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Research Article

Genetic characterization of the population of São Luís, MA, Brazil

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Abstract

Five loci (vWA1, F13A1, D12S67, Apo-B and D1S80) were investigated by polyacrylamide gel electrophoresis followed by silver staining in a sample of 177 individuals from the population of São Luís, State of Maranhão, Brazil. A total of 70 different alleles were identified. A statistically significant deviation from the Hardy-Weinberg equilibrium was observed in a single locus (F13A1, p = 0.0075). The average heterozygosity (H) was estimated at 77.7%, the mean number of alleles per locus as 14. The PD (capacity of genotype differentiation at each locus) ranged from 88.9% (vWA1) to 96.7% (F13A1). The combined PE (power of exclusion) of these five loci was 99.8%. In terms of racial admixture (42% European, 39% Indian, and 19% African Black ancestry), São Luís presented an estimate similar to Belém, another trihybrid Amazonian population.

Keywords: Amazon region, DNA, polymorphisms, VNTRs, STRs, interethnic admixture.

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Introduction

The city of São Luís, capital of the State of Maranhão, Brazil, was founded in 1612 by the French, the first Europeans who had contact with the native people of the region, *i.e.*, Indians of the Jê and Tupi-Guarani groups. Three years later came the Portuguese, who expelled the French and settled in the city, bringing along their model of manufacturing colonization with the use of slave labor (Tavares, 1979; Mota and Mantovani, 1998). Dutch colonizers also participated in the colonization of the city in 1641, but were expelled by the Portuguese in 1643 (Moraes, 1987).

The first record of African slaves trading to Maranhão dates back to 1655. Those people were compulsorily introduced from Guinea-Bissau, Togo, Benin, Nigeria and Angola, and, to a lesser extent, from Senegal, Gambia, Guinea, Upper Volta, Ghana, Congo and the Cabo Verde, São Tomé and Principe archipelagoes (Meireles, 1994). Slave trading lasted a total of two hundred years in Maranhão. In 1822, the population of the Province of São Luís consisted of 152,893 inhabitants, 77,914 (51%) of which were slaves. The final estimate of the number of African slaves brought to Maranhão during the period from 1655 to 1822 was 187,000, corresponding to 8.3% of the 2,250,000 slaves imported to Brazil during that period (Meireles, 1994).

Santos and Guerreiro (1995) investigated 13 genetic markers (HP, CP, TF, ALB, CHE1, CHE2, ABO, RH, ESD, CA2, ACP, GLO and HBB) in a total of 5,417 individuals from 11 populations of the Brazilian Amazon Region (Manaus, Parintins, Caari, Oriximiná, Óbidos, Santarém, Castanhal, Belém, Alenquer, Monte Alegre and Bragança). Their results showed that in most towns the Amerindian contribution (41%) was greater than the African Black (12%), but smaller than the Caucasian (47%). The authors suggested the existence of approximately four million Amerindians amalgamated into the urban population of the Amazon Region.

Since 2002, many papers have been published describing the variability of STRs and VNTRs in the Brazilian population: for the Northeast, data from the States of Alagoas and Bahia (Ferreira-da-Silva *et al.,* 2002; Santos *et al.*, 2004); for the Southeast, from São Paulo and Rio de Janeiro (Bydlowski *et al.*, 2003; Góes *et al.*, 2004), and for Brazil as a whole (Whittle *et al.*, 2004). However, there was only one paper (Callegari-Jacques *et al.*, 2003) reporting a spaciotemporal analysis to estimate the relative proportions of continental ancestral contributions to the Brazilian population. These authors studied 12 STR markers in 1,037 individuals living in different regions of Brazil (North, Northeast, Midwest, Southeast and South). The relative

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proportions of ancestral contributions showed values between 68%-71% of European, 12%-14% of African and 12%-17% of Amerindian ancestry in the Northern Region, values which are different from those estimated by Santos and Guerreiro (1995).

In the present study, genomic DNA markers (variable number of tandem repeats - VNTRs - and short tandem repeats - STRs) were analyzed in a Brazilian population, with determination of heterozygosity, power of discrimination (PD), power of exclusion (PE) and polymorphism informative content (PIC), and interethnic admixture estimations were made.

