Genetic microstructure in two spanish cat populations. I: genic diversity, gene flow and selection.

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The genetic microstructure of two Spanish cat populations (in Barcelona and Alicante) was studied. These populations were in Hardy-Weinberg equilibrium at the locus O. There was significant genetic heterogeneity for most of the loci studied at the colony level, especially, and the subpopulation level in Barcelona, and at the subpopulation level in Alicante, although the amount of heterogeneity was relatively small compared with that found in other mammal species. Therefore, the major part of the gene diversity found was at the level of the small population structure (colonies). This agrees quite well with high theoretical gene flow estimates.

The Lewontin-Krakauer test showed, in some cases, significant F tests, which appears to indicate the presence of several selective events on some of the loci studied (diversifying and/or unifying), although some other causes, such as differential gene flow, some different historical and demographic parameters at the time of the introduction of the different alleles within the cities studied, as well as different mutation rates of the loci analyzed, could produce significant Lewontin-Krakauer test values, as well.

INTRODUCTION

There are a good number of publications describing the genetic profiles of more than 400 cat populations worldwide. These profiles use genetic markers which code for coloring, pattern, length of fur, and certain skeletal anomalies. Nevertheless, there has been a noteworthy absence of data on the genetic structure of the cat populations at the microgeographic level. Only in recent years has there begun to be a detailed analysis at the microgeographic level as to what type of events could be directing the allelic frequencies in different cat populations in several cities of the world (Ruiz-Garcia, 1988, 1989, 1991, 1993, 1994b, 1998). Notwithstanding, we still do not possess a clear understanding of all of the evolutionary events which could be acting at this microgeographic level, and if they are always of the same nature in cat populations of different cities. Therefore, we were carrying out an in depth analysis of the genetic structure of two cat populations in two Spanish mediterranean cities (Barcelona and Alicante). The analysis of

Barcelona's population is especially detailed, since it was possible to identify different subpopulations of cats that were isolated from each other there, making it possible to divide them on a still smaller geographical scale, using such defined sample structures as "colonies". In the case of the city of Alicante, it was only possible to perform the analysis at the subpopulational level, because the sampling was insufficient to determine the colony structure. The main microgeographic aspects which interested us in relation to the genetic structure of the cat populations of these two cities were as follows:

1/ To analyze the Hardy-Weinberg equilibrium at the O locus, at the colonial (in Barcelona), as well as at subpopulation levels (Barcelona and Alicante). 2/ To analyze the genetic diversity, heterogeneity, and the gene flow in the different defined sample structures in both these cities. 3/ To analyze by means of Lewontin and Krakauer's test (1973) the possible differential variance of the distributions of the allele frequencies of the genes studied, and, therefore, the possibility of the existence of diversifying and/or unifying selection affecting in various ways the alleles analyzed. In order to do this, we used different allele sets. 4/ To analyze whether the results obtained by this analysis were in agreement with the reported data

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from studies of the ecology, ethology and reproductive biology of this species.

Previous studies of the distribution of genetic variation in natural populations showed that differences are often greater at the microgeographic level than at coarser levels of structure. In fact, it may well be that in some mammalian (and other) species differentiation at the microgeographic level is the norm rather than the exception (Smith et al. 1978; Varvio-Aho and Pamilo, 1980; Lorenzini et al. 1993; Preleuthner and Pinsker, 1993). The populations of many mammals appear to share a higher demic structure, with subpopulations divided into lineages. This has the effect of producing significant endogamy and largely limits the gene flow between these lineages (for instance, see Nevo et al. 1992). Some of these studies have demonstrated that the effective number of these lineages (or demes or colonies) can be very small (DeFries and McClearn, 1972; Patton & Feder, 1981) and the relative importance of the stochastic processes in determining allelic frequencies can be very important. Immigrants introduced into such population structures have relatively little chance to influence the reproductive outcome, and so there is very little gene flow. The opposite conclusions have been reached in other studies (Jackson and Pounds, 1979; Baker, 1981; Lidecker and Patton, 1987; Berry et al. 1990), where gene flow was observed to be important in social species. In populations of domestic cats, social interactions appear to have great importance (Kerby and Macdonald, 1988; Liberg and Sandell, 1988; Natoli and De Vito, 1988) and together with the reproductive structure of this species and with the actions of human beings as a powerful dispersion vector, it would be expected that the microgeographic structure of cat populations would conform with this second option. Nevertheless, although the human species can actively disperse cats, in order for the gene flow to be effective, it is necessary that the individuals that come to the new locality be able to interact reproductively with the individuals peculiar to the place. Therefore, the cats' social reproductive structure has to be flexible and "open" to accept foreign animals as reproductive elements. The genetic results obtained seem to agree quite well with this second kind of structure.

