2. F-statistics analysis: Table 6 and Table 7 present the F-statistics analyses of genetic differentiation both regionally and by nuclei of origin. Regionally there is a deficit of heterozygotes averaging 7% ($P<0.001$) and averaging 8.3% ($P<0.001$) in the population as a whole. At the nucleus of origin level the average deficit of heterozygotes was 4.9% ($P<0.001$), whereas the estimated heterozygote deficit for the global population was 6.4% ($P<0.001$).
3. Inbreeding degree: F_{IS} values for each *locus* and Region are shown in Table 8. Only the Ripollès Region, with 19.0% ($P<0.05$), and the Solsonès Region, with 6.6% ($P<0.05$), showed a significant deficit of heterozygotes. The Nucleus of Origin results are presented in Table 9. All the subpopulations showed non-significant values.
4. Direct comparison among OTUs (Operational Taxonomic Units): Statistics of genetic differentiation, F_{ST}, are presented in Table 10 (by region) and 11 (by nucleus of origin). The degree of genetic diferentiation among regions range from 0.1 to 3.0% with only the Pallars Jussà (PJ) Region significantly different compared with all the others. At the nucleus of origin level, F_{ST} statistics range from 0.1 to 2.2%. Only two Nuclei, Pallars Jussà (PJ) and Isil (IS), showed significant differences with all the other groups and between themselves.

Genetic relationships

Using cluster analysis methods and by applying the UPGMA algorithm to F_{ST} estimates (Table 10 for Regions and Table 11 for Nuclei), the dendrograms of Fig. 2 and Fig. 3, respectively, were obtained.

REGIONS: There are two large clusters; one formed by the Pallars Jussà (PJ) region and a second including the rest of the regions. Of this second group, the Pallars Sobirà (PS), Solsonès (SO), Alta Ribagorça (AR) and Osona-Berguedà (OB) regions, form a group differentiated from members of the Ripollès (RI) and Cerdanya (CE) regions.

TABLE 4. STATISTICS OF GENETIC VARIABILITY AT THE REGION LEVEL

Region	Mean sample size per locus	Mean no. of alleles per locus	Mean heterozygosity observed	Mean heterozygosity expected(*) (**)
(AR) Alta Ribagorça	83.0	2.2	0.284	0.295
	(0.0)	(0.2)	(0.073)	(0.074)
(CE) Cerdanya	23.0	2.2	0.261	0.290
	(0.0)	(0.2)	(0.060)	(0.058)
(OB) Osona-Berguedà	102.0	2.2	0.327	0.327
	(0.0)	(0.2)	(0.069)	(0.068)
(PJ) Pallars Jussà	52.0	2.2	0.231	0.287
	(0.0)	(0.2)	(0.058)	(0.079)
(PS) Pallars Sobirà	170.0	2.2	0.339	0.327
	(0.0)	(0.2)	(0.061)	(0.055)
(RI) Ripollès	56.0	2.2	0.246	0.307
	(0.0)	(0.2)	(0.061)	(0.066)
(SO) Solsonès	57.0	2.2	0.305	0.330
	(0.0)	(0.2)	(0.067)	(0.063)

(Standard errors in parentheses.)

(*) Unbiased estimate (Nei, 1978).

(**) ANOVA test: F ratio = 0.083; not significant.

TABLE 5. STATISTICS OF GENETIC VARIABILITY AT THE NUCLEUS OF ORIGIN LEVEL

Nucleus of origin	Mean sample size per locus	Mean no. of alleles per locus	Mean heterozygosity observed	Mean heterozygosity expected(*) (**)
(VA) Vall d'Aran	44.0	2.2	0.250	0.291
	(0.0)	(0.2)	(0.048)	(0.058)
(AR) Alta Ribagorça	85.0	2.2	0.287	0.288
	(0.0)	(0.2)	(0.075)	(0.072)
(VF) Vallferrera	42.0	2.0	0.314	0.303
	(0.0)	(0.0)	(0.065)	(0.061)
(EL) Espot-Llessui	83.0	2.2	0.316	0.308
	(0.0)	(0.2)	(0.071)	(0.066)
(IS) Isil	69.0	2.2	0.362	0.359
	(0.0)	(0.2)	(0.069)	(0.054)
(DS) Unknown Pallars Sobirà	87.0	2.2	0.338	0.328
	(0.0)	(0.2)	(0.071)	(0.066)
(PJ) Pallars Jussà	42.0	2.2	0.252	0.313
	(0.0)	(0.2)	(0.069)	(0.082)
(CE) Cerdanya	32.0	2.2	0.269	0.309
	(0.0)	(0.2)	(0.064)	(0.051)
(RI) Ripollès	41.0	2.0	0.263	0.315
	(0.0)	(0.3)	(0.077)	(0.081)