Material and Methods

The present study was conducted in the population of São Luís, capital of the State of Maranhão, in the Amazon Region of Northeastern Brazil. Peripheral blood (10 mL) was collected from 177 unrelated individuals born in that city, with Vacutainer tubes containing EDTA as anticoagulant. DNA was extracted by the phenol-chloroform method (Sambrook *et al.*, 1989). The following DNA loci were analyzed by PCR: i) STRs (vWA1, F13A1 and D12S67); ii) VNTRs (Apo-B and D1S80). PCR was carried out with a model PTC 100 apparatus (MJ Research). All reactions were performed in a total volume of 25 µL, the solutions containing 100 ng of genomic DNA template. The PCR protocols used for the amplification of the five loci have been described elsewhere (Nakamura *et al.*, 1988; Boerwinkle *et al.*, 1989; Peake *et al.*, 1990; Hegele *et al.*, 1996; Polymeropoulos *et al.*, 1991; Thymann *et al.*, 1993; Santos, 1999). The VNTRs and STRs were genotyped by polyacrylamide gel electrophoresis (PAGE), using silver nitrate stain.

Statistical analysis

Allele and genotype frequencies were assessed by gene counting, using the GENEPOP program, version 3.4 (Raymond and Rousset, 1995). The same software was used to calculate the observed and the expected number of homozygotes and heterozygotes. The allele frequencies for each locus were compared with those found in the population of Alagoas, another northeastern state of Brazil (Ferreira-da-Silva *et al.*, 2002), and with the weighted means of previously observed European, Asian, African and Amerindian frequencies, using the CLUMP program (Sham and Curtis, 1995).

The genetic distances between the population of São Luís and the others that were compared were estimated by the method of Nei *et al.* (1983), using the DISPAN program.

Admixture estimates were calculated by the gene identity method (Chakraborty, 1985), using the ADMIX95 (Admixture Analysis for Hybrid Populations - www.genetica.fmed.edu.uy) program. Heterozygosity (H), PIC (Polymorphism Informative Content), PE (Power of Exclusion) and PD (Power of Discrimination, capacity of genotype differentiation at each locus) were calculated according to the methods described by Nei *et al.* (1983), Boldstein *et al.* (1980), Chakraborty and Stivers (1996), and Guo and Elston (1999). Each locus was tested for the Hardy-Weinberg equilibrium using Fisher's Exact Test as calculated by the Genetic Data Analysis (GDA) program (Lewis and Zavkin, 2001).

Results and Discussion

A total of 70 different alleles, an average of 14 alleles per locus, were identified in the 177 individuals. The allele frequencies at the five loci analyzed are given in Table 1 and Figure 1. To compare the present study with that from Alagoas, mention should be made that, in that investigation, 598 individuals were tested and 85 different alleles were observed, an average of 9.4 alleles per locus (Ferreira-da-Silva *et al.*, 2002).

VWA1

Ten alleles were detected for the vWA1 locus in the São Luís population (Table 1), which showed a bimodal pattern of distribution, with frequency peaks for the vWA1*16 and vWA1*20 alleles (Figure 1a). In the Alagoas population, only one modal peak, in vWA1*16, was observed.

We observed the presence of vWA1*14, an allele as yet not described in Brazil, which shows relevant frequencies among Portuguese (11.8%) and West-Africans (6.9%) (Gamero *et al.*, 2003; Whittle *et al.*, 2004). This allele was also observed by Rangel-Villalobos *et al.* (1999) in a Mexican population (Jalisco), at a frequency of 7.5%.

F13A1

Nineteen different alleles were found. The electrophoretic migration pattern revealed the presence of three alleles as yet not described in Brazilian populations, F13A1*18, F13A1*19 and F13A1*22 (Table 1).

F13A1*5 and F13A1*7 are the most frequent alleles in West-African populations (Gamero *et al.*, 2003). In Portuguese populations, F13A1*7 and F13A1*6 are the most frequent (Gamero *et al.*, 2003; Bell *et al.*, 2000), while F13A1*3, and F13A1*6 and F13A1*4 are the most frequent alleles in Asian and Amerindian populations, respectively (Hammond *et al.*, 1994; Robertson *et al.*, 1995; Péres-Lezaun *et al.*, 1997; Santos, 1999; Halos *et al.*, 1999).

In the São Luís population, the frequency distribution showed a bimodal pattern, with frequency peaks in the F13A1*3, F13A1*2 and F13A1*6 alleles, and with the F13A1*3 allele being the most frequent (Figure 1b). In Alagoas, however, a unimodal distribution was found, with F13A1*6 being the most frequent allele.

Table 1 - Allele frequencies and variability measures at the five loci analyzed in the São Luís population.

