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Research Article

A biogeographical population genetics perspective of the colonization of cats in Latin America and temporal genetic changes in Brazilian cat populations

Manuel Ruiz-García and Diana Alvarez

Departamento de Biología, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, Colombia.

Abstract

We used nine morphological genes to analyze cat populations from Mexico, the Dominican Republic, the Colombian, Brazilian and Peruvian Amazon, Bolivia and Brazil. Most populations were in Hardy-Weinberg equilibrium at the O locus. The highest allele frequencies so far detected at world level for alleles I (inhibitor) and L (long hair) were found at La Paz (Bolivia). The analyses revealed at least five cat gene pools in Latin America: These findings suggest that the current genetic distribution of cats in Latin America correlates with the colonization of the Americas during the XIV to XVIII centuries. Additionally, the cat populations of São Paulo, Rio de Janeiro and Manaus were sampled, to compare their 1996-2003 genetic profiles with those obtained in 1983. Generally, these genetic profiles seem temporally stable, which is important for comparing cat populations sampled in different years and decades.

Key words: domestic cats, Latin American cats, population genetics, genetic changes.

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Introduction

Since Searle (1949) published the first population genetics study on the domestic cats of London, more than 400 populations have been analyzed from a biogeographical population genetics perspective in different parts of the world, using coat characteristics and skeleton abnormalities. Multiple studies of cat populations have been conducted in Europe (Lloyd and Todd, 1989; Ruiz-Garcia, 1994, 1997) and North America (Shostell *et al.*, 2005). Given the large number of studies devoted to cat population genetics, it is surprising that few studies have included information about Latin America. The only Latin American cat populations studied before the 1990s included some populations from Mexico, Caracas (Venezuela), Willemstadt (Curaçao), and 16 populations from Brazil (Todd et *al.*, 1974, 1976; Watanabe, 1981, 1983).

Studies of Latin American cat populations with emphasis on population genetics began in the 1990s and included the cities of Buenos Aires (Argentina) and Havana (Cuba), Bogota, Ibague, Bucaramanga, Cali, Popayan and Pasto (Colombia), San Jose (Costa Rica), Asunción (Paraguay) and Santiago (Chile) (Kajon *et al.*, 1992; Ruiz-Garcia, 2000; Ruiz-Garcia and Alvarez, 1996, 1999, 2003; Ruiz-Garcia et *al.*, 2002).

However, the recent Latin American study results have left a number of unanswered questions. We focused on cat populations from Acapulco, Mexico D.F., Veracruz, and La Heroica Alvarado (Mexico), Leticia (Colombia) and Tabatinga (Brazil) and neighboring small villages and communities (Colombian-Brazilian-Peruvian Amazon border), Santa Cruz de la Sierra and La Paz (Bolivia), Santo Domingo (Dominican Republic), and new samples from Rio de Janeiro, São Paulo and Manaus (Brazil), in order to resolve three main questions: 1) How many cat gene pools are there in Latin America? 2) If there are different cat gene pools in Latin America, how are they associated with human migration events from Europe? 3) Are the genetic profiles of the Latin American cat populations temporally stable? If the cat gene profiles are temporally stable, we can compare cat populations sampled in different years and decades in Latin America and in Europe. To bring new data to bear on the "Historical migration hypothesis" (Todd, 1977), it is important to determine whether the Latin American cat genetic profiles were "frozen" at the moment or in the first years or decades after Europeans founded different cities in Latin America. If these genetic profiles are continuously changing, many of the comparisons between Latin American and European cat populations make no sense from a historical-genetic perspective, a fact that has not been previously determined for any Latin American cat population.

Send correspondence to Manuel Ruiz-García. Departamento de Biologia, Facultad de Ciencias, Pontificia Universidad Javeriana, CRA 7A n. 43-82, Bogota, Colombia. E-mail: mruiz@javeriana.edu.co.

Materials and Methods

A total of 2613 domestic cats (*Felis catus*) were analyzed from August 1996 to December 2003, sampled in populations of Acapulco (n = 310), Mexico D.F. (n = 443), Veracruz (n = 241), Alvarado (n = 123), Mexico; Santo Domingo (n = 251), Dominican Republic; Santa Cruz de la Sierra (n = 146) and La Paz (n = 133), Bolivia; Leticia, Colombia; and Tabatinga, Brazil (87% of the sample), and several neighboring villages and Indian communities (Puerto Nariño, Macedonia and Amacayacu National Park in Colombia, Benjamin Constant and Atalaya do Norte and other small villages of the Javarí River in Brazil, and Santa Rosa and Islandia in Peru) near the Colombian-Brazilian-Peruvian Amazon border (n = 423), plus some new samples from three Brazilian cities: Rio de Janeiro (n = 276 in 1996 and n = 81 in 1999; total n = 357); São Paulo (n = 70) and Manaus (n = 116) (Figure 1). The sampling design employed consisted of scoring the phenotypes of cats observed in streets and houses of non-overlapping routes in several areas of each city, so as to survey a large section of each site. Cats from the Abandoned Animals Refuge and from the Center for Animal Protection were also screened in Mexico City. Each route was used only once, in order to avoid sampling the same animals twice. A large percentage of animals were sexed, and the sex ratios of these populations were calculated.

In accordance with the Committee on Standardized Genetic Nomenclature for Cats (1968), the analyzed genetic characteristics were: sex-linked (O, o; Orange [epistatic for the observation of the A locus] *vs.* non-orange) and the autosomal loci, A (A, a; Agouti *vs.* Non-agouti [epistatic for the T locus]), T (t^+, t^b, T^a; striped or mackerel tabby *vs.* blotched tabby *vs.* Abyssinian tabby), D (D, d; non-dilution *vs.* dilution), L (L, l; short hair *vs.* Long hair), S (S, s; Piebald white spotting *vs.* non- white spotting), W (W, w; Dominant white [epistatic for the other colors] *vs.* normal color). Other loci analyzed were I (I, i; Inhibitor or "silver" *vs.* non-silver color) and C (C, c^s; normal color *vs.* Siamese pattern). The characteristics of these genes are described in Robinson (1977).

Figure 1 - Map of the Latin American cat populations sampled.

The frequency of the sex-linked allele was calculated from direct counts of surveyed cats that were sexed, and also estimated with the maximum-likelihood procedure (Robinson and Silson, 1969) for samples that were not sexed. A 1:1 sex ratio was assumed for non-sexed samples, using the equation $p(O) = (2a+b)/2N$, where a is the number of orange cats (O/O and O/-), b is the number of tortoiseshell females, or calico (O/o), and N is the total sample size for this locus. The direct and theoretical calculated sex ratios were compared using Robinson's equation (1972): $m = (4ac-b^2)/((2a+b)(b+2c))$, where c is the number of animals that do not have the orange allele. Two versions of the chi-square test were used in the analysis of the Hardy-Weinberg equilibrium at the O locus. One test utilized the observed frequencies of the genotypes O/O (& O/-), O/o, and o/o (& o/-) in addition to the allele frequency calculated via the maximum likelihood method, assuming a sex ratio of 1:1. The second test utilized only the genotypes of the sexed female cats (O/O, O/o, & o/o).

