Brazilian Journal of Genetics, 20, 2, 225-236 (1997)
(Revista Brasileira de Genética)

Spatial genetic structure of the "Gos d'Atura" dog breed in Catalonia (Spain)

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ABSTRACT

Six "Gos d'Atura" dog populations were studied in Catalonia (Spain) using 21 allozymic loci. Of these loci, 11 were polymorphic (Sod, Lap, Mpi, Alb, Pep-D, Tf, α1B, Pi-1, Prt-1, Prt-2 and Pa-1). The levels of expected average heterozygosity and the percentage of polymorphism were high compared to other mammals, and all populations were in Hardy-Weinberg equilibrium. The overall genetic heterogeneity was relatively small (F_{st} = 0.04), although significant. Only one locus (Pi-1) showed clear significant heterogeneity. This locus also showed a significant monotonic cline when a spatial autocorrelation analysis was conducted. Other loci (Sod A, Alb S, Tf B, Pi-1 F, Mpi A, Pep-D A) showed significant spatial structure but were not monotonic clinal. Some of them, such as Tf B and Pep-D, showed differentiation between distant populations and Mpi A showed significant similarity between the closest populations.

INTRODUCTION

Although several studies of genetic variability in different dog breeds have been made (e.g. Tanabe *et al.*, 1978; Juneja *et al.*, 1981; Jordana, 1989; Jordana *et al.*, 1992), little is known about the spatial structure of genetic variation in dogs. It is important to know if the intrabreed genetic variability, in a domestic species, can have a spatial structure because it would help understand the evolutionary history of the species as well as the effect of human influence on both the reproductive and genetic structure of a particular species.

The "Gos d'Atura" breed occurs in Catalonia (Spain). It is a shepherd dog, and was the symbol of the Barcelona 1992 Olympic Games. Six populations were sampled. Four of them were sampled in little rural villages where dogs roam free, taking care of the flocks of sheep. The samples of these rural villages come from free-ranging dog populations of this breed in which the animals themselves make selections of mates. The other two populations were sampled in cities (Barcelona and Girona) and the individuals were obtained from professional dog breeders. In these two populations, most selection of mates is made on the basis of pedigrees and enforced artificial selection by humans. The spatial structure of genetic variation of the domestic cat *Felis catus*, distributed in the same area of the Iberian Peninsula (Ruiz-Garcia, 1991, 1993, 1994, 1997; Ruiz-Garcia *et al.*, 1995) has been comparatively studied. In the case of the cats, they were stray, feral or "pseudo-wild".

Knowledge about the genetic variability, differentiation, and spatial structure of genetic differentiation in domestic animal populations (and breeds) can

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contribute in a decisive way to their rational improvement and conservation. This knowledge would help define new "genetic sources" for future generations and to establish better strategies in selection programs of domestic animals. This kind of information should be integrated into the FAO Global Data Bank on Domestic Animal Diversity (Barker *et al.*, 1993).

MATERIAL AND METHODS

Eighty-six blood samples were obtained from different individuals that belong to the dog breed "Gos d'Atura". These samples came from six localities in Catalonia: Vallferrera (N = 22), Vall d'Assua (N = 12), Urgell (N = 10), Conca de Barbera (N = 17), Barcelona (N = 18) and Girona (N = 7). The localization of these populations can be seen in Figure 1. The individuals were randomly chosen. The blood samples were taken with Na_2 EDTA (1 mg per ml blood). The samples were separated into the three blood components, plasma, red blood cells and white blood cells and stored at -20°C. By means of different electrophoretic techniques (horizontal electrophoresis in starch gel, polyacrylamide and bidimensional agarose-polyacrylamide gels) 21 loci were studied. These loci were: superoxide dismutase (Sod), glucose phosphate isomerase (Gpi), 6-phosphogluconate dehydrogenase (6-Pgd), phosphoglucomutase-1 (Pgm1), and glucose 6-phosphate dehydrogenase (G6pd) (five red blood cell systems), mannose phosphate isomerase (Mpi), malate dehydrogenase soluble form (Mdhs), malate dehydrogenase mitochondrial (Mdhm), acid phosphatase (Pac) (four leucocyte systems), leucine amino-peptidase (Lap), albumin (Alb), peptidase D (Pep-D), transferrin (Tf), prealbumin (Pr), Gc protein (Gc), α1B-glycoprotein (α1B), α1-protease inhibitor (Pi-1), protease inhibitor-3 (Pi-3), postalbumin-1 (Pa-1), pretransferrin-1 (Prt-1) and pretransferrin-2 (Prt-2) (12 plasma systems). Of these 21 loci, 11 were polymorphic (Sod, Lap, Mpi, Alb, Pep-D, Tf, α1B, Pi-1, Prt-1, Prt-2 and Pa-1). For a detailed description of electrophoretic techniques, buffer systems, and staining recipes used, see Jordana (1989).

Genic variability statistics

The study of unbiased average expected heterozygosity (Nei, 1978), the percentage of polymorphism, and the number of alleles per locus are important criteria in determining the existence of a possible diversification of the stochastic processes in this breed and between the populations of this breed. The unbiased average expected heterozygosity is especially important due to its independence from evolutionary events, such as selection favoring (or not favoring) homozygotes and/or heterozygotes, or the reproductive system used. On the other hand, the average expected heterozygosity can be influenced by certain stochastic processes (for example, gene drift; Nygren and Rasmusson, 1980).

Figure 1 - Map with the origin of the six "Gos d'Atura" dog populations studied in Catalonia (Iberian Peninsula).