Alleles	VWA1	D12S67	F13A1	APOB3'	D1S80
1			0.114		
2			0.168		
3		0.032	0.171		
4		0.067	0.117		
5		0.087	0.144		
6		0.163	0.161		
7		0.240	0.040		
8		0.208	0.017		
9		0.119	0.003		
10		0.064	0.003		
11		0.016	0.000		
12		0.003	0.003		
13			0.003		
14	0.003		0.007		
15	0.127		0.010		
16	0.480		0.013		
17	0.034		0.013		
18	0.011		0.003		0.235
19	0.096		0.003		0.000
20	0.136		0.000	0.006	0.018
21	0.107		0.000	0.000	0.055
22	0.003		0.003	0.003	0.037
23	0.003			0.000	0.007
24				0.003	0.246
25				0.000	0.070

Alleles	VWA01	D12S67	F13A1	APOB3'	D1S80
26				0.000	0.018
27				0.000	0.048
28				0.016	0.085
29				0.000	0.063
30				0.068	0.055
31				0.000	0.004
32				0.058	0.029
33				0.000	0.018
34				0.211	0.011
36				0.299	
38				0.036	
40				0.058	
42				0.029	
44				0.042	
46				0.091	
48				0.071	
50				0.006	

	Total number of chromosomes				
Indices	354	312	298	308	272
HO	0.723164	0.807692	0.758389	0.818182	0.781022
HE	0.715082	0.843641	0.869315	0.839841	0.85933
PIC	0.684303	0.821429	0.851788	0.820672	0.842435
PD	0.889023	0.955417	0.967471	0.957026	0.965529
PE	0.451976	0.682371	0.733269	0.674943	0.713335
P	0.8578	0.0525	0.0075	0.1612	0.0653

HO: observed heterozygosity; HE: expected heterozygosity; PIC: polymorphic information content; PD: power of discrimination; PE: power of exclusion; P: Hardy-Weinberg equilibrium. exact test.

D12S67

Ten alleles were identified at the D12S67 locus in the São Luís population (Table 1). The allele distribution showed a unimodal pattern, the highest frequency being observed for D12S67*7 (Figure 1c). These results disagree with those reported for a European sample, which showed 11 alleles and a bimodal distribution, with frequency peaks for D12S67*6 and D12S67*5 (Falcone *et al*., 1995). Studies conducted on Brazilian indigenous tribes from Maranhão, Awá-Guajá and Urubu-Kaapor, presented bimodal distributions, with peaks for the D12S67*9 and D12S67*7 alleles, and for the D12S67*9 and D12S67*10 alleles, respectively (Santos, 1999).

The lack of similarity between the São Luís population and other populations investigated previously may be related to the small number of studies using this marker, despite the fact that it represents one of the most polymorphic systems. Especially lacking are data from African populations, which significantly contributed to the formation of the population studied here.

Apo-B

Apo-B is one of the most widely studied VNTRs. In general, African and European populations show a unimodal distribution for the Apo-B*36 allele (Ludwig *et al.*, 1989; D'Aloja *et al.*, 1992; Hixon *et al.*, 1993; Latorra *et al.*, 1994; Pinheiro *et al.*, 1996; Maviglia *et al.*, 2001), while in Asian populations the most frequent allele is Apo-B*34 (Deka *et al.*, 1992; Renges *et al.*, 1992; Evans *et al.*, 1993). Amerindian populations show two frequency peaks, one for the allele Apo-B*36 and one for Apo-B*46 (Zago *et al.*, 1996; Vallinoto, 1996).

The São Luís population showed 15 alleles at this locus. These alleles and their frequencies are shown in Table 1. The allele distribution presented a bimodal pattern with peaks for the alleles Apo-B*36, Apo-B*34 and Apo-B*46, with Apo-B*36 being the most frequent (Figure 1d). The São Luís frequencies (Apo-B*34, 21%; Apo-B*36, 30%)

