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Note

Spatial Structure and Gene Flow from Biochemical Markers in the "Pyrenean Brown" Breed, a Rare Cattle Race in Catalonia (Spain)

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INTRODUCTION

Recently several researchers have established that major genetic resources in domestic animals are being lost at an accelerated rate due to genetic mixing caused by humans and the constant movement of these animals from one place to another (Baker *et al*., 1993). In addition, since the beginning of animal domestication, many local or regional forms of cattle have appeared on the face of the earth. Over centuries, many of these local cattles adapted themselves perfectly to their surrounding environmental conditions (see, for example, a lot of African and Asian cattle breeds; Hall and Ruane, 1993). This mixing has also resulted in the loss of genetic adaptations to strictly local conditions. One example is the local race of cattle, called Pyrenean Brown, found in the Catalonia Region of the Pyrenees Mountains. The origin of this local race apparently dates back to the fusion of two different European races about a century ago. Different ecotypes of the Pyrenean race were crossed with the Brown Swiss. This had been introduced mainly via France through the d'Aran Valley (Vall d'Aran). Since its appearance, this local race was confined to an area about 150 km long in the Catalonia Region of the Pyrenees Mountains. The number of individuals pertaining presently varies between 6,000–8,000; thus it can be classified either as rare (Hodges, 1992) or as having an insecure status.

The Pyrenean Brown race is interesting for several reasons. First, the origin

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of the race occurred at a specific point in the Pyrenees Mountains of the Catalonian region (Vall d'Aran). Second, the race has a limited geographic distribution in which different ecotypes of Pyrenean race that were probably geographically isolated could have contributed to the formation of the Pyrenean Brown. These characteristics suggest the possibility of an increase of some type of significant spatial structure in this race for five blood markers that were analyzed electrophoretically. Some questions were researched as follows.

1. Is there a cline of genetic differentiation from north to south between pure populations of this race of cattle due to the fact that the introduction of the Brown Swiss component initially and mainly was in the extreme northwest point of the Catalonian Pyrenees Mountains? Due to the steep and inaccessible terrain of this part of the Pyrenees, could isolation by distance have developed between these populations (Wright, 1943, 1969)?
2. The isolation by distance affects the genome globally. Given the possibility that this event did not take place, or that it had a relatively small impact, have all the markers analyzed the same spatial structure?
3. Sokal and Wartenberg (1983), Sokal *et al*. (1986, 1987, 1989), and Sokal and Jacquez (1991) showed that gene drift originates specific correlogram forms when a spatial autocorrelation analysis is applied. Would it be possible to detect this spatial structure in a statistic, as is the expected heterozygosity (Ruiz-Garcia, 1994b), which is sensitive to genetic drift?
4. If it is necessary to undertake reintroductions or reconstitute new stocks of Brown Pyrenean, based on what can be determined about the spatial structure of these cattle, from which of the study populations should individuals be selected? Would it make a difference?

Our spatial analyses provide concrete answers to these questions.

MATERIALS AND METHODS

Populations and Biochemical Markers Analyzed

A total number of 525 blood samples were obtained in nine pure "Brown Pyrenean" cattle populations. Blood samples were taken with EDTA 2Na (1 mg per ml of blood) and separated into plasma and red blood cell components, and stored at −20 C.

The populations are shown in Fig. 1. The populations sampled were Vall d'Aran (n = 44), Alta Ribagorca (n = 85), Pallars Jussa (n = 42), Cerdanya (n = 32), Ripolles (n = 41), Vallferrera (n = 42), Espot-Llessui (n = 83), Isil (n = 69) and Pallars Sobira (n = 87). The mean number of individuals sampled per population was 60. These sample sizes are large enough to be representative (Nei and Roychoudhury, 1974; Nei, 1978). Five loci were analyzed by diverse