To establish the genetic relationships between the cat populations reported here and other cat populations, a group composed of 78 American and European cat populations was analyzed. The European populations selected may have been the origin of the American cat populations studied (Spain, n = 7; Portugal, n = 2; Italy, n = 2; France, n = 3; and Great Britain, n = 4). The American and Canary Island samples were composed of 60 populations, including 10 North American populations of Anglo origin (Atlantic coast and U.S. Midwest) and three Canadian populations, 21 (including those reported here) Latin American and one Canary Island sample (Tenerife 2) directly obtained by the authors, four populations from the US (Richmond, California; Denver, Colorado; and Dallas and Houston, Texas) that may have been influenced by the Spaniards during their settlement process (Lamoreux and Robinson, 1971; Blumenberg, 1976; Blumenberg and Blumenberg, 1976; Morrill and Todd, 1978), 16 populations from Brazil, two from Mexico, Venezuela (Caracas), Curaçao (Willemstadt), Jamaica (Kingston) (Lloyd *et al.*, 1981), and one population from Santa Cruz de Tenerife (Canary Islands) (Lloyd, unpublished), referred to as Tenerife 1.

Two approaches were used to analyze the Latin American cat populations reported here with regard to other cat populations: similarity and phylogenetic analysis. For the first one, DA (Nei *et al.*, 1983) and Prevosti's (1974) genetic distance matrices among pairs of populations were obtained, and the UPGMA and neighbor-joining (Saitou and Nei, 1987) algorithms were applied. The trees with the best cophenetic correlation coefficients (Sneath and Sokal, 1973) and the lowest Fitch and Margoliash (1967) standard deviations were analyzed. The bootstrap method (Felsenstein, 1985) using Efron's (1982) resample technique was used for several trees (500 resamples), to analyze the reliability of each tree cluster. However, the bootstrap percentages were low to very low in all the trees analyzed.

The second approach was a maximum parsimony analysis. The branch-swapping technique was employed using the mhennig* command of the HENNIG86 v. 1.5 program where each character (= mutant allele) presented multiple states (Felsentein, 1983), as follows: when the allele was not found in a population, the corresponding state was 0; when the frequencies were between 0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.5-0.6, 0.6-0.7, 0.7-0.8, 0.8-0.9, and 0.9-1.0, the corresponding multiple states were 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, respectively. Leticia-Tabatinga (on the Colombian-Brazilian border in the Amazon) and Duitama (in the Colombian Andean highlands) were used as outgroups, because previous partial analyses indicated that they behaved differently from other Latin America populations. This analysis was developed for 21 populations sampled exclusively by the authors in Latin America. The minimum number of steps attained in the most-parsimonious trees, consistence, and retention coefficients (Kluge and Farris, 1969) were estimated. In addition, the possible states of each hypothetical ancestral population for each genetic trait studied for each phylogenetic tree were obtained by using the routine steps of the HENNIG86 v. 1.5 program.

Finally, we studied the temporal genetic stability of three Brazilian cat populations (São Paulo, Rio de Janeiro and Manaus). Previously, Watanabe (1983) had studied large samples of these cat populations. We sampled these populations again about 13-20 years later (1996-2003), which represents about 13 to 40 generations in the natural history of this species. A chi-square test was applied to determine if there were significant temporal changes in the allele frequencies of these cat populations. If a high temporal stability in the genetic profiles of the Latin America cat populations can be demonstrated, this could be an important proof in favor of the hypothesis that these current cat genetic profiles represent the original profiles of the cat populations upon the foundation of cities in Latin America by Europeans.

Results

The allele frequencies and the corresponding standard deviations of the nine morphologic loci studied in the cat populations of Acapulco, Mexico D.F., Veracruz, Alvarado, Leticia-Tabatinga and neighboring small villages, São Paulo, Rio de Janeiro, Manaus, Santa Cruz, La Paz, and Santo Domingo are listed in Table S1. The Hardy-Weinberg equilibrium analyses applied at the <u>O</u> locus are shown in Table S2. When they were applied to the complete samples using the maximum likelihood method, they proved to be in Hardy-Weinberg equilibrium, with the exception of La Paz, where an excess of homozygotes was found ($\chi^2 = 3.987$, 1 df, $p < 0.05$), although with the Bonferroni correction this value was not significant. Some sex ratios showed a favorable value towards males by this method (m = 0.691, La Paz; m = 0.567, Veracruz; m = 0.643, Alvarado; m = 0.734, Manaus), and in one case it was favorable towards females (m = 0.317, Rio de Janeiro).

However, the Hardy-Weinberg equilibrium analysis of the sexed females showed different results. Mexico D.F. ($\chi^2 = 5.079$, 1 df, $p < 0.05$) and Acapulco ($\chi^2 = 32.01$, 1 df, $p < 0.001$) had a significant excess of heterozygotic females (O/o), while La Paz showed an excess of homozygotic females. The other populations were in Hardy-Weinberg equilibrium for this genetic marker in the females. The direct sex ratio showed discrepancies from those obtained theoretically by using the maximum likelihood method. With the Bonferroni correction, Mexico D.F. (m = 0.364; $\chi 2 = 8.25$, 1 df, $p < 0.001$) and Acapulco (m = 0.286; $\chi^2 = 12.8$, 1 df, $p < 0.001$) were the only populations that presented an excess of females. These significant differences imply that the maximum likelihood method used to estimate <u>m</u> can misrepresent the reality. However, the excess of homozygotes in La Paz can rather be attributed to a lack of heterozygous than to an excess of homozygous males. La Paz is probably the Latin American cat population most influenced by endogamy in this study. However, the estimation of the total frequency for the <u>O</u> allele was not particularly different when using either method. The values calculated by the maximum likelihood and direct counter methods for each population were, respectively: Mexico D.F. (0.24 *vs.* 0.29); Acapulco (0.38 *vs.* 0.43); Veracruz (0.31 *vs.* 0.29); Alvarado (0.24 *vs.* 0.25); Leticia-Tabatinga (0.16 *vs.* 0.15); Manaus (0.13 *vs.* 0.18); São Paulo (0.19 *vs.* 0.18); Rio de Janeiro (0.14 *vs.* 0.23); Santa Cruz (0.27 *vs.* 0.25); La Paz (0.19 *vs.* 0.17); and Santo Domingo (0.31 *vs.* 0.32). On the other hand, in the populations of Mexico D.F., Acapulco, Veracruz, Alvarado, Leticia-Tabatinga, Manaus, São Paulo and Santo Domingo, the <u>O</u> allele frequencies for males and females were practically identical. The two populations from Bolivia and Rio de Janeiro showed the largest differences for p(O) between the sexes. In Santa Cruz, the frequencies for males and females were 0.295 and 0.235, while in La Paz the frequencies were 0.231 and 0.139, respectively.

Similarity and phylogenetic analyses of the Latin American cat populations

A neighbor-joining (NJ) analysis using the DA distance (Figure 2) was performed with 78 North American, Latin American and European cat populations. Two large

