Genetic Polymorphism and Forensic Parameters of Nine Short Tandem Repeat Loci in Ngöbé and Emberá Amerindians of Panama

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Abstract Nine STR loci (*CSF1PO*, *TPOX*, *TH01*, *F13A01*, *FESFPS*, *VWA*, *D16S539*, *D7S820*, and *D13S317*) were analyzed in unrelated Ngöbé and Emberá Amerindians of Panama. The chi-square test demonstrated statistically significant differences ($P < 0.001$) in the allele frequencies for all markers except one (*D16S539*; $P < 0.01$). Both populations shared their alleles with the highest frequencies in seven loci. However, there were also noticeable differences at the *TPOX* locus, which showed its highest frequencies at alleles 11 (0.48) and 6 (0.54) for the Ngöbé and Emberá, respectively. Interestingly, these alleles are present in one population and are absent in the other, suggesting that they could be distinctive for each population. These results demonstrate that, despite the fact that each population belongs to a different linguistic stock [Chibchan (Ngöbé) and Chocoan (Emberá)], both retain strong similarities in their allele-frequency distributions. Three loci (*TPOX*, *VWA*, and *F13A01*) in the Ngöbé and two loci (*TH01* and *TPOX*) in the Emberá departed from Hardy-Weinberg equilibrium. The analysis of the STR markers demonstrates that, despite their low levels of genetic polymorphisms, most of them could be informative for forensic purposes, showing a combined power of discrimination of 0.9999 for both Amerindian populations. However, powers of exclusion in the Ngöbé were very low, particularly at the *TH01* (0.04) and *FESFPS* (0.08) loci. The combined powers of exclusion were 0.9338 and 0.9890 for the Ngöbé and the Emberá, respectively. Furthermore, the combined typical paternity index in the Ngöbé was considerably low (2.58), and in the Emberá it was 40.44, which is also very low. The low genetic polymorphism levels suggest that the use of additional loci supplementing the battery of the nine loci is recommended for paternity and forensic tests in both populations, particularly for the Ngöbé.

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Short tandem repeats (STRs) have been demonstrated to be excellent polymorphic markers for genetic characterization of both individuals and populations. Because of their large interethnic diversity in world populations, STRs have contributed to an understanding of genetic relationships, evolution, and migration among human populations (Rangel-Villalobos et al. 2000). Moreover, the fast development of PCR technology has made possible the wide use of STRs in forensics and paternity testing (Mastana and Singh 2002). More recently, these markers have been used in ethnic admixture studies (Cerda-Flores et al. 2002a, 2002b) in Mestizo (trihybrid-admixed) populations. The application of these markers to the study of Amerindian populations is an exciting area of population genetics because Amerindians represent the most recent human colonization of a previously uninhabited continental landmass (30,000–12,000 years B.P.; Batista et al. 1995).

Archeological, ethnohistoric, linguistic, and genetic evidence indicates that the Ngöbé (also known as the Ngawbe-Guaymi) and Emberá tribes developed in situ for several millennia, originating from migrating bands of Paleo-Amerindians that came to populate the Americas from northeastern Asia (Kolman et al. 1995). These Paleo-Amerindians crossed the Bering land bridge from the Mongolia/Manchuria/southeastern Siberia region (Neel et al. 1994). Although the Ngöbé and Emberá live in close proximity, they represent two distinct linguistic families: Chibchan and Chocó/Paezan, respectively (Kolman and Bermingham 1997). Recently the authorities of Costa Rica have reported that the Ngöbé have been emigrating from Panama. Constant movement of the Ngöbé has been well documented between the frontiers of both countries.

The Ngöbé population originated, developed, and still inhabit western Panama, especially in the Ngöbé-Buglé *comarca*, which is a territory encompassing 6,673 km^2 (Figure 1). The Ngöbé population is composed of approximately 142,986 members (Direccion de Estadistica y Censo 2000) and constitutes the largest unadmixed Amerindian tribe existing today (Jorge et al. 1999). The Emberá tribe is considered a population of Amazonian origin that settled in eastern Panama in the last 100 years through constant westward migration from the province of Chocó in Colombia (Jorge-Nebert et al. 2002). They mainly inhabit the Emberá-Wounaan *comarca*, a territory of 4,398 km^2 (Figure 1), with a population of approximately 19,422 individuals (Direccion de Estadistica y Censo 2000). However, they are also distributed in the Republic of Colombia, with a population of 40,000 individuals (Jorge-Nebert et al. 2002).