(Standard errors in parentheses.)
(*) Unbiased estimate (Nei, 1978).
(**) ANOVA test: F ratio = 0.265; not significant.

TABLE 6. ESTIMATED F-STATISTICS PER *LOCUS* AT THE REGION LEVEL

Locus	F_{IS} (1)	F_{IT} (2)	F_{ST} (3)	χ^2 (1)	χ^2 (2)	χ^2 (3)
Hb	0.035	0.042	0.007	0.66	0.96	7.60
Tf	0.071	0.091	0.022	5.47	8.99*	47.78***
Alb	−0.058	−0.046	0.011	1.83	1.15	11.95
Gc	0.193	0.204	0.014	20.23***	22.60***	15.20*
Ptf2	0.043	0.056	0.013	1.00	1.70	14.12*
MEAN	0.070	0.083	0.014	29.19***	35.40***	96.65***

(*) $P<0.05$; (**) $P<0.01$; (***) $P<0.001$.

TABLE 7. ESTIMATED F-STATISTICS PER *LOCUS* AT THE NUCLEUS OF ORIGIN LEVEL

Locus	F_{IS} (1)	F_{IT} (2)	F_{ST} (3)	χ^2 (1)	χ^2 (2)	χ^2 (3)
Hb	0.016	0.033	0.017	0.13	0.57	17.85*
Tf	0.017	0.038	0.022	0.30	1.52	46.20***
Alb	−0.080	−0.052	0.026	3.36	1.42	27.30***
Gc	0.183	0.197	0.017	17.58***	20.37***	17.85*
Ptf2	0.052	0.058	0.006	1.42	1.76	9.92
MEAN	0.049	0.064	0.016	22.80***	25.64***	119.12***

(*) $P<0.05$; (**) $P<0.01$; (***) $P<0.001$.

NUCLEUS OF ORIGIN: Two large clusters are observed. One of the groups is formed by two Nuclei: Pallars Jussà (PJ) and Isil (IS); the other group includes the rest of the Nuclei. Within the last group are also two clusters, one including Espot Llessui (EL), PS-Unknown (DS) and Cerdanya (CE), and the other including Vall d'Aran (VA), Alta Ribagorça (AR) and Vallferrera (VF). The Nucleus of Ripollès (RI) seems more closely related to this cluster than to the cluster formed by Isil (IS) and Pallars Jussà (PJ).

TABLE 8. ESTIMATED F_{IS} VALUES FOR EACH *LOCUS* AND REGION

Region (Locus)	AR (83)	CE (23)	OB (102)	PJ (52)	PS (170)	RI (56)	SO (57)
Hb	0.095	0.098	−0.007	0.120	−0.012	0.338*	−0.310*
Tf	0.040	0.113	−0.056	0.302*	−0.120	0.030	0.176
Alb	−0.018	−0.070	−0.036	−0.020	−0.083	−0.037	−0.065
Gc	0.041	0.327	0.028	0.209	0.039	0.366**	0.340*
Ptf2	−0.030	−0.027	0.029	0.086	−0.013	0.143	0.132
MEAN	0.031	0.081	−0.006	0.187	−0.040	0.190*	0.066*

Analyzed sample size within parentheses.(*) $P<0.05$; (**) $P<0.01$; (***) $P<0.001$.