Analysis of Hardy-Weinberg equilibrium

The observed genotypic frequencies were compared with the expected frequencies, assuming Hardy-Weinberg equilibrium, in those loci that showed genetic variability. Two different analytical procedures were used. One was the Wright's F (1965) whose expression is:

$$F = (H_e - H_o)/H_e = 1 - H_o/H_e$$

where H_o is the number of observed heterozygotes and H_e is the number of expected heterozygotes. As F maintains a relationship with the statistic χ^2, a significant deviation from zero can be tested:

$$\chi^2 = F^2 N (m - 1)$$

with m (m - 1)/2 degrees of freedom, where N is the sample size and m is the number of alleles at each locus studied (Li and Horvitz, 1953). The F variance was measured in the same way, as was done by Rasmussen (1964). The second analytical procedure was an unbiased estimator (f) based on the proportion of homozygotic alleles, and it can be used as a measure of the endogamy coefficient. This procedure was developed by Robertson and Hill (1984). For each *i* allele, the unbiased estimation of the homozygote frequency (*ii*) deviations with respect to the expected values, according to Hardy-Weinberg equilibrium is:

$$T_{ii} = [2(2n - 1) n_{ii} - n_i (n_i - 1)]/[4(n - 1)]$$

where n is the sample size, n_{ii} is the number of observed homozygotes and n_i is the number of *i* alleles in the sample. For each allele, the f estimation is expressed by:

$$f_{ii} = 4nT_{ii}/[n_i (2n - n_i)].$$

The estimations obtained for each *i* allele are used to obtain a global estimator for each locus studied, weighting f_{ii} with $(1-p_i)$, where p_i is the frequency of that allele in a given population. The total statistic (f_T) is expressed by:

$$f_T = 2 (T_{ii}/n_i)/(m - 1)$$

where m is the number of alleles at a given locus. The variances of the f_{ii} estimation for each allele and the f_T estimation for each locus are respectively:

$$VAR\ f_{ii} = 1/n \text{ and } VAR\ f_T = 1/[n(m - 1)].$$

Both, the Wright's F and the Robertson and Hill's f estimations were calculated for each allele and for each locus in each of the populations, and also for the group of six Catalan populations. The significance level in each of the tests carried out was $\alpha = 0.05$. However, we also used Bonferroni's multiple method (e.g. Vrijenhoek and Graven, 1992). With this criteria of multiple tests, the significance level chosen was $\alpha' = 0.00094$ (= 0.05/53) which corresponded to an F-value ≥ 0.8692.

Genetic differentiation statistics

To analyze the degree of genetic differentiation among the six localities studied, an analysis of genic diversity was used (Nei, 1973, 1975). The following statistics were calculated: H_t (genic diversity in the total population), D_{st} (average gene diversity between populations) and G_{st} (genetic differentiation between populations relative to the gene diversity in the total population). We also calculated $F_{st}' (= F_{st} - 1/2N_t)$. This is the estimate of the genetic heterogeneity between populations (standardized variance), using one of the fixation indexes of Wright (1965), corrected for sampling error (Workman and Niswander, 1970), where N_t is the sample size. We tested the null hypothesis $F_{st}' = 0$ because $\chi^2 = 2N_t F_{st}' (k - 1)$ with (k - 1) (s - 1) degrees of freedom, where *k* is the number of alleles studied and *s* is the number of populations studied. Both means and variances used were weighted according to sample size.

Genetic relationships between localities

Two genetic distances with different mathematical properties were used to analyze the genetic relationships between these dog localities: the Nei's (1972) genetic distance and the Cavalli-Sforza and Edwards' (1967) chord distance. With both genetic distance matrices, a principal coordinates analysis using the Gower (1966) procedure was developed. In this paper, we show the three-dimensional axis relationship between the populations. To see the probable local distortion generated by this process of dimensional reduction, a graphic matrix ("Minimum spanning tree") was superimposed (Gower and Ross, 1969; Rohlf, 1970). The genetic distance matrices between the six samples were also clustered with a UPGMA algorithm (Sneath and Sokal, 1973).

Spatial autocorrelation analysis

To determine if there was some significant spatial structure in the patterns of allozyme variation among these six "Gos d'Atura" samples, a spatial autocorrelation analysis was carried out. There are four analytical techniques applied to perform a global spatial autocorrelation analysis (Sokal, 1979; Sokal and Wartenberg, 1983; Sokal *et al.*, 1989): a) estimation of statistical heterogeneity. To calculate the genetic heterogeneity of each studied loci, we used the χ^2 values arising from F_{st} statistics. b) Calculation of autocorrelation coefficients and correlograms. We used two different statistics: Moran's I index (Moran, 1950) and Geary's c coefficient (Durbin and Watson, 1950). Both, Moran's and Geary's coefficients were calculated for all possible pairs of populations separated by geographic distances within a range of distance classes arbitrarily

chosen by the investigators. In this study, we chose correlograms with three distance classes (DC): 1 DC: 0-102 km; 2 DC: 102-137 km; 3 DC: 137-160 km. These particular distances were chosen to optimize the allocation of locality pairs (an equal number of point pairs) within each distance class. This minimizes comparisons between distance classes represented by inadequate samples. The connection matrix was binary. In addition, a single autocorrelation coefficient was obtained for each genetic variable studied. In this case, the point pairs were weighted as the inverse square separation distance between the localities. Only one allele was examined in order to reduce the stochastic dependence of gene frequencies in the same locus (in the case of diallelic loci). In the case of triallelic loci, only two alleles are submitted to spatial autocorrelation analysis. The percentage of significant autocorrelation coefficients was obtained to determine if it is superior to the 5% error margin. This percentage was compared with the percentage found in a group of domestic cat populations in the same area of Spain. The Bonferroni procedure was used to determine the statistical significance for autocorrelation coefficients (Oden, 1984). c) Similarity analysis between the variable surfaces. The similarity of the gene surfaces was studied using the product-moment correlation coefficient of Pearson (r). We calculated the percentage of correlations higher than $|0.7|$ to verify if they were higher than 5% (Sokal *et al.*, 1987). This percentage was compared with the percentage of correlations higher than $|0.7|$ calculated for seven genetic variables which code for color, tabby, and length of coat in the group of domestic cat populations in the same area of Spain (Ruiz-Garcia, 1991, 1993). d) Similarity analysis of the correlograms. To determine the similarity between the correlograms, we calculated the average Manhattan distance matrices (Sneath and Sokal, 1973) between the autocorrelation coefficients estimated for variable pairs of correlograms. This analysis can determine if each of the genetic variables studied was subjected to the same spatial evolutionary event, or if they were under pressure from different spatial evolutionary agents. Sokal and Wartenberg (1983) and Sokal *et al.*, (1986, 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 Moran's I index, and smaller than 0.2 in the case of Geary's c coefficient. For this reason, we considered the percentage of Manhattan distance values smaller than 0.1 and 0.2, respectively. These values were compared with the data obtained in our study of domestic cat populations (Ruiz-Garcia, 1991, 1993). To determine the similarity of the spatial relationships among the allozymic variables studied, we applied the UPGMA algorithm to the Manhattan distance matrices.

