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Mitochondrial and Nuclear DNA Diversity in the Chocó and Chibcha Amerinds of Panamá

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ABSTRACT

Mitochondrial and nuclear DNA diversities were determined for two Chocó-speaking Amerind populations, the Emberá and Wounan, sampled widely across their geographic range in eastern Panamá. These data were compared with mitochondrial and nuclear diversities determined here and previously for neighboring Chibcha-speaking Ngöbé and Kuna populations. Chocoan groups exhibited mitochondrial diversity levels typical for Amerind populations while Chibchan groups revealed reduced mitochondrial diversity. A slight reduction in autosomal levels of heterozygosity was determined for the Chibcha while *X* and *Y* variation appeared equivalent in all populations. Genetic distinctiveness of the two linguistic groups contradicts the anthropological theory that Paleoindians migrated repeatedly through the isthmian region and, instead, supports the idea of cultural adaptation by endogenous populations. Reduced genetic diversity in Chibchan populations has been proposed to represent a population bottleneck dating to Chibchan ethnogenesis. The relative sensitivities of haplotype pairwise difference distributions and Tajima's *D* to detect demographic events such as population bottlenecks are examined. Also, the potential impact of substitution rate heterogeneity, population subdivision, and genetic selection on pairwise difference distributions are discussed. Evidence is presented suggesting that a larger effective population size may obscure the historical signal obtained from nuclear genes while the single mitochondrial locus may provide a moderately strong signal.

PANAMÁ occupies a unique geographic position as the land bridge between North and South America. Archaeological evidence demonstrating cultural transitions and hiatuses has been used to suggest that the Panamanian isthmus was a dynamic migration corridor through which Paleoindians traveled repeatedly during colonization of the New World (BARTLETT and BARGHOORN 1973; LINARES 1977; ICHON 1980; PIPERNO 1988). Recent archaeological, linguistic, and genetic evidence, however, appears to discount the thesis that late Pleistocene-era Panamá represented a human landscape in flux (YOUNG 1971; BARRANTES *et al.* 1990; CONSTENLA-UMAÑA 1991; COOKE and RANERE 1992). Instead, this alternate viewpoint supports the continuous presence of Amerind groups in the isthmian region since their arrival 7000–10,000 years before present (YBP) and diminishes the role of population replacement as the cause of cultural transitions in the archaeological record. Against this complex historical backdrop rests the distribution of Panamá's contemporary indigenous groups. To the east along the rivers of the Darien jungle, the Emberá and Wounan represent the Chocó linguistic family that extends south to Ecuador. To the west and along the eastern Caribbean versant are the Ngöbé and Kuna, members of the Chibchan linguistic family extending north to Nicaragua (see Figure 1) (CONSTENLA-UMAÑA 1991).

Our collaboration with Panamá's indigenous people provides an opportunity to determine the degree of genetic disjunction between two linguistic families living in geographic proximity. Through comparison to the rich archaeological record and language, the relative movement of ideas and genes across Paleoindian people can be explored. The empirical value of our human population analyses across the Panamanian landscape is enhanced through study of two groups within each linguistic family. Within this empirical framework, the parallels and asymmetries in the mitochondrial and nuclear genetic records can also be investigated. It has been suggested that the mitochondrial DNA (mtDNA) genome may be more sensitive to demographic changes such as population bottlenecks due to its smaller effective population size (WILSON *et al.* 1985; MOORE 1995). Our mtDNA-based studies of Panamanian Amerinds provide no evidence of admixture with Caucasoids or Negroids (BATISTA *et al.* 1995; KOLMAN *et al.* 1995) and support the contention that mtDNA may be especially suited to tribal studies owing to the probable asymmetry in the introduction of mtDNA genes relative to nuclear genes to indigenous populations (BATISTA *et al.* 1995). In the case of the Chibcha at least, admixture rates of nuclear genes are also very

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FIGURE 1.—Geographic distribution of Emberá, Wounan, Ngöbé, and Kuna Amerinds and location of collection sites for Emberá and Wounan populations. Sample sizes were as follows: E1, $n = 2$; E2, $n = 9$; E3, $n = 7$; E4, $n = 4$; E5, $n = 10$; E6, $n = 5$; E7, $n = 1$ (birthplaces of six Emberá were unknown) and W1, $n = 10$, W2, $n = 5$; W3, $n = 4$; W4, $n = 6$; W5, $n = 6$. Approximately 21% of the Emberá and 29% of the Wounan reported birthplaces in Colombia.

low. Using blood group markers, BARRANTES (1993) demonstrated mean admixture rates of 2.4 and 2.45% with Caucasoids and Negroids, respectively.

Herein we present mtDNA and nuclear (nDNA) data on four groups of Panamanian Amerinds. In the Emberá and Wounan populations, mitochondrial control region I sequence was determined [360 base pairs (bp)], seven mtDNA restriction/deletion sites were scored, and 13 nuclear microsatellite and *Alu* insertion loci were assayed. Control region I sequence and three restriction/deletion sites had been previously reported for the Ngöbé and Kuna (BATISTA *et al.* 1995; KOLMAN *et al.* 1995). In the present study, four additional mtDNA restriction fragment length polymorphisms (RFLPs) and 13 nuclear microsatellite and *Alu* insertion loci were scored for the same set of Ngöbé and Kuna samples.

MATERIALS AND METHODS

Population samples: Representing the two linguistic families present in Panamá, the Emberá, Wounan, Ngöbé, and Kuna are also the largest Panamanian groups both in terms of geographic range (Figure 1) and numerical size: Emberá, 30,000; Wounan, 5,000; Ngöbé, 125,000; and Kuna, 65,000 members (1990 Panamanian government census Panama City, Panama). For the present study, seven Emberá and five Wounan settlements distributed throughout eastern Panamá were sampled (Figure 1). Eighty-one Emberá (74 blood samples and seven hair samples) and 38 Wounan (38 blood samples) samples were collected. Extensive biographical information was recorded for each individual including names, birthplaces, and languages spoken by parents and grandparents. Approximately 21% of the Emberá and 29% of the Wounan reported birthplaces in Colombia, reflecting the recent and ongoing migration of Emberá and Wounan into Panamá.

Ultimately, 44 Emberá and 31 Wounan individuals were chosen for mtDNA sequence analysis based on family histories suggesting no obvious, *i.e.*, recent, admixture with other Amerind or non-Amerind populations. An additional two Emberá and one Wounan, each known to be maternally related to one of the chosen samples, were included in the study as controls. For analysis of nuclear loci, a subset of 25 Emberá and 25 Wounan samples were chosen based on lack of maternal and paternal relatedness. Forty-six samples from the previously studied Ngöbé (KOLMAN *et al.* 1995) and 55 samples from the previously studied Kuna (BATISTA *et al.* 1995) were scored for four mtDNA RFLPs and a subset of 27 Ngöbé and 25 Kuna samples were chosen for nDNA analysis.

TABLE 1

Mitochondrial restriction/deletion sites with corresponding PCR primers and conditions

Site	Primers	Annealing temperature (°)
*Hae*III:bp 663	L577:H743 (KOLMAN *et al.* 1995)	62
*Alu*I:bp 5176	L5099:H5333 (BATISTA *et al.* 1995)	62
*Dde*I:bp 10394, *Alu*I:bp 10397	L10284:H10484 (KOLMAN *et al.* 1996)	61
*Alu*I:bp 13262	L13232:H13393 (WARD *et al.* 1991)	49
*Hae*III:bp 16517	L16453:H408 (KOLMAN *et al.* 1996)	57
COII/tRNA^{Lys} intergenic deletion	L8215:H8297 (WARD *et al.* 1991)	55

Isolation of mtDNA and nDNA: Blood (20–30 ml) was collected into Vacutainer tubes (Becton Dickinson) as described previously (KOLMAN *et al.* 1995). mtDNA and nDNA were isolated from blood samples using one of two methods described in KOLMAN *et al.* (1995). mtDNA and nDNA were isolated from hair samples following the procedure of BATISTA *et al.* (1995).