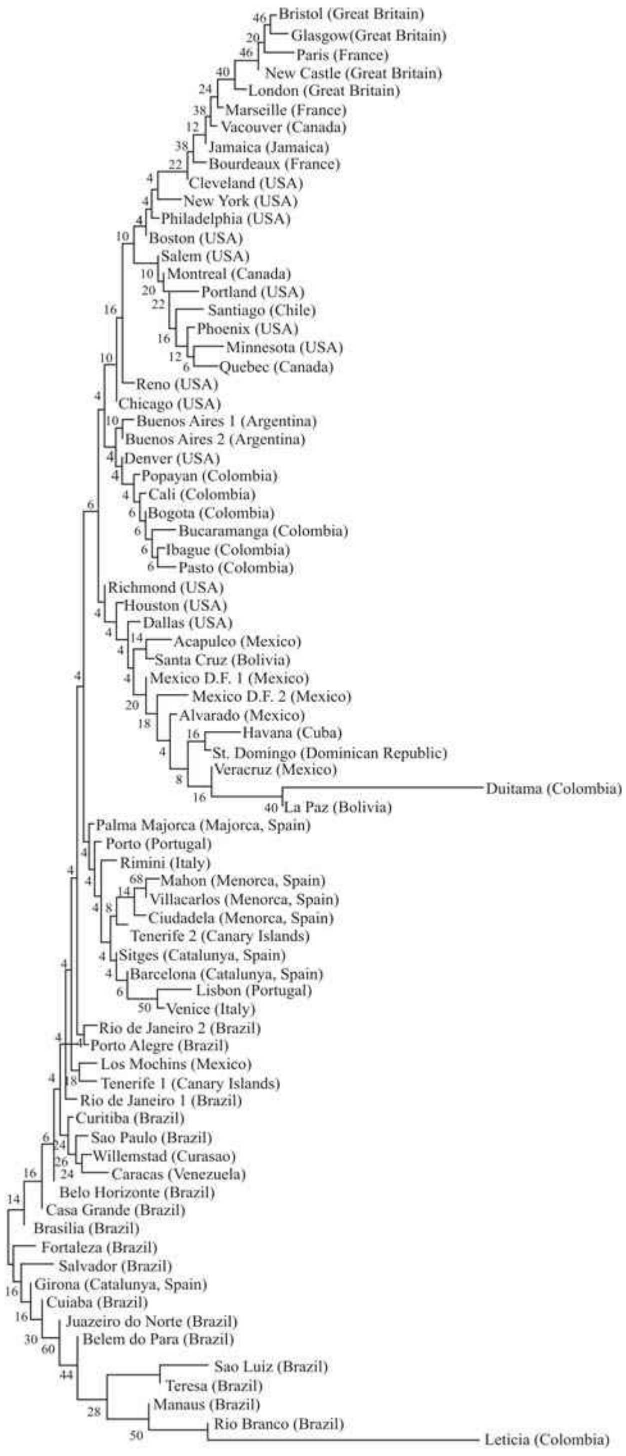

Figure 2 - Phenetic analysis of 78 Latin American, North American and European cat populations, estimated by using the neighbor-joining algorithm with the DA genetic distance (Nei *et al.*, 1983). Numbers on the tree nodes represent bootstrap percentages.

clusters were differentiated. One was composed of the populations from Brazil (Northern and Amazonian), including those of Leticia-Tabatinga and Manaus reported here. In fact, the two Amazonian populations were significantly different only at W ($\chi^2 = 8.79$, 1 df, $p < 0.01$). The other eight genes did not present significant differences between these two Amazonian localities separated by 1500 km. The second cluster was formed by 5 groups: (1) southern Brazil populations along with one sample from Tenerife, Los Mochis (Mexico), and the populations of Caracas and Curaçao (North Atlantic South America); (2) the populations from Catalonia and the Balearic Islands, the other sample from Tenerife, and populations from Portugal and Italy; (3) the populations from California, Texas, Acapulco, Santa Cruz, Mexico D.F., La Paz, Duitama, and those of the Spanish-speaking Caribbean countries; (4) a U.S. cat population of presumed Spanish origin (Denver), Buenos Aires, six Colombian populations, and some U.S. Midwest populations (Reno, Nevada, and Chicago, Illinois); (5) the U.S. populations of presumed Anglo origin, such as the populations of Canada, Great Britain, France, Santiago (Chile) and Jamaica. Additionally, other analyses ratified the existence of at least five different gene ensembles for Latin American cats. For instance, a UPGMA tree using the Prevosti distance with a set formed by cat populations from 50 Latin American sites and from the U.S. with Spanish influence showed the following picture: the most divergent population was Duitama (Colombia), with probable founder effect and extensive genetic drift. The other populations were grouped into five well delimited groups. The most divergent group comprised the Amazonian and North Brazilian populations, including Leticia-Tabatinga and Manaus. The second group of populations comprised the Santiago and Jamaica populations, which historically received more direct and indirect British influence. The third group was composed of populations from Spanish-speaking Caribbean countries, including Mexico, Cuba and the Dominican Republic, in addition to the populations of Mexico D. F. (the oldest sample not sampled by us) and the population of La Paz (Bolivia). The last two clusters were the highest ones. The first cluster was composed of the populations of Bogota, Ibague, Bucaramanga, and Pasto (all from Colombia), Buenos Aires (Argentina) along with Colorado and California (USA) and Cali and Popayan (both in Colombia), Mexico D.F (the new sample obtained by us), Santa Cruz, Acapulco, and the populations of Texas (USA). The last cluster comprised the populations of South Brazil, including the samples from São Paulo and Rio de Janeiro sampled by us, Los Mochis (Mexico), Tenerife (Canary Islands), Curaçao, and Caracas (Venezuela) (Figure 3).

The phylogenetic analysis generated two maximum parsimony trees with minimum length (56 steps), a consistency index of 48, and a retention index of 61. The tree, using Duitama (Colombia) as an outgroup element, showed that Pasto was its closest Colombian population (Figure 4a). Four well-delimited groups were observed: (1) the populations of Spanish-speaking Caribbean countries; (2) the populations of Buenos Aires, Acapulco, Santiago and Bolivia; and (3) Mexico D.F. These three groups were closer related among each other than to the fourth group,

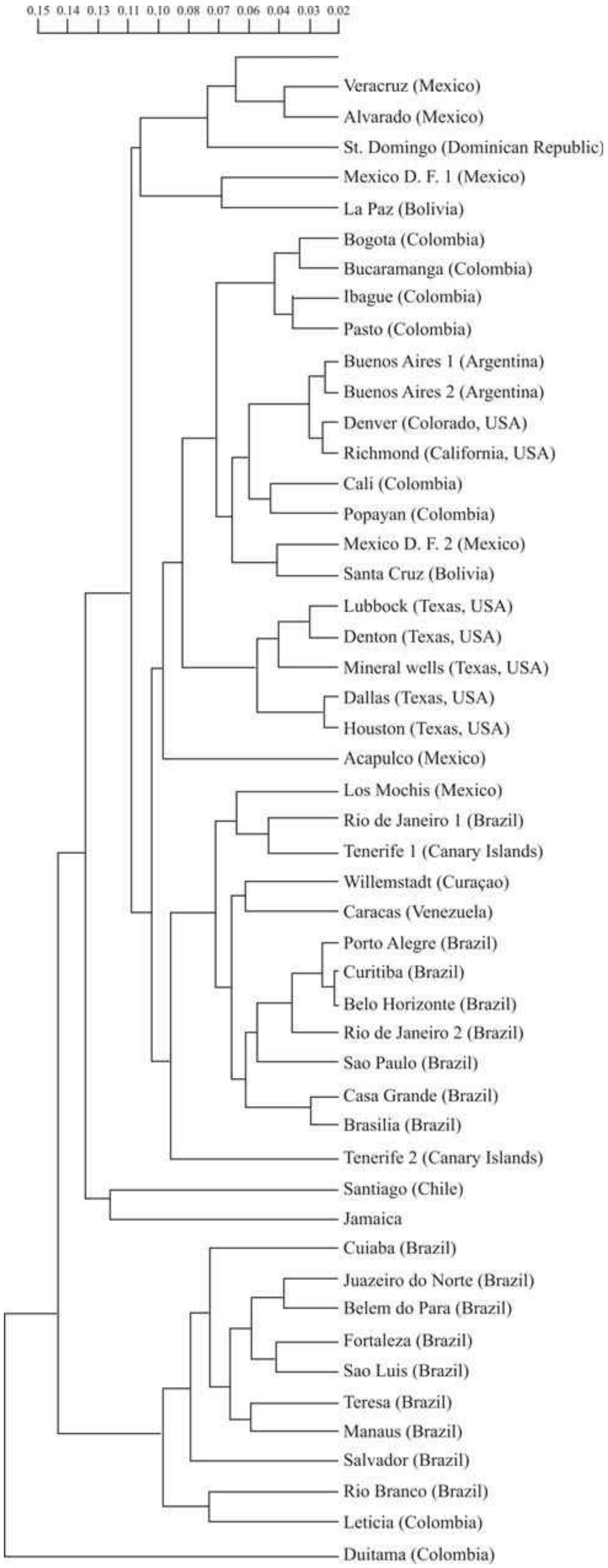

Figure 3 - Phenetic analysis of 50 Latin American and U.S. Southwest cat populations, estimated by using the UPGMA algorithm with the Prevosti genetic distance.