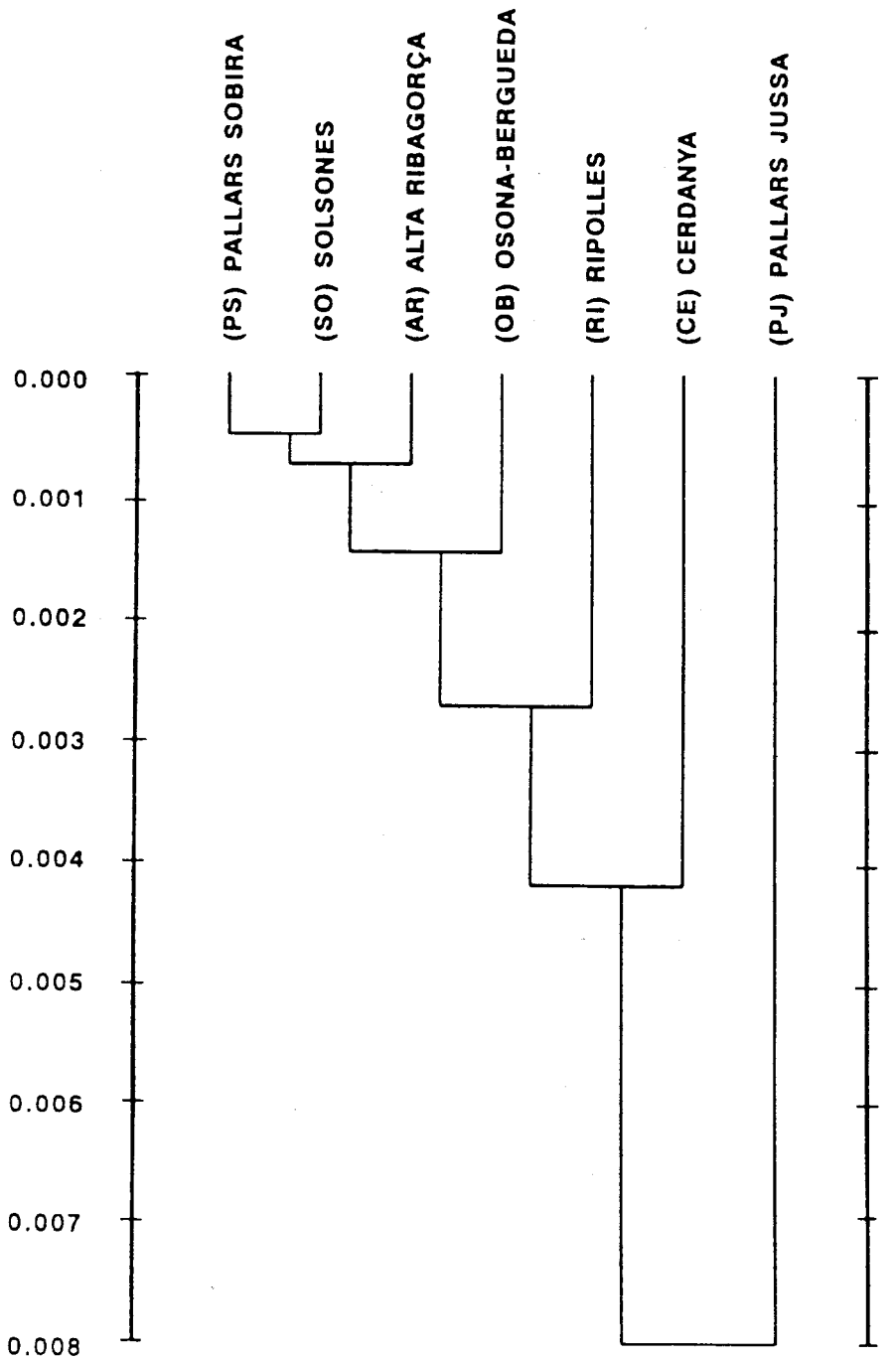

FIG. 2. PHENOGRAM OBTAINED BY THE UPGMA METHOD USING F_{ST} DISTANCE VALUES, AT THE REGION LEVEL.