MATERIALS AND METHODS

Characters studied and areas sampled. The phenotypes of the cats were recorded directly from observations of animals. The genetic nomenclature used is in accordance with the Committee on Standardized Genetic Nomenclature for Cats (1968). The genetic characteristics studied here included: a gene sex-linked (O, o ; Orange [Mutant, all pigment is orange; epistatic to the A locus] vs non-orange [Wild; pigmentation no orange]) and the autosomal loci, A (A, a ; Agouti [Wild; Agouti color]

vs Non-agouti [Mutant recessive; Non-agouti color, black colour; epistatic to the T locus]), T (t^+ , t^b , T^a ; striped or mackerel tabby [Wild; recessive to T^a but dominant to t^b] vs blotched tabby [Mutant recessive] vs Abyssinian tabby [Mutant dominant]), D (D, d ; Dense colour [Wild] vs Dilution [Mutant recessive; dilute colour]), L (L, l ; Short hair [Wild] vs Long hair [Mutant recessive]), S (S, s ; Piebald white spotting [Mutant dominant] vs Non-piebald white spotting [Wild]), W (W, w ; Dominant white [Mutant dominant; epistatic to all the other colours] vs normal colour [Wild]), C (C, c^s ; full colour [Wild] vs Siamese colour [Mutant recessive]) and M (M, m ; Manx [without tail; Mutant dominant; lethal homozygote] vs normal tail [Wild]). The inheritance of all of these characters follows simple Mendelian patterns which permits the easy calculation of allelic frequencies at the respective loci and they have been previously discussed in detail by Robinson (1959, 1977) and Wright and Walters (1982).

Most of this work was done using the seven most commonly studied loci (O, A, T, D, L, S and W), which allows for comparison with previous studies. We introduce the use of the M and C loci (where they were observed) in certain analyses, as these two loci represent recent "exotic" additions to the genetic variance of the observed populations, and allow us to observe the introgression of foreign genes. Data on the phenotypes of the cats of Barcelona and Alicante were obtained by direct observations of individual animals in their "home" territories. Each cat was identified with a particular colony or subpopulation without any doubt. A photographic register of every cat was compiled. Each cat sampled was a stray, an alley-cat, a feral cat or "pseudo-wild". The colonial level in Barcelona was defined as the cats observed at fixed locations and which probably consisted of a few phylopatric female groups inhabiting a small ranging and clearly separated from other such populations by geographical distances ranging from 1000 to 7500 m, approximately, with a mean value of 4212 m. Defined in this way, the number of colonies studied in Barcelona was eleven. The subpopulational level was defined as the groups of colonies inhabiting neighbouring areas clearly separated from each other by geographic barriers (average separation distance was about 5-6 Km). For instance, there were subpopulations separated by 30 or more roads with very intense traffic as an artificial barrier. Defined in this way, we were able to define five subpopulations in Barcelona and four in Alicante. In Alicante, no further division of the population was possible (see Figure 1).

Hardy-Weinberg equilibrium. The Hardy-Weinberg equilibrium was analyzed at the O locus, because at this locus, one can determine the genotypic frequencies of homo- and heterozygotes. To test random mating at this locus, a G test was performed (Sokal and Rohlf, 1981).

Fig. 1. Maps of cat colonies and subpopulations in Barcelona and in Alicante

Analysis of genic diversity and gene flow. For this purpose, we carried out a Nei's (1973, 1987) genic diversity analysis. For each of the sampled loci at the subpopulation level in Barcelona and Alicante, we calculated the following statistics: H_t (genic diversity in the total

population), H_s (average gene diversity within subpopulations), G_{st} (Gene differentiation between subpopulations relative to the gene diversity in the total population), and D_m (the absolute intersubpopulational gene diversity).

A hierarchical gene diversity analysis was conducted in

Table 1. Genetic profiles of cat subpopulations and colonies studied in Barcelona and Alicante. * Alleles: O = orange (sex-linked), a = non-agouti, t^b = blotched tabby pattern, d = dilute color, l = long hair, S = piebald white spotting, W = dominant white. # s = subpopulations, c = colonies.

POPULATIONS		ALLELES*						
BARCELONA subpopulations and colonies	Sample Size	O	a	t ^b	d	l	S	W
A (s.c)#	64	0.24	0.73	0.37	0.22	0.12	0.19	0.008
B(s)	179	0.16	0.69	0.28	0.28	0.07	0.27	0.003
B1(c)	31	0.27	0.65	0	0.37	0	0.28	0
B2(c)	66	0.14	0.71	0.34	0.18	0	0.27	0
B3(c)	82	0.12	0.68	0.30	0.31	0.11	0.26	0.006
C(s.c)	50	0.10	0.67	0.33	0.32	0	0.39	0
D(s)	100	0.11	0.68	0.27	0.25	0.10	0.25	0
D1(c)	39	0.13	0.68	0.31	0.32	0	0.28	0
D2(c)	61	0.10	0.68	0.25	0.18	0.13	0.23	0
E(s)	316	0.17	0.70	0.23	0.26	0.17	0.27	0.005
E1(c)	34	0.25	0.65	0	0.25	0	0.44	0
E2(c)	30	0.07	0.65	0.42	0.33	0.32	0.33	0.035
E3(c)	143	0.20	0.74	0.12	0.31	0.17	0.23	0
E4(c)	109	0.13	0.68	0.29	0.17	0.14	0.26	0.005
ALICANTE subpopulations								
A	67	0.32	0.85	0.34	0.28	0	0.15	0
B	96	0.18	0.82	0.67	0.11	0	0.27	0
C	52	0.18	0.76	0.31	0.28	0	0.34	0.01
D	120	0.24	0.79	0.46	0.24	0	0.44	0.008

Table 2. G test (Sokal and Rohlf 1981) for Hardy-Weinberg equilibrium at the Orange locus in Barcelona and Alicante cat populations. * Population identifiers as in Table 1. O/? = orange color, sex unknown; O/+ = orange and black females; +/? = black, sex unknown. p(O) = Orange frequencies.