formed by: (4) the Colombian populations, Rio de Janeiro, Leticia-Tabatinga, and Tenerife. Santiago was more similar to the geographically closest Latin American populations when using the maximum parsimony method than when the distance method was employed. The two populations of Bolivia were more similar to each other by the distance method, and Buenos Aires showed a strong similarity with the Latin American Pacific coast populations. In contrast with other analyses, the Caribbean populations were more similar to other Latin American populations, while the Colombian populations were very similar to the populations of Tenerife (Canary Islands) and South Brazil. The other maximum parsimony tree, using the population of Leticia-Tabatinga as an outgroup (Figure 4b), supported the existence of the relationships mentioned above. The genetic profiles of the hypothetically ancestral populations of the most important current groups of Latin American cat populations are listed in Table S3.

We compared the allele frequency profiles of the cat populations of São Paulo, Rio de Janeiro and Manaus sampled prior to those reported here (Table S4). In the case of Manaus, none of the loci (7) showed significant differences in the last 13-20 years with regard to the Watanabe (1983) sample. The two loci which showed greater temporal variation were O (0.18 *vs.* 0.13) and S (0.47 *vs.* 0.36). Therefore, these two genes seem to have decreased over time, but without reaching statistical significance. In the case of São Paulo, our sample did not differ for any locus from the first genetic profile obtained by Todd and Jeanne (1972), although their sample was very small and came from a single site of the city. Our sample only differed at the S locus (0.42 *vs.* 0.31; $\chi^2 = 5.68$, 1df, $p = 0.025$) from that of Watanabe's (1983) genetic profile, although this significance disappeared with the Bonferroni correction. Thus, taken together Manaus and São Paulo, no case out 14 loci studied was clearly statistically significant. This clearly points toward genetic stability in the cat populations of Manaus and São Paulo.

The case of Rio de Janeiro is different. In a first approach, we compared the two genetic profiles that we obtained in 1996 (n = 276) and 1999 (n = 81) from all over the city. No significant differences were detected at the nine loci examined. The only locus close to a significant change (but without reaching it) was c^s (Siamese), which was not detected in the first sample (0 *vs.* 0.11). Therefore, both samples were taken together and the overall genetic profile was compared to the Watanabe (1983) profile. With the Bonferroni correction, three out of seven loci showed significant differences: O (0.24 *vs.* 0.14; $\chi^2 = 25.84$, 2df, $p < 0.001$), l (0.19 *vs.* 0.29; $\chi^2 = 17.63$, 1df, $p < 0.001$), and W (0.04 *vs.* 0.01; $\chi^2 = 16.87$, 1df, $p < 0.001$). Orange (O) and Dominant White (W) decreased over time, while long hair (l) increased over time. The genetic instability of the Rio de Janeiro profile compared to those of Manaus and São Paulo is explained in the Discussion.

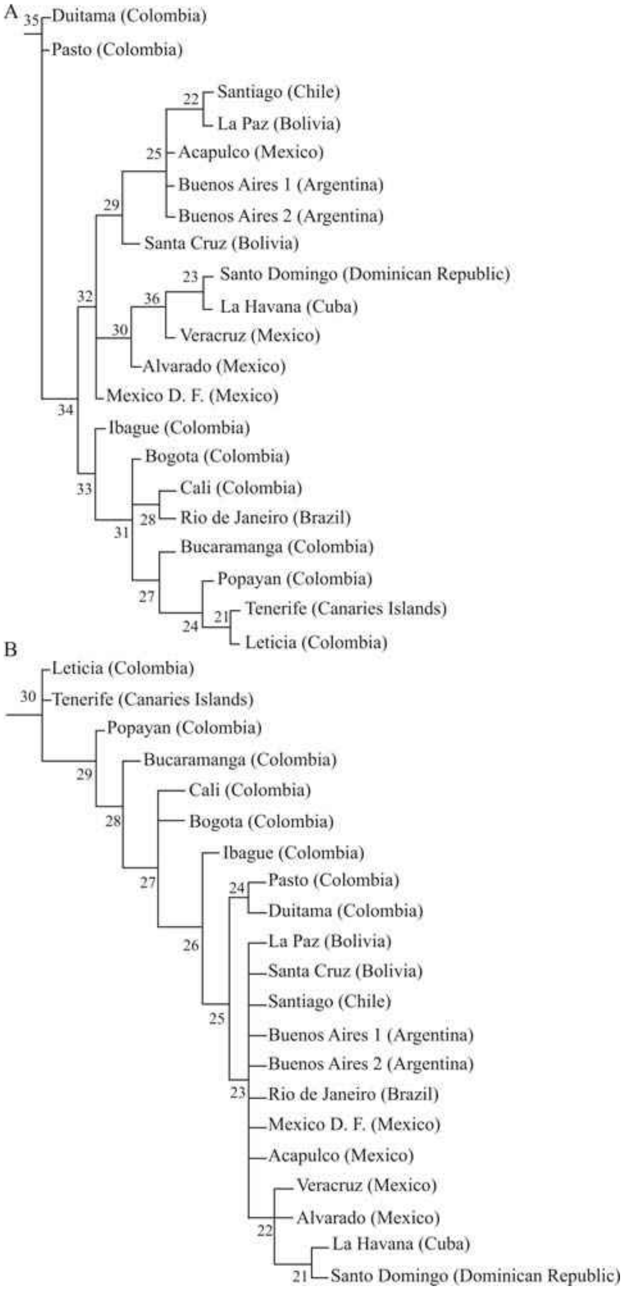

Figure 4 - Cladogenetic analysis with maximum parsimony methods, using allele frequency intervals as character states. A - Maximum parsimony tree using the Duitama cat population as outgroup. B - Maximum parsimony tree using the Leticia-Tabatinga cat population as outgroup.

Discussion

Some considerations on Hardy-Weinberg equilibrium and sex ratio in Latin American populations of cats

The cat population of La Paz showed beyond any doubt the existence of an excess of homozygotes at the O locus. Moreover, we observed showy genetic characteristics in this population. The dominant mutants curled-ear (Cu) (3/131, p(Cu) = 0.0115) and Manx (M) (1/131, p(M) = 0.004) were present. La Paz, together with Leticia-Tabatinga, also had the lowest q(a) frequency of all the Hispanic American cat populations studied and the highest frequencies of long hair (l) and inhibitor (I) so far detected in the world. These findings, in addition to the geographic isolation of this city at 4000 meters above sea level, make it likely that the original population was established by a small number of cats with uncommon genetic traits (founder effect). Of course, the existence of any natural and/or artificial selective pressure in the current genetic makeup cannot be ruled out. In contrast, the two Mexican populations of Acapulco and Mexico D.F showed a large excess of heterozygous females at the O locus. Acapulco may have had multiple points of origin that are now reflected in its current genetic makeup. Although many of these cat immigrants were probably Spanish, some were Asian (Ruiz-Garcia and Alvarez, 1999). A population shows an excess of heterozygotes if it presents inner groups that have been mixing and yet differ in their allele frequencies. It is also possible that cats from other cities in Mexico or the United States, genetically different from Acapulco, contributed to the excess of observed heterozygotes in this Mexican Pacific population. Although the excess of heterozygous females was significant in both cities, in Mexico D. F. it was smaller than in Acapulco. For other characteristics, such as the d allele, Mexico D. F. was placed between the Mexican Pacific and the Mexican Caribbean populations, although it was more similar to Acapulco on the Pacific coast. These two genetic pools (Pacific and Caribbean) may have converged in Mexico D.F. and caused the excess of heterozygotes at O observed in the Mexican capital. It was also noteworthy that the O frequencies in males and females of the two populations of Bolivia were extremely different. In both cases, the male frequencies p(O) were higher than the female frequencies. This result might generate several hypotheses. For example, these two populations could have been established more recently than other Latin American populations, and therefore did not have sufficient genetic homogenization time for the frequencies at this locus to become equal between the sexes.