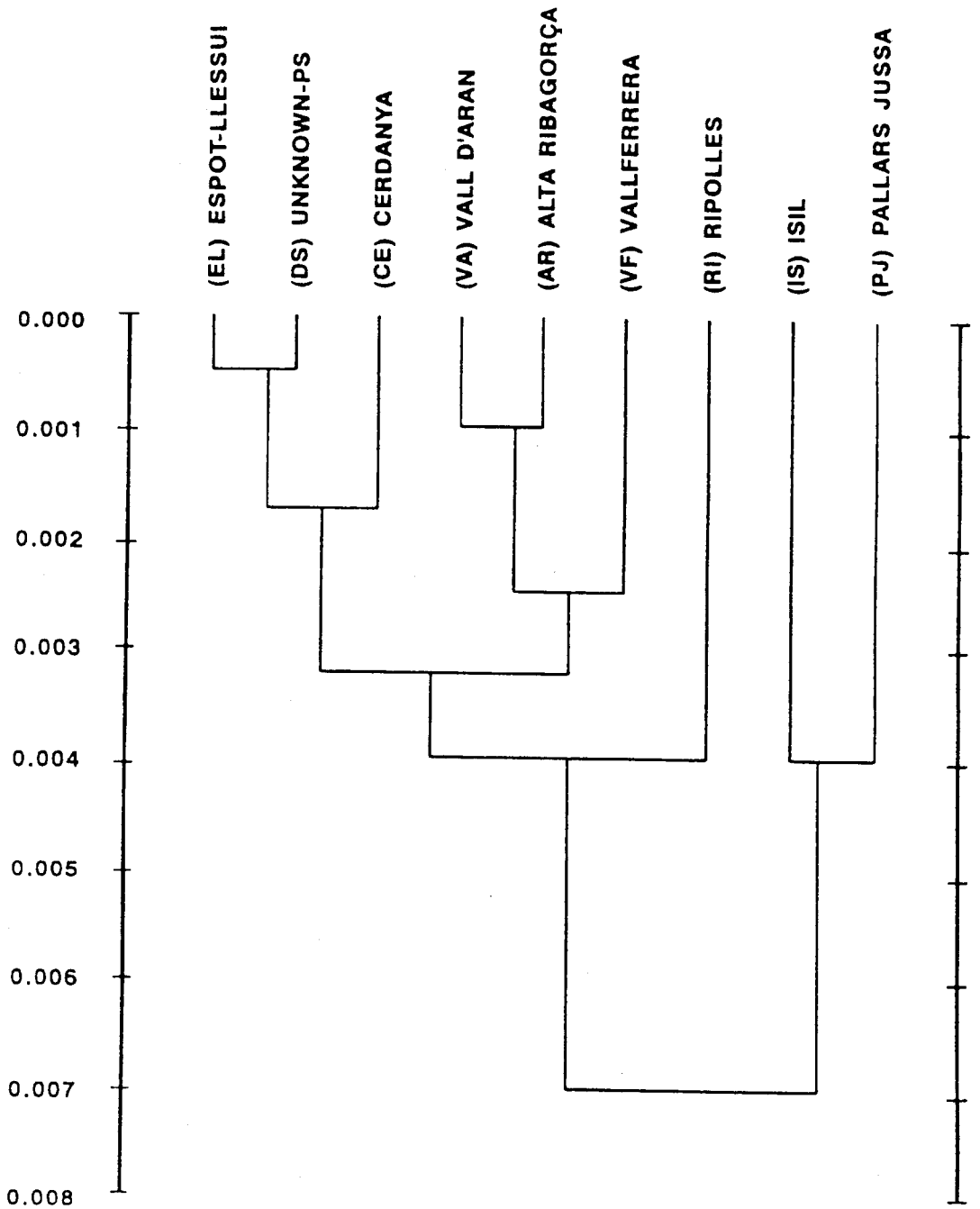

FIG. 3. PHENOGRAM OBTAINED BY THE UPGMA METHOD USING F_{ST} DISTANCE VALUES, AT THE NUCLEUS OF ORIGIN LEVEL.