Amplification and sequencing of mtDNA: Primers L15997: H16401 (WARD *et al.* 1991) and L00029:H00408 (KOLMAN *et al.* 1995) were used to amplify regions I and II, respectively, of the mtDNA control region (VIGILANT *et al.* 1989). Reaction components were as described in KOLMAN *et al.* (1995) except that the final bovine serum albumin concentration was reduced to 100 μg/ml. Control region I sequence was generated from λ exonuclease-digested single-stranded DNA following the protocol of BATISTA *et al.* (1995). Primers L15997 (WARD *et al.* 1991), L16106 (BATISTA *et al.* 1995), and L16191 (BATISTA *et al.* 1995) were used to sequence the light strand and primers H16401 (WARD *et al.* 1991) and H16226 (BATISTA *et al.* 1995) were used to sequence the heavy strand of control region I. Owing to a T-to-C mutation at position 16189, which caused the *Taq* polymerase to stutter, sequence could only be read in each direction until the location of the transition for the 25 Emberá and 11 Wounan individuals who carried this mutation. An internal primer downstream of the mutation (L16191) allowed additional sequence of the light strand to be read from position 16230 to 16400. In the remaining 39 individuals lacking the T-to-C transition, the sequences analyzed were completely verified through full overlap of heavy and light strands. For control region II sequence, single-stranded DNA was generated by a second, asymmetric PCR performed as previously described (KOLMAN *et al.* 1995). PCR primers L00029 and H00408 (WARD *et al.* 1991) were used to sequence the light and heavy strands, respectively, of region II, with complete overlap of the resultant sequences. The sequences reported in this article have been deposited in the GenBank data base (accession nos. U95647–U95691).

RFLP analysis: Samples were assayed for seven polymorphic sites located outside of the mtDNA control region I se-

TABLE 2

Microsatellite and *Alu*-insert loci with corresponding PCR conditions

Locus[a]	Concentration (μM)	Multiplex group[b]	Chromosome	Reference
HUMCSF1PO[AGAT]$_n$	0.09	B	5q33.3-q34	HAMMOND *et al.* (1994)
D6S366[3bp]$_n$	0.40	C	6q21-qter	HAMMOND *et al.* (1994)
HUMF13A01[AAAG]$_n$	0.12	C	6p24-p25	HAMMOND *et al.* (1994)
HUMTH01[AATG]$_n$	0.08	A	11p15.5	HAMMOND *et al.* (1994)
HUMPLA2A1[AAT]$_n$	0.20	B	12q23-qter	HAMMOND *et al.* (1994)
D13S126[CA]$_n$	0.25	Single-locus PCR	chrom.13	BOWCOCK *et al.* (1994)
HUMFESFPS[AAAT]$_n$	0.80	B	15q25-qter	HAMMOND *et al.* (1994)[c]
HUMARA[AGC]$_n$	0.40	Single-locus PCR	Xcen-q13	HAMMOND *et al.* (1994)
HUMHPRTB[AGAT]$_n$	0.56	A	Xq26	HAMMOND *et al.* (1994)
DYS19[4bp]$_n$[d]	0.40	Single-locus PCR	Yp	SANTOS *et al.* (1993) ROEWER *et al.* (1992)
(YAP) DYS287	0.12	Single-locus PCR	Yq11	HAMMER *et al.* (1995)[e]
YCAIIIa/b[2bp]$_n$[f]	0.40	Single-locus PCR	Y chrom.	MATHIAS *et al.* (1994)

[a] Locus designations are the GenBank locus name with the repeat sequence in brackets. Exceptions are D6S366, D13S126, DYS19, YAP, and YCAIII, which are Genome Database or common designations. In the text, loci are referred to by the first three letters of the locus designations, with the following exceptions: HPRTB, D6, YAP, and DYS19.

[b] Seven primer pairs were multiplexed in three sets of PCR reactions.

[c] Correct sequence for the forward primer is 5′-GCTGTTAATTCATGTAGGGAAGGC-3′ (H. HAMMOND, personal communication).

[d] Also referred to as 27H39LR (ROEWER *et al.* 1992; SANTOS *et al.* 1993).

[e] Annealing temperature for these primers was raised to 54°.

[f] Primers for this locus appeared to amplify two distinct *Y*-derived products (MATHIAS *et al.* 1994).

quence, using six sets of primers (Table 1). Balanced PCR reactions (run for 29 cycles) utilized thermal cycler and PCR conditions as described in KOLMAN *et al.* (1995) except that primer pairs L577:H743 and L5099:H5333 used the following temperature profile: 94° for 30 sec, 62° for 15 sec, and 72° for 15 sec. PCR products were electrophoresed through 15% polyacrylamide gels or 4% MetaPhor (FMC BioProducts) agarose gels.

Analysis of nuclear loci: Twelve sets of primers were used to amplify seven autosomal, two *X*-chromosome and four *Y*-chromosome microsatellite and *Alu* insertion loci (Table 2). With the exception of the YAP primers, all forward primers were labeled with one of three fluorescent dyes: FAM, HEX, and TET (Research Genetics, Inc., Huntsville, AL). Optimal primer concentrations for amplification reactions were determined empirically. Seven primer pairs were multiplexed, *i.e.*, amplified simultaneously, in three sets of PCR reactions while the remaining primer pairs were amplified individually (Table 2). Ten microliter amplification reactions contained components as described in KOLMAN *et al.* (1995) with the noted exception of primer concentrations. Thermocycler conditions were as described in the referenced literature with the exception of the YAP primers, which were annealed at 54°. Three additional *Y*-chromosome microsatellite loci [SPY-1 (T. KARAFET and M. HAMMER, unpublished results), DYS199 (UNDERHILL *et al.* 1996) and DXYS156Y (CHEN *et al.* 1994)] were assayed by T. KARAFET and M. HAMMER (unpublished results) and were utilized in heterozygosity and *F*-statistic calculations.

All amplification reactions using fluorescently labeled primers were electrophoresed through 6% (19:1) acrylamide:bisacrylamide denaturing (8 M urea) sequencing gels run on an Applied Biosystems 373 DNA Sequencer. One-half to one microliter of each reaction was combined with formamide, loading dye, and fluorescently labeled molecular-weight marker (Genescan-350, Applied Biosystems, Inc.) in a 4:1:1 ratio. Electrophoresis at 26–28 W and data collection for 3.5 hr detected fragments as large as 350 bp. Genescan 672 software (Applied Biosystems, Inc.) was used to determine the size of all locus alleles. Computer-calculated allele sizes for loci CSF, FES, and DYS were +1, +2, and +2, respectively, relative to published allele sizes (SANTOS *et al.* 1993; HAMMOND *et al.* 1994). To confirm allele designations, control DNAs RJK1094 and RJK1258 with published allele sizes (HAMMOND and CASKEY 1994) were amplified and electrophoresed on all relevant gels. Amplification reactions using the YAP primers were electrophoresed on 4% MetaPhor (FMC BioProducts) agarose gels and visualized by ethidium bromide staining.