Although these two Amerindian groups represent less than 8% of the total population of Panama (Direccion de Estadistica y Censo 2000), it is important to note that their genetic presence in the Mestizo population of Panama is about 36% (Arias et al. 2002). (The Mestizo population is a mixture of Caucasians, blacks, and Amerindians and represents most of Panama, approximately 85%. This high level of Amerindian admixture is significant because of its biomedical implications with some disease susceptibilities and metabolic disorders known as New World syndrome (Salzano and Callegari-Jacques 1988).

Previous genetic studies on these two Amerindian populations of Panama were mainly conducted on classical markers (Barrantes et al. 1990), mitochondrial

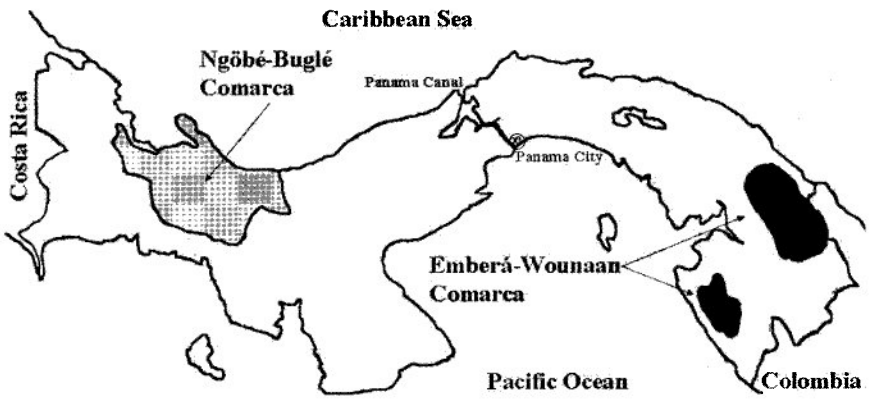

Figure 1. Geographic distribution of the Ngöbé and Emberá Amerindians in the Republic of Panama. Each population is located mainly in its corresponding *comarca*, where samples were collected.

DNA, and nuclear DNA sequences (Kolman and Bermingham 1997), and they reported low genetic diversity and focused on the ethnohistoric and microevolutionary context of the groups. However, despite some STR markers having been analyzed, no allele-frequency data were obtained, limiting the knowledge and application of these data. Other genetic studies included pharmacogenetic research on polymorphisms of N-acetyltransferase-2 (*NAT2*) and cytochrome P450 CYP2D6 (debrisoquine 4-hydroxylase) enzymes and showed different proportions of phenotypes and genotypes for slow and rapid metabolizers (Jorge et al. 1999; Jorge-Nebert et al. 2002). Therefore more studies are necessary on the nature and extent of the population genetic diversity at STR loci in these Amerindian groups.

The Amerindian tribes of Panama and those from other Latin American countries are being constantly absorbed into nonnative communities, thus changing the population genetic structure (Rangel-Villalobos et al. 2000). This trend toward acculturation and ethnic admixture observed among Amerindians will probably be accelerated in the near future. The question is whether the groups will lose their biological (genetic) and cultural identity, or whether they will resist this homogenizing process (Salzano and Callegari-Jacques 1988). Consequently, more information about the gene pools of these native populations is urgently needed to help to solve and prevent biomedical problems of Amerindian and admixed populations. This genetic information could also be useful in reconstructing the details of the historical events leading to the peopling of the Americas.

The nine STR loci described in this study have been highly useful in Panama in solving crimes and determining paternity. Despite this use, however, no population genetic studies of these nine STR loci have been conducted in the Panamanian population. Although a large number of studies on STRs have been reported from different world populations (Mastana and Singh 2002; Yunis et al. 2000), the databases are largely restricted to broadly defined population groups (e.g., US

whites, US blacks, and Hispanics) and ethnically defined populations; isolated populations with smaller effective sizes are relatively scarce (Sun et al. 2003). Moreover, because the Ngöbé and Emberá also inhabit Costa Rica and Colombia, respectively, the impact of these results is also relevant for those countries.

In this study we analyze the genetic diversity of the Ngöbé and Emberá Amerindian populations using nine STR markers. Using the obtained allele frequencies, we compare statistically the differences between the two populations. We also calculate some population parameters to evaluate their allelic and heterozygosity deficiency. In addition, we have included some forensic parameters that will allow accurate calculations for genetic identification purposes in these populations. These data will lead to a better understanding of admixture estimates of the Mestizo population of Panama, which represents most of the country.