This hypothesis is supported by the divergent genetic profile of La Paz, but it is not supported by the genetic profile of Santa Cruz and the short generational time in this species (1 year). A second hypothesis (Pontier *et al.*, 1995) assumes that in rural habitats the orange male cats are selectively favored over non-orange males because of their larger size. In contrast, larger males would be negatively selected in urban habitats. This second hypothesis does not apply to female cats, because there is no evidence to suggest that coat color is correlated with body size. The higher p(O) in males compared to females in Bolivia supports Pontier *et al.*'s hypothesis, because a high percentage of its human population was born in the countryside, having only recently moved to the cities. A third possibility would be the existence of positive artificial selection for orange males. There is anecdotal evidence suggesting that the native Indians of Bolivia prefer orange males.

Analysis of some of the mutant allele frequencies estimated in Latin American cat populations

The frequencies of the a allele in many Latin American cat populations were within the range found in the Spanish populations. Other populations, such as Colombia, Curaçao, Venezuela, the Canary Islands, and Colorado in the U.S., presented higher frequencies of q(a). According to Todd (1978), such an increase in the q(a) frequencies is expected under specific conditions. Based on studies with rodents (Keeler, 1942) and other carnivores (Keeler *et al.*, 1968, 1970), Todd (1978) hypothesized that there is a pleiotropic association between melanizing genes and docile behavior. Therefore, black animals (aa) would have been better able to tolerate shipping, contact with humans, and conditions of high densities with other cats during travel. However, this hypothesis was not supported by the Caribbean and other populations, such as the Leticia-Tabatinga and the Bolivian populations, which were the result of multiple migration steps. For instance, the populations of Mexico D.F. and Acapulco likely originated from Spanish Mediterranean populations with the following intermediate steps: Canary Islands, Santo Domingo, Havana, and Veracruz. Cat propagules that originated in Spain underwent a large number of intermediate selection steps before they settled in Bolivia. In addition to the steps previously cited for Mexico D.F. and Acapulco, cats destined for Bolivia underwent other steps, including different ports on the Pacific coast of Central America (Panama, Balboa), Ecuador (Guayaquil), Peru (Lima and El Callao, the most important commercial port on the Pacific used by the Spaniards) and Chile (Valparaiso). However, Santa Cruz did not support Todd's hypothesis, because its q(a) value was similar to that of several Spanish populations. Even the value of this character found in La Paz (0.635) was lower than that found in Spain. If Todd's hypothesis was true, a population located in an area of difficult access, 4000 meters above sea level in the Andes, would have a very high q(a) frequency. The same could be said for Leticia-Tabatinga (0.628). The cats imported from Portugal and Spain must have arrived at the mouth of the Amazon, later being transported the nearly 3000 km up the Amazon River to found the current populations of Leticia and Tabatinga. However, this does not seem to increase the frequency of this allele as postulated by Todd's hypothesis.

The frequencies of $\underline{t}^{\underline{b}}$ in all the Latin American populations that we studied were low, or very low, supporting little contact with populations of British origin. Furthermore, the Spanish cat populations considered as ancestral, such as the Catalonian and Balearic ones, have low frequencies of this genetic trait. On the other hand, the populations of southern Spain (for example, the two Andalusian cities Cadiz and Malaga), from where most of the ships that colonized and conquered America sailed, showed higher frequencies of $\underline{t}^{\underline{b}}$. This could be the result of an increase of $q(t^b)$ in southern Spain during the last centuries. However, the size of the samples and the number of cat populations from southern Spain analyzed are still very limited. An analysis of the populations of Seville and Huelva is essential for the confirmation of a possible change in the $\underline{t}^{\underline{b}}$ frequencies in southern Spain. However, there are some historical reasons that could explain the increase of $\underline{t}^{\underline{b}}$ in some Spanish ports relating to the conquest of America. For instance, Cadiz was an important colonization point for the Americas during the epoch of Philip V until the epoch of Charles III. Although Seville was the most important port for the Americas until 1717, by 1654 strong competition from Cadiz began to be recognized. During the XVIII century, Cadiz became the port most commonly used by ships headed for the Americas. During this time, three-quarters of all Spanish exports in terms of value originated from this port (Fisher, 1988). For example, between 1785 and 1796, Veracruz and Havana in the North Caribbean received 42.5% of all exports from Cadiz. The genetic profile of the cats of Cadiz, however, does not show a conspicuous similarity to those of Latin America cat populations. The current high levels of $q(t^b)$ in Cadiz are similar to other West European cat populations and could be the result of an influx of more than three-quarters of its inhabitants since 1750, especially of French, Dutch and Italian origin. For instance, in 1654, France began to dominate the commerce of the ports of Cadiz, Alicante and San Sebastian (Spain). This commercial domination of the Cadiz port could have introduced many cats from French populations to this city. At the same time (XVII and XVIII centuries), the port of Malaga was dominated by the Genovese (Italians), who transported English textiles between Spain and North Africa and formed more commercial routes throughout the inner region of Andalusia (especially Cordoba, Jaen and Antequera) than from Spain to America. Therefore, the cats exported to Latin America were those of the first centuries of the conquest (XV, XVI and XVII), prior to an increase of $\underline{t}^{\underline{b}}$ in the southern Spanish ports.

White spotting, S, also provided one of the most interesting genetic trait distributions in Latin America. While the majority of the Spanish populations has p(S) frequencies between 0.20-0.30, some Minorcan (Mahon, Villacarlos, Mercadal and Alaior) and Latin American populations (Colombia: Bogota, Ibague, and Bucaramanga; Bolivia: La Paz and Santa Cruz) presented lower frequencies. These populations might reveal the frequencies of this allele that originated in Spain and did not become distorted during their establishment in Latin America. In contrast, other populations presented much higher S frequencies. This was the case of Santo Domingo, Havana and Tenerife and of some populations of Brazil (especially the tropical Amazon ones; 0.35-0.55). These populations are located in areas that receive intense solar radiation and are exposed to high temperatures. Therefore, the p(S) distributions may be linked to habitat variations resulting from intensive solar radiation and high temperature (a selective hypothesis). This could be the best example of a gene submitted to *in situ* natural selection in the new colonized habitats.