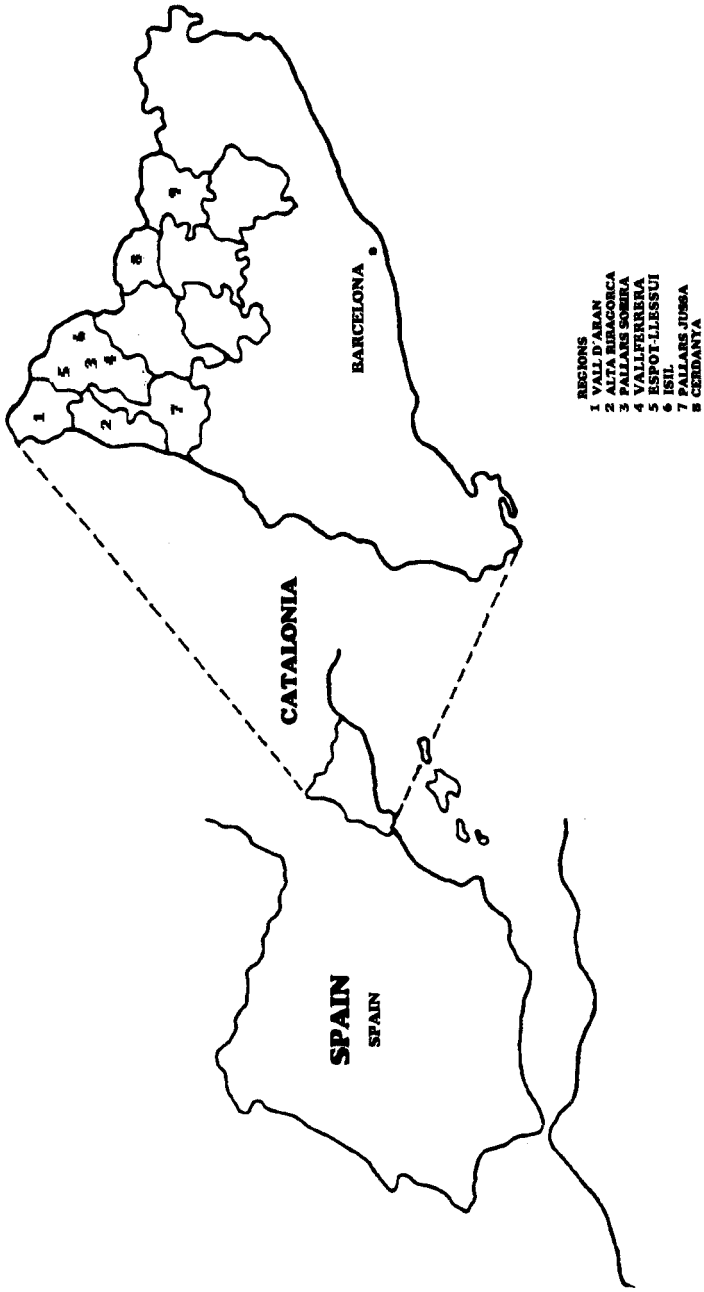

Fig. 1. Map of the Pyrenean Brown breed cattle populations analyzed in Catalonia (Spain).

electrophoretic techniques. The haemoglobin (Hb) red-blood-cell system was analyzed by horizontal electrophoresis in agarose gels (Gahne and Juneja, 1985). The plasma systems analyzed were transferrin (Tf), post-transferrin 2 (Ptf-2), group-specific component (Gc) studied by horizontal electrophoresis in polyacrylamide gels (Gahne *et al*., 1977) and albumin (Alb) studied by horizontal electrophoresis in starch gels (Bortolozzi, 1983).

Genetic and Spatial Statistics

To analyze if the populations studied were in Hardy-Weinberg equilibrium, Robertson and Hill's (1984) *f* statistic was applied to all the blood markers used.

The first spatiae technique employed was a global spatial autocorrelation analysis (Sokal *et al*., 1986, 1987, 1989; Sokal and Jacquez, 1991; Ruiz-Garcia, 1994a, b, 1997a, b; Ruiz-Garcia and Jordana, 1997; Ruiz-Garcia and Klein, 1997). There are, at least, three different procedures which must be applied to obtain evolutionary consequences.

(A) Analysis of Genetic Heterogeneity. From the cases of concordance and discordance between the observed genotypic frequencies for each locus in the populations studied, the following statistics were calculated: the Cochran-Mantel-Haenszel statistic (Cochran, 1954; Mantel and Haenszel, 1959) and the likelihood ratio square statistic (Fienberg, 1977; Everitt, 1992). It is important to distinguish among significant heterogeneity and significant spatial structure.