Materials and Methods

Selection of Amerindians and Sample Collection. Individuals selected for the study were interviewed to record biographic information, which included names, birthplaces, and languages spoken by their parents and grandparents. Appropriate consents were obtained from relevant community authorities and the selected individuals. The individuals considered for this study had Amerindian phenotypic characteristics, were born in their *comarca*, spoke the native language, and had parents and grandparents belonging to the same Amerindian group.

The Ngöbé samples were obtained from individuals in four population clusters located in widely separated regions across the Ngöbé-Buglé *comarca*, which is a vast territory in Bocas del Toro Province, in western Panama (see Figure 1). The Emberá samples were obtained from individuals in five Emberá settlements distributed throughout the Emberá-Wounaan *comarca* in Darien Province in eastern Panama (see Figure 1).

All the blood samples from both populations were collected from unrelated (to at least the fourth degree of consanguinity) and healthy volunteers of both sexes. Venous blood samples (10 mL) were obtained by venipuncture and were collected into tubes containing EDTA or ACD anticoagulant buffers. Tubes were gently rotated to ensure that the EDTA and blood were adequately mixed. After extraction, the blood was stored at approximately 4°C, for a maximum of 3–4 days and was either frozen at −20°C or immediately processed.

For the Emberá, blood was extracted from the tubes (125 μL) using plastic pipettes and placed onto the center of each of four circles of commercially prepared FTA Cards (DNA Testing Center Inc., Euless, Texas). An FTA paper punch was used to cut a 1-mm-diameter circle of paper, and the paper was washed according to the procedure specified by the manufacturer. Amplification was performed directly on the treated paper.

DNA from Ngöbé samples was extracted from peripheral leukocytes by a modification of the procedure described by Miller et al. (1988). DNA quality was

verified by agarose gel electrophoresis using the K562 DNA marker as a reference (Promega Corp., Madison, Wisconsin).

This study was approved by the Ethics Committee of the University of Panama and complies with the laws of the Republic of Panama. Individuals who did not speak an Amerindian language, presented any obvious evidence of recent admixture (e.g., curly hair) with other Amerind or non-Amerind populations, and were not born in Amerindian regions or individuals whose parents did not meet these criteria were excluded from the study.

STR Amplification and Genotyping. Amplifications were performed with a Perkin Elmer model 2400 Thermal Cycler (Perkin Elmer, Waltham, Massachusetts). All polymerase chain reactions were carried out in a 25-μL total volume containing approximately 50 ng of genomic DNA template (Ngöbé) or a 1-mm-diameter circle of FTA paper (Emberá). The nine STR loci (*CSF1PO*, *TPOX*, *TH01*, *F13A01*, *FESFPS*, *VWA*, *D16S539*, *D7S820*, and *D13S317*) were typed using the GenePrint STR Systems kit (Promega). Multiplex PCR amplifications, polyacrylamide electrophoresis, silver nitrate staining detection, and allele identification were performed according to the manufacturers' recommended protocols. The recommendations of the DNA Commission of the International Society for Forensic Genetics were followed (DNA Commission 1994; Bar et al. 1997).

Statistical Analysis. Statistical analyses were carried out using the software PopGene, version 1.32 (available at http://www.ualberta.ca/~fyeh/), to compute allele frequencies. Observed and expected genotype frequencies were determined using the algorithm of Levene (1949) by two methods: chi-square and likelihood ratio (G^2) tests for Hardy-Weinberg expectations per locus per population. We also determined, as measures of gene diversity, the effective number of alleles (N_e) (Hartl and Clark 1989) per locus and combined, the observed number of alleles (N_a), and Shannon's information index (I) (Shannon and Weaver 1949) per locus and combined. In addition, we used the software Statistica, version 6.6, to compare allele frequencies per locus of both populations by Hedrick's method (1985). The levels of observed heterozygosity (H_o) and the number of alleles per locus (N_a) were compared using the Wilcoxon-Mann-Whitney test (Siegel and Castellán 1998).

The Microsoft Excel workbook template PowerStatsV12 (Promega Corp.) was used to calculate forensic parameters, which include matching probability and power of discrimination. Also, we estimated several paternity parameters: power of exclusion, typical paternity index, and percentage of homozygotes and heterozygotes.