	Observed			Expected			p(O)	G
	O/?	O/+	+/?	O/?	O/+	+/?		
BARCELONA								
TOTAL	65	90	539	63.72	92.56	537.72	0.159	0.0872
A*	9	12	42	9.28	11.43	42.29	0.238	0.0402
B	15	25	137	15.88	3.22	137.90	0.155	0.1872
B1	7	3	21	5.41	6.17	19.42	0.274	2.5619
B2	4	10	51	5.12	7.75	52.13	0.138	0.8860
B3	4	12	65	5.61	8.76	66.62	0.123	1.6260
C	2	6	42	2.75	4.50	42.75	0.100	0.6980
D	7	8	84	6.11	9.78	83.11	0.111	0.4760
D1	4	2	33	2.82	4.36	31.82	0.128	1.0410
D2	3	6	52	3.35	5.49	52.15	0.100	0.0845
E	32	39	234	30.09	42.79	232.12	0.168	0.4760
E1	6	4	22	5	6	21	0.250	0.9906
E2	1	2	24	1.07	1.85	24.07	0.074	0.0166
E3	17	21	101	16.47	22.06	100.48	0.198	0.0687
E4	8	12	87	7.91	12.16	86.92	0.131	0.0007
ALICANTE								
TOTAL	48	52	223	45.58	57.05	220.48	0.229	0.6196
A	13	16	37	13.84	14.32	37.85	0.318	0.2625
B	12	8	70	9.42	13.15	67.43	0.177	3.0976
C	5	8	37	5.31	7.38	37.31	0.180	0.0710
D	18	19	80	16.98	21.03	78.99	0.235	0.2746

Barcelona, where we defined three levels of structure (total population, subpopulation and colony). We calculated the statistical values of H_c (average gene diversity within colonies), D_{cs} ($= H_s - H_c$; average gene diversity between colonies relative to the gene diversity in the subpopulations), $G_{cs(t)}$ ($= D_{cs}/H_t$; the relative degree of gene differentiation attributable to colonies within subpopulations relative to the gene diversity in the total population), G_{cs} ($= D_{cs} / H_s$; genic differentiation attributable to colonies within subpopulations relative to the subpopulational gene diversity). These statistical values satisfy the following relationships:

$$H_t = H_c + D_{cs} + D_{st} \text{ and } (1-G_{cs})(1-G_{st})H_t = H_c \text{ and } H_c/H_t + D_{cs}/H_t + D_{st}/H_t = 1.$$

In Barcelona, for those subpopulations which could be divided into colonies, we calculated the statistical values of H_{si} (gene diversity in the total i subpopulation), H_{csi} (average gene diversity of the colonies within a i subpopulation), G_{csi} (gene diversity between colonies relative to the total genetic diversity within a subpopulation), and Dm_{csi} (absolute gene diversity of colonies within single subpopulations).

In addition in this study, the F_{st} statistical value, cor-

Table 3. Analysis of genetic diversity and gene flow within Barcelona and Alicante cat subpopulations. O= orange (sex-linked), a= non-agouti, t^b= blotched tabby pattern, d= dilute color, l= long hair, S= piebald white spotting, W= dominant white, M= manx, c^s= Siamese.*

A. BARCELONA									
	H_t	H_s	D_{st}	G_{st}	$F_{st'}$	χ^2	df	D_m	$Nm\alpha$
O	0.2667	0.2643	0.0024	0.0089	0.0082	11.387*	4	0.0029	19.342
a	0.4231	0.4225	0.0005	0.0013	0.0005	0.644	4	0.0007	311.260
tb	0.3959	0.3923	0.0036	0.0090	0.0077	5.901	4	0.0046	20.608
d	0.3896	0.3886	0.0010	0.0026	0.0019	2.662	4	0.0013	83.013
L	0.2113	0.2058	0.0055	0.0260	0.0253	35.335**	4	0.0069	6.170
S	0.3916	0.3884	0.0032	0.0082	0.0075	10.417*	4	0.0040	21.158
W	0.0071	0.0071	0.0000	0.0014	0.0007	1.073	4	0.0000	208.153
Mean (7 loci)	0.2979	0.2955	0.0023	0.0082	0.0074	67.422**	28	0.0029	—
	± 0.0751	± 0.0749	± 0.0009	± 0.0043	± 0.0043			± 0.0012	—
M	0.0071	0.0071	0.0000	0.0036	0.0029	4.078	4	0.0000	54.677
c ^s	0.1865	0.1798	0.0067	0.0358	0.0351	49.440**	4	0.0083	4.403
Mean (9 loci)	0.2532	0.2507	0.0025	0.0108	0.0099	120.940**	36	0.0032	—
	± 0.0819	± 0.0817	± 0.0012	± 0.0060	± 0.0060			± 0.0015	—
Direct value for 7 loci				0.0078				0.0029	21.422
Direct value for 9 loci				0.0100				0.0032	15.862
B. ALICANTE									
	H_t	H_s	D_{st}	G_{st}	$F_{st'}$	χ^2	df	D_m	$Nm\alpha$
O	0.3515	0.3460	0.0054	0.0155	0.0140	9.064*	3	0.0073	9.882
a	0.3179	0.3163	0.0016	0.0052	0.0034	1.893	3	0.0022	40.710
tb	0.4981	0.4630	0.0350	0.0703	0.0668	19.127*	3	0.0467	1.857
d	0.3408	0.3306	0.0102	0.0299	0.0284	18.351*	3	0.0136	4.810
L	0	0	0	0	0	—	3	0	—
S	0.4321	0.4084	0.0237	0.0548	0.0532	34.422*	3	0.0315	2.498
W	0.0092	0.0091	0.0000	0.0043	0.0028	1.807	3	0.0000	50.594
Mean (7 loci)	0.2785	0.2677	0.0108	0.0257	0.0241	84.665*	21	0.0145	—
	± 0.0984	± 0.0933	± 0.0067	± 0.0137	± 0.0133	—	—	± 0.0090	—
c ^s	0.2556	0.2469	0.0087	0.0340	0.0325	21.567*	3	0.0116	3.997
Mean (8 loci)	0.2756	0.2650	0.0106	0.0268	0.0252	106.232*	24	0.0141	—
	± 0.0912	± 0.0864	± 0.0063	± 0.0128	± 0.0124	—	—	± 0.0084	—
Direct value for 7 loci				0.0390					5.690
Direct value for 8 loci				0.0384					5.448