(B) Calculation of the Spatial Autocorrelation Coefficients and Correlograms. For continuous variables, two different statistics were used to determine the possible existence of some type of spatial structure in the allele frequencies of the five loci analyzed and the expected heterozygosity. These were Moran's *I* index (Moran, 1950) and Geary's *c* coefficient (Durbin and Watson, 1950). One spatial autocorrelation analysis with 4 DC is shown here (1 DC: 0–32 Km; 2 DC: 32–64 Km; 3 DC: 64–95 Km; 4 DC: 95–127 Km). All these particular geographic distances were chosen to optimize the allocation of population pairs. The graphs of the relation between spatial autocorrelation coefficients and geographic distances are called correlograms. The resulting correlogram summarized the pattern of geographic variation exhibited by the surface of a determined allele and is a simple analog of a spectral analysis of the surface. Correlograms of each allele describe the underlying spatial relation for a surface rather than its appearance. For this reason (Sokal, 1986), they are probably closer guides to the processes that have generated the surfaces than are the surfaces themselves.

(C) Similarity Analysis of the Correlograms. To determine the similarity between the correlograms for each allele analysed, we calculated the average Manhattan distance matrices (Sneath and Sokal, 1973) between the autocorrelation coefficients estimated for variable pairs of correlograms using Moran's *I* index. This analysis is useful to determine if each of the genetic loci studied was

subjected to the same spatial evolutionary event. Sokal *et al*. (1986, 1987, 1989) showed by means of simulation studies that correlogram pairs generated by the same evolutionary spatial processes have Manhattan distances smaller than 0.1 in the case of the Moran's I index.

A recent method to analyze the existence of isolation by distance for a set of genes studied in a population group was applied (Slatkin, 1993). This method is based on the regression equations between the estimate gene flow matrices (Nm = M; Cockerham and Weir, 1993) among locality pairs from the F_{st} and G_{st} statistics corrected by sampling sizes (Nei and Chesser, 1983; Weir and Cockerham, 1984) and the geographic distances among the same localities. The expression used is

$$\log_{10}(M) = a + b \log_{10}(\text{geographic distance})$$

If the *b* value is high and significantly negative, it is concluded that there is strong isolation by distance. If not, isolation by distance is not accepted as a parsimonious explanation.

The last analysis applied was a comparison of multiple regression equations between the genes analyzed and the geographic latitudes and longitudes of the populations. This technique let us analyze which spatial factor (latitude or longitude) played a more determinant role.

RESULTS

In Table I is shown the allele frequencies for the five genetic markers used in the nine cattle populations analyzed. The application of Robertson and Hill's (1984) *f* statistic manifested that global and individually, populations are in Hardy-Weinberg equilibrium. With the multiple Bonferroni's test, only the Gc locus in Cerdenya ($F = 0.6381$, var $= 0.0313$; $\chi^2 = 14.56$, 1 df, $P = 0.0003$) showed a significant deviation from that expected in Hardy-Weinberg equilibrium. The individual populations, and as a unique set, were also in Hardy-Weinberg equilibrium.

Analysis of Spatial Autocorrelation

(A) Analysis of Genetic Heterogeneity. The Cochran-Mantel-Haenszel statistic based on the contingency table results showed two loci exhibited significant heterogeneity (Tf, $\chi^2 = 28.9$, df $= 16$, $P = 0.025$; Alb, $\chi^2 = 29.3$, df $= 8$, $P < 0.001$), while the loci Hb, Gc, and Prtf-2 did not. Clearly, the locus which discriminates the most between these populations of cattle is Albumen.

(B) Autocorrelation Coefficients and Correlograms. Basically the same

Table I. Allele Frequencies of the Five Blood Allozymes Studied in Nine Populations[a] of the Pyrenean Brown Cattle Breed in Catalonia (Spain)

Locus[b]	VA (44)	AR (85)	VF (42)	EL (83)	IS (69)	PS (87)	PJ (42)	CE (32)	RI (41)
Hb-A	0.841	0.800	0.810	0.723	0.761	0.701	0.655	0.734	0.793
Hb-B	0.159	0.200	0.190	0.227	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
Tf-D	0.727	0.712	0.702	0.741	0.565	0.678	0.571	0.797	0.695
TF-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
Alb-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	0.163	0.203	0.172	0.131	0.141	0.268
Gc-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
Ptf2-F	0.727	0.700	0.667	0.729	0.754	0.741	0.798	0.703	0.732