Discussion

Genetic variability

The most important conclusion of this study, both at the Region level and at the Nucleus of Origin level, is the great genetic uniformity of the *Bruna dels Pirineus* breed. The great similarity in allelic distribution and number of alleles per *locus* (ranging between 2.0 and 2.2) suggests that the population size has stayed constant over time. It is known that a primary effect of a reduction in the population size is the loss of alleles, not reduction in the levels of heterozygosity (Nei, 1987). Moreover, according to Hartl and Pucek (1994), average heterozygosity alone is not a reliable indicator of population bottlenecks and genetic depletion, especially when only a small number of *loci* are investigated.

The observed genetic uniformity may be due to elevated gene flow between these subpopulations, a finding corroborated by the high, similar levels of heterozygosity seen in all subpopulations, ranging from 0.287 (PJ-Region) to 0.359 (Isil-Nucleus of Origin). Differences in heterozygosity levels between subpopulations were not statistically significant. It has been previously shown that elevated gene flow maintains high levels of heterozygosity (Waples, 1987; Terauchi, 1990; Ruiz-García, 1994) which suggests the potential importance of gene flow in this breed.

The F_{IS} value for the Nucleus level is lower than the F_{IS} at the Region level (4.9 vs 7.0), which is consistent with the fact that individuals of several regions have the same source of origin. In a parallel, but contradictory sense, the F_{ST} value is less for the Regions than for the Nucleus of Origin subdivisions since individuals of several sources are included in each Region; this tends to randomize the sample, leading subsequently to a minor degree of genetic differentiation between these subpopulations.

Degree of inbreeding and theoretical gene flow

Inbreeding is known to increase the frequency of homozygotes. We had a theoretical "maximum" value of inbreeding for the *Bruna* breed, ranging between 6.4 and 8.3% (highly significant F_{IT} values, Tables 6 and 7). Nevertheless, inbreeding should affect all *loci*, but the observed heterozygote deficit is attributed almost exclusively to the group-specific component *locus* (*Gc*). For this reason, inbreeding is not believed to be the principal cause of the observed heterozygote deficit.

Regionally (Table 8), F_{IS} values were significant only for Ripollès (RI) and Solsonès (SO), and at the level of Nucleus of Origin (Table 9) all the OTUs showed non-significant values, although the *Gc* system for Cerdanya (CE) showed very high significant differences. Selection (the "genetic hitchhiking" effect over *Gc locus*) might be the cause of the heterozygote deficit in these subpopulations if this *Gc locus* were linked to some morphological or productive trait of selective interest. The possible existence of null alleles is another possible cause of a heterozygote deficit.

The Wahlund Effect also may be a cause of the heterozygote deficit observed Ripollès and Solsonès. The breeding cows would have been introduced into these "receiver" regions in groups coming from several origins (mainly from the Pyrenean zones), with little or no genetic exchange among farms of the same area, contributing to the observed heterozygote deficit.

To obtain a more reliable estimate of the inbreeding coefficient of the *Bruna* population, the number of analyzed *loci* should have been increased and studied separately. Excluding the group-specific component system (*Gc*) from the analysis, the F_{IT} value equals 0.057 (5.7%) for the Region level, and F_{IT} is 0.036 (3.6%) for the Nucleus of Origin level. These values are substantially lower and less significant than those found previously ($\chi^2 = 12.80$, $df = 6$, $P<0.05$; $\chi^2 = 5.27$, $df = 6$, NS, respectively).

This lack of statistical significance of the heterozygote deficit found in the *Bruna* population confirms that the subpopulations are behaving as a single population with random matings. All the subpopulations show close agreement with HWE at the Nucleus of Origin level. In an "island" model, Wright (1978) showed that at equilibrium, F_{ST} values allow the estimation of the effective number of individuals, N_em, exchanged between populations in each generation (gene flow). Using Wright's formula, as modified by Takahata (1983) to take into account the number of populations (n), the theoretical gene flow (N_em) between these sub-

populations was 12.1, even when group-specific component (*Gc*) *locus* was excluded.

Trexler (1988) showed that if $N_e m > 1$ (in an infinite island model), gene flow is enough to attenuate the genetic differentiation between populations, balanced for migration and genetic drift. If an independent estimate of either N_e or m (migration rate) were available, the other could then be inferred. Both N_e and m are notoriously difficult to estimate although N_e is sometimes estimated from census data. Unfortunately, the sizes of breeding populations (census of males and females) were not known.