Results

The allele frequencies at each STR locus as well as the percentage of homozygotes and heterozygotes are presented in Table 1. Because some DNA samples

Table 1. Allele Frequencies of Nine STR Loci in the Ngöbé (NG) and Emberá (EM) Amerindians of Panama

	CSF1PO		*TPOX*		*TH01*		*F13A01*		*FESFPS*		*VWA*		*D16S539*		*D7S820*		*D13S317*	
Allele	*NG*	*EM*	*NG*	*EM*	*NG*	*EM*	*NG*	*EM*	*NG*	*EM*	*NG*	*EM*	*NG*	*EM*	*NG*	*EM*	*NG*	*EM*
3.2							0.48	0.38										
3.3								0.01										
4							0.31	0.11										
5	0.00	0.00		0.01	0.00	0.00	0.13	0.24					0.00	0.00				
6	0.00	0.00	0.00	0.54	0.86	0.00	0.07	0.02					0.00	0.00	0.00	0.00		
7	0.00	0.00	0.00	0.39	0.13	0.01	0.01	0.23	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.05	0.40	0.02	0.00	0.55	0.00	0.01	0.00	0.00			0.01	0.00	0.00	0.05	0.00	0.01
9	0.06	0.04	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00			0.15	0.18	0.01	0.01	0.56	0.33
9.3				0.05	0.01	0.00												
10	0.03	0.27	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.10			0.37	0.37	0.18	0.25	0.04	0.13
11	0.33	0.14	0.48	0.00	0.00	0.20	0.00	0.00	0.77	0.65	0.00	0.00	0.25	0.19	0.43	0.45	0.05	0.10
12	0.46	0.43	0.11	0.00		0.23	0.00	0.00	0.21	0.14	0.00	0.00	0.22	0.22	0.37	0.21	0.10	0.22
13	0.13	0.07	0.01	0.00		0.01	0.00	0.00	0.01	0.11	0.00	0.00	0.00	0.03	0.01	0.04	0.17	0.14
14	0.00	0.00					0.00	0.00	0.00	0.00	0.06	0.06	0.00	0.01	0.00	0.00	0.09	0.07
15	0.00	0.00					0.00	0.00			0.09	0.09	0.00	0.00			0.00	0.00
16							0.00	0.00			0.60	0.56						
17											0.23	0.19						
18											0.01	0.11						
19											0.01	0.00						
20											0.00	0.00						
21											0.00	0.00						
Homozygosity (%)	23.3	28.4	39.3	50.5	75.8	38.2	51	32.6	64.6	41.3	54	42.2	43.9	27.1	31.6	36.5	43.9	15.6
Heterozygosity (%)	76.7	71.6	60.7	49.5	24.2	61.8	49	67.4	35.4	58.7	46	57.8	56.1	72.9	68.4	63.5	56.1	84.4
Total chromosomes	120	176	122	186	124	178	98	92	96	92	100	90	114	192	114	192	114	192

Table 2. Allele-Frequency Distribution Patterns per Locus for the Ngöbé and Emberá Populations

Group	*Locus*	*Allele Frequency Pattern*
I	*TPOX, TH01*	The most frequent alleles and other alleles for each population are different except for one.
II	*FESFPS, D13S317, F13A01*	Similar predominant alleles in both populations, but they differ in their frequencies (>0.1); the same for other alleles.
III	*D7S820, CSF1PO, VWA*	Fewer differences in predominant allele frequencies for both populations (<0.1); others alleles differ but with low frequencies.
IV	*D16S539*	All alleles show few differences between populations.

For all loci the chi-square test showed statistically significant differences ($P < 0.001$) among the allele frequencies of both populations, except for the *D16S539* locus ($P < 0.01$).

could not be optimally amplified at certain loci, the number of chromosomes analyzed differ to some extent from one locus to another. For seven of the STR loci the alleles with the highest frequencies were the same in both Amerindian populations; these alleles were *CSF1PO*12*, *F13A01*3.2*, *FESFPS*11*, *VWA*16*, *D16S539*10*, *D7S820*11*, and *D13S317*9*. However, there were differences at locus *TPOX*, in which the highest frequencies were found at alleles **11* (0.48) and **6* (0.54) for the Ngöbé and Emberá, respectively. Similarly, there were differences at locus *TH01*, in which the highest frequencies for the Ngöbé and Emberá were found at alleles **6* (0.86) and **8* (0.55), respectively. With the exception of *D16S539*, the other eight loci showed differences that were statistically significant in their allele frequencies between both populations. These differences generated specific patterns, giving rise to four clusters (Table 2). The first cluster contains the loci that best differentiate both populations (*TPOX* and *TH01*). Cluster groups II and III do the same but at a minor level, because they have the same predominant alleles.