[a]VA, Vall d Aran; AR, Alta Ribagorca; VA, Vallferrera; EL, Espot-Llessui; IS, Isil; PS, Pallars Sobira; PJ, Pallars Jussa; CE, Cerdanya; RI, Ripolles. Sample sizes are within parentheses.
[b]Hb, haemoglobin; Tf, transferrin; Alb, albumin; Gc, specific component Gc; Ptf2, pretransferrin-2.

results were obtained using Moran's I index and the c coefficient of Geary. None of the five genetic markers used exhibited significant overall spatial structure. Neither did the expected heterozygosity (Table IIA). Only 8.3% (2/24) of the spatial autocorrelation coefficient exhibited significant values with $\alpha = 0.05$. This percentage does not differ from a Type I error of 5%. The only variable which showed some similar spatial structure between the closest populations was Alb. The average correlogram also showed that no spatial structure was present. This is the first indication of high gene flow.

(C) Similarity of the Correlograms. In the analysis with four DC, the percentage of Manhattan's distances between the correlograms with lower values than 0.1 did not significantly differ from 5% type I error. This fact allows us to deduce that the evolutionary agents operating spatially were different for each of the variables implicated.

Slatkin's (1993) Isolation-by-Distance Test

Slatkin's (1993) statistic to detect isolation by distance was negative. It can be noted that the estimates of gene flow were extremely high. The mean values of M were 78.06 ± 97.31 and 43.14 ± 19.09, by using Fst and Gst respectively. The geographic distances explained, for both methods, between 0.000025% and 3.6% of the variation of the M estimates between population pairs. The regression equations between $\log_{10}$ (M) and $\log_{10}$ (geographic distance) were as follows:

$$\log_{10}(\mathrm{M}) = 1.490 - 0.005 \log_{10}(\text{geographic distance}), \text{ and}$$

Table II. (A) Spatial Autocorrelation Analysis; (B) Basic Multiple Regression Statistics Between the Five Blood Allozymes Studied and the Latitudinal and Longitudinal Geographic Components[a]

A

Variable	Hb	Tf-A	Tf-D	Alb	Gc	Ptf2	Average	Heter.$
Moran's I-4 DC								
1 (32 Km)	−0.36	−0.17	−0.22	0.13	−0.11	−0.34	−0.18	−0.23
2 (64 Km)	−0.06	0.03	0.04	−0.32	0.01	0.02	−0.05	−0.16
3 (95 Km)	0.19	−0.77*	−0.79*	−0.27	−0.65	−0.43	−0.45	−0.07
4 (127 Km)	−0.15	0.09	0.17	0.00	−0.03	0.12	0.03	0.06
CGRAMPROB	0.553	0.175	0.154	0.481	0.269	0.634		0.974

B

r (correlation coefficient)

	Latitude	Longitude
Hb	0.4675	−0.0237
Tf-D	0.1348	−0.3310
Tf-E	0.0016	−0.1826
Alb	−0.6865	−0.0341
Gc	0.2183	0.4664
Ptf-2	−0.3917	0.2021

Multiple regression equations

Hb = −0.1771 (latitude) + 0.02922 (longitude) + 6.798
Tf-D = −0.1259 (latitude) + 0.06864 (longitude) + 4.742
Tf-E = −0.0072 (latitude) + 0.0067 (longitude) + 0.2909
Alb = 0.1546 (latitude) − 0.02055 (longitude) − 7.538
Gc = −0.01095 (latitude) − 0.04641 (longitude) − 0.4292
Ptf-2 = 0.1088 (latitude) − 0.03105 (longitude) − 5.385

	R (Residual variances)	te (Typical errors)	P^2 (Square correlation multiple coefficients)
Hb	0.0025	0.0507	0.2701
Tf-D	0.0046	0.0680	0.1932
Tf-E	0.0002	0.0153	0.0398
Alb	0.0006	0.0253	0.5318
Gc	0.0021	0.0458	0.2189
Ptf-2	0.0009	0.0307	0.3046

[a]Only Moran's *I* index was used. Four distance classes were defined. Here we only show the analysis with distance classes of the same size. $, expected heterozygosity; CGRAMPROB, overall correlogram probability; Allozyme abbreviations as in Table I. *$P < 0.05$.

$\log_{10}(M) = 1.838 - 0.190 \log_{10}(\text{geographic distance})$, respectively.

It can be concluded that there was no significant distance isolation in the present model.