Diversity, statistical, and phylogenetic analyses: Haplotype diversity, h (equation 8.4, NEI 1987), nucleotide diversity, π (equation 2, NEI and JIN 1983), and variances due to sampling and stochastic processes were calculated for mitochondrial control region I DNA sequence data using Arlequin, v. 1.0 (S. SCHNEIDER, J.-M. KUEFFER, D. ROESSLI and L. EXCOFFIER; http://anthropologie.unige.ch/arlequin). Calculations of Tajima's D (TAJIMA 1989), number of segregating sites, and mean number of pairwise differences were also determined using Arlequin, v. 1.0. Heterozygosity measures and standard errors based on nuclear loci were calculated using BIOSYS-1, v. 1.7 (SWOFFORD and SELANDER 1981). For *X* loci, only data from females were used.

Minimum-spanning trees (MSTs) depicting the relationship of mtDNA control region haplotypes were constructed by hand following the recommendations of EXCOFFIER *et al.* (1992) and used in the analysis of molecular variance (AMOVA), which partitioned genetic variability among and between the studied populations. AMOVA analyses (AMOVA, v. 1.5, L. EXCOFFIER) were performed using a distance matrix calculated from the evolutionarily parsimonious network shown in Figure 4. Significance of variance terms derived in the AMOVA was tested by permuting the original data 1000 times. *F* statistics for the microsatellite data were calculated using GENEPOP, v. 2.0 (RAYMOND and ROUSSET 1995).

Calculations of D_{SW}, D_A, and derived phylogenies based on the nine polymorphic microsatellite loci presented here were provided by M. SHRIVER (SHRIVER *et al.* 1995). Pairwise genetic difference analyses were computed using Arlequin, v. 1.0. Population expansion estimates from these analyses were based on the divergence rate for mtDNA control region I as calculated by HARPENDING *et al.* (1993). Simulation of pairwise difference genetic data were performed using programs provided by H. HARPENDING.

RESULTS

mtDNA sequence analysis in Emberá and Wounan populations: Forty-four Emberá and 31 Wounan samples were analyzed by determining the DNA sequence of mitochondrial control region I (bp 16040–16400, numbered according to ANDERSON *et al.* 1981) (Figure 2). Twenty Emberá and 14 Wounan haplotypes were observed with five sequences in common for a total of 29 mtDNA sequence haplotypes. Of the 361 nucleotide positions sequenced, 32 were polymorphic. Thirty-one transitions, with a strong bias towards pyrimidine transitions, and a single A-to-C transversion were observed. Five of the Emberá and Wounan haplotypes had been detected previously in Amerind populations while the remaining 24 mtDNA sequence haplotypes were described for the first time.

Fifteen Emberá and 10 Wounan individuals were also sequenced for nucleotide pairs 39–380 of mitochondrial control region II (Figure 3). Nine Emberá and seven Wounan DNA sequence haplotypes were observed with three sequences in common for a total of 13 control region II DNA sequence haplotypes. Of the 341 nucleotide positions determined, 15 were found to be polymorphic, including 11 transitions and three deletions. Relative to the human reference sequence (ANDERSON *et al.* 1981), monomorphic mutations were observed at bp 73, 263, 303.1, and 311. The A-to-G transition at bp 263 has been observed in all Amerind populations studied to date (MERRIWETHER 1993; SANTOS *et al.* 1994; KOLMAN *et al.* 1995; BATISTA *et al.* 1995) and has been proposed to represent an Amerind-specific mutation (KOLMAN *et al.* 1995). In terms of haplotype counts, regions I and II showed similar levels of variability: 13 region I and 13 region II haplotypes were observed. Similar levels of polymorphism between regions I and II have also been detected in the Mandenkalu of West Africa (EXCOFFIER *et al.* 1996). Owing to the limited number of control region II sequences determined, no region II data were used in any of the following analyses.

As a control for accurate DNA sequencing, two pairs of siblings known to be maternally related were sequenced for control region I and one pair of maternally related siblings were sequenced for control region II. In all cases, identical sequences were obtained for each pair of siblings (Figures 2 and 3). Only one individual from each sibling pair was used in the following analyses.

Mitochondrial RFLP analysis of Chocoan and Chibchan populations: Six restriction/deletion haplotypes were observed in the Wounan, with five of the haplotypes also occurring in the Emberá, for a total of six restriction/deletion haplotypes in Chocoan populations (Figure 2). Analysis of additional restriction sites (*Alu*I:bp 5176, *Dde*I:bp 10394, *Alu*I:bp 10397, and *Hae*III:bp 16517) in the Ngöbé and Kuna samples revealed three restriction/deletion haplotypes in both Chibchan populations as previously reported (BATISTA *et al.* 1995; KOLMAN *et al.* 1995) (Table 3).

Four haplogroups, labeled A, B, C, and D, were initially defined by TORRONI *et al.* (1993) to represent founding New World lineages based on four restriction/deletion polymorphisms: *Hae*III:bp 663, the 9-bp deletion; *Alu*I:bp 13262; and *Alu*I:bp 5176, respectively. Recently, MERRIWETHER and FERRELL (1996) divided haplogroups A–D into two subsets based on the *Hae*III:bp 16517 polymorphism; A1, B1, C1, and D1 possess this polymorphism while A2, B2, C2, and D2 do not. All four originally defined haplogroups (A, B, C, and D) and six of the newly defined haplogroups (A1, A2, B1, C1, C2, and D2) were detected in the Emberá/Wounan data set; no unclassifiable haplotypes were observed (Figure 2). As previously reported, Ngöbé and Kuna populations revealed lower levels of mitochondrial diversity with only two of the originally defined haplogroups (A and B) and three of the newly defined founding haplogroups (A1, A2, and B1) identified in the Chibchan data set (Table 3). Previous analysis of six additional Chibchan groups (Teribe, Cabecar, Bribri, Guatuso, Huetar, and Boruca) also determined significant frequencies only of haplogroups A and B, with low levels of haplogroup D detected in two populations (SANTOS *et al.* 1994; TORRONI *et al.* 1994).

nDNA analysis of Chocoan and Chibchan populations: Subsets of the samples assayed for mtDNA polymorphisms were selected for nDNA analysis based on an additional criterion stipulating no paternal relationship. Of the 12 loci assayed, six autosomal, two *X*-chromosomal, and one *Y*-chromosomal microsatellite repeat were polymorphic in the Panamanian populations and are presented in the Appendix. Three additional loci were determined to be uninformative for the study and are not presented; D13S126 was monomorphic in all individuals tested and a single primer pair simultaneously amplified similarly sized alleles from loci YCAIIIa and YCAIIIb, making it impossible to distinguish one locus from the other. We provided DNAs representing the same individuals analyzed herein to T. KARAFET and M. HAMMER for their study of *Y*-chromosomal variation in human populations. They have kindly provided us their unpublished results on three additional *Y* microsatellite loci; SPY-1, DYS199, and DXYS156Y.