* $p < 0.05$, ** $p < 0.001$

rected for sampling error by using Workman and Niswander's (1970) method (F_{st}'), was calculated as well. The estimates of F_{st} were tested for significant deviation from 0 using the equation:

$\chi^2 = 2 N_t F_{st} (k-1)$ with $(k-1)(s-1)$ degrees of freedom, where N_t is the total sample size, k is the number of alleles, and s is the number of subpopulations. To quantify the possible differences in heterogeneities introduced by each locus, the F-test of Fisher and Snedecor (Workman and Niswander, 1970) was used.

By using the F_{st} statistic, we obtained indirect estimates of Nm , the product of effective population size for the migratory rate per generation, by the n -dimensional island model (Takahata, 1983; Crow and Aoki, 1984), where $Nm_\alpha = [(1/G_{st}) - 1] / 4[n/(n-1)]^2$, n being the number of subpopulations, or colonies, considered.

Selection analysis. In order to verify globally whether any type of selection has any sort of important effect on the genetic structure in the different microgeographic levels analyzed in the cat populations of Barcelona and Alicante, the Lewontin-Krakauer test (1973) was used. The theoretical basis of this test depends on the non-existence of significant heterogeneity in the F_{st} values analyzed in the sample groups under consideration if this variation is due to stochastic processes affecting the same parameters and/or interpopulational gene flow, since those events would have to affect all the loci equally (Wood, 1978). In principle, on the contrary, the existence of significant heterogeneity would suggest the presence of selective agents differentially affecting the analyzed

loci. We used the value $k=2$ since this preserves a magnitude that is appropriate for a binomial distribution and for F_{st} values between 0 and 0.05 (Nevo, 1973). We applied this test to different loci arrays, including and excluding W, which is apparently under the control of uniformly negative selection-mutation equilibrium (Bergsma and Brown, 1971; Todd et al. 1977), an including and excluding the C and M loci, which are considered to be genetic characteristics recently introduced in the cat populations through selective human action. However, the criticisms of this test should not be forgotten (Robertson, 1975ab).

RESULTS

Phenotypic frequencies, gene frequencies and Hardy-Weinberg equilibrium. The genetic profiles of the cat populations of Barcelona and Alicante, broken down into subpopulations and colonies, is given in Table 1. In the case of Alicante, the M locus was not scored. Analysis of these data for Hardy-Weinberg equilibrium at the O locus with the application of a G test is summarized in Table 2. There was no significant statistical deviation between the observed proportions and those expected for Hardy-Weinberg equilibrium at the O locus for any of the colonies studied in Barcelona or for any of the subpopulations studied in Barcelona and Alicante.

Genetic diversity, heterogeneity and theoretical gene flow. In Table 3, we present the genic diversity analysis of the cat subpopulations in Barcelona and

Table 4. Gene diversity attributable to colonies within subpopulations and total population and gene flow between colonies in the cat population of Barcelona. df= degree of freedom. O= orange (sex-linked), a= non-agouti, t^b= blotched tabby pattern, d= dilute color, l= long hair, S= piebald white spotting, W= dominant white, M= manx, c^s= Siamese.

	H _c	D _{cs}	G _{cs}	G _{cs(t)}	D _{ct}	G _{ct}	F _{ct'}	χ^2	df	Nm α
O	0.2606	0.0037	0.0139	0.0138	0.0061	0.0227	0.0220	30.6**	10	9.2
a	0.4210	0.0015	0.0036	0.0036	0.0021	0.0049	0.0041	5.1	10	50.6
tb	0.3679	0.0244	0.0622	0.0617	0.0279	0.0707	0.0736	56.4**	10	2.6
d	0.3805	0.0081	0.0209	0.0208	0.0092	0.0235	0.0232	32.1**	10	8.7
L	0.1840	0.0074	0.0358	0.0349	0.0129	0.0609	0.0686	95.9**	10	2.8
S	0.3847	0.0037	0.0094	0.0093	0.0069	0.0176	0.0168	23.3**	10	12.1
W	0.0070	0.0001	0.0129	0.0129	0.0001	0.0144	0.0136	19.0*	10	14.9
Mean (7 loci)										
	0.2886	0.0069	0.0227	0.0225	0.0093	0.0307	0.0317	262.5**	70	—
	±0.0735	±0.0041	±0.0101	±0.0099	±0.0046	±0.0125	±0.0138			
Direct value for 7 loci	0.0236	0.0234		0.0312					6.3	
M	0.0071	0.0000	0.0017	0.0017	0.0000	0.0054	0.0046	6.5	10	44.2
c ^s	0.1761	0.0037	0.0208	0.2006	0.0104	0.0558	0.0647	91.3**	10	2.9
Mean (9 loci)										
	0.2448	0.0058	0.0202	0.0199	0.0084	0.0306	0.0324	360.3**	90	—
	0.1600	0.0058	0.0200	0.0188	0.0084	0.0250	0.0283			
Direct value for 9 loci	0.0233	0.0231		0.0332					6.2	

* p<0.05, ** p<0.001

Table 5. Genetic diversity and gene flow in each individual Barcelona cat subpopulation, where a number of colonies were defined. Gene diversity statistics are defined as in other tables, in this case, for individual subpopulations. df = degree of freedom. ** = $p < 0.001$, * = $p < 0.05$.