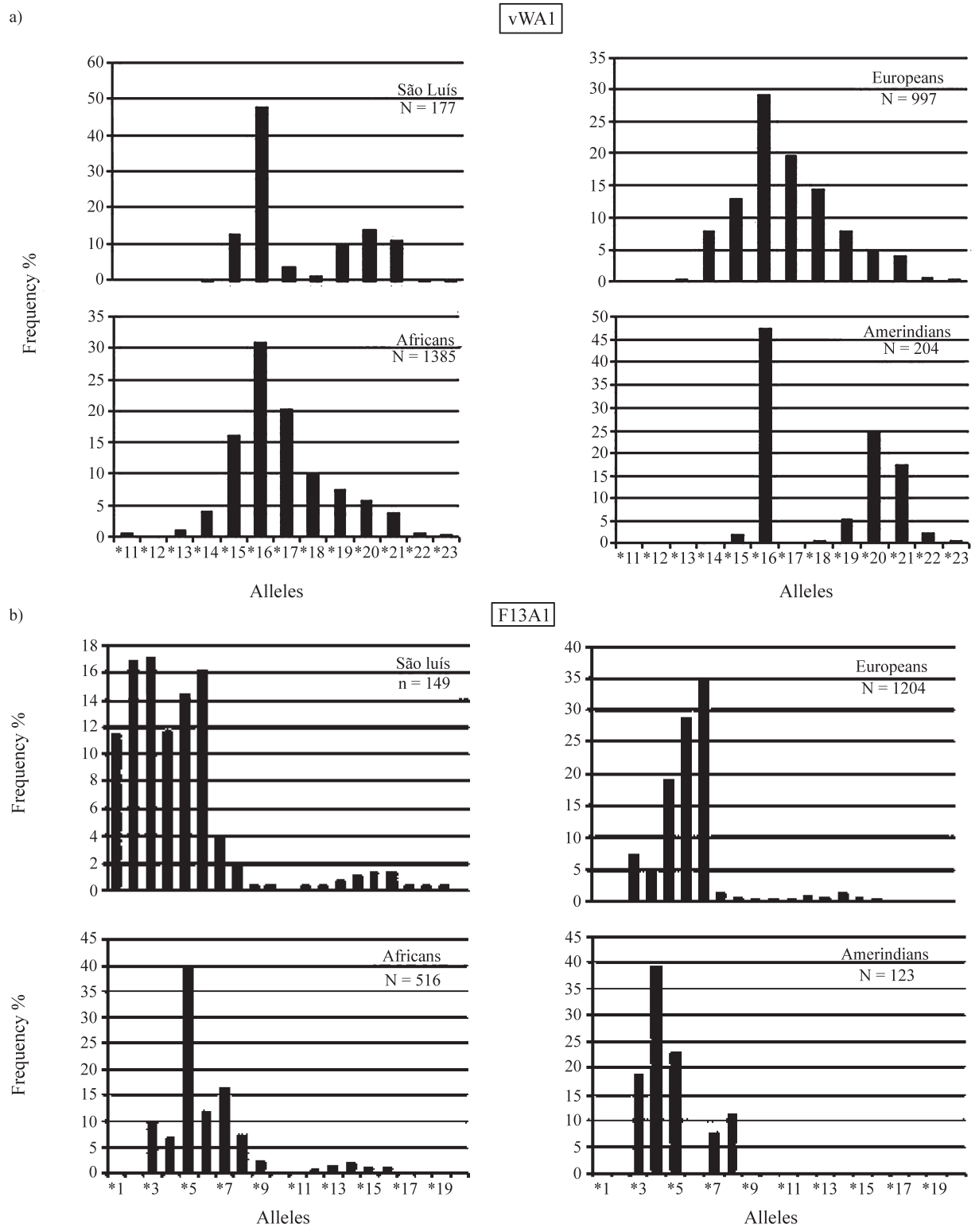

Figure 1 - Allele frequencies in the São Luís population and in the main ethnic groups that contributed to its formation.

are very similar to those of African (Apo-B*36, 22.1%; Apo-B*34, 13.8%) and European (Apo-B*36, 37.9%; Apo-B*34, 21.2%) populations.

Although the Apo-B locus showed variable allele frequencies among different human populations, homogeneity was observed for the most frequent alleles, suggesting