ID	n Em	n Wo	Collection sites	16051	16086	16092	16094	16111	16126	16129	16150	16166	16187	16189	16193	16217	16218	16223	16234	16242	16265	16274	16278	16290	16291	16295	16298	16300	16301	16311	16319	16322	16325	16327	16362	HaeIII 663	AluI 5176	DdeI 10394	AluI 10397	AluI 13262	HaeIII 16517	9 bp 8272/8289	New World haplogroup	References for previously reported sequences
Reference				A	T	T	T	C	T	G	C	A	C	T	C	T	C	C	C	C	A	G	C	C	C	C	T	A	C	T	G	A	T	C	T									
EW1	9		E2,E3,E4,E5	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	1	0	0	0	1	1	B1	1,2,3,4,5
EW2	1		E5	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	0	1	0	0	0	1	1	B1	
EW3	2		E2,E5	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	1	0	0	0	1	1	B1	
EW4	2	2	E3,E4,W1,W5	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	1	0	0	0	1	1	B1	
EW5	2		E4,E5	.	C	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	1	0	0	0	1	1	B1	1
EW6	2		E2,E8	.	.	.	.	.	.	A	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	1	0	0	0	1	1	B1	
EW7	1		E5	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	0	1	0	0	0	1	1	B1	
EW8	2		E2	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	0	1	0	0	0	1	1	B1	
EW9	2		E5,E7	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	C	0	1	0	0	0	1	1	B1	
EW10		2	W3,W4	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	.	0	1	0	0	0	1	1	B1	
EW11		1	W3	.	.	.	.	.	.	.	T	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	0	1	0	0	0	1	1	B1	
EW12		1	W3	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	0	1	0	0	0	1	1	B1	
EW13		4	W1,W2,W4	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	C	1	1	0	0	0	0	2	A2	
EW14		1	W5	.	.	.	.	T	.	.	.	.	.	C	.	.	T	T	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	C	1	1	0	0	0	1	2	A1	
EW15	2		E2,E5	.	.	.	.	T	C	.	.	.	.	C	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	C	1	1	0	0	0	0	2	A2	
EW16	2	1	E2,E3,W1	.	.	.	.	T	C	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	C	1	1	0	0	0	1	2	A1	
EW17	2		E3,E5	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	C	1	1	0	0	0	0	2	A2	1,2,4,5
EW18		2	W4,W5	.	.	.	.	T	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	C	1	1	0	0	0	0	2	A2	
EW19		1	W5	.	.	C	.	T	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	C	1	1	0	0	0	0	2	A2	
EW20	2		E8	.	.	.	.	T	.	A	.	.	.	.	.	.	.	T	.	.	.	A	.	T	.	.	.	.	.	.	A	.	.	.	.	1	1	0	0	0	0	2	A2	
EW21	2		E1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	C	A	.	.	.	C	1	1	0	0	0	0	2	A2	
EW22	2	1	E1,E5,W3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	T	.	0	1	1	1	1	1	2	C1	4,6
EW23	1		E5	G	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	T	.	0	1	1	1	1	0	2	C2	6
EW24	2	7	E8,W1,W2,W5	G	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	T	.	0	1	1	1	1	1	2	C1	
EW25	4	5	E2,E5,E8,W1,W4	G	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	T	.	0	1	1	1	1	0	2	C2	
EW26	1		E4	G	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	C	.	.	.	.	.	C	T	.	0	1	1	1	1	0	2	C2	
EW27	1		E3	G	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.	0	1	1	1	1	0	2	C2	
EW28		2	W1,W4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	C	.	.	.	.	.	C	T	C	0	1	1	1	1	0	2	C2	
EW29		1	W4	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	G	C	.	C	0	0	1	1	0	0	2	D2	

FIGURE 2.—Polymorphic nucleotide, restriction, and deletion sites determined for Emberá and Wounan populations. Sites are numbered according to ANDERSON *et al.* (1981). Collection sites are illustrated in Figure 1. The designation of New World haplogroups is defined in MERRIWETHER and FERRELL (1996). References for previously reported sequences are as follows: 1, KOLMAN *et al.* 1995; 2, BATISTA *et al.* 1995; 3, SANTOS *et al.* 1994, 4, WARD *et al.* 1991; 5, WARD *et al.* 1993; and 6, GINTHER *et al.* 1993. EW4 and EW25 represent the two pairs of maternally related siblings that were sequenced for control region I.

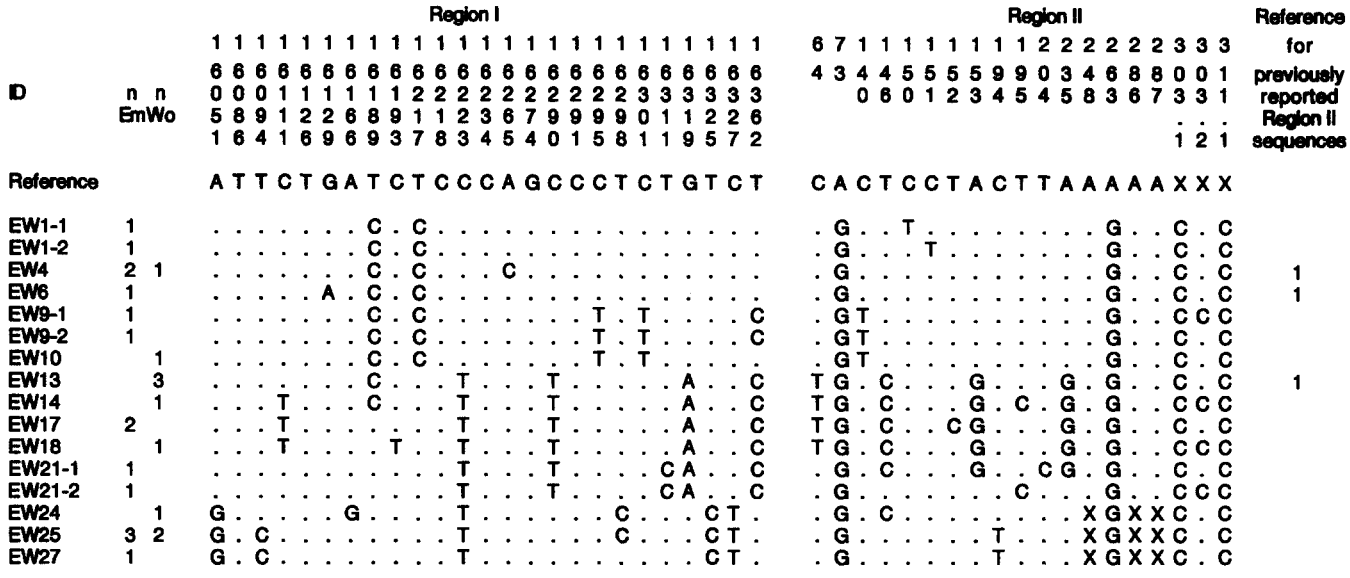

FIGURE 3.—Polymorphic nucleotides of Emberá and Wounan individuals sequenced for mtDNA control regions I and II. Sites are numbered according to ANDERSON *et al.* (1981). Deletions are represented by X. Previously reported sequences are from SANTOS *et al.* 1994. EW18 represents the pair of maternally related siblings that were sequenced for control region II.

In general, the Chibchan and Chocoan groups resembled Asian or Asian-derived populations assayed for the same nuclear markers. At autosomal loci FES, PLA, and THO, all four Panamanian populations exhibited allele frequencies that placed them firmly with the Asian or Mexican-American populations analyzed by HAMMOND *et al.* (1994) and EDWARDS *et al.* (1992). The Panamanian populations also resembled the Amerind groups assayed by DEKA *et al.* (1995a) more closely than any other populations in the study, and the Emberá, Ngöbé, and Kuna shared virtually identical allele frequencies with the neighboring Cabecar. As expected, all Panamanian populations lacked the *Y*-chromosomal *Alu* insertion, which has not been detected in any Asian or Asian-derived population except the Japanese (HAMMER and HORAI 1995).