In the Ngöbé the highest observed heterozygosity was 76.7% at the *CSF1PO* locus and the lowest was seen at the *TH01* locus (24.2 %). In contrast, in the Emberá the highest and the lowest heterozygosities were 84.4% and 49.5% at loci *D13S317* and *TPOX*, respectively. For all loci the Emberá showed higher levels of observed and expected heterozygosity than the Ngöbé (for H_o there were significant differences, Wilcoxon-Mann-Whitney test $P = 0.025$) (Table 3). There were few differences between both estimates in average value per population and per locus. The Emberá also showed a minor proportion of heterozygote deficit per locus, measured by the positive values of the F_{IS} index. This tendency toward more elevated levels of heterozygosity in the Emberá population was also evidenced by analyzing their allelic diversity using three measures (Table 4). The Emberá showed average values of all the indexes that were more elevated in the Ngöbé; the N_a differences were statistically significant (Wilcoxon-Mann-Whitney test $P = 0.031$). These differences are mainly caused by the *TPOX*, *F13A01*, and *D13S317*

Table 3. Average Values and Typical Deviations of Observed and Expected Heterozygosity Levels for the Ngöbé and Emberá Populations

Population	H_o[a]	H_e[b]	*Average Difference*	*Difference Per Locus*	*Heterozygosity Deficiency (%)*
Ngöbé	0.522 (0.158)	0.567 (0.160)	0.045	0.071	66.7
Emberá	0.656 (0.102)	0.665 (0.087)	0.007	0.043	55.5

a. Observed heterozygosity (typical deviation in parentheses).
b. Expected heterozygosity (typical deviation in parentheses).

loci, which have seven alleles each. On the other hand, the Ngöbé have only two loci (*VWA* and *D13S317*) with six alleles each.

The distribution of genotype frequencies for most loci (six in the Ngöbé and eight in the Emberá) did not show deviation from Hardy-Weinberg equilibrium. For both analyzed tests the *F13A01* locus in the Ngöbé and the *TPOX* locus in the Emberá showed genetic disequilibrium ($P < 0.05$). Two other loci in the Ngöbé also showed disequilibrium (*VWA* and *D16S539*), but at $P < 0.010$. These loci showed significant heterozygote deficiencies, with differences of more than 0.10 between the observed and expected values as a result of low levels of diversity, probably caused by population bottleneck and genetic disequilibrium.

Forensic and paternity parameters are shown in Table 5. The matching probability in the Ngöbé ranged from 0.124 to 0.602 at the *D16S539* and *TH01* loci, respectively. Similarly, in the Emberá population the matching probability varied from 0.085 to 0.279 at loci *D13S317* and *TPOX*. The power of discrimination

Table 4. Allele Diversity Parameters per Locus for the Ngöbé (NG) and Emberá (EM) Populations

	N_a[a]		N_e[b]		I[c]		*FIS*[d]	
Locus	*NG*	*EM*	*NG*	*EM*	*NG*	*EM*	*NG*	*EM*
CSF1PO	5	6	2.9777	3.2103	1.2619	1.3828	−0.1543	−0.0688
TPOX	5	7	2.6278	2.5351	1.1019	1.1042	0.0208	−0.0205
TH01	3	5	1.3135	2.2638	0.4303	0.9582	−0.0136	0.114
F13A01	5	7	2.8858	3.7518	1.218	1.4698	0.2505	0.0812
FESFPS	4	4	1.5679	2.1428	0.6225	1.0239	0.0222	−0.1006
VWA	6	5	2.354	2.7931	1.1221	1.2612	0.2003	0.0654
D16S539	5	6	3.6805	3.9343	1.3721	1.4801	0.2188	0.0223
D7S820	5	6	2.8203	3.2022	1.1255	1.3417	−0.0601	0.0912
D13S317	6	7	2.7476	4.7665	1.3343	1.7003	0.1173	−0.0678
AV/TypDev[e]	4.89	5.89	2.55	3.1778	1.0654	1.3076	–	–
	(0.92)	(1.05)	(0.73)	(0.86)				

a. Observed number of alleles per locus.
b. Effective number of alleles per locus.
c. Shannon's information index.
d. Fixation index.
e. Average and typical deviation (in parentheses) for each parameter per population.