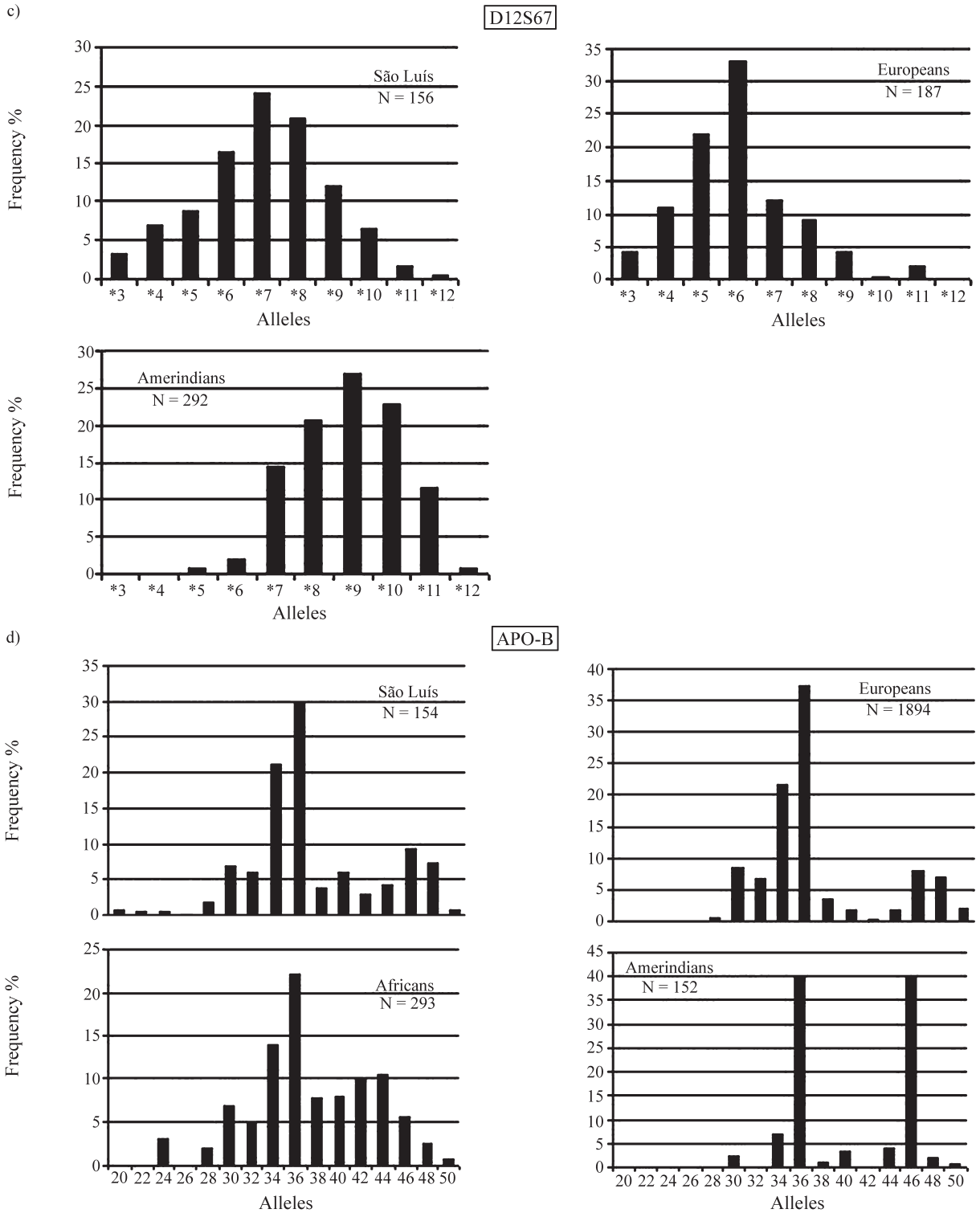

Figure 1 (cont.) - Allele frequencies in the São Luís population and in the main ethnic groups that contributed to its formation.

that the polymorphism at this locus preceded the geographic dispersal of the main ancestral population groups (Deka *et al.*, 1992).

D1S80

The pattern of allele distribution at this locus is generally polymodal in African populations, with frequency