Genetic diversity in Chocoan and Chibchan populations: Based on mitochondrial control region I DNA sequence data, haplotype diversity, *h*, was determined to be 0.94 for the Emberá and 0.91 for the Wounan and nucleotide diversity, π, was 0.017 for the Emberá and 0.019 for the Wounan (Table 4). mtDNA diversity values for the Ngöbé and Kuna were also calculated: *h* was 0.76 in the Ngöbé and 0.59 in the Kuna and π was 0.012 in the Ngöbé and 0.009 in the Kuna. Chocoan mtDNA diversity was similar to that of other Amerind groups [*e.g.*, π = 0.016 for the Nuu-Chah-Nulth and Mapuche and *h* = 0.95 and 0.91 for the Nuu-Chah-Nulth and Mapuche, respectively (KOLMAN *et al.* 1995)] but was elevated relative to Chibchan mtDNA diversity. However, when standard errors of the two diversity measures were taken into account, only haplotype diversities were significantly different between Chocoan and Chibchan populations. Total number of haplotypes, percentage of rare haplotypes, and number of segregating sites were also elevated in Chocoan populations (Table 4). Tajima's *D* (TAJIMA 1989) values were nearly zero in the Emberá and Wounan and positive for the Chibchan populations, although none of the values varied significantly from zero (Table 4).

Heterozygosity values were also calculated for each population by pooling autosomal, *X*-chromosomal, and *Y*-chromosomal loci separately (Table 4). Autosomal heterozygosities were slightly elevated in Chocoan pop-

TABLE 3

Polymorphic restriction sites and frequency of haplotypes in Ngobe and Kuna (Chibchan) populations[a]

ID Ngobe	ID Kuna	N Ng	N Ku	*Hae*III 663	*Alu*I 5176	*Dde*I 10394	*Alu*I 10397	*Alu*I 13262	*Hae*III 16517	9 bp 8272/8289	New World haplogroup[b]
NG1-3, 6-8	K1, 3, 4	24	30	1	1	0	0	0	0	2	A2
NG4-5	K1, 5	7	8	1	1	0	0	0	1	2	A1
NG9-15	K7, 21, 28	15	17	0	1	0	0	0	1	1	B1

[a] Sites are numbered according to ANDERSON *et al.* (1981). *Hae*III/663, *Alu*I/13262, and the 9-bp deletion were assayed previously by BATISTA *et al.* (1995) and KOLMAN *et al.* (1995).

[b] Haplogroup nomenclature and description according to MERRIWETHER and FERRELL (1996).

TABLE 4

Measures of mitochondrial and nuclear diversity in Chocoan and Chibchan populations

	Emberá (Cc)[a]	Wounan (Cc)	Ngöbé (Cb)	Kuna (Cb)
mtDNA				
Haplotype diversity, *h*	0.94 ± 0.021	0.91 ± 0.030	0.76 ± 0.039	0.59 ± 0.060
Nucleotide diversity, π	0.017 ± 0.0090	0.019 ± 0.010	0.012 ± 0.0068	0.009 ± 0.0054
Tajima's *D*	0.364 (NS)[b]	−0.250 (NS)	1.68 (NS)	1.52 (NS)
No. of haplotypes	20	14	7	7
Rare haplotypes (%)[c]	90	78	28	43
S (no. of segregating sites)	23	29	12	10
nDNA				
Autosomal				
Heterozygosity	0.59 ± 0.048	0.65 ± 0.039	0.52 ± 0.092	0.56 ± 0.040
Average no. of alleles/locus	5	5.2	3.8	3.7
Rare alleles (%)[c]	30	16	22	14
X-chromosomal				
Heterozygosity	0.69 ± 0.092	0.71 ± 0.097	0.72 ± 0.093	0.64 ± 0.047
Average no. of alleles/locus	7	7.5	7	6
Rare alleles (%)[c]	50	47	34	33
Y-chromosomal				
Heterozygosity	0.12 ± 0.12	0.23 ± 0.098	0.10 ± 0.069	0.20 ± 0.10
Average no. of alleles/locus	2.5	2.5	2.25	2.25
Rare alleles (%)[c]	30	20	22	22

[a] Cc, Chocoan populations; Cb, Chibchan populations.
[b] NS, not significant.
[c] Haplotypes or alleles with a frequency of one or two.

ulations relative to Chibchan groups, although the difference was not significant. Average number of alleles at autosomal loci was reduced 50% in the Chibcha in comparison with the Chocó. In contrast, *X*- and *Y*-chromosomal heterozygosities and allele counts appeared equivalent across all four populations (Table 4).

Statistical analyses of Chocoan and Chibchan populations: Two separate analyses of genetic diversity were used to detect substructure among the populations studied here. An AMOVA was performed on the mitochondrial control region I DNA sequence data (EXCOFFIER *et al.* 1992). The nuclear loci were analyzed using a standard determination of *F* statistics (WEIR and COCKERHAM 1984).

Mitochondrial interpopulation variances were based on the MST-network pictured in Figure 4, which provided a graphical summary of the 37 mtDNA haplotypes determined for the four Panamanian groups. AMOVAs revealed no significant associations within Emberá or Wounan populations. For example, we compared coastal and inland communities of both Emberá and Wounan populations (E4, E5, E6, and E7 *vs.* E1, E2, E3 and W1, W2, W3, and W5 *vs.* W4) and found no statistical support for a genetic distinction between subpopulations ($P = 0.84$ and 0.37, respectively). Previous AMOVAs had revealed no population subdivision within Ngöbé or Kuna populations (BATISTA *et al.* 1995; KOLMAN *et al.* 1995).

AMOVAs were also performed to test for associations between Emberá, Wounan, Ngöbé, and Kuna populations. Significant interpopulation variances were determined for all population pairs (Table 5). Levels of interpopulation variance ranged from 6.9% for the Chocó-speaking Emberá and Wounan and 12% for the Chibcha-speaking Ngöbé and Kuna to 24% for the Emberá and Kuna. An average interpopulation variance of 18% for all four groups was determined. When a nested analysis was performed, the only combination that yielded a significant interpopulation variance was the analysis that grouped Emberá and Wounan populations in one region and Ngöbé and Kuna populations in a second region, *i.e.*, according to the linguistic classification (13% intergroup variance, $P < 0.001$).

Interpopulation variances, calculated as F_{ST}s, were also determined for the Panamanian populations based on autosomal, *X*-chromosomal, and *Y*-chromosomal loci (Table 5). No significant differences were determined between Emberá and Wounan populations. Interpopulation variances based on autosomal loci were significant in all remaining populations pairs and *X*-chromosome-based interpopulation variances were significant between Emberá/Kuna and Ngöbé/Kuna population pairs. Analysis by *F* statistics supported the AMOVA results in that the Emberá and Wounan were most closely related in both analyses. However, AMOVAs determined that the Ngöbé and Kuna were the next most closely population pair related whereas *F* statistics suggested that the Ngöbé and Kuna were as distant from each other as they were from the Chocoan groups.