SUBPOPULATION B								
	H_{sB}	H_{csB}	D_{csB}	$F_{csB(=GcsB)}$	χ^2	df	D_{mcsB}	$Nm\alpha$
O	0.2583	0.2519	0.0063	0.02444	8.652**	2	0.0094	4.435
a	0.4286	0.4275	0.0010	0.00237	0.768	2	0.0015	46.733
tb	0.3799	0.3484	0.0315	0.08295	16.425**	2	0.0473	1.228
d	0.3956	0.3841	0.0115	0.02903	10.218**	2	0.0172	3.716
L	0.0965	0.0905	0.0060	0.06273	22.333**	2	0.0091	1.660
S	0.3917	0.3916	0.0001	0.00022	0.080	2	0.0001	487.72
W	0.0056	0.0055	0.0000	0.00329	1.174	2	0.0000	33.567
Mean (7 loci)								
	0.2794	0.2714	0.0080	0.02929	59.652**	14	0.0121	—
	± 0.0853	± 0.0819	± 0.0055	± 0.01616			± 0.0083	—
c ^s	0.0865	0.0795	0.0070	0.08133	29.118**	2	0.0105	1.255
Mean (9 loci)								
	0.2554	0.2474	0.0079	0.03579	88.771**	16	0.0119	—
	± 0.0845	± 0.0831	± 0.0051	± 0.01756	± 0.0077			—
Direct value for 7 loci			0.0288				3.68	
Direct value for 9 loci			0.0311				2.99	
SUBPOPULATION D								
	H_{sD}	H_{csD}	D_{csD}	$F_{csD(=GcsD)}$	χ^2	df	D_{mcsD}	$Nm\alpha$
O	0.1975	0.1971	0.0004	0.00192	0.381	1	0.0007	32.44
a	0.4382	0.4382	0.0000	0.00000	0.000	1	0.0000	—
tb	0.3945	0.3927	0.0018	0.00470	0.508	1	0.0037	13.22
d	0.3614	0.3524	0.0090	0.02501	4.952*	1	0.0180	2.43
L	0.1442	0.1362	0.0079	0.05517	10.924*	1	0.0159	1.07
S	0.3734	0.3717	0.0016	0.00436	0.863	1	0.0032	14.26
W	0.0000	0.0000	0.0000	—	—	—	—	—
Mean (6 loci)								
	0.3182	0.3147	0.0034	0.01519	17.629*	6	0.0069	—
	± 0.0591	± 0.0598	± 0.0019	± 0.01079	± 0.0039	—		
c ^s	0.1439	0.1361	0.0077	0.05414	10.829*	1	0.0105	1.09
Mean (9 loci)								
	0.2933	0.2892	0.0041	0.02076	28.459*	7	0.0119	—
	± 0.0632	± 0.0642	± 0.0019	± 0.01229			± 0.0077	—
Direct value for 7 loci			0.0109			0.0069	5.657	
Direct value for 9 loci			0.0139			0.0081	4.415	
SUBPOPULATION E								
	H_{sE}	H_{csE}	D_{csE}	$F_{csE(=GcsE)}$	χ^2	df	D_{mcsE}	$Nm\alpha$
O	0.2816	0.2772	0.0043	0.01550	9.457*	3	0.0058	8.93
a	0.4172	0.4143	0.0029	0.00693	3.773	3	0.0038	20.13
tb	0.3165	0.2852	0.0313	0.09890	32.637*	3	0.0417	1.28
d	0.3791	0.3701	0.0089	0.02358	14.337*	3	0.0119	5.82
L	0.2612	0.2506	0.0106	0.05673	25.142*	3	0.0142	3.30
S	0.3958	0.3875	0.0082	0.02088	12.739*	3	0.0110	6.59
W	0.0097	0.0095	0.0000	0.02027	12.491*	3	0.0003	6.79
Mean (6 loci)								
	0.2944	0.2849	0.0095	0.03241	110.579*	21	0.0126	—
	± 0.0693	± 0.0681	± 0.0051	± 0.01552			± 0.0068	—
M	0.0128	0.0128	0.0000	0.00218	1.345	3	0.0000	64.24
c ^s	0.2484	0.2458	0.0026	0.01054	6.603*	3	0.0034	13.19
Mean (9 loci)								
	0.2580	0.2504	0.0077	0.02662	118.527*	27	0.0102	—
	± 0.0759	± 0.0742	± 0.0048	± 0.01465			± 0.0064	—
Direct value for 7 loci			0.0323			0.0126	4.209	
Direct value for 9 loci			0.0298			0.0103	4.473	

Alicante. The mean value of F_{st}' for the subpopulations of Barcelona (obtained using seven loci) was 0.0074 ± 0.0043 . Therefore, each subpopulation has, on average, 99.26% of the total genic diversity found in the total cat population of Barcelona. Nevertheless, although some F_{st}' and G_{st} values were very small, the average value of the seven loci ($F_{st}' = 0.0074$; $\Sigma \chi^2 = 67.4$, 28 df, $P < 0.001$) and those for the $\underline{O}$ ($F_{st}' = 0.0082$), $\underline{l}$ ($F_{st}' = 0.0253$) and $\underline{S}$ ($F_{st}' = 0.007$) loci, indicated significant heterogeneity.