The inhibitor allele, I, has very low and residual frequencies in Spanish and most other populations across the world and is almost residual in some populations of Latin America. However, four of the populations studied (Alvarado, Santa Cruz, La Paz and Islandia, a Peruvian Amazon island included in the Leticia-Tabatinga sample) presented higher frequencies than those of other populations analyzed. In fact, the p(I) value of La Paz (0.236) was the highest found in the world. For this locus, it is more difficult to determine if there was pre-migrational selection, at least for some populations, or if these p(I) values merely reflect a founder effect with or without *in situ* natural and/or artificial selection. The La Paz cat population could show a strong *in situ* artificial selection. The Spaniards founded many of the present cities of Bolivia, because they were considered optimal sites from which to search for and to obtain silver. Spain considered the location of La Paz to be critical, because it was between the southern silver mines and those on the Pacific coast. The Spaniards established a second Bolivian city (Potosi) in 1545, after having discovered abundant silver deposits. Five thousand mine entrances were created and eight million Mitayo Indians died during the extraction process (Buero, 1994). By 1651, the number of inhabitants had reached 160,000, three times the size of the current population and, at the time, second only to London in the world. An astonishingly large amount of silver (16,000 tons) was extracted from Potosi and shipped to Seville during the time period of 1545-1660. Perhaps the high frequency of silver cats in silver-mining Bolivian cities is not coincidental, but *in situ* natural selection can be not ruled out either. The Andean cat (*Oreailurus jacobita*) that lives in this region also has a silver coat, and possible hybridization among both felines during the formation of the domestic cat population could not be discarded. On the other hand, the presence of the inhibitor trait together with the Abyssinian tabby character only in the Peruvian Amazon island of Islandia (alleles not detected in other Amazonian cat populations) revealed a founder effect in this cat population.

Diverse Latin American cat gene pools and possible historical causes

Analysis of the Latin American cat populations shows the existence of at least five differentiable gene pools, four of them presumed to have an Iberian origin:

(1) the populations of northern Brazil and the Brazilian Amazon, including the Leticia-Tabatinga sample; (2) some populations spread across Latin America which present some British influence, such as Jamaica and Santiago de Chile; (3) the populations of the Spanish-speaking countries of the northern Caribbean (Mexico, Cuba, and the Dominican Republic); (4) a group of populations found from California and Colorado (U.S) to Buenos Aires (Argentina) that principally, but not exclusively, follow a route along the Pacific coast. The Spaniards sailed from southern Spain and touched the Canary Islands, Santo Domingo (Dominican Republic), and Havana (Cuba) prior to their arrival at Veracruz (Mexico). A second route used by the Spaniards was Santo Domingo – Panama (Puerto de Dios, Colon, Balboa and Portobello). From those Pacific ports they traveled to the Pacific coast of Colombia (Buenaventura), Ecuador (Guayaquil), Peru (Lima, El Callao), and Chile (Valparaiso, Concepcion, and Arica). During the XVII century, two expeditions left Spanish ports every year, one in January and another in August. When they reached these latitudes, they crossed the Andes to exploit the phenomenal resources of gold and silver found mainly in nowadays' Bolivia. After the silver was obtained, two routes were used. By the first route, the Spaniards returned to the port of El Callao (Peru), where they shipped the silver and gold to Portobello (Panama) previous to the crossing to Europe. The second route crossed southern Bolivia, part of Paraguay, and went deep into the deserts of northern Argentina. Several cities, including Salta, Jujuy, Tucuman, Santa Fe and Rosario, were established to provide provisions before reaching Buenos Aires, the city from which silver and other precious metals were shipped across the Atlantic to Spain. These commercial routes that extend across large geographical distances help to explain why there is a common gene pool for cat populations located from Colorado (U.S) to Buenos Aires (Argentina). The enormous and significant impact that the Pacific connection exerted on Spain can be put into perspective with the following historical data. From 1785 to 1796, exports from the port of Cadiz to the Pacific coast (El Callao, first route) and to Rio de la Plata (second route) together accounted for 33% of all commercial exports from Spain, although the first route seemed to be more important than the second (52 million *vs.* 26 million reales, in 1778). Moreover, some Pacific ports such as Acapulco were also used to colonize the coasts of California. These diverse historic facts could explain the existence of some genetic subgroups of cats that are connected across the Pacific coast. Within this large geographical area, there were some subgroups which presented small differences: (4.1) most of the Colombian populations (with the exception of Leticia-Tabatinga on the Amazon River) seem to have purer and stronger contributions from the Pacific route than detected in other populations; (4.2) Buenos Aires (Argentina), Cali and Popayan (Colombia), California and Colorado (U.S.) constitute a subgroup that clearly demonstrates the Pacific connection, because a direct relationship among them could not have been established via the Atlantic Ocean: Cali and Popayan (Buenaventura port and Guapi bay), geographically closer

to the Pacific coast than the other Colombian populations studied, showed direct contributions from the Pacific route more clearly than the other Colombian populations; Pasto, founded by Spanish expeditions from Ecuador (Caillavet and Pachón, 1996), sometimes also showed a stronger relationship to the Pacific route than to the other Colombian populations; (4.3) the populations of Acapulco, Mexico D.F., Santa Cruz and La Paz (Bolivia) constitute another group that confirmed this Pacific connection, but with some small divergent influences. However, the samples from La Paz and the ancient sample from Mexico D.F showed inconsistent relationships with some distances and algorithms. In the first case, the strong founder effect that affected this population could be the explanation for the scarce relationships seen with other Latin America populations. In the second case, the first genetic profile of Mexico D.F. (Todd *et al*., 1976) could be non-representative of this population due of inadequate way of sampling. Group (4.4) comprised the populations of Texas, which were related to this Pacific connection, but with their own genetic characteristics. Group (5), comprising the populations of southern Brazil, Tenerife and Puerto de la Cruz (Canary Islands), Curaçao and Venezuela, was found to be an exclusively Atlantic group. It is important to mention that the Colombian populations (Bogota, Ibague, and Bucaramanga, for instance), which must have been originally formed by cats from the Atlantic ports of Cartagena de Indias, Barranquilla, and Santa Marta, did not show genetic profiles that were very different from those of the Pacific connection. However, they did differ from the Caribbean populations (Atlantic constitution), from which they were thought "a priori" to have originated. For example, Santo Domingo (Dominican Republic) was visited immediately preceding Cartagena and Santa Marta, and the commerce between Santa Marta and Havana was very active (Fisher, 1981, 1988). However, in genetic terms, the populations of Colombia were not very similar to those from the Caribbean area. This could be the result of selection acting on some genes within the Caribbean populations of Santo Domingo and Havana, or of a varied origin of the Colombian and Caribbean populations. Only a study of the populations of Cartagena, Barranquilla and Santa Marta could clarify this issue. The cat population of Los Mochis in Mexico was inexplicably associated to this group. Probably the sample studied by Todd *et al.* (1976) did not adequately represent this population. Additionally, the cat population of Duitama (Colombian Andean highlands) was marked by a strong founder effect with gene drift, and its association with other Colombian cat populations is distorted.

Although there is no consensus for the degree of relationship among these five groups, the maximum parsimony analysis showed some interesting traits, as follow. (1) The Colombian populations were the most divergent from the other Latin American populations analyzed, and the populations of the Canary Islands were closer to the Colombian populations than to the rest of the Latin American populations. This means that in this analysis the Colombian populations were most similar to the Spanish populations. (2) The populations of Pacific and Caribbean influence were closer related to each other than to the Colombian populations. (3) There was a strong relationship between the two populations of Bolivia, and (4) Santiago, Chile, behaved essentially as a Latin American Pacific population. However, using the genetic distance methods, some of these trends obtained with the maximum parsimony methods were not observed.