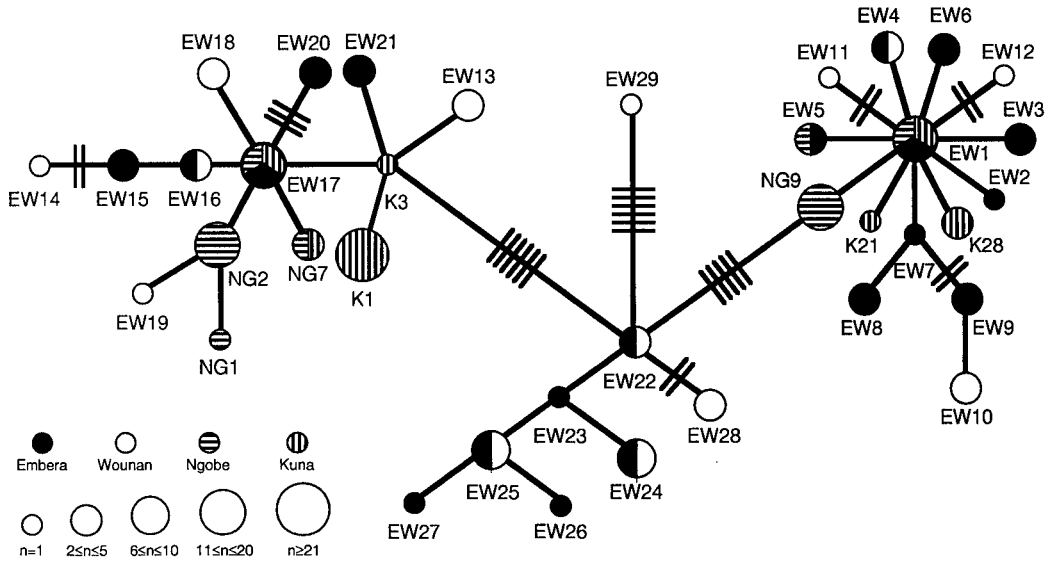

FIGURE 4.—MST illustrating the relationships between mitochondrial haplotypes of the Emberá and Wounan (Chocó) and the Ngöbé and Kuna (Chibcha). The size of each circle reflects the frequency of that haplotype and different fill patterns represent each of the four populations. Each line connecting the circles represents a single mutation except where the cross-hatches enumerate mutations greater than one. The MST is based on the 29 Emberá and Wounan sequences presented here in addition to Ngöbé sequences NG1, 2, 7, and 9 (KOLMAN *et al.* 1995) and Kuna sequences K1, 3, 21, and 28 (BATISTA *et al.* 1995). The four clusters centered on haplotypes EW17, EW1, EW22, and EW29 correspond to haplogroups A, B, C, and D, respectively, from TORRONI *et al.* (1992).

Phylogenetic analysis of Chocoan and Chibchan nuclear loci: Phylogenetic analyses of the nuclear data compared the resolving power of a traditional calculation of genetic distance with a newly derived distance measure designed to more accurately model the mutational mechanism of microsatellite loci. Based on the allele frequencies of nine polymorphic nuclear loci, the two genetic distances were D_A, a traditional distance measure that assumed an infinite-alleles model (NEI 1987), and D_{SW}, a stepwise-weighted distance measure designed for a single-step stepwise mutation mechanism such as that proposed for microsatellite loci (SHRIVER *et al.* 1995). Phylogenetic trees based on each distance were generated and are shown in Figure 5. For compar-

TABLE 5

Interpopulation variances for Chocoan and Chibchan populations

	Wounan (Cc)[a]	Ngöbé (Cb)	Kuna (Cb)
mtDNA-AMOVA results			
Emberá (Cc)	0.069*	0.22**	0.24**
Wounan (Cc)		0.18**	0.20**
Ngöbé (Cb)			0.12**
nDNA-F_{st} values			
Emberá (Cc)			
Autosomal	0.0092	0.037***	0.045***
X chromosome	−0.016	0.050	0.11****
Y chromosome	0.018	0.0034	0.22
Wounan (Cc)			
Autosomal		0.068***	0.045***
X chromosome		−0.0098	0.0087
Y chromosome		0.024	−0.014
Ngöbé (Cb)			
Autosomal			0.063***
X chromosome			0.039*****
Y chromosome			0.15

[a] Cc, Chocoan populations; Cb, Chibchan populations. * P = 0.014; ** P = 0.001; *** P = 0.0001; **** P = 0.0003; ***** P = 0.023.

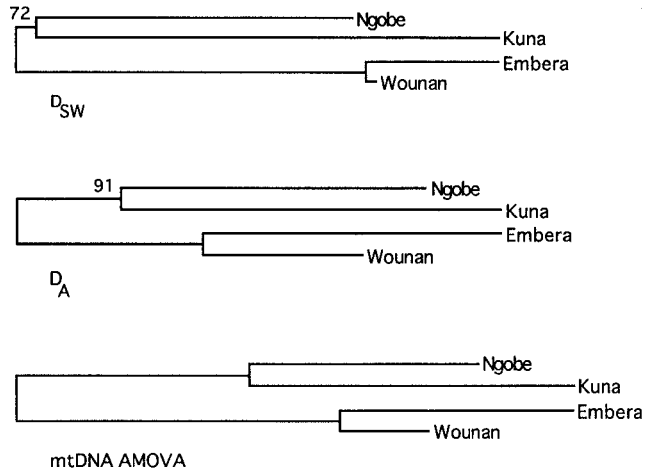

FIGURE 5.—Neighbor-joining trees, using midpoint rooting, of the nine presented polymorphic nuclear loci. Trees are based on D_{SW}, a stepwise-weighted genetic distance (SHRIVER *et al.* 1995), and D_A, an infinite-alleles genetic distance (NEI 1987). Bootstrap values are indicated. An additional tree based on mtDNA interpopulation variances is also included.