The average gene flow estimate (N_m) was 21.4. Greater mean genetic differentiation was observed among the cat subpopulations of Alicante ($F_{st} = 0.0241 \pm 0.0136$).

Each subpopulation had, on average, 97.59% of the total genic diversity found in the total Alicante cat population. The average F_{st}' value ($F_{st}' = 0.0241$, $\Sigma \chi^2 = 84.7$, 21df, $P < 0.001$) as well as those of the $\underline{O}$ ($F_{st}' = 0.014$), $\underline{t}^b$ ($F_{st}' = 0.0668$), $\underline{d}$ ($F_{st}' = 0.0284$) and $\underline{S}$ ($F_{st}' = 0.05321$) alleles were statistically significant. The gene flow estimate (N_m) was 5.7. The introduction in these analyses of "exotic" genes such as $\underline{c}^s$ and $\underline{M}$ did not appreciably modify the mean values obtained for the other seven loci.

The gene diversity and the gene flow estimates for colonies within subpopulations of Barcelona can be seen in Table 4. For instance, genic diversity analysis of the cat colonies in Barcelona showed a mean value of $F_{ct}' = 0.031 \pm 0.014$. This value was slightly greater than that at the subpopulational level, but was low compared with the values in other organisms (Ruiz-Garcia, 1991). Each colony had, on average, 96.86% of the total genic diversity found in the total cat population of Barcelona. At this level, we observed significant heterogeneity in all alleles, except in the $\underline{a}$ allele, and for the total value of the seven analyzed loci ($F_{ct}' = 0.0317$; $\Sigma \chi^2 = 262.5$, 70 df, $P < 0.001$). Some alleles, such as $\underline{t}^b$ and $\underline{l}$, showed significantly more heterogeneity than other alleles such as $\underline{a}$ and $\underline{W}$ (F-test of Fisher and Snedecor). The directly estimated gene flow at this sampled level was 6.3.

The especially detailed analysis of the contribution of the defined cat colonies in Barcelona in subpopulations B, D and E turned out to be interesting (Table 5). The over-

all genetic heterogeneity in those subpopulations due to the contribution of their respective colonies was significant in these three cases (for seven loci in subpopulation B: $F_{csB} = 0.0293 \pm 0.0161$; subpopulation D: $F_{csD} = 0.0152 \pm 0.0123$; subpopulation E: $F_{csE} = 0.0324 \pm 0.015$). Nevertheless, the loci which contributed to this heterogeneity in each case turned out to be very different. In subpopulation B, the loci which yielded significant heterogeneity were $\underline{O}$, $\underline{t}^b$, $\underline{d}$ and $\underline{l}$ (also $\underline{c}^s$). The F_{csB} values were especially elevated for the $\underline{t}^b$ (0.0829), $\underline{l}$ (0.0627) and $\underline{c}^s$ (0.0813) alleles. In subpopulation D, the alleles which showed significant heterogeneity were $\underline{d}$, $\underline{l}$ and $\underline{c}^s$, with the last two being those which had the highest F_{csD} values (0.0552 and 0.0541, respectively). In subpopulation E, all of the loci except $\underline{a}$ yielded significant heterogeneity, with alleles $\underline{t}^b$ (0.0989) and $\underline{l}$ (0.0567) being especially outstanding. This interesting finding established that a recently introduced allele like $\underline{c}^s$ showed an appreciable heterogeneity in these subpopulations. It is therefore at the colonial level where the stochastic processes can be greater (genetic drift, founder effects), and it is at this level that greater genetic heterogeneity was found.

Selection The selective neutrality of the loci analyzed was tested by the Lewontin and Krakauer (1973) test (Table 6). The results of this test, which is sensitive to heterogeneity in the allele frequencies, are given for various combinations of loci at different levels of structure. In the cat population of Barcelona, at the subpopulation level, the loci $\underline{O}$, $\underline{a}$, $\underline{t}^b$, $\underline{d}$, $\underline{l}$, and $\underline{S}$ showed no significant heterogeneity. When the $\underline{W}$ locus was added to these, the heterogeneity was marginally significant ($F(6, \infty) = 2.67$, $P < 0.05$) as it was when the $\underline{C}$ and $\underline{M}$ loci were added. This same analysis at the subpopulation level in Alicante did not show statistical significance independently from the groups of loci employed. That is to say, at the subpopulation level in both cities, the loci $\underline{O}$, $\underline{A}$, $\underline{T}$, $\underline{D}$, $\underline{L}$, and $\underline{S}$ did not show significant heterogeneity. In contrast to this was the heterogeneity observed at the colonial level in Barcelona. Tests using the six loci above showed significant heterogeneity and the addition of the $\underline{W}$, $\underline{M}$ and $\underline{C}$ loci

Table 6. Lewontin-Krakauer test applied to Barcelona and Alicante at the subpopulation level. S = Significant value. NS = not significant.