Genetic differentiation and relationships

In spite of the narrow geographical vicinity, the relatively low degree of genetic differentiation among subpopulations, the observed high level of gene flow, and the unlikely action of differential selective agents, we have observed a significant genetic heterogeneity between OTUs in the two levels of subdivision (Regions: $F_{ST} = 0.014$; $P < 0.001$; Nucleus of Origin: $F_{ST} = 0.016$; $P < 0.001$), indicating that significant subpopulation structure could exist within this population (Tables 6 and 7).

These F_{ST} values among subpopulations within the same breed are similar to the values reported for other organisms, for example: black-tailed prairie dogs (*Cynomys ludovicianus*) from different wards within a population ($F_{ST} = 0.05$; Chesser, 1983); Indians from different villages ($F_{ST} = 0.04$; Nei, 1975); Spanish dogs within different breeds ($F_{ST} = 0.037$; Jordana *et al.*, 1992); and human populations from Lowland South America and Highland New Guinea, subdivided by dialect groups ($F_{ST} = 0.025$ and $F_{ST} = 0.017$, respectively; Smouse and Long, 1988).

Theoretically, the differentiation or divergence between two populations can be the result of one or more causes: mutation, migration, natural and/or artificial selection and genetic drift, so it is difficult to determine precisely the factors causing the observed intra-breed differentiation in *Bruna dels Pirineus*. Nevertheless, migration and genetic drift could be the factors which have contributed the most to the observed intra-breed differentiation, owing to the low effective population size in the subpopulations studied. Furthermore, in most domestic species the drift process is accelerated, because both sexes are not equally represented, which is especially common in beef cattle.

The genetic uniformity observed in the *Bruna* breed should be mainly due to gene flow among its subpopulations. However, the amount of gene flow is not the same among all OTUs. The differences between the levels of genetic exchange and, consequently, between the genic differentiation values among subpopulations (F_{ST}) allow us to infer in greater detail the main migratory paths that have existed in this breed.

REGIONS: The Region level has been defined purely on geographical criteria, including animals of different origins that matured in that area. The study of this subdivision permits us to infer the "present" amount of dispersion of the breed, the trade of cattle among regions, and the current reproductive history of this population. The results of this study concur with the productive history of the breed, according to information from "Breeder's Society of the Bruna dels Pirineus breed".

For brevity, we will only consider the case of the Pallars Jussà (PJ) Region. Most matings in that area occur among the individuals of this Region, maintaining on average, a larger degree of reproductive isolation than that found among the other Regions. The first consequence of genetic drift is the differentiation between subpopulations; only Pallars Jussà (PJ) shows significant F_{ST} differences

(Table 10). The second consequence is the decrease in genetic variability of this subpopulation; in Table 4, we observe that (PJ) shows the least heterozygosity $H_e = 0.287$. The third consequence is an increase of homozygotes at the expense of heterozygotes ($F_{IS} = 0.187$; Table 8). The lack of significance of F_{IS} is not attributable to a small sample size, but rather to the uniform distribution of the deficit of heterozygotes across *loci*.

NUCLEUS OF ORIGIN: The Nuclei of Origin correspond to the geographical zones from which the animals came. The study of this subdivision allows us to infer the "original" migratory relationships of the breed. A generalized migratory trend from the Pyrenean mountain areas (Vall d'Aran, Alta Ribagorça, Espot-Llessui, Vallferrera) toward the pre-Pyrenean areas (Cerdanya, Ripollès), and particularly the NW–SE trend, clearly emerges. Incidentally, most of the animals included in the Unknown-Pallars Sobirà (DS) subpopulation might belong to the Espot-Llessui (EL) Nucleus of Origin, geographically located in the Pallars Sobirà Region. This hypothesis is supported in a Nei's distance of zero between both OTUs, and the smallest F_{ST} value ($F_{ST} = 0.001$; NS).