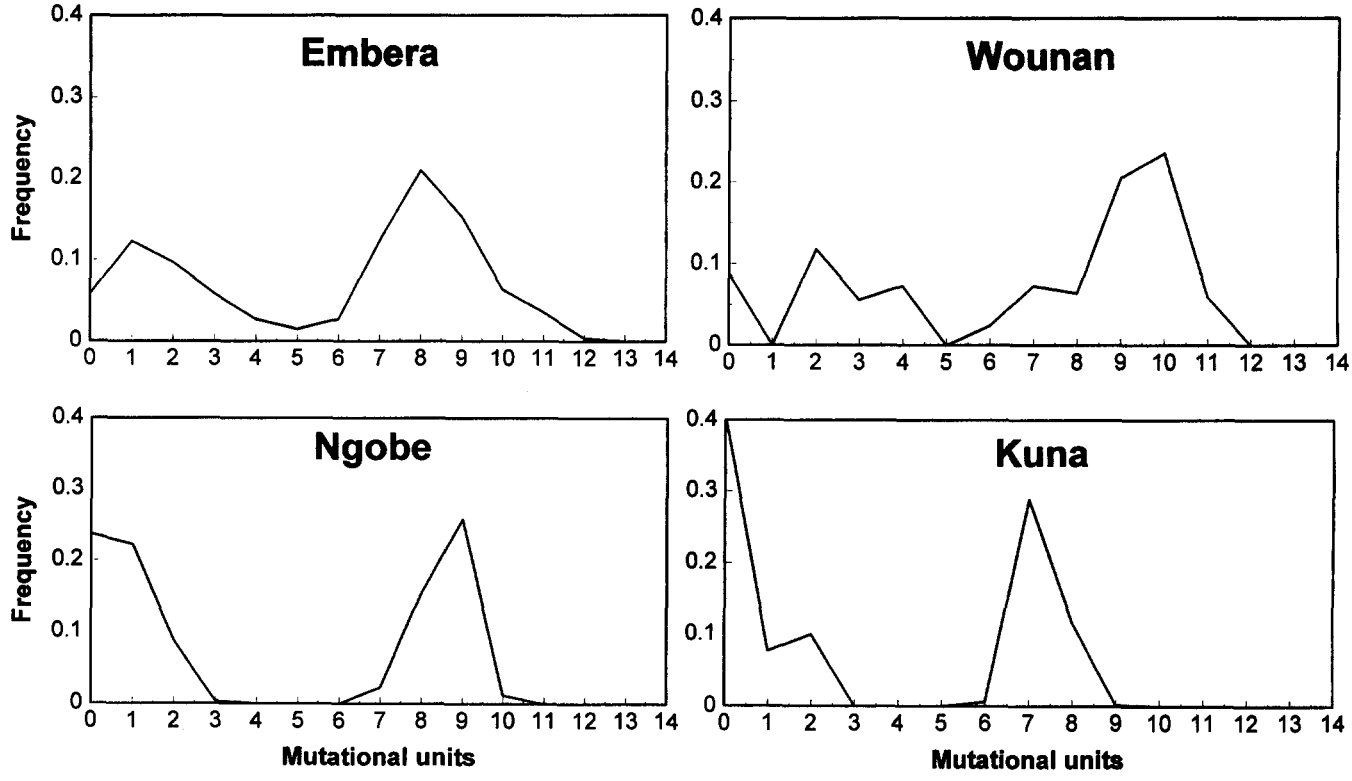

FIGURE 6.—Pairwise difference distributions of mtDNA control region I sequence data from Emberá, Wounan, Ngöbé, and Kuna populations. The *X*-axis represents the number of pairwise differences in units of mutational difference (1 unit = 9300 years; HARPENDING *et al.* 1993) and the *Y*-axis represents the frequency of each pairwise difference.

ative purposes, a tree based on mtDNA interpopulation variances is also presented. The D_A-based tree depicted the Ngöbé-Kuna divergence as ~1.5 times older than the Emberá-Wounan divergence while the D_{SW}-based tree depicted the Chocoan and Chibchan divergences as temporally separated by a factor of 5.5.

Pairwise genetic difference distributions: Pairwise genetic difference distributions were calculated for the four Panamanian populations (Figure 6). All groups shared a peak at 7–10 units of mutational time, suggesting a common expansion between 65,000 and 93,000 YBP. A second major peak was revealed in the Chibchan populations, suggesting a second, more recent expansion. This expansion occurred during 0 and 1 unit of mutational time and had been previously estimated at 6800 YBP with a range of 1850–14,000 YBP (KOLMAN *et al.* 1995). However, the previous analysis dated the population expansion based on a bimodal distribution with no attempt made to distinguish between the two peaks, *i.e.*, the two expansions. Therefore, in the present study, an additional analysis was performed based only on group A haplotypes, which produced a unimodal distribution with a peak at 0 units of mutational time (Ngöbé and Kuna data were pooled to increase the sample size). In this analysis, the Chibchan expansion was dated at 10,900 YBP (τ = 1.17 mutational units), a date consistent with the range of dates previously reported (KOLMAN *et al.* 1995).

DISCUSSION

Mitochondrial and nuclear markers were assayed in four Panamanian populations of Amerinds who live in close proximity but represent two distinct linguistic families. The Chocó-speaking Emberá and Wounan exhibited mtDNA diversity values similar to other Amerind groups and carried all four New World founding haplogroups proposed by TORRONI *et al.* (1993). Chibcha-speaking Ngöbé and Kuna populations, on the other hand, displayed the lowest mtDNA diversity yet reported for Amerind groups and carried only two of the four New World founding haplogroups. Levels of heterozygosity, as measured by autosomal loci, were slightly elevated in Chocoan populations relative to Chibchan populations while *X* and *Y* variation appeared equivalent across all four populations. mtDNA-based interpopulation variances were significant for all population pairs while ~40% of nuclear-derived interpopulation variances were significant.

Differences in the mitochondrial and nuclear record: Differences in diversity levels between Chibchan and Chocoan groups were significant at the mitochondrial level but not at the nuclear level. This result was further reflected in the fact that all mitochondrial interpopulation variances were significant in contrast to nuclear interpopulation variances, which were significant in fewer than half the cases. At first glance, the lack of

accord between the mitochondrial and nuclear results might be interpreted as a difference in maternal and paternal patterns of migration. However, the presence of private alleles in each of the two linguistic groups argues against this idea and, instead, supports a lack of migration by both men and women between the linguistic groups. The conflicting diversity comparisons may instead reflect differences in the mitochondrial and nuclear genomes themselves. The fact that the mtDNA genome has one quarter the effective population size of the autosomal genome implies that a population could lose all of its mtDNA variability while retaining a significant fraction of its nuclear variability (WILSON *et al.* 1985), a conclusion consistent with the results presented here. Although historical and cultural differences between the Chibcha and Chocó obviously contribute to their intergroup variances, these influences can be, in a sense, controlled for by only comparing Chibcha to Chibcha or Chocó to Chocó. Using only within-linguistic group comparisons, more mtDNA-based interpopulation variances were significant compared to those based on nuclear loci (2/2 mtDNA and 2/6 nDNA variances were significant). Thus, it appears that mtDNA may be better able to resolve population differences than nDNA (based on 13 nuclear loci).

Support for a Chibchan population bottleneck: Chibchan populations exhibited reduced levels of genetic diversity relative to the Chocó as demonstrated by mitochondrial diversities and, to a lesser extent, by nuclear diversity measures. KOLMAN *et al.* (1995) have proposed that reduced Chibchan diversity is the result of a population bottleneck at Chibchan ethnogenesis wherein only a fraction of the diversity carried in the migration wave that colonized the Americas was sampled. This idea is supported by the fact that a survey of six additional Chibchan populations revealed similarly reduced levels of mitochondrial diversity (SANTOS *et al.* 1994; TORRONI *et al.* 1994).

The potential of autosomal, *X*, and *Y* loci to reveal past population bottlenecks depends on these loci carrying sufficiently high levels of diversity that detection of a bottleneck as reflected in a reduction in diversity would be possible. High levels of heterozygosity across autosomal loci have been documented by various laboratories (BOWCOCK *et al.* 1994; DEKA *et al.* 1995a,b). Although the mutation rate of *Y* loci is thought to be quite low, by combining the five loci studied here on the nonrecombining portion of the *Y* chromosome, we obtained the following haplotype diversities: Emberá, 0.62; Wounan, 0.56; Ngöbé, 0.32; and Kuna, 0.59. These values are similar to the haplotype diversities calculated for Chibchan mtDNA, suggesting that the levels of *Y* variation determined in our study are sufficiently high to allow detection of a population bottleneck. Since *Y* loci and mtDNA have equivalent effective population sizes in that a single copy of each is transmitted from parent to offspring, it might be predicted that a population bottleneck would similarly impact the levels of diversity measured by both loci. Therefore, the fact that *Y* variation is similar across Chibchan and Chocoan groups and does not mirror the dichotomy seen in mtDNA diversities suggests that the proposed Chibchan population bottleneck may not have cut across both male and female populations. It has been suggested that polygamy (practiced by all Panamanian groups) may result in larger genetic variance at *Y* loci (EXCOFFIER *et al.* 1996) and the contrasting marriage practices of the Chibcha and Chocó might also impact levels of variation at *Y* loci. While these effects, and others, may play a role in determining *Y* variation, the dichotomy in mtDNA diversity levels and the equivalence of *Y* variation across Chibchan and Chocoan groups are consistent with a mild population bottleneck at ethnogenesis of the Chibcha that was limited to the maternal line.

Chibchan populations were further distinguished from Chocoan populations at the mitochondrial level by the bimodal nature of their pairwise difference distributions and the high frequency of identical haplotypes. These results also suggest a relatively recent population bottleneck and subsequent expansion in Chibchan populations. Pooling of Ngöbé and Kuna data placed the proposed bottleneck and expansion at ~10,900 YBP.