BARCELONA			
LEVEL	# of loci	test L-K (S^2fst/s^2fst)	significance
subpopulation	6 ($\underline{O}$, $\underline{A}$, $\underline{T}$, $\underline{D}$, $\underline{L}$, $\underline{S}$)	2.149	$F(5,8)$ NS
	7 ($\underline{O}$, $\underline{A}$, $\underline{T}$, $\underline{D}$, $\underline{L}$, $\underline{S}$, $\underline{W}$)	2.678	$F(6, \infty)$ S $p < 0.05$
	9 ($\underline{O}$, $\underline{A}$, $\underline{T}$, $\underline{D}$, $\underline{L}$, $\underline{S}$, $\underline{W}$, $\underline{M}$, $\underline{C}$)	2.925	$F(8, \infty)$ S $p < 0.005$
ALICANTE			
LEVEL	# of loci	test L-K (S^2fst/s^2fst)	significance
subpopulation	6 ($\underline{O}$, $\underline{A}$, $\underline{T}$, $\underline{D}$, $\underline{L}$, $\underline{S}$)	1.462	$F(5, \infty)$ NS
	7 ($\underline{O}$, $\underline{A}$, $\underline{T}$, $\underline{D}$, $\underline{L}$, $\underline{S}$, $\underline{W}$)	1.832	$F(6, \infty)$ NS
	8 ($\underline{O}$, $\underline{A}$, $\underline{T}$, $\underline{D}$, $\underline{L}$, $\underline{S}$, $\underline{W}$, $\underline{C}$)	1.464	$F(7, \infty)$ NS

to these increased the value of F and made the tests more significant ($P < 0.001$).

DISCUSSION

Hardy-Weinberg equilibrium. The analysis of the O locus in these two Spanish cities at the microgeographic level showed the probable existence of Hardy-Weinberg equilibrium at this locus. As has been verified in multiple studies in other cat populations (for instance, Lloyd, 1985; Ruiz-Garcia, 1990abcd, 1991; Ruiz-Garcia et al., 1995, 1999), the genetic structure of the cat populations reported here was characterized by not showing clear evolutionary events that cause deviations in the observed genotypic frequencies from those expected in Hardy-Weinberg equilibrium. Therefore, factors such as selection in favor and/or against the heterozygotes, or endogamy due to breeding among relatives, or positive or negative Wahlund effect due to geographical and/or temporal causes, can be ruled out as events with a determining influence on the cat populations analyzed at the O locus.

Our findings do not necessarily mean that these populations are in perfect Hardy-Weinberg equilibrium (Smith, 1970). Indeed, several tests of the existence of Hardy-Weinberg equilibrium showed a weak ability to detect type II errors, which, in some cases, do not detect the existence of effective endogamy in the samples analyzed (Ward and Singh, 1970; Pamilo and Varvio-Aho, 1984). It has been shown that in populations where there is not panmixia, multinomial distributions which are very similar to the distributions in perfect Hardy-Weinberg equilibrium can appear (Li, 1988). Furthermore, it was shown that in loci strongly affected by selection, this is not detectable using several current Hardy-Weinberg equilibrium analysis tests (Wallace, 1958; Lewontin and Cockerham, 1959; Li, 1959; Workman, 1969). In addition, inability to reject the null hypothesis does not necessarily indicate that the hypothesis holds, only that the sample size was insufficient for its rejection (Lessios, 1992).

Nevertheless, samples of hundred and thousands of individuals had been analyzed in more than 400 cat populations for these loci and, generally, no significant deviations from the Hardy-Weinberg equilibrium were detected. Some studies with blood allozymes in cat populations of the USA, Israel, Japan and Sweden also showed Hardy-Weinberg equilibrium (O'Brien, 1980; Saliternick-Vardy et al. 1980; Nozawa et al., 1985; Juneja et al., 1991).

Genic diversity, heterogeneity, gene flow and population structure. In these Iberian populations, at the microgeographic level, the genic diversity of the total populations (H_t) differed only slightly from the genic diversity within the subpopulations (H_s) or within colonies

(H_c). In Barcelona, the amount of diversity introduced by the structure at the colonial level was slightly higher than that at the subpopulation level ($F_{st}=0.0074$ vs. $F_{ct}=0.031$). It is straightforward to note that the genetic heterogeneity of several loci among the colonies within subpopulation B (O , t^b , l , and d) and within subpopulation E (t^b , d , l and S), in the same city, was relatively high. This shows that it is in the colonies that to some relative degree the founder effects and the genetic drift could have a chance to modify the allele frequencies. Nevertheless, the majority of genic diversity was contained within the smallest sample population structures. The observed differentiation in these cat populations was much smaller than that observed for other mammals (Massey and Joule, 1981; Patton and Feder, 1981; Chesser, 1983; Chesser et al, 1982; Navajas y Navarro and Britton-Davidian, 1989; Hartl et al, 1990, 1991; Daley, 1992; Bigalke et al, 1993; Preleuthner and Pinsker, 1993; Lorenzini et al, 1993; Corbet and Robinson, 1991; Corbet et al, 1994). From our results, the smallest sampling unit of population structure contained 95% of the total genetic variation. Indeed, there seems to be a certain uniformity of the distribution of allelic frequencies which would be hard to explain using strong stochastic processes (drift, founder effects, population bottle-necks), although certain significant genetic heterogeneity was found especially at the colony level in Barcelona. This suggested that the effective population size must be large enough to resist the strong effects of drift. This may be explained by the relatively high levels of gene flow among the populations studied. It is known that populations with high levels of gene flow (Nm) have relatively little population structuring (i.e: Soltis and Soltis, 1989; Ruiz-Garcia, 1994b).