Multiple Regression Analysis for the Determination on the Importance of Latitudinal and Longitudinal Components

In general, important simple correlations between latitude, longitude, and the genes studied were not noted. Similarly, highly elevated values were not observed for the multiple correlation coefficients between each gene and the two geographic variables. The multiple regression equations, residual variances, multiple correlation coefficients (square transformed), and simple correlation coefficients between the genetic variables and each of the geographic variables are shown in Table IIB. The individual loci frequently exhibited different behaviors. As in previous analyses, although a significant conclusion cannot be made, the locus Alb showed the greatest dependence on geographic variables. There was a negative relation between latitude and genetic variation (46.6% of the variation explained). Both geographic variables together explained 53.2% of the genetic variation for this locus. This locus was the most informative to differentiate between cattle populations not only from the point of view of genetic heterogeneity, but also to show a slight tendency toward spatial structure.

DISCUSSION

The answers to the questions are as follows.

1. No cline of genetic differentiation was observed for this race of cattle in the Catalonian Pyrenees Mountains. The Pyrenean Brown race appears to have originated at the beginning of the century. If there was a significant structural cline at the moment when this race originated, enough time has passed to allow an elevated gene flow that appears to characterize this race, thus eliminating any cline. Moreover, the more or less complicated topography of the area has not been sufficient to generate isolation by distance for these populations. Further, for some genetic systems, there was greater genetic similarity between distant populations than between nearby populations. This could possibly suggest that between certain populations in the northwest Pyrenees and others situated more toward the southeast (Cerdenya and Ripolles) traditionally (or recently) there could have been more gene flow than between populations in the northwest Pyrenees (Vall d'Aran and Pallars Sobira). For the areas adjacent to the Pyrenees, it is unusual to buy or sell cattle within the region. The regions with high

mountains would be cattle producers, while the Prepyrenees or regions with low mountains would be the buyers (Jordana and Piedrafita, 1996).

2. There are no variables that exhibit significant spatial structures, nor are there any that are markedly different from each other. Thus there are no diversifying selective agents affecting any of the genes that were analyzed. In addition, the Manhattan distance analysis between correlogram pairs suggested that there were no unique evolutionary events affecting simultaneously the five genetic markers that were analyzed. This is one proof more of the wideranging and extensive rate at which gene flow took place in all directions and at various levels of intensity between the populations of these races of cattle.

3. The expected level of heterozygosity did not present any type of significant spatial structure, and was not even significant statistically. The absence of autocorrelation and the homogeneity of means of this genetic statistic confirm that stochastic processes are not very important as evolutionary agents in these cattle populations. The expected level of heterozygosity, individually as well as collectively, was quite high.

4. One would incorrectly conclude, due to the limited geographical extension of this race, that this is highly threatened due to genetic impoverishment through genetic drift (Kimura, 1964; Crow and Kimura, 1970; Crawford, 1984; Roughgarden, 1996), bottlenecks (O'Brien *et al*., 1983, 1985, 1987; Hedrick, 1995a, b), or a pronounced founder effect (Mani, 1984; Preleuthner and Pinsker, 1993). Likewise, one might also expect that many of the genetic characteristics of this race could easily be lost through genetic introgression as a result of mixing with other nearby races of cattle. This loss would be especially severe for populations at great distances from the point of origin of this race, especially if genetic flow was minimal. However, none of these possibilities is correct. This race of cattle, on the contrary, is characterized by a high level of genetic diversity and by a high level of random gene flow that has resulted in homogenized populations. Despite the small geographical range, no imminent genetic dangers are apparent, and none that would threaten the race with extinction. The result of long-distance shipping by cattle ranchers imposes differences compared with large wild mammals (Bonell and Selander, 1974; Scribner, 1993; Randi, 1993; Lorenzini *et al*., 1993; Roelke *et al*., 1993; Taylor *et al*., 1994; O'Brien, 1994; Gotelli *et al*., 1994). Small populations of domestic animals can possess high levels of genetic variability (Ruiz-Garcia and Jordana, 1997). Some ideas exposed here should be useful in the management of wild Bovidae or Cervidae (to maintain in captivity little groups of animals with high levels of genetic diversity). For this race, in case it is necessary to initiate repopulation of an area or to reconstitute new stocks for ranching purposes, the selection of one population or another as the source would not have any effect on the genetic makeup of the resulting populations. The high level of genetic exchange and the highly homog-

enized nature of the populations makes them quite similar genetically. For this reason, it would not make any difference which population was selected.

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