Mantel test

The Mantel (1967) test was used to find relationships between genetic distances and the geographical distances separating the dog populations. The Mantel statistic was normalized using Smouse *et al.*'s (1986) technique, which transforms that statistic into a correlation coefficient. We generated two different types of matrices with the genetic variables. In the first case, the possible dependence between the Nei (1972) and Cavalli-Sforza and Edwards (1967) genetic distance matrices and the geographic distance matrices were analyzed. The data were used with and without logarithmic transformations. The existence of a significant association between both types of matrices could reveal a global trend of spatial autocorrelation between the localities studied. In the second case, we obtained the individualized Mantel statistic for each of the genetic variables studied. To do this, the matrices of absolute differences between gene frequencies of each variable and the geographic distances between localities were compared. The correlation significance was tested by means of a Monte Carlo simulation (with 2,000 random permutations), and with an approximate Mantel *t*-test.

RESULTS

The gene frequencies of the 11 electrophoretic loci demonstrating genetic variability are shown in Table I.

Genetic variability

The mean value of the expected heterozygosity for the six localities analyzed was $\overline{H} = 0.1647$ (± 0.0055) and was $\overline{H} = 0.167$ (± 0.048) for the global group of dogs (Table II). There were no significant differences in the mean values of expected heterozygosity between the localities studied (pairs of localities; Student *t*-test) nor for the global group of localities (Kruskal-Wallis H test). The percent polymorphism (P) ranged from 38.1% (Girona and Vall d'Assua) to 47.6% (Vallferrera), with a mean of 42.86%. Comparisons of the percentages between locality pairs showed no significant differences for the entire group. The mean number of alleles per locus ranged from 1.5 ($\pm$ 0.1) to 1.6 ($\pm$ 0.2). There were no significant differences between localities, or for the entire group.

Table I - Values of the gene frequencies and sample size (N) for each of the eleven polymorphic loci studied in the six populations of the "Gos d'Atura" dog breed.

Locus	Alleles	Populations					
		VF	VA	UR	CB	BA	GI
Sod	(N)	22	12	10	17	18	7
	A	0.932	1.000	1.000	1.000	0.972	1.000
	B	0.068	0.000	0.000	0.000	0.028	0.000
Lap	(N)	22	12	10	17	18	7
	A	0.932	1.000	1.000	1.000	0.944	0.929
	B	0.068	0.000	0.000	0.000	0.056	0.071
Alb	(N)	22	12	10	17	18	7
	S	0.568	0.542	0.350	0.500	0.694	0.786
	F	0.432	0.458	0.650	0.500	0.306	0.214
Tf	(N)	22	12	10	17	18	7
	B	0.364	0.542	0.600	0.592	0.472	0.214
	C	0.636	0.458	0.350	0.471	0.582	0.786
	E	0.000	0.000	0.050	0.000	0.000	0.000
Pi-1	(N)	22	12	10	17	18	7
	S	0.045	0.208	0.350	0.265	0.305	0.500
	I	0.250	0.250	0.150	0.147	0.028	0.000
	F	0.705	0.542	0.500	0.588	0.667	0.500
α1B	(N)	22	12	10	17	18	7
	S	0.386	0.625	0.600	0.618	0.722	0.714
	F	0.614	0.375	0.400	0.382	0.278	0.286
Mpi	(N)	22	12	8	17	18	7
	A	0.795	0.875	0.938	0.912	1.000	1.000
	B	0.205	0.125	0.063	0.088	0.000	0.000
Prt-1	(N)	21	12	10	17	18	7
	S	0.071	0.083	0.050	0.000	0.083	0.071
	F	0.667	0.500	0.650	0.765	0.778	0.786
	D	0.262	0.417	0.300	0.235	0.139	0.143
Prt-2	(N)	21	12	10	15	18	7
	S	0.024	0.000	0.050	0.100	0.000	0.000
	F	0.976	1.000	0.950	0.900	1.000	1.000
Pep-D	(N)	22	12	10	15	18	7
	A	0.886	0.917	0.850	0.941	0.833	0.643
	B	0.114	0.083	0.150	0.059	0.167	0.357
Pa-1	(N)	11	12	10	15	13	7
	S	0.591	0.545	0.750	0.733	0.538	0.500
	F	0.409	0.455	0.250	0.267	0.462	0.500

VF = Vallferrera, VA = Vall d'Asua, UR = Urgell, CB = Conca de Barbera, BA = Barcelona, GI = Girona. Sod = Superoxide dismutase; Lap = leucine amino peptidase; Alb = albumin; Tf = transferrin; Pi-1 = α1 protease inhibitor; α1B = α 1B-glycoprotein; Mpi = mannose phosphate isomerase; Prt-1 = pretransferrin-1; Prt-2 = pretransferin-2; Pep-D = peptidase D; Pa-1 = postalbumin-1.

Table II - Expected mean heterozygosity (E.M.H.), percentage of polymorphic loci and number of alleles per locus (± SD) in six dog populations of the "Gos d'Atura" breed in Catalonia (Spain).

Populations	E.M.H. ± SD	% Polymorphism	# of alleles per locus
Vallferrera	0.179 ± 0.047	47.6	1.6 ± 0.1
Vall d'Assua	0.174 ± 0.054	38.1	1.5 ± 0.1
Urgell	0.170 ± 0.051	42.9	1.5 ± 0.1
Conca de Barbera	0.159 ± 0.048	42.9	1.6 ± 0.1
Barcelona	0.152 ± 0.046	42.9	1.5 ± 0.1
Girona	0.155 ± 0.046	38.1	1.5 ± 0.1

Hardy-Weinberg equilibrium analysis

In the localities of Girona, Conca de Barbera, Vall d'Assua and Vallferrera, there were no significant differences between observed and expected genotypic frequencies for all loci. In the Barcelona sample, only one locus (Tf) showed a significant departure according to the Robertson and Hill's f statistic (f = 0.4852; VAR f = 0.0566; χ^2 = 4.237, 1 d.f., P = 0.0396). In Urgell, only the Pep-D locus showed a significant excess of homozygotes using the same statistic (f = 0.6972; VAR f = 0.1000; χ^2 = 4.860, 1 d.f., P = 0.0275). For the overall group of six localities no locus showed significant departure from Hardy-Weinberg equilibrium, using Wright's F statistic, as well as Robertson and Hill's f statistic. None of the 53 tests made to assess the fit to Hardy-Weinberg equilibrium with the criteria of standard Bonferroni technique (α' = 0.05/53 = 0.00094; F ≥ 0.8692) showed significant deviations.