Table 5. Forensic and Paternity Parameters of Nine STR Loci in the Ngöbé and Emberá Amerindian Populations

	Ngöbé				*Emberá*			
Locus	*MP* [a]	*PD* [b]	*PE* [c]	*TPI* [d]	*MP*	*PD*	*PE*	*TPI*
CSF1PO	0.2	0.8	0.539	2.14	0.131	0.869	0.453	1.76
TPOX	0.255	0.745	0.299	1.27	0.279	0.721	0.183	0.99
TH01	0.602	0.398	0.042	0.66	0.23	0.77	0.313	1.31
D16S539	0.124	0.876	0.247	1.14	0.115	0.885	0.475	1.85
D7S820	0.202	0.798	0.404	1.58	0.154	0.846	0.336	1.37
D13S317	0.173	0.827	0.247	1.14	0.085	0.915	0.683	3.2
F13A01	0.184	0.816	0.179	0.98	0.133	0.867	0.389	1.53
FESFPS	0.465	0.535	0.088	0.77	0.254	0.746	0.276	1.21
VWA	0.245	0.755	0.155	0.93	0.172	0.828	0.265	1.18
Combined	–	0.9999	0.9338	2.58	–	0.9999	0.989	40.44

a. Matching probability.
b. Power of discrimination.
c. Power of exclusion.
d. Typical paternity index.

in the Ngöbé ranged from 0.398 to 0.876 for the *TH01* and *D16S539* loci, respectively, whereas in the Emberá population the corresponding STRs were *TPOX* and *D13S317*, ranging from 0.721 to 0.915. The combined power of discrimination was 0.9999 in both Amerindian populations. The power of exclusion in the Ngöbé population ranged from 0.042 to 0.539 for *TH01* and *CSF1PO*, respectively, and the combined power of exclusion for nine loci was 0.9338. In the Emberá the power of exclusion varied from 0.183 to 0.683 at the corresponding loci *TPOX* and *D13S317*, and the combined power of exclusion was 0.9890. The typical paternity index in the Ngöbé varied from 0.66 to 2.14 for *TH01* and *CSF1PO*, in that order; and the combined value was 2.58. For the Emberá, the typical paternity index was between 0.99 and 3.2 at *TPOX* and *D13S317*, and the combined typical paternity index was 40.44.

Discussion

Allele comparisons between the Ngöbé and the Emberá revealed some similarities and discrepancies among their frequencies. The significance of these results can be better appreciated when they are contrasted with those of other representative populations of Amerindians from North and South America, populations from Asia (northeast Asians), and reference populations from the United States (Table 6). For simplicity, in Table 6 we include only the highest frequencies for these populations. Although we did not perform statistical analyses, we found some interesting similarities and discrepancies among the populations. Seven of the nine STR loci shared the same alleles with the highest frequencies in both populations (Ngöbé and Emberá). These similarities are also frequent in other

Table 6. Comparisons of Alleles with the Highest Frequencies for Nine STR Loci in the Ngöbé, Emberá, and Other Representative Amerindian Populations from South and North America and in Asian and Other Reference Populations from the United States

	Allele with the Highest Frequency (and Frequency)								
Population	*CSF1PO*	*TPOX*	*THO1*	*F13A01*	*FESFPS*	*VWA*	*D16S539*	*D7S820*	*D13S317*
Amerindians from Central America									
Ngöbé (Panama)	12 (0.46)	11 (0.48)	6 (0.86)	3.2 (0.48)	11 (0.77)	16 (0.60)	10 (0.37)	11 (0.43)	9 (0.56)
Emberá (Panama-Colombia)	12 (0.43)	6 (0.54)	8 (0.55)	3.2 (0.38)	11 (0.65)	16 (0.56)	10 (0.37)	11 (0.45)	9 (0.33)
Amerindians from South America									
Kichwa (Ecuador)	10 and 12 (0.31)	8 (0.51)	6 (0.48)	–	–	16 (0.60)	10 (0.29)	11 (0.37)	9 (0.24)
Terena (Brazil)	11 (0.35)	8 (0.47)	7 (0.48)	–	–	16 (0.41)	11 (0.33)	11 (0.39)	12 (0.33)
Mapuche (Argentina)	12 (0.36)	–	6 (0.53)	7 (0.34)	11 (0.50)	16 (0.58)	–	–	–
Tehuelche (Argentina)	12 (0.41)	–	7 (0.43)	3.2 (0.35)	11 (0.71)	16 (0.42)	–	–	–
Wichi (Argentina)	12 (0.58)	–	7 (0.72)	5 (0.35)	11 (0.54)	16 (0.51)	–	–	–
Amerindians from North America									
Otomi (Sierra Madre, Mexico)	13 (0.42)	8 (0.47)	7 (0.41)	–	–	16 (0.40)	12 (0.35)	11 (0.33)	9 (0.35)
Otomi (Ixmiquilpan Valley, Mexico)	13 (0.52)	8 (0.47)	7 (0.43)	–	–	16 (0.36)	10 and 12 (0.29)	11 (0.42)	9 (0.30)
Huasteco (Mexico)	13 (0.41)	8 (0.54)	7 (0.43)	–	–	16 (0.56)	11 (0.30)	11 (0.31)	9 (0.36)
Asian populations									
Han (China)	12 (0.37)	8 (0.51)	9 (0.51)	–	–	14 (0.25)	9 (0.28)	11 (0.34)	8 (0.28)
Tu (China)	12 (0.45)	8 (0.50)	9 (0.47)	–	–	17 (0.28)	–	11 (0.30)	11 (0.24)
Sala (China)	12 (0.38)	8 (0.51)	9 (0.51)	–	–	16 (0.31)	–	11 (0.31)	8 and 11 (0.25)
Japan	12 (0.42)	8 (0.43)	9 (0.39)	–	–	17 (0.28)	9 (0.34)	11 (0.35)	8 (0.26)
Reference populations from the United States									
African Americans	10 (0.27)	8 (0.35)	7 (0.38)	5 (0.34)	11 (0.36)	16 (0.26)	11 (0.32)	10 (0.35)	12 (0.41)
Caucasian Americans	12 (0.33)	8 (0.53)	9.3 (0.33)	7 (0.33)	11 (0.44)	17 (0.26)	11 (0.32)	10 (0.30)	11 and 12 (0.31)
Hispanic Americans	12 (0.36)	8 (0.50)	7 (0.31)	7 and 5 (0.23)	11 (0.45)	16 (0.30)	11 (0.30)	10 (0.28)	12 (0.24)