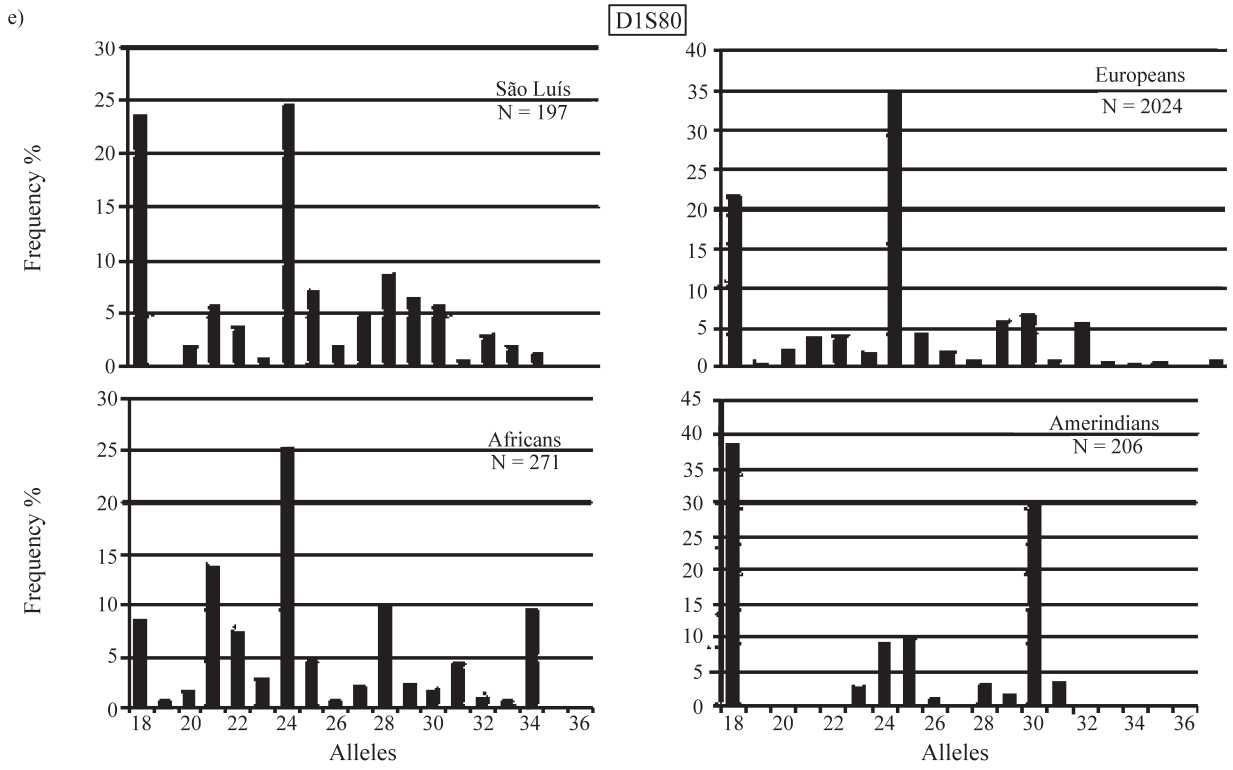

Figure 1 (cont.) - Allele frequencies in the São Luís population and in the main ethnic groups that contributed to its formation.

peaks for D1S80*18, D1S80*21, D1S80*24, D1S80*28 and D1S80*34. Together, these alleles account for approximately 70% of the alleles observed there (Vallinoto, 1996; Heidrich *et al.*, 1995). Among European populations, D1S80*18 and D1S80*24 are the most frequent (Falcone *et al.*, 1995; Flores *et al.*, 2001). Amerindians show a trimodal pattern, with peaks for D1S80*18, D1S80*24 and D1S80*30 (Budowle *et al.*, 1991; 1995; Latorra *et al.*, 1994; Zago *et al.*, 1996).

In the São Luís population, 16 alleles were identified at the D1S80 locus (Table 1), showing a trimodal pattern of distribution with peaks for D1S80*18, D1S80*24 and D1S80*28, the most frequent being D1S80*24 (Figure 1e). The greatest similarity was observed with Asian populations.

Genetic distances

The allele frequencies obtained in the present study were used to generate a genetic distance matrix (Table 2) that revealed a great similarity between the populations of São Luís and Belém. This finding was expected, since both populations live in the same geographic region (Amazon) and therefore share similar social, interethnic and demographic processes.

Genotype distribution

The 10 alleles detected at the vWA1 locus allowed the identification of 22 different genotypes. The most frequent were 6-6 (22.6%), 6-10 (12.4%), 5-6 (11.9%), 6-11 (10.2%) and 6-9 (9.6%), corresponding to 67% of the sample. The F13A1 locus showed 19 alleles, with 49 genotypes, the most frequent being 2-5 (8.1%), 3-6 (7.4%), 2-4 (6.0%), 3-3 (6.0%) and 6-6 (5.5%), which accounted for 33% of all individuals in the sample. At the D12S67 locus, 10 alleles were identified, in a total of 33 genotypes, the most frequent being 7-8 (11.5%), 6-7 (9.0%), 8-9 (6.4%), 7-7 (5.8%) and 6-8 (5.1%), corresponding to 38% of all individuals analyzed. The combination of 49 genotypes for Apo-B was obtained based on 15 alleles, the most frequent being 34-36 (11.7%), 36-36 (9.7%), 36-40 (5.8%) and 36-46 (5.8%), corresponding to 33% of all individuals analyzed. Sixteen alleles were identified at the D1S80 locus, which corresponded to a total of 56 genotypes, the most frequent being 18-24 (10.9%), 24-24 (8.0%), 24-28 (4.4%) and 18-29 (4.4%), representing 35% of the individuals analyzed.