Genetic differentiation statistics

Only one of the 11 loci that had genetic variability showed a clearly significant heterogeneity (Pi - 1: F_{st}' = 0.0584; $\chi^{2'}$ = 20.108, 10 d.f., P < 0.05; G_{st} = 0.0642) (Table III). This heterogeneity was principally produced by allele Pi - 1 S (F_{st}' = 0.0919; $\chi^{2'}$ = 15.81, 5 d.f., P < 0.001). Another two loci exhibited borderline statistically significant heterogeneity (α_1B and Mpi). If we used the F_{st} statistic without correction for sample size, then there would be significant values of heterogeneity for α_1B and Mpi (see Table III). However, if the F_{st}' is used with correction for sample size, neither of the two loci shows significant heterogeneity. The other loci (Sod, Lap, Alb, Tf, Prt-1, Prt-2, Pep-D, Pa-1) did not show significant heterogeneity in any case.

There was significant genetic heterogeneity for the total value of the 11 analyzed polymorphic loci ($\overline{F}_{st'}$ = 0.0431 ± 0.013; $\Sigma\chi^{2'}$ = 101.98, 70 d.f., P < 0.001). Thus, each population had, on average, 95.7% of the total genic diversity found in the overall area studied.

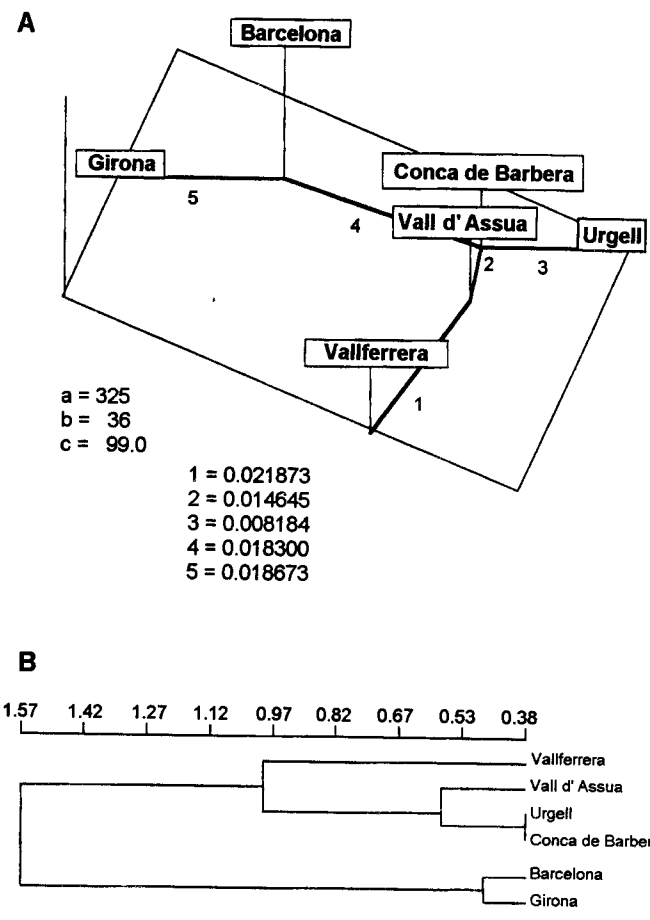
Principal coordinates and phenetic analyses

The Nei's and Cavalli-Sforza and Edwards' distances offered similar results when a principal coordinates analysis was applied (Figure 2a). It was evident that the Barcelona and, especially, the Girona population were slightly different from the remainder of the populations studied. Using both genetic distances, the values of the graphic matrix (MST) showed the close connection between the localities of Vall d'Assua and Conca de Barbera, Urgell and Conca

Table III - Analysis of genic diversity and genetic differentiation in the six populations of the "Gos d'Atura" dog breed studied in Catalonia.

Locus	H_t	D_{st}	G_{st}	$F_{st'}$	χ^2	d.f.	
Sod A	0.0454	0.0016	0.0357	0.0299	5.138	5	NS
Lap A	0.0673	0.0021	0.0307	0.0249	4.285	5	NS
Alb S	0.4903	0.0275	0.0560	0.0502	8.637	5	NS
Tf B	0.4966	0.0229	0.0462	0.0404	6.954	5	NS
Tf C	0.4975	0.0267	0.0538	0.0479	8.248	5	NS
Tf E	0.0115	0.0005	0.0444	0.0386	6.644	5	NS
Tf (average)	-	-	0.0481	0.0423	14.564	10	NS
Pi-1 I	0.2566	0.0178	0.0694	0.0636	10.944*	5	SIG
Pi-1 F	0.4756	0.0122	0.0256	0.0198	3.407	5	NS
Pi-1 S	0.3631	0.0355	0.0977	0.0919	15.811**	5	SIG
Pi-1 (average)	-	-	0.0642	0.0584	20.108*	10	SIG
α1B F	0.4849	0.0317	0.0654	0.0595	10.240	5	NS***
Mpi A	0.1723	0.0121	0.0704	0.0644	10.020	5	NS***
Prt-1 F	0.4246	0.0178	0.0419	0.0361	6.129	5	NS
Prt-1D	0.3721	0.0157	0.0422	0.0363	6.169	5	NS
Prt-1 S	0.1107	0.0019	0.0174	0.0115	1.963	5	NS
Prt-1 (average)	-	-	0.0338	0.0279	9.508	10	NS
Prt-2 F	0.0584	0.0027	0.0460	0.0400	6.641	5	NS
Pep-D	0.2316	0.0117	0.0508	0.0450	7.743	5	NS
Pa-1 S	0.4720	0.0184	0.0389	0.0315	4.292	5	NS
Average	0.2959 ± 0.0928	0.0152 ± 0.0056	0.0489 ± 0.0096	0.0431 ± 0.0068	101.988****	70	SIG

Direct G_{st} value = 0.0514, *P < 0.05, **P < 0.01, ****P < 0.001.
***If F_{st} values are not corrected by sample size, α1B F and Mpi-A show significant heterogeneity (χ^2 = 11.24, 5 d.f. P < 0.05 and χ^2 = 11.82, 5 d.f. P < 0.05, respectively).



de Barbera and Barcelona and Girona. The most important relationship was found between Urgell and Conca de Barbera (Nei: MST = 0.00818; Cavalli-Sforza and Edwards: MST = 0.37606). Also the UPGMA phenogram with Nei's and Cavalli-Sforza and Edwards' distances offered identical results. There were two principal clusters: one with Barcelona and Girona and the other with the remaining populations, where Vallferrera was the most differentiated (Figure 2b).