Amerindian populations from Mexico (Barrot et al. 2005), Colombia (Yunis et al. 2005), Ecuador (González-Andrade and Sánchez 2004), Brazil (da Silva et al. 2004), and Argentina (Sala et al. 1998) and even in some northeast Asian populations from China (Chen and Li 2004; Wang et al. 2003) and Japan (Hashiyada et al. 2003). However, there were also noted differences at the *TPOX* locus, showing the highest frequencies for alleles **11* (0.48) and **6* (0.54) for the Ngöbé and Emberá, respectively. It is interesting to note that these alleles are present in one population and absent in the other, suggesting that they could be distinctive for each population. Moreover, the most frequent *TPOX* allele in all other populations was **8* (Table 6). The *TPOX*11* allele showed a relatively high frequency (approximately 0.32) in African Americans, Hispanic Americans, Caucasian Americans (data from Promega Corp.), Tu and Sala (two Chinese populations; Chen and Li 2004), Japanese (Hashiyada et al. 2003), and the Amerindian Terena from Brazil (da Silva et al. 2004). Moreover, the *TPOX*6* allele had low frequencies in these same populations.

At the *TH01* locus the predominant allele for the Ngöbé was **6* (which is also present in two South Amerindian populations), whereas in the Emberá it was **8*; incidentally, the Emberá are the only population with the highest frequency of this allele (see Table 6). It is important to note that the *TH01*6* allele in the Ngöbé showed a remarkably higher frequency (0.86) than in other populations. The *TH01*8* allele had low frequencies in Hispanic Americans (0.01) and in the Tu (0.09) and Sala (0.03) populations, but it was the most frequent allele in Terena Amerindians (0.48). There were also several other exclusive alleles in the Ngöbé or Emberá, although these occurred at low frequencies (see Table 1).

In all Amerindian and Asian populations allele **11* was the predominant allele for the *D7S820* locus, whereas in the reference populations the predominant allele was allele **10*. Although there are some missing data points, at the *FESFPS* locus all populations shared the same predominant allele (**11*). The *CSF1PO*12* allele was the most common allele in the Ngöbé (0.46) and the Emberá (0.43) and also in all Asian populations, Caucasian Americans, Hispanic Americans, and most South Amerindians, differing from North Amerindians (**13*). At the *F13A01* locus allele **3.2* was the most common in the Ngöbé (0.48), Emberá (0.38), and Tehuelche (0.35), differing from the Mapuche and Wichi, both of which showed their highest frequencies for alleles **7* (0.34) and **5* (0.35), respectively.

The *VWA* locus revealed its most common frequencies for allele **16* for all Amerindian populations and for the Sala and African and Hispanic Americans, although in minor proportions. On the other hand, the *D16S539* locus presented its highest frequencies at allele **10* with the same value (0.37) for the Ngöbé and the Emberá and a frequency of 0.29 for the Kichwa and Otomi from Ixmiquilpan Valley. However, in the Terena the highest frequency was 0.33 for allele **11*, which is similar to African Americans (0.32), Caucasian Americans (0.32), and Hispanic Americans (0.30).