Table 2 - Genetic distance matrix calculated according to Nei *et al.* (1983). based on the allele frequencies for the vWA1. F13A1. Apo-B and D1S80 loci.

Populations	São Luís	Belém	Europeans	Asians	Africans
Belém	0.0992				
Europeans	0.1214	0.1237			
Asians	0.1760	0.2129	0.0814		
Africans	0.1263	0.1205	0.0743	0.1351	
Amerindians	0.1861	0.1013	0.2559	0.3191	0.2646

Table 3 - Gene frequencies in the parental populations used to estimate the ethnic admixture in the São Luís population.

Populations and systems	Observed Alleles																									
vWA1	#-13	*14	*15	*16	*17	*18	*19	*20	*21	*22	*23															
São Luís	0.0000	0.0030	0.1270	0.4800	0.0350	0.0110	0.0960	0.1350	0.1070	0.0030	0.0030															
European	0.0014	0.1221	0.1148	0.2224	0.2762	0.1817	0.0669	0.0102	0.0043	0.0000	0.0000															
Amerindian	0.0000	0.0000	0.0190	0.4700	0.0000	0.0050	0.0530	0.2500	0.1730	0.0250	0.0050															
African	0.0300	0.0450	0.1600	0.3100	0.1950	0.0920	0.0700	0.0600	0.0320	0.0050	0.0010															
F13A	*1	*2	*3	*4	*5	*6	*7	*8	*9	*10	*11	*12	*13	*14	*15	*16	*17	*18	*19	*22						
São Luís	0.1150	0.1700	0.1710	0.1200	0.1450	0.1610	0.0400	0.0210	0.0030	0.0030	0.0000	0.0030	0.0030	0.0100	0.0100	0.0130	0.0030	0.0030	0.0030	0.0030						
European	0.0000	0.0000	0.0843	0.0291	0.1817	0.3052	0.3575	0.0116	0.0000	0.0000	0.0000	0.0000	0.0000	0.0102	0.0000	0.0160	0.0044	0.0000	0.0000	0.0000						
Amerindian	0.0000	0.0000	0.1880	0.3920	0.2300	0.0000	0.0790	0.1110	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000						
African	0.0000	0.0000	0.0980	0.0670	0.3940	0.1200	0.1640	0.0710	0.0230	0.0020	0.0020	0.0060	0.0140	0.0170	0.0100	0.0120	0.0000	0.0000	0.0000	0.0000						
ApoB	*22	*24	*26	*28	*29	*30	*31	*32	*33	*34	*35	*36	*37	*38	*40	*42	*44	*46	*47	*48	*50	*52	*54	*56	*58	
São Luís	0.0100	0.0030	0.0030	0.0170	0.0000	0.0700	0.0000	0.0600	0.0000	0.2010	0.0000	0.2900	0.0000	0.0410	0.0610	0.0300	0.0420	0.0910	0.0000	0.0710	0.0100	0.0000	0.0000	0.0000	0.0000	
European	0.0000	0.0000	0.0000	0.0030	0.0000	0.0800	0.0000	0.0650	0.0000	0.2300	0.0000	0.3800	0.0000	0.0400	0.0240	0.0020	0.0100	0.0700	0.0000	0.0750	0.0210	0.0000	0.0000	0.0000	0.0000	
Amerindian	0.0000	0.0000	0.0000	0.0000	0.0000	0.0229	0.0000	0.0000	0.0000	0.0664	0.0000	0.4036	0.0000	0.0098	0.0328	0.0000	0.0398	0.4017	0.0000	0.0165	0.0065	0.0000	0.0000	0.0000	0.0000	
African	0.0000	0.0310	0.0000	0.0180	0.0006	0.0680	0.0001	0.0490	0.0001	0.1474	0.0000	0.2314	0.0000	0.0770	0.0790	0.1010	0.1060	0.0560	0.0150	0.0200	0.0000	0.0001	0.0000	0.0000	0.0001	
D1S80	#-17	*18	*20	*21	*22	*23	*24	*25	*26	*27	*28	*29	*30	*31	*32	*33	*34	*35	*36	*37	*38	*39	*40	*41		
São Luís	0.0000	0.2400	0.0180	0.0550	0.0360	0.0070	0.2410	0.0730	0.0200	0.0500	0.0840	0.0620	0.0550	0.0040	0.0300	0.0140	0.0110	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		
European	0.0110	0.2210	0.0230	0.0400	0.0380	0.0130	0.3400	0.0450	0.0200	0.0100	0.0570	0.0660	0.0090	0.0600	0.0080	0.0050	0.0050	0.0010	0.0140	0.0120	0.0000	0.0000	0.0010	0.0010		
Amerindian	0.0000	0.3920	0.0000	0.0003	0.0000	0.0250	0.0970	0.1000	0.0010	0.0000	0.0360	0.0150	0.3000	0.0330	0.0000	0.0007	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		
African	0.0330	0.0860	0.0180	0.1360	0.0730	0.0280	0.2520	0.0450	0.0080	0.0210	0.1000	0.0220	0.0200	0.0430	0.0100	0.0070	0.0940	0.0020	0.0000	0.0000	0.0000	0.0020	0.0000	0.0000		