Spatial autocorrelation analysis and Mantel's test

Spatial autocorrelation analysis and individualized Mantel's test for each variable studied

The results of these analyses can be seen in Tables IV and V.

The Lap A, Tf C, α_1B F, Prt-1 F, Prt-1 D, Prt-2 F and Pa-1 S alleles did not show any spatial genetic structure. The following genes did show significant spatial genetic patterns:

Figure 2 - (A) Principal coordinates analysis with Gower (1966) procedure of the six "Gos d'Atura" dog populations studied using the Nei (1972) distance with the Minimum Spanning tree superimposed. (B) UPGMA phenetic analysis with the Cavalli-Sforza and Edwards (1967) distance.

Table IV - Spatial autocorrelation analysis with both the Moran's I index and Geary's c coefficient for the 14 allozymic alleles studied in the "Gos d'Atura" samples, and average coefficients for each distance class. Distance classes are identified only by the upper limit.

Allele	Distance classes			
	102.4 km	136.9 km	159.8 km	CGRAMPROB
Moran's I				
Sod A	-0.357	-0.615	0.372*	0.062
Lap A	-0.134	0.218	-0.684	0.334
Alb S	0.066	0.101	-0.767*	0.142
Tf B	0.034	0.253	-0.886*	0.054
Tf C	0.051	-0.250	-0.400	0.675
Pi-1 I	0.540*	-0.223	-0.917*	0.050
Pi-1 F	-0.609	-0.529	0.538*	0.070
α1B F	0.127	-0.364	-0.363	0.355
Mpi A	0.493*	-0.455	-0.638	0.063
Prt-1 F	0.251	-0.499	-0.353	0.238
Prt-1 D	0.200	-0.247	-0.554	0.352
Prt-2 F	-0.028	-0.344	-0.228	0.869
Pep-D A	0.098	0.066	-0.764*	0.048
Pa-1 S	0.161	-0.315	0.445	0.501
Average Morans'I	**0.064**	**-0.229**	**-0.435**	
Geary's c				
Sod A	0.901	1.791	0.308*	0.137
Lap A	0.860	0.716	1.424	0.311
Alb S	0.808	0.596	1.596	0.172
Tf B	0.610	0.541	1.849*	0.041
Tf C	0.629	0.974	1.397	0.447
Pi-1 I	0.265*	0.917	1.818*	0.032
Pi-1 F	1.287	1.358	0.355*	0.105
α1B F	0.536	1.406	1.031	0.432
Mpi A	0.303*	1.321	1.376	0.095
Prt-1 F	0.474	1.517	1.010	0.264
Prt-1 D	0.582	1.135	1.282	0.405
Prt-2 F	0.969	1.182	0.849	1.000
Pep-D A	0.502	0.600	1.898*	0.047
Pa-1 S	0.765	1.103	1.132	0.761
Average Geary's c	**0.679**	**1.082**	**1.237**	

CGRAMPROB = Global Correlogram Probability.
Significant at *P < 0.05.

Sod A: The spatial autocorrelation analysis, with 3 DC, showed that this allele, together with Pi-1 F, followed a spatial dynamic different from the remaining alleles studied. The overall correlogram was non-significant but showed a significantly positive autocorrelation coefficient in the 3 DC (with Moran's I index as well as with Geary's c coefficient). This means that the most distant localities were the most similar for the frequency of this allele.

Alb S: The overall 3 DC correlogram was not significant. However, the 3 DC was significantly negative, by Moran's I index (P = 0.047), while Geary's c coefficient approached statistical significance (P = 0.057). There was a trend towards long distance differentiation. The single autocorrelation coefficient showed insignificant positive values. The individualized Mantel test showed a positive value of r = 0.4029, close to statistical significance (Mantel *t*-test: P = 0.0457; Monte Carlo: P = 0.064). In this case, the geographic distance explained 16.2% of the genetic variability in this allele (r^2).

Table V - Mantel's test for every individual locus (M.I.T.), Mantel *t*-test probability (M.T.P.), Monte Carlo simulation probability (M.C.S.), and individual autocorrelation coefficients with Moran's I index (I) and with Geary's c coefficient (c).

Locus	M.I.T. (r)	M.T.P.	M.C.S.	I	Prob	c	Prob
Sod A	-0.135	0.271	0.335	-0.66	0.138	1.80	0.083
Lap A	0.068	0.409	0.405	-0.38	0.384	1.17	0.369
Alb S	0.403	0.046*	0.064	0.19	0.229	0.32	0.115
Tf B	0.276	0.122	0.154	-0.12	0.437	0.64	0.265
Tf C	0.226	0.168	0.202	-0.09	0.417	0.63	0.259
Pi-1 I	0.831	0.0013*	0.0015*	0.74	0.051	0.17	0.062
Pi-1 F	-0.297	0.861	0.135	-0.52	0.285	1.32	0.276
α1B F	0.230	0.145	0.184	-0.16	0.459	1.23	0.349
Mpi A	0.508	0.022*	0.040*	0.58	0.069	0.48	0.176
Prt-1 F	0.199	0.204	0.189	0.17	0.223	0.92	0.448
Prt-1 D	0.297	0.144	0.114	0.10	0.278	0.82	0.376
Prt-2 F	-0.002	0.504	0.417	0.26	0.166	0.53	0.211
Pep-D A	0.408	0.028*	0.070	0.16	0.182	0.29	0.122
Pa-1 S	0.277	0.155	0.159	0.46	0.127	0.28	0.091

Significant at *P < 0.05.