Several comments should be made about the gene flow estimates obtained here. Wright (1943) stated that in an island model if $Nm > 1$ then gene flow is important enough to erase the genetic heterogeneity between the populations in equilibrium. In a stepping-stone model, this value must be larger than 4 (Trexler, 1988). In both models, $Nm < 0.5$ means that the populations are highly isolated. All the estimates of Nm in this study were very high (colonial level in Barcelona: $Nm = 6.2$; subpopulation level in Barcelona: $Nm = 21.4$; and subpopulation level in Alicante: $Nm = 5.7$). However, a significant genetic heterogeneity was observed in all cases. This might be explained according to Allendorf and Phelps (1981), who argued that the most correct interpretation of $Nm > 1$ is that the populations share the same alleles, although not necessarily with the same allele frequencies. By means of simulation models, they showed that significant allele divergence occurred in 50% of the generations with gene flow of $Nm = 50$ and that significant allele divergence occurred on most occasions when $Nm = 10$. The estimates obtained for gene flow at this microgeographic level were probably underestimates of the true values (Ruiz-Garcia, 1991, 1993,

1997a). These estimates were obtained with highly polymorphic loci and without consideration of the amount of local extinction and recolonization per generation (e_o , extinction rate per generation, k number of colonists, etc) which can occur in these populations (Ruiz-Garcia, unpublished observations). This has been demonstrated to augment the gene flow in the case of model I of Slatkin (1977) and Wade and McCauley (1988) with the migrant pool. In this model I, migrants move from an external source with a constant gene frequency to an infinite number of local colonies, with migrant pool, where new colonists (k) are randomly sampled from the entire population, and if $k > 2Nm + 0.5$, the F_{st} value obtained is smaller (and Nm is larger) than the F_{st} expected without local extinction and recolonization. On the contrary, if the local extinction and recolonization process was generated by model II, where migrants are drawn at random from within a finite array of subdivided populations, with propagule pool, where the new colonies are founded by choosing colonists at random from a single randomly chosen colony, this process will generate F_{st} larger than the F_{st} expected without extinction and recolonization. Thereby, our values of gene flow (Nm) will be overestimations of the reality.

Selection. The Lewontin-Krakauer test applied to different geographical levels showed diverse results. For example, in Alicante and Barcelona at the subpopulation level, six loci did not present significant values. In the case of Barcelona at the subpopulation level, there were statistically significant differences due to the introduction of W_1 on the one hand, and c^s and M , on the other hand. This could be attributable to uniform selection against W and M , because they are alleles, which introduce viability problems in their carriers (Robinson, 1977; Todd et al., 1977; Adalsteinsson, 1980). In all the cases, the addition of W increased the values of the ratios S^2_{Fst} / s^2_{Fst} showing that the heterogeneity of this locus is much lower than those of the remaining six loci, confirming the possible action of uniform selection upon this allele. Nevertheless, significant values were observed at the smallest level, colonies in Barcelona. This should be an indication of selection differentially affecting the loci analyzed. However, the explanation of these the significant values may be of the same nature as some explanations previously expressed. The existence of historic-stochastic factors differentially affecting these loci and the existence of differential gene flow throughout the colonization history of a determined area should produce significant values of the ratio S^2_{Fst} / s^2_{Fst} , as well. For this reason, significant values in the Lewontin-Krakauer test do not necessarily indicate the existence of differential selection within a loci array analyzed. We agree with the critical comments made about this test (Robertson, 1975ab; Ruiz-Garcia, 1996; Taylor et al, 1995).

Ethological and ecological concerns. The ethology of cats in urban populations has been well studied (Waser and Wiley, 1979; Dards, 1979; Natoli, 1985; Natoli and De Vito, 1988; Kerby and MacDonald, 1988). The correspondence between these studies and the microgeographical data presented here was good. Many of the behavioural characteristics of cats provide good mechanisms to explain the observed levels of gene flow. Cats are territorial but do not hold exclusive territories. Rather, both males and females have overlapping home ranges, with the males having much ranges than the females. In fact, it is primarily males which are responsible for gene flow, as they breed promiscuously with many females over large areas (Leyhausen, 1965; Liberg, 1980, 1983; Izawa et al, 1982; Liberg and Sandell, 1988).

A very important factor in the spatial distribution of cats (and many other carnivores) is the distribution of food sources for the species. As both males and females will congregate near food sources (in the case of cats, there are largely tied to the actions of humans), the availability of food only at specific locations will influence the amount of gene flow. As males enlarge their territories considerably during breeding periods to include many such locations (Corbett, 1979; Izawa et al., 1982), they act as effective agents of gene flow.

Augmenting this behavior is that of the females toward male offspring. The females, and sometimes the resident males, will force pubescent males to leave the colonies of their birth (Liberg, 1980; Izawa et al, 1982; Warner, 1985). These behaviors, coupled with the fact that there is no effective dominance hierarchy for mating success by males (Natoli and De Vito, 1988) tend to increase the amount of gene flow, reduce endogamy, and increase the effective population size. This is in clear contrast to the situation in other mammals with male dispersion (i.e: Scribner et al., 1991, Kurt et al., 1993, Nelson, 1993).

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