#- the vWA1*13 and D1S80*17 less frequent alleles have been collapsed for this analysis.

[Alonso *et al.* (1993); Bell *et al.* (2000); Boerwinkle *et al.* (1989); Budowle *et al.* (1995); Buresi *et al.* (1995); Chakraborty *et al.* (1991); D'Aloja *et al.* (1992); Deka *et al.* (1992; 1994); Destro-Bisol *et al.* (1994; 2000); Evans *et al.* (1993); Falcone *et al.* (1995); Flores *et al.* (2001); Gamero *et al.* (2000; 2003); Gené *et al.* (1993; 1995); Gusmão *et al.* (1997; 2001); Gutowski *et al.* (1995); Halos *et al.* (1999); Hammond *et al.* (1994); Heidrich *et al.* (1995); Hixon *et al.* (1993); Latorra *et al.* (1994); Maviglia *et al.* (2001); Peake *et al.* (1990); Pena *et al.* (1994); Péres-Lezaun *et al.* (1997); Pinheiro *et al.* (1996); Rangel-Villalobos *et al.* (1999); Renges *et al.* (1992); Robertson *et al.* (1995); Sala *et al.* (1998); Santos (1999); Schnee-Griese *et al.* (1993); Trabetti *et al.* (1992); Vallinoto (1996); Zago *et al.* (1996)].

A total of 70 alleles, and 209 of the 556 expected genotype combinations, were identified.

Variability measures

Heterozygosity values are shown in Table 1. The F13A1 frequencies were not in Hardy-Weinberg equilibrium ($p = 0.0075$), due to an excess of homozygotes. Since the number of alleles was high (19) and the number of individuals studied relatively small (171), this result is probably due to sampling.

The highest heterozygosity value (81.8%) was observed for Apo-B, and the lowest (72.3%) for the vWA1 locus. The average heterozygosity for the analyzed markers was 77.7%, with a mean number of 14 alleles per locus. These values are higher than those obtained for another northeastern population (Alagoas), which showed an average heterozygosity of 75.4% and a mean number of alleles of 9.4. High heterozygosity is expected in miscegenated populations (Byard *et al.*, 1985), and this was confirmed by the present study.

PD is defined as the capacity of genotype differentiation at each locus. The PD observed for the São Luís population ranged from 88.9% for vWA1 to 96.7% for F13A1. The median power of exclusion (PE) ranged from 45.2% for vWA1 and 73.3% for F13A1. The combined PE for these five loci was 99.8%.

Paternity and forensic applications require markers with high average heterozygosity, cumulative PE and cumulative PIC values. Therefore, the five DNA markers analyzed in the present study can be safely employed for these purposes.

Interethnic admixture

Estimates of interethnic admixture were based on four loci, due to lack of previous studies on D12S67 in African populations. The comparisons made are displayed in Table 3. The results indicated a high contribution of European genes (42% ± 1%), followed by Amerindian (39% ± 7%), and a low contribution of African genes (19% ± 7%). These results are similar to those obtained for the average of the Brazilian Amazon Region (47% Caucasian, 41% Amerindian, and 12% African) by Santos and Guerreiro (1995). The values reported by Callegari-Jacques *et al.* (2003) are different (as detailed in a previous section), which could be due to the fact that they studied a different Amazonian population (Manaus) or to the fact that the bulk of their samples was composed of individuals who could pay for paternity determinations. The difference, therefore, could reflect the marked socio-economic differentials that exist among people of different ethnies in Brazil.

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