The populations of the Canary Islands were highly related to several Balearic populations. In some analyses, Barcelona was related to Tenerife in the Canary Islands. This relationship was very important for identifying the basic genetic characteristics of the Iberian populations that originated many of the current Latin American cat populations. The port of Barcelona was extremely valuable for continued commerce with America during the XVIII century, especially with New Granada and specifically with Cartagena and Santa Marta (although not with the Pacific region), being the second in importance after Cadiz (Fisher, 1988). These recognized trade routes help to explain direct and indirect connections and relationships of some Catalan cat populations, such as Girona, with some Amazonian populations, such as Leticia-Tabatinga and Cuiabá (Brazil).

The genetic relationships among some Catalan and Amazonian cat populations deserve a detailed historical discussion. Europe's "Great Depression" at the end of the XIX century motivated a massive emigration of European workers to Latin America. Simultaneously, some Catalan religious orders intensified their emigrations to the Colombian Amazon to exploit natural sources, such as "caucho" and "quina" (Alzate, 1993). In 1902, the Catalan Fray Lorenzo de Pupiales founded Amazonian settlements such as San Francisco only for whites. The first imported Europeans were 10 rural workers, 10 carpenters, two textile workers and two cattlemen, all Catalans. After a short time, many other Catalans migrated into the Colombian Amazon. These Catalans founded villages such as Puerto Leguízamo and Puerto Asís. In 1905, the Catalans brought 19 cattle from Catalonia to this Amazonian region, and by 1916 there were a significant number of cattle as well as horses and other domestic animals that were also imported from Catalonia. Catalans also built the roads connecting Sibundoy and Puerto Asis (Colombian Amazon). Puerto Asís was an important port for the Amazonian navigation and for the export and import of goods transported across the Amazon River to the Atlantic Ocean, North America and Europe. Another Catalan, Gaspar de Pinell, established commercial relationships that connected Leticia, Puerto Asis and Manaus with the Amazon River enterprise. From Manaus, many industrial goods, textiles, tools and domestic animals were sent to the Colombian Amazon. Therefore, the strong genetic relationship between the cat populations of Leticia-Tabatinga and Manaus (separated by 1500 km of the Amazon River) is explained by this commercial route. The Catalans who migrated into the Amazon region provided a dispersal mechanism with countless opportunities for the transport of other domestic animals such as cats. This also provides an explanation as to why there is a genetic resemblance between Leticia and some Catalan cat populations.

Explanations for some temporal genetic changes in some Brazilian cat populations

As previously discussed, the new samples of Manaus and São Paulo did not show significant temporal changes for the gene frequencies of seven loci compared to the gene profiles obtained by Watanabe (1983). The sole marginal exception was the case of S in São Paulo, which decreased. However, our 1999 sample was small (n = 70); therefore, this significant difference could have no biological reason. Basically, the result confirms that both populations are large enough to be insensitive to gene drift effects, because the effective numbers are important, and there are no selective patterns affecting these loci during the 13-40 generations of the cat populations of these Brazilian cities. Identically, these cat populations did not receive important influxes from other foreign cat gene pools. This probably means that the genetic profiles of these cat populations were established at the origin of these cities and especially when they reached a population size of 15,000-30,000 human inhabitants (Ruiz-Garcia, 1990).

Contrarily, the Rio de Janeiro cat population seemed to change genetically for some loci over this time. O and W significantly decreased, and l and, partially, d increased. There are two hypotheses to explain this finding. The first one is neutral. The same both authors of this paper obtained two samples in Rio de Janeiro, one in 1996 (n = 276) and the other in 1999 (n = 81). Although they did not show significant differences for nine loci, many of the cat communities sampled in 1996 had disappeared in 1999 because of human actions. It is possible that the effective sizes have been reduced in the last decades in this Brazilian city. Therefore, gene drift could be more important and more possibilities for gene changes occurred. Furthermore, the opportunity for an introduction of new alleles from other immigrant foreign pools to the cats of Rio de Janeiro becomes possible. Precisely the two alleles (l and d) more positively affected by pre-migrational selection in the cat colonization of Latin America from Spain and Portugal were those whose gene frequencies increased over time. Additionally, the Siamese (c^s) allele was not present in the first sample but present in the second sample, even though its size was smaller than that of the first one. This agrees quite well with the fact that new and foreign alleles were introduced into the Rio de Janeiro cat gene pool. A second, selective explanation is also possible. The increase of major urban pressures could negatively affect some alleles such as O and W. Moreover, both events could act together. Nevertheless, if a major urban pressure developed in Rio de Janeiro, the question is why this change has not also affected the cat populations of Manaus and São Paulo.

More Latin American cat populations must be analyzed for a complete understanding of the population genetics dynamics of the colonization process from Europe and the internal evolution of this species within Latin America.

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Supplementary Material

The following online material is available for this article:

- Table S1. Allele frequencies.
- Table S2. Hardy-Weinberg analysis.
- Table S3. Allele frequencies of ancestral populations.
- Table S4. Temporal allele frequency changes.

This material is made available as part of the online article from http://www.scielo.br/gmb.

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Table S1. Allele frequencies and standard deviations of *O* (Orange), *a* (non-agouti), t^b (blotched tabby), T^A (Abyssinian tabby), *d* (dilution), *l* (long hair), *S* (white spotting), *W* (dominant white), c^s (Siamese) and *I* (Inhibitor) of the 11 Latin American cat populations analyzed. N = sample size.

	n	*O*	*a*	t^b	T^A	*d*	*l*	*s*	*W*	c^S	*I*
Acapulco	310	0.377±0.027	0.707±0.024	0.245±0.034	0	0.477±0.026	0.441±0.026	0.349±0.022	0.022±0.005	0.153±0.028	0.029±0.007
Mexico D.F.	443	0.237±0.020	0.739±0.019	0.169±0.034	0	0.359±0.025	0.497±0.023	0.264±0.018	0.024±0.006	0.385±0.022	0.007±0.003
Veracruz	241	0.310±0.019	0.742±0.026	0.119±0.041	0.004±0.004	0.204±0.033	0.472±0.031	0.347±0.025	0.011±0.005	0.291±0.031	0.014±0.006
Alvarado	123	0.235±0.035	0.711±0.036	0.197±0.056	0.006±0.006	0.131±0.046	0.473±0.041	0.327±0.034	0.008±0.006	0.182±0.045	0.071±0.017
Leticia-Tabatinga	423	0.159±0.034	0.628±0.021	0.325±0.029	0.012±0.044	0.123±0.025	0	0.298±0.033	0.003±0.034	0.239±0.024	0.012±0.036
Manaus	116	0.133±0.069	0.707±0.037	0.189±0.066	0	0	0	0.361±0.065	0.044±0.065	0.275±0.047	0
São Paulo	70	0.188±0.081	0.762±0.041	0	0	0	0.396±0.055	0.308±0.078	0.007±0.084	0.170±0.059	0
Rio de Janeiro	357	0.139±0.036	0.755±0.014	0.292±0.036	0	0.318±0.025	0.294±0.025	0.379±0.034	0.010±0.037	0.053±0.027	0.012±0.037
Santa Cruz	146	0.275±0.036	0.739±0.032	0.153±0.053	0	0.471±0.039	0.411±0.039	0.234±0.028	0.011±0.006	0.267±0.041	0.079±0.017
La Paz	133	0.189±0.031	0.635±0.037	0.151±0.053	0	0.415±0.041	0.668±0.033	0.216±0.027	0.015±0.007	0.124±0.044	0.236±0.029
Santo Domingo	251	0.306±0.027	0.734±0.024	0.232±0.040	0	0.259±0.031	0.534±0.027	0.538±0.028	0.006±0.003	0	0.017±0.006

Table S2. Hardy-Weinberg analysis applied to the Orange (*O*) locus using a Maximum likelihood method, with the females directly sexed in the 11 Latin American cat populations. O/- = Orange males; O/O = Orange females; O/+ = calico (= heterozygote) females; +/- = wild males; +/+ = wild females; X^2 = chi-square test; P = probability; - = not estimated.