Pallars Jussà (PJ) and Isil (IS) nuclei do not maintain any relationship with the rest (Fig. 3). This lack of relationship (in the context of a high uniformity of the

TABLE 9. ESTIMATED F_{IS} VALUES FOR EACH *LOCUS* AND NUCLEUS

Nucl. Locus	VA (44)	AR (85)	VF (42)	EL (83)	IS (69)	DS (87)	PJ (42)	CE (32)	RI (41)
Hb	−0.019	0.044	0.074	−0.083	0.004	−0.097	−0.001	−0.041	0.332*
Tf	0.097	0.026	−0.082	−0.067	−0.175	−0.058	0.333	0.147	−0.045
Alb	−0.048	−0.018	−0.105	−0.031	−0.122	−0.048	−0.024	−0.085	—
Gc	0.277	0.045	−0.091	0.055	0.104	0.033	0.268	0.612***	0.255
Ptf2	0.198	−0.092	−0.071	−0.006	0.141	0.011	0.189	0.027	0.130
MEAN	0.130	−0.003	−0.050	−0.032	−0.016	−0.035	0.185	0.118	0.154

Analyzed sample size within parentheses. RI for the Albumin system was monomorphic.(*) $P<0.05$; (**) $P<0.01$; (***) $P<0.001$.

TABLE 10. F-STATISTICS ANALYSIS OF THE REGIONS COMPARED IN PAIRS

Regions	F_{IS}	F_{IT}	F_{ST}	Chi-square*
AR-CE	0.055	0.063	0.008	13.78*
AR-OB	0.012	0.015	0.003	6.29
AR-PJ	0.018	0.119	0.013	17.28**
AR-PS	−0.006	-0.004	0.002	12.14
AR-RI	0.112	0.117	0.005	7.51
AR-SO	0.050	0.051	0.001	4.20
CE-OB	0.034	0.044	0.010	17.25**
CE-PJ	0.134	0.160	0.030	29.10***
CE-PS	0.016	0.024	0.007	21.62**
CE-RI	0.137	0.151	0.016	14.85*
CE-SO	0.073	0.080	0.008	9.44
OB-PJ	0.084	0.095	0.011	20.33**
OB-PS	−0.023	−0.021	0.002	7.07
OB-RI	0.089	0.092	0.004	5.37
OB-SO	0.030	0.033	0.003	4.77
PJ-PS	0.066	0.075	0.010	28.86***
PJ-RI	0.189	0.203	0.017	19.87**
PJ-SO	0.122	0.136	0.015	17.66**
PS-RI	0.071	0.077	0.006	15.82*
PS-SO	0.013	0.015	0.001	3.18
RI-SO	0.126	0.132	0.007	7.23

*Significance of F_{ST} indicated by the chi-square value: (*) $P<0.05$; (**) $P<0.01$; (***) $P<0.001$. All the pairs were analyzed with 6 degrees of freedom.

TABLE 11. F-STATISTICS ANALYSIS OF THE NUCLEI COMPARED IN PAIRS

Nuclei	F_{IS}	F_{IT}	F_{ST}	Chi-square[a]
VA-AR	0.064	0.065	0.002	3.10
VA-VF	0.038	0.043	0.005	5.85
VA-EL	0.047	0.051	0.005	6.10
VA-IS	0.049	0.059	0.010	16.05*
VA-DS	0.042	0.049	0.007	8.38
VA-PJ	0.159	0.177	0.022	19.26**
VA-CE	0.124	0.129	0.006	5.93
VA-TI	0.143	0.147	0.005	7.31
AR-VF	−0.027	−0.023	0.004	9.65
AR-EL	−0.018	−0.015	0.003	5.04
AR-IS	−0.010	0.001	0.011	26.80***
AR-DS	−0.020	−0.015	0.005	9.29
AR-PJ	0.095	0.111	0.017	21.84**
AR-CE	0.059	0.064	0.005	10.30
AR-RI	0.079	0.085	0.006	9.32
VF-EL	−0.041	−0.032	0.008	14.50*
VF-IS	−0.032	−0.020	0.012	15.98*
VF-DS	−0.043	−0.033	0.009	13.42*
VF-PJ	0.069	0.089	0.021	19.49**
VF-CE	0.034	0.041	0.007	4.88
VF-RI	0.054	0.069	0.016	19.26**
EL-IS	−0.023	−0.012	0.011	25.84***
EL-DS	−0.034	−0.032	0.001	3.74
EL-PJ	0.077	0.090	0.013	20.25**
EL-CE	0.043	0.045	0.002	5.06
EL-RI	0.062	0.067	0.006	10.17
IS-DS	−0.025	−0.019	0.006	13.42*
IS-PJ	0.077	0.085	0.008	12.65*
IS-CE	0.046	0.061	0.061	23.84***
IS-RI	0.063	0.072	0.010	20.96**
DS-PJ	0.072	0.078	0.007	18.33**
DS-CE	0.039	0.044	0.005	9.28
DS-RI	0.057	0.063	0.006	12.29
PJ-CE	0.152	0.171	0.022	22.50***
PJ-RI	0.170	0.186	0.019	18.59**
CE-RI	0.136	0.146	0.011	13.72*