Finally, the *D13S317* locus exhibited its highest frequencies for allele **9* for the Ngöbé (0.56), Emberá (0.33), Kichwa (0.24), and North Amerindians (about

0.33) and for allele *12 for the Terena (0.33), African Americans (0.41), and Caucasian Americans (0.31). In the Tu population the most common allele at the *D13S317* locus was *11 (0.24), and alleles *8 and *11 had the same high frequency (0.25) in the Sala population.

The analysis of forensic and paternity parameters for these nine STR loci demonstrates that despite their low levels of genetic polymorphisms, most of them could be informative for forensic purposes, showing a combined power of discrimination of 0.9999 for both Amerindian populations. However, the powers of exclusion in the Ngöbé were very low, particularly at the *TH01* and *FESFPS* loci, which exhibited values of 0.04 and 0.08, respectively. The combined powers of exclusion were 0.9338 and 0.9890 in the Ngöbé and the Emberá, respectively. On the contrary, the powers of exclusion for African Americans, Caucasian Americans, and Hispanic Americans were 0.9993, 0.9985, and 0.9986, respectively, demonstrating that the use of additional loci supplementing the battery of nine STR loci is recommended for paternity and forensic purposes, particularly in the Ngöbé population. Similar recommendations were suggested by Sun et al. (2003) using other STR markers on isolated populations. Furthermore, an acceptable value for the combined typical paternity index is about 90; however, for the Ngöbé it was considerably lower (2.58) and for the Emberá it was 40.44, which is also quite low.

In summary, despite their well-known dissimilar origins (the Ngöbé belonging to the Chibchan linguistic family and the Emberá to the Chocoan), both Amerindian populations retain strong similarities in their allele-frequency distributions; however, they differ in the presence of certain specific genetic variations, and they also show unusually high allele frequencies in some polymorphic loci, as was previously reported by Barrantes et al. (1990) using classical markers. These similarities are also evidenced by their affinities with other Amerindian populations and with some northeast Asian populations that belong to Asian human groups that are similar to Amerindians; these Asian groups are phylogenetically close and share common ancestry. In fact, the large allele sharing, low mean values for the number (N_a) and effective number (N_e) of alleles (see Tables 1, 2, and 4), and heterozygote deficiencies are consistent with the low levels of genetic diversity in these Amerindian populations, both of which lived for many years in isolation from outside society (Young 1971). This phenomenon was confirmed by estimating Shannon's information index (I) in the Ngöbé and Emberá separately; combined values of 1.06 and 1.31, respectively, were found. In addition, the Ngöbé population showed three loci (*TPOX*, *VWA*, and *F13A01*) and the Emberá showed two loci (*TH01* and *TPOX*) that departed from Hardy-Weinberg equilibrium, which probably was caused by their heterozygote deficiencies.

The low levels of diversity are also shown by the average heterozygosity, which was 65% in the Emberá and 52.2% in the Ngöbé. This is also closely related to their high levels of homozygosity, especially at the *TH01* (75.8%) and *FESFPS* (64.6%) loci in the Ngöbé and the *TPOX* locus (50.5%) in the Emberá. The average heterozygosity level observed in the Emberá was similar to the level in the Tarahumara (66.8%), a Mexican Amerindian population (Rangel-Villalobos

et al. 2000), and the Wichi (63.5 %), an Argentinean Amerindian population (Sala et al. 1998). Kolman et al. (1995) also reported similar low levels of diversity in the Ngöbé using mitochondrial DNA, and they proposed that this population had recently undergone a population bottleneck. In fact, ethnohistoric evidence supports the hypothesis that populations of the Ngöbé suffered a drastic reduction followed by an increase in population size with an accompanying loss of genetic diversity or variability (Jorge et al. 1993).

These characteristics are typical for most Amerindian populations, which are considered small isolated populations that have undergone recent bottlenecks (Sun et al. 2003). The historical records suggest that disease and mass exterminations may have caused a demographic collapse during the European conquest (Kolman et al. 1995; Jorge et al. 1993). An alternative interpretation posits that bottlenecks originated from ethnogenesis from small founding populations of Paleo-Amerindians and rapid in situ fragmentations into separate tribal groups with low levels of genetic admixture among them (Kolman et al. 1995). These are processes that promote the fast fixation or extinction of genes or alleles in the presence of small population effective sizes, as is the case for Amerindians (Barrantes 1990). Differences in genetic variability between the two populations could be due to either of these microevolutionary processes with the negative effects more apparent in the Ngöbé Amerindians.

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