Tf B: For this allele, the overall correlogram (3 DC) was significant according to Geary's c coefficient (P = 0.041), and approached statistical significance according to Moran's I index (P = 0.054). The 2 DC was more positive than the 1 DC, and the 3 DC was significantly negative (Moran: P = 0.018; Geary: P = 0.014). In our analysis this allele showed a significant long distance differentiation.

Pi-1 I: The 3 DC autocorrelation analysis showed a significant overall correlogram using Geary's coefficient (P = 0.032) and approached statistical significance according to Moran's I index (P = 0.070). The correlogram had a clear trend of a significant monotonic cline (at least with the Geary's statistic). The 1 DC was significantly positive and the 3 DC significantly negative (with both spatial autocorrelation statistics). This was the only genetic variable that had a clear monotonic cline pattern. The single autocorrelation coefficient gave positive values which exhibited borderline statistical significance (I = 0.736, P = 0.051; c = 0.171, P = 0.062). The individual Mantel test showed a significant positive value (r = 0.8308; Mantel *t*-test: P = 0.0013; Monte Carlo: P = 0.0015). Sixty-nine percent of the genetic variation of this allele can be explained by the geographic distance. This was the only

variable that clearly showed a significant genetic heterogeneity and a significant monotonic cline pattern as well.

Pi-1 F: Together with the Sod A allele, this allele displayed a spatial pattern completely different from the remaining genetic variables studied. The autocorrelation analysis with 3 DC showed that the 3 DC was significantly positive. The most similar localities were those located at the greatest distance. However, the overall correlogram was not significant.

Mpi A: The autocorrelation analysis with 3 DC showed overall correlograms close to statistical significance (I: P = 0.063; c: P = 0.095), and the defined 1 DC was significantly positive. This means that there was a strong genetic similarity between the nearest localities. The single spatial autocorrelation coefficient also showed positive values close to statistical significance (I = 0.578; P = 0.069). The Mantel test showed a significantly positive value (r = 0.5079). This means that 25.8% of genetic variation was explained by geographic distance.

Pep-D A: The autocorrelation analysis with 3 DC showed a significant overall correlogram for Moran's I index as well as for Geary's c coefficient (I: P = 0.048; c: P = 0.047). The 3 DC was significatively negative. This is a case of differentiation at long distance. The single autocorrelation coefficient was slightly but not significantly positive. The individual Mantel test exhibited a significant positive value (r = 0.4084) with the approximate Mantel *t*-test. Thus, geographic distance explained 16.7% of the genetic variation.

Average spatial autocorrelation analysis and the global Mantel test

The proportion of significant autocorrelation coefficients was 8/42 for Moran's I index and 7/42 for Geary's c coefficient. This is slightly superior to the error criterion of 5%. It indicated that there was an observable spatial structure in the genetic data. This was evident when the average overall correlogram was analyzed. The average autocorrelation coefficients for every distance class became more negative when the geographic distances increased. The average correlogram with Moran's I index was: 1 DC: 0.064; 2 DC: -0.229; 3 DC: -0.435. The same happened when Geary's c coefficient was used: 1 DC: 0.679; 2 DC: 1.083; 3 DC: 1.238. Globally, there was a decrease in the genetic similarity when the geographic distance increased between populations, although the amount of genetic heterogeneity was relatively small. Also, the global Mantel test using the Nei and the Cavalli-Sforza and Edwards distances showed that there was a significantly positive dependence between the genetic and geographic distances. For the Nei and for the Cavalli-Sforza and Edwards distances, the values obtained were r = 0.6353 (Mantel *t*-test: P = 0.0037; Monte Carlo: P = 0.0045) and r = 0.6621 (Mantel *t*-test: P = 0.0044; Monte Carlo: P = 0.0130), respectively. This means that for each of these genetic distances, the geographic distance explained 40.4% and 43.8% of the genetic variation. This positively significant dependence was also observed for the logarithmic data (Nei: r = 0.5795; Mantel *t*-test: P = 0.0079; Monte Carlo: P = 0.0090; Cavalli-Sforza and Edwards: r = 0.5806; Mantel *t*-test: P = 0.0145; Monte Carlo: P = 0.0350), where the geographic distance explained 33.6% and 33.7% of the genetic variation, respectively.

Similarity analysis between the surfaces of the variables

The fraction of correlations above |0.7| between the allele frequencies was 20/91, this value being above the statistical error of 5%. When some alleles were excluded from the comparisons within the same loci (Pi-1, Prt-1, Tf), the fraction obtained was similar 19/88.

Similarity analysis between the correlograms

The fraction of correlogram pairs that gave a Manhattan distance below 0.1 (Moran's I) or 0.2 (Geary's c) was 9/91 and 13/91 which was not significantly above the type error of 5%. When the spatial relationships between variables were compared by means of a cluster made with a UPGMA algorithm, and using Manhattan distances between pairs of correlograms generated with the Moran's I index as well as with Geary's c coefficient, it was observed that the only relationship which remained constant was the one found between Sod A and Pi-1 F. Both variables were clearly differentiated from the spatial patterns adopted by the remaining variables (Figure 3). This evidence supports the weak relationship between the spatial patterns of each of the genetic variables studied.