[a] Significance of F_{ST} indicated by the chi-square value: (*) $P<0.05$; (**) $P<0.01$; (***) $P<0.001$. All the pairs were analyzed with 6 degrees of freedom.

Bruna dels Pirineus breed) is confirmed by the comparative study of the F_{ST} values as both subpopulations, (PJ) and (IS), showed significant differences with all the rest and between themselves (Table 11). As in Region level analyses, genetic drift may be the factor which has most influenced the process of differentiation. With regard to the Isil nucleus (IS), we suspect that genetic drift has occasioned the differentiation with the remaining subpopulations (PJ included); however, the effects or consequences of the dispersive process are not manifested so clearly in this subpopulation. The only visible effect is the amount of differentiation (high and significant F_{ST} values). The observed heterozygosity is highest among all the nuclei, $H_e = 0.359$ (Table 5), which could indicate a high gene flow with the rest of the nuclei; furthermore, the observed F_{IS} value ($F_{IS} = -0.016$) is not indicative of an increase of homozygosity. These possible discrepancies suggest that the nucleus (IS) does not behave as a "small population" subjected to a dispersive process (Falconer, 1983). The (IS) nucleus seems to maintain reproductive isolation from the rest but maintains a sufficient population size with random matings. The results are a high heterozygosity and low F_{IS} values.

In conclusion, we propose the animals of the Pyrenean mountain areas (Vall d'Aran, Alta Ribagorça, Pallars Sobirà and Pallars Jussà) be the main nucleus for conservation and maintenance of the genetic variability of the breed.

The gene flow from the Pyrenean areas to the pre-Pyrenean ones (Cerdanya, Ripollès, Solsonès, Osona and Berguedà) contributes to the genetic uniformity of the *Bruna dels Pirineus* breed, and the maintenance of a particular gene pool, avoiding a possible genetic introgression from alien populations or breeds (for example Brown Swiss). The knowledge of the past and recent reproductive history of the breed ("original" migratory relationships) confirms that the past and current efforts to conserve the breed are on the right track.

The allele frequencies of the genetic *loci* analyzed add information to establish the genetic relationships that this population maintains with other Spanish and European cattle breeds in a similar way as do the studies carried out in this sense by Altarriba *et al.* (1977), Kidd *et al.* (1980), Aupetit (1985), González *et al.* (1987), Grosclaude *et al.* (1990), Moazami-Goudarzi *et al.* (1994), Arranz (1994) and Medjugorac *et al.* (1994).

This study constitutes a first approach to the genetic structure and molecular characterization of an endangered and small population. This study should be extended in the near future by using an adequate number of highly polymorphic microsatellite markers following the FAO recommendations (Barker *et al.*, 1993) in order to integrate this information into the FAO Global Data Bank on Domestic Animal Diversity.

Acknowledgements—The authors gratefully acknowledge "Departament d'Agricultura, Ramaderia i Pesca" of the "Generalitat de Catalunya" and "Fundació Caixa de Pensions" who financed this study; the reviewers for their suggestions and improvements; and Chuck Simmons for assistance with preparation of the manuscript.

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