		Maximum Likelihood method					Directly sexed females				
Population		O/- & O/O	O/+	+/- & +/+	χ^2	p	O/O	O/+	+/+	χ^2	p
Mexico D.F.	observed	54	63	243	0.1	NS	7	70	63	5.08	<0.05
	expected	52.9	65.19	241.9			12.6	58.8	68.6		
Acapulco	observed	73	72	144	0.33	NS	4	73	17	32.01	<0.0001
	expected	75.04	67.88	146.08			17.44	46.09	30.45		
Veracruz	observed	47	40	129	1.13	NS	9	41	55	0.12	NS
	expected	43.89	46.21	125.89			8.28	42.42	54.29		
Alvarado	observed	20	15	82	2.38	NS	4	15	30	1.07	NS
	expected	16.98	21.03	78.99			2.69	17.6	28.71		
Leticia-Tabatinga	observed	39	48	309	0.11	NS	3	15	51	0.23	NS
	expected	36.48	52.95	306.54			1.59	17.79	49.62		
Manaus	observed	10	6	82	1.03	NS	2	4	16	0.63	NS
	expected	7.38	11.3	79.32			0.72	6.54	14.72		
São Paulo	observed	7	12	50	0.32	NS	-	-	-	-	-
	expected	8.92	10.53	50.76			-	-	-		
Rio de Janeiro	observed	20	57	271	3.69	NS	2	11	19	0.03	NS
	expected	27.55	41.65	278.89			1.75	11.47	18.78		
Santa Cruz	observed	23	25	81	0.03	NS	3	25	38	0.19	NS
	expected	22.61	25.72	80.66			3.64	23.72	38.64		
La Paz	observed	18	12	97	3.98	<0.05	3	11	47	3.76	<0.05
	expected	14.26	19.46	93.28			1.18	14.63	45.19		
Santo Domingo	observed	52	47	148	1.11	NS	15	47	61	1.53	NS
	expected	49.27	52.41	145.31			12.05	52.89	58.05		

Table S3. Allele frequencies of ancestral populations (nodes) of several Latin American cat populations estimated by means of maximum parsimony trees for the alleles *O, a* , t^b, *d, l, S,* and *W*, with (a) the population of Duitama as outgroup and (b) Leticia-Tabatinga as outgroup. Numbers are the nodes of the trees of Figure 4.

a. Maximum Parsimony Tree

Duitama outgroup

O	*a*	t^b	*d*	*l*	*S*	*W*
210.1-0.2	0.8-0.9	0.2-0.3	0.3-0.4	0.1-0.2	0.2-0.3	0
220.1-0.2	0.7-0.8	0.2-0.3	0.4-0.5	0.5-0.6	0.2-0.3	0 - 0.1
230.3-0.4	0.7-0.8	0.2-0.3	0.2-0.3	0.5-0.6	0.3-0.4	0 - 0.1
240.1-0.2	0.8-0.9	0.2-0.3	0.3-0.4	0.2-0.3	0.2-0.3	0 - 0.1
250.2-0.3	0.7-0.8	0.2-0.3	0.4-0.5	0.4-0.5	0.2-0.3	0 - 0.1
260.3-0.4	0.7-0.8	0.1-0.2	0.2-0.3	0.4-0.5	0.3-0.4	0 - 0.1
270.1-0.2	0.8-0.9	0.1-0.2	0.3-0.4	0.2-0.3	0.2-0.3	0 - 0.1
280.1-0.2	0.8-0.9	0.1-0.2	0.3-0.4	0.3-0.4	0.3-0.4	0 - 0.1
290.2-0.3	0.7-0.8	0.1-0.2	0.4-0.5	0.4-0.5	0.2-0.3	0 - 0.1
300.2-0.3	0.7-0.8	0.1-0.2	0.2-0.3	0.4-0.5	0.3-0.4	0 - 0.1
310.1-0.2	0.8-0.9	0.1-0.2	0.3-0.4	0.3-0.4	0.2-0.3	0 - 0.1
320.2-0.3	0.7-0.8	0.1-0.2	0.3-0.4	0.4-0.5	0.2-0.3	0 - 0.1
330.2-0.3	0.8-0.9	0.1-0.2	0.3-0.4	0.3-0.4	0.2-0.3	0 - 0.1
340.2-0.3	0.8-0.9	0.1-0.2	0.3-0.4	0.4-0.5	0.2-0.3	0 - 0.1
350.2-0.3	0.8-0.9	0 - 0.1	0.3-0.4	0.4-0.5	0.2-0.3	0

b. Maximum Parsimony Tree

Leticia - Tabatinga Outgroup

O	a	t^b	d	l	s	W
210.3-0.4	0.7-0.8	0.2-0.3	0.2-0.3	0.5-0.6	0.3-0.4	0 - 0.1
220.2-0.3	0.7-0.8	0.1-0.2	0.2-0.3	0.4-0.5	0.3-0.4	0 - 0.1
230.2-0.3	0.7-0.8	0.1-0.2	0.3-0.4	0.4-0.5	0.2-0.3	0 - 0.1
240.2-0.3	0.8-0.9	0 - 0.1	0.3-0.4	0.4-0.5	0.2-0.3	0
250.2-0.3	0.8-0.9	0 - 0.1	0.3-0.4	0.4-0.5	0.2-0.3	0 - 0.1
260.2-0.3	0.8-0.9	0.1-0.2	0.3-0.4	0.3-0.4	0.2-0.3	0 - 0.1
270.3-0.4	0.7-0.8	0.1-0.2	0.2-0.3	0.4-0.5	0.3-0.4	0 - 0.1
280.2-0.3	0.7-0.8	0.2-0.3	0.4-0.5	0.4-0.5	0.2-0.3	0 - 0.1
290.3-0.4	0.7-0.8	0.2-0.3	0.2-0.3	0.5-0.6	0.3-0.4	0 - 0.1
300.1-0.2	0.8-0.9	0.2-0.3	0.3-0.4	0.1-0.2	0.2-0.3	0

Table S4. Temporal allele frequency changes in the cat populations of Manaus, São Paulo and Rio de Janeiro (Brazil), sampled throughout time for 9 morphological loci. * = borderline significant differences; # = statistically significant differences; N = sample size.

Populations	n	*O*	*a*	t^b	*d*	*l*	*S*	*W*	*I*	c^S
Manaus										
Watanabe, 1983	993	0.18*	0.68	0.32	0.06	0.06	0.47*	0.03	-	-
Present Work	116	0.13*	0.71	0.19	0	0	0.36*	0.04	0	0.28
São Paulo										
Todd & Jeanne, 1972	90	0.19	0.71	0.26-0.40	0.-0.15	0.11-0.18	0.35	0.01	-	-
Watanabe, 1983	1644	0.22	0.71	0.26	0.18	0.35	0.42#	0.03	-	-
Present Work	70	0.17	0.76	0	0	0.40	0.31#	0.01	0	0.17
Rio de Janeiro										
Watanabe, 1983	1545	0.24#	0.74	0.32	0.27*	0.19#	0.38	0.04#	-	-
Present Work overall	357	0.14#	0.75	0.29	0.32*	0.29#	0.38	0.01#	0.012	0.053
Present Work 1996		0.15	0.76	0.26	0.33	0.31	0.36	0.01	0.013	0
Present Work 1999		0.12	0.75	0.36	0.25	0.25	0.44	0.01	0.006	0.112