DISCUSSION

Hardy-Weinberg equilibrium and genetic variability

There was no significant departure between the observed and expected genotypic frequencies in each

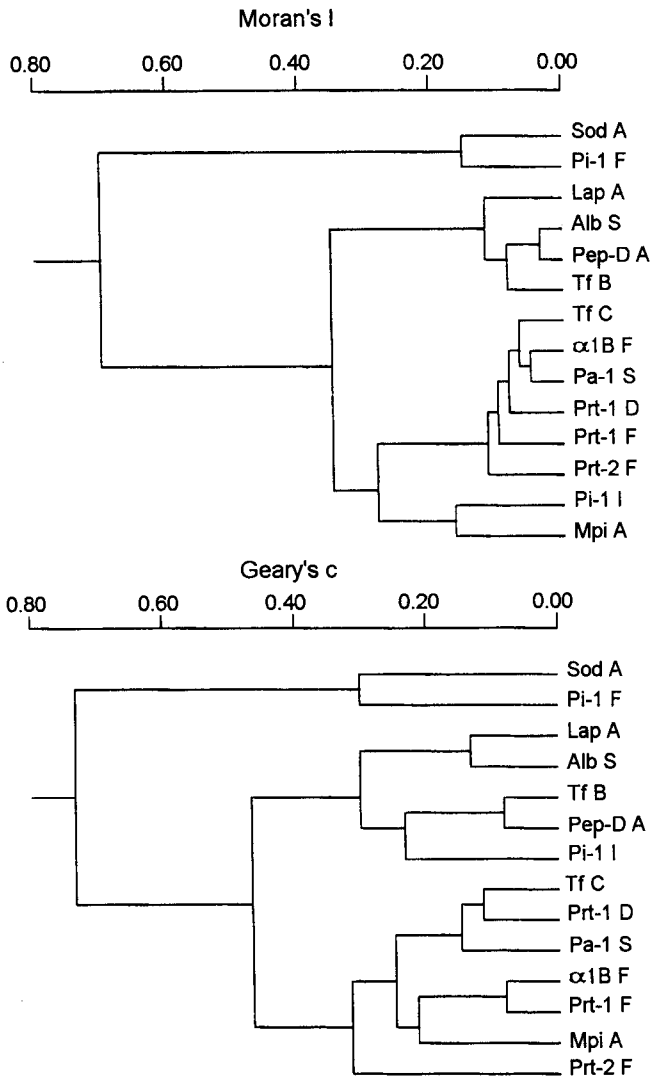


Figure 3 - UPGMA analysis of the spatial autocorrelation coefficients (Moran's I and Geary's c) of the genetic variables that showed variability using the Manhattan distance (city block) between pairs of correlograms.

individual locality and at a global level. Thus, the "Gos d'Atura" dog breed was in Hardy-Weinberg equilibrium. Therefore, the group of localities studied can be considered part of a single large and relatively homogeneous population. This means that there is and/or there was in the recent past, enough genetic exchange between the populations to prevent a marked genetic divergence between them. The results obtained, for these allozymic characters, indicate that reproduction is panmictic and that human action does not introduce bias and/or human action accounts for panmixia. Also, we observed that there were no net causes of selective origin in favor and/or against homozygotes and/or heterozygotes for these genetic variables. The consanguinity and Wahlund effect had no importance as evolutionary agents for this dog breed because there was not a significant excess of homozygotes (Nygren, 1980; Nygren and Rasmusson, 1980).

The levels of genetic variability in this dog breed were similar ($\overline{H}$ = 0.167 ± 0.048) to those of other Spanish dog breeds (Jordana, 1989; Jordana *et al.*, 1992). The expected genetic variability for nine dog breeds studied in Spain were: "Mastin pirineos" breed ($\overline{H}$ = 0.158 ± 0.044), "Mastin español" ($\overline{H}$ = 0.176 ± 0.049), "Perdiguero de Burgos" ($\overline{H}$ = 0.139 ± 0.044), "Galgo español" ($\overline{H}$ = 0.184 ± 0.049), "Sabueso español" ($\overline{H}$ = 0.163 ± 0.044), "Podenco Canario" ($\overline{H}$ = 0.139 ± 0.045), "Ca de Bestiar" ($\overline{H}$ = 0.130 ± 0.039), "Podenco eivissenc" ($\overline{H}$ = 0.146 ± 0.045) and "Podenco Ibérico" ($\overline{H}$ = 0.161 ± 0.049). These comparative results show that the "Gos d' Atura" dog breed has not been submitted to important stochastic events with a consequent loss of genetic variability. Moreover, the mean value of expected heterozygosity was above those found in other mammals studied (Nevo, 1978; Nevo *et al.*, 1984): *Felis catus* ($\overline{H}$ = 0.066, domestic type; $\overline{H}$ = 0.040, wild type; Randi and Ragni, 1991), *Canis latrans* ($\overline{H}$ = 0.009; Hamilton and Kennedy, 1986), *Procyon lotor* ($\overline{H}$ = 0.014; Beck and Kennedy, 1980) and *Acynonix jubatus* ($\overline{H}$ = 0; O'Brien *et al.*, 1983). Generally, the wild carnivores studied have a very low genetic variability (Simonsen, 1976). However, other wild mammals had mean heterozygosis values as high as those found in this dog breed, e.g. *Peromyscus maniculatus* (Garcia *et al.*, 1990); *Mus musculus* (Berry *et al.*, 1981); *Calomys laucha* (Garcia *et al.*, 1990); and *Odocoileus virginianus* (Kennedy *et al.*, 1987). Thus, it can be said that humans transporting dogs from one locality to another and controlling matings in some instances is enough to maintain a high degree of genetic variability, while in wild mammals species the situation is different.

Differentiation, spatial patterns and genetic relationships between the populations studied

Globally, the amount of genetic heterogeneity found was statistically significant. However relatively speaking, the amount of heterogeneity (F_{st}) was small (4.3%) (Hartl, 1980). When each locus was individually analyzed, this fact was evident. Only one locus showed a clear and significant genetic heterogeneity (Pi-1), and another two loci were nearly significant (α_1B and Mpi). Other mammals have shown higher F_{st} values. Some examples are: *Canis latrans* (F_{st} = 0.08; Hamilton and Kennedy, 1986), *Cynomis ludovicianus* (F_{st} = 0.10; Chesser, 1983), *Alces alces* (F_{st} = 0.09; Ryman *et al.*, 1980), *Mus musculus* in the western Mediterranean area (F_{st} = 0.274; Navajas y Navarro and Britton-Davidian, 1989),

and *Mus musculus* in Europe (F_{st} = 0.465; Britton-Davidian, 1990). This shows the relatively high genetic homogeneity typical of this dog breed from Catalonia. However, although there was high genetic homogeneity, the small degree of genetic heterogeneity had a certain degree of spatial structure. In a global manner, this fact was evident in the progressive decrease of genetic similarity when the geographic distance was increased between the localities studied (average correlogram and global Mantel test). When each locus was individually analyzed, the spatial autocorrelation analysis showed that only the Pi-1 I allele had a significant monotonic cline. The Tf B and Pep-D A alleles showed a significant spatial structure with differentiation at long distance, and the Mpi A allele showed a significant similarity between the closest populations. Also, the individualized Mantel test showed spatial structures for Pi-1 I, Mpi A, and Pep-D A. However, there was no classical isolation by distance. There was a nuclear group of populations formed by Vallferrera, Vall d'Assua, Urgell, and Conca de Barbera that were genetically very similar, while the remaining localities, Barcelona and Girona, exhibited a slight differentiation from this highly similar nucleus (see the UPGMA phenogram with Cavalli-Sforza and Edwards distance in Figure 2). The strongest genetic relationship in this nucleus was formed by Urgell with Conca de Barbera and Vall d'Assua (two central and one north Pyrenean populations). This result was evident in the correlograms of the variables that showed differentiation at long distance, although this divergence was not extraordinarily important. The slight differentiation of Barcelona and Girona could be due to professional dog breeders from those localities which mate dogs with pedigree (mating between championship dogs). Most dogs of these two populations are included in the "Spanish origin Book" (LOE), in the "Canine Race Register" (RRC) and in the "Catalonian Gos d'Atura Club". This would produce a higher number of stochastic fluctuations in their allele frequencies due, principally, to reproductive isolation motivated by economic factors, because mating between individuals with and without pedigree is rejected and/or because of small effective population sizes. Probably in these two urban Gos d'Atura populations, the sex-ratio in favor of females is important, and produces accelerated gene drift. In the remaining populations, this phenomena does not exist because they are rural free-ranging shepherd dog populations not controlled by human selection. However, the most differentiated sample (Girona) only had seven individuals. Thus, the sample error could be very high.

The 2 DC, in several correlograms, had more positive values than the 1 DC. This can be explained because Vall d'Assua was more similar to Urgell and Conca de Barbera than to Vallferrera, although both were close to Pyrenean populations (in agreement with the UPGMA phenogram). This fact could be due to greater gene flow, produced by human movements, from Vall d'Assua and the central populations (Urgell and Conca de Barbera). The flocks of sheep from central localities, together with their shepherd dogs (Gos d'Atura), go up to Vall d'Assua in summer, and go down again to the central localities in winter. During these periods, genetic exchanges between these dogs could occur.

Also, two variables, Sod A and Pi-1 F, showed the highest similarity between the most distant localities, in contrast to the rest of variables. The reasons for these results were not apparent with the data that we currently have.

Comparison between the spatial genetic structure found in the "Gos d'Atura" dog breed and that found in a group of Spanish cat populations

Three concepts were compared: 1) *Amount of genetic heterogeneity.* In the same way that is observed in the dog populations studied, the cat populations analyzed from the Catalonia, Balearics and other Spanish Mediterranean localities showed a very small genetic differentiation, although it was statistically significant ($\overline{F}_{st}$ = 0.017, $\overline{F}_{st}$ = 0.02, and $\overline{F}_{st}$ = 0.03, respectively, Ruiz-Garcia, 1991, 1993, 1994, 1997). This small heterogeneity (in comparison with that observed in other mammals) can be explained by the gene flow that is established between localities due to human transport of domestic animals from one locality to another. Both species seem to have a common element: human action. 2) *Percentages of significant autocorrelation coefficients.* Using the Moran's I index, it can be observed that the percentage of significant autocorrelation coefficients in the cat populations was higher than the "Gos d'Atura" dog populations studied (31.4% vs. 19.0%). However, the cat area studied was larger than the extension of the area where Gos d'Atura dog breed was studied. 3) *Percentages of Manhattan distances lower than 0.1 with the Moran's I index.* In both cases, the percentage of Manhattan distances between correlograms of genetical variable pairs using the autocorrelation coefficients was very small and similar and did not differ significantly from the 5% type error (9.5%, in the case of cat populations, and 9.9% in the case of Gos d'Atura populations). This means that, in both

cases, the spatial pattern for each variable with respect to the others was highly divergent. This fact allows us to deduce that the evolutionary agents operating spatially were different for each of the variables implicated. Thus, a single cause (or few causes) does not explain the spatial patterns of these variables neither for the "Gos d"Atura" dog populations studied here, nor for the natural cat populations in the Spanish area analyzed. We can conclude that the rural populations should be a potential "new" genetic source for future generations of this dog breed.

ACKNOWLEDGMENTS

We thank Dr. E. Nevo (Haifa, Israel), Dr. A. Sanjuan (Vigo, Spain), Dr. K.K. Klein (Mankato, USA), Dr. R. Fagen (Alaska, USA) and the referees for their respective comments. Also our thanks to Dr. B.H. Zimmermann (Michigan, USA) for help with the English syntax of this manuscript, and especially to Diana Alvarez (Bogotá, DC, Colombia) for her absolutely magnificent assistance.

This work was partially supported by "Gallina Blanca Purina" (one of the authors, J.J.) and by grant Nos. 139-94 and 140-96 (Decreto 1742 de 1994) of COLCIENCIAS to M.R.-G.

RESUMO

Seis populações de cães "Gos d'Atura" foram estudadas na Catalunha (Espanha) usando 21 loci alozímicos. Destes 21 loci, 11 foram polimórficos (Sod, Lap, Mpi, Alb, Pep-D, Tf, α1B, Pi-1, Prt-1, Prt-2 e Pa-1). Os níveis de heterozigozidade média esperada e a porcentagem de polimorfismo foram altos em relação a outros mamíferos. Todas as populações estavam em equilíbrio de Hardy-Weinberg. A heterogeneidade genética global foi relativamente pequena ($F_{st} = 0,04$), embora significante. Apenas um locus (pi-1) mostrou heterogeneidade nitidamente significante. Este locus (pi-1 I) também mostrou uma inclinação monotônica significante quando uma análise de autocorrelação espacial foi empregada. Outros loci (Sod A, Alb S, Tf B, Pi-1 F, Mpi A e Pep-D A) mostraram estrutura espacial significante mas sem inclinação monotônica. Alguns desses loci, como Tf B e Pep-D, mostraram diferenciação a longa distância e Mpi A exibiu uma significativa semelhança entre as populações mais próximas.

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(Received August 16, 1995)