Recently, the use of pairwise difference distributions to reconstruct the evolutionary history of populations has come under scrutiny by a number of researchers who have suggested that a multimodal distribution can be caused by any factor that creates a lack of correlation between DNA sequences, such as population substructure, mutation rate heterogeneity, or selection for a particular haplotype (MARJORAM and DONNELLY 1994; ARIS-BROSOU and EXCOFFIER 1996; BERTORELLE and SLATKIN 1995). The AMOVA results presented here demonstrate that, although all four Panamanian populations are genetically distinct, no population structure exists within any of the four populations. ARIS-BROSOU and EXCOFFIER (1996) showed that mutation rate heterogeneity could produce a multimodal pairwise difference distribution when α (γ shape parameter) = 0.01. However, α = 0.01 implies an extremely high level of rate heterogeneity even for the human mitochondrial control region. Using four different substitution models, the lowest α calculated for the data set presented here was 0.0933 (data not shown) and, in ARIS-BROSOU and EXCOFFIER's (1996) study, α = 0.1 generated a multimodal distribution only when accompanied by a population expansion. Furthermore, LUNDSTROM *et al.* (1992) showed that exclusion of the seven most hypervariable sites from a sample data set of New World control region I DNA sequences (WARD *et al.* 1991) did not significantly change their pairwise difference distribution. The issue of selection is more difficult to address. Calculations of Tajima's *D* for all four populations did

not deviate significantly from zero, suggesting that selection was not present. However, it has been proposed that population expansion and mutation rate heterogeneity have synergistic effects on *D* values and may reduce the chance of rejecting the hypothesis of selective neutrality (ARIS-BROSOU and EXCOFFIER 1996). No other appropriate test exists for detecting selection between closely related populations such as those studied here. Nevertheless, a visual inspection of the data reveals inconsistencies with a selection mechanism for generation of the multimodal distribution. The two haplogroups present in the Chibcha, A and B, are two of the most divergent found in the New World (KOLMAN *et al.* 1995) whereas effects such as selection and genetic hitchhiking (BALLARD and KREITMAN 1995) would be expected to act on a single haplotype or group of related haplotypes. Furthermore, due to their geographic proximity, the Chibcha and Chocó would have experienced similar environments and opportunities for a hyperadapted mtDNA type to spread throughout their populations but this apparently did not occur.

Elimination of all factors other than historical demographics supports the interpretation that the multimodal distribution and elevated number of identical haplotypes exhibited by Chibchan groups may indeed reflect a population bottleneck and subsequent expansion. Simulation analyses also demonstrated that only a mild population bottleneck (n = 1500–3000) at 1 unit of mutational time produced a major peak between 0 and 1 mutational unit while retaining a major peak at 7–10 mutational units (data not shown). Moreover, the estimated date of 10,900 YBP for the Chibchan expansion is consistent with the 10,000–7000 YBP time frame for ethnogenesis of the Chibchan linguistic family that is supported by archaeologic, linguistic, and standard genetic marker data (BARRANTES *et al.* 1990; CONSTENLA-UMAÑA 1991; COOKE and RANERE 1992).

Statistics such as Tajima's *D* (TAJIMA 1989), a decrease in rare variation, and the number of segregating sites have also been used to document past population bottlenecks and expansions, but these measures may not be sufficiently sensitive to detect events as recent as 10,000 YBP. For example, Chibchan populations exhibit a positive *D* value and a reduction in mitochondrial rare variation and number of segregating sites. These values are consistent with a population currently in a bottleneck. However, mutation rate heterogeneity will push Tajima's *D* to positive values and is also consistent with a reduction in number of segregating sites (ARIS-BROSOU and EXCOFFIER 1996). Furthermore, historical records document the expansion of Chibchan populations in the 20th century (1990 Panamanian government census; GJORDING 1991). The question of whether a population is currently in a bottleneck or has passed through one and the contrasting influences of population expansion and mutation rate heterogeneity on the measures discussed here may revolve around the amount of time elapsed since a proposed bottleneck and the relative sensitivities of these measures to detect recent events. For example, an analysis of pairwise difference distributions may be more sensitive to the effects of a recent bottleneck since generation of a bimodal distribution depends only on an increase in the number of identical haplotypes, *i.e.*, common variation. Measures such as Tajima's *D*, on the other hand, require the generation of rare variation (to produce a negative *D* value), which will occur subsequent to the increase in common variation seen immediately following a population bottleneck. The number of segregating sites will also increase with the generation of rare variation. Using one of the highest estimates for the control region mutation rate yet reported [11% per 100,000 years (LUNDSTROM *et al.* 1992)], 10,900 years is likely insufficient time to generate the rare variation necessary to affect Tajima's *D* and the number of segregating sites. It is interesting to note that, in ARIS-BROSOU and EXCOFFIER's (1996) study using both pairwise difference distribution and Tajima's *D* and related measures, none of the population bottlenecks and subsequent expansions detected in several human populations were recent events but dated to 40,000–70,000 YBP using HARPENDING *et al.*'s (1993) conversion factor (9,300 years per mutational unit).

Evolutionary relationship between the Chocó and Chibcha as detected by phylogenetic analysis: Phylogenetic trees are only as valid as the distance measures on which they are based and the recent surge in analysis of nuclear microsatellite repeats has spurred the development of genetic distance measures designed to reflect the unique mutational mechanism of these loci. Specifically, the new distance measures assume a single-step stepwise mutation model as opposed to the more common infinite-alleles model (GOLDSTEIN *et al.* 1995; 1995b; SHRIVER *et al.* 1995; SLATKIN 1995). SHRIVER *et al.* (1993) found that 3–5 bp repeat loci were modeled most accurately by a stepwise mutation process while 1–2 bp repeat loci were described less well (all loci presented here were 3 or 4 bp repeats). Figure 5 shows phylogenies of the Panamanian groups based on D_{SW}, a stepwise-weighted measure (SHRIVER *et al.* 1995), D_A, an infinite-alleles distance measure (NEI 1987), and, for comparative purposes, mtDNA interpopulation variances. Although all trees shared similar topologies, they differed in the time scale estimated for divergence of the four groups: the D_{SW}-based tree predicted that the Chibchan divergence predated the Chocoan divergence by a factor of 5.5 while the D_A- and mtDNA-based trees showed the two divergences occurring on the same time scale. Based on linguistic evidence, KAUFMAN (1990) reported minimum time depths of 5,600 years for the Chibcha and 700 years for the Chocó. This translates to a factor of eight differences between the two

divergences, a number most consistent with the D_{SW} based tree. Smaller mtDNA- and nDNA-based interpopulation variances were detected for the Emberá and Wounan than for the Ngöbé and Kuna, which also support a more recent divergence of the Chocó relative to the Chibcha. SHRIVER *et al.* (1995) has reported that D_A-based phylogenies of microsatellite loci tend to exaggerate recent divergences and underestimate ancient ones, a conclusion that appears consistent with the results presented here.

Migration history of lower Central America: Genetic distinctiveness of the two linguistic groups presented here supports the idea of a fragmentation of ancestral populations into separate tribal groups with very low levels of subsequent migration between neighboring groups. Lack of mtDNA haplogroups C and D in the Chibcha provides evidence against migration of Chocoan women into the Chibchan populations and nuclear markers suggest a lack of migration in either direction between Chibchan and Chocoan populations. Despite the geographic proximity of the Panamanian populations, there appears to have been very little intermarriage and genetic admixture between the two linguistic groups.

The frequencies of haplogroups A–D throughout the Chibcha and Chocó, more so than a comparison of diversity levels, make the most compelling argument against the role of lower Central America as a continuous migration corridor between cultures to the north and south. On the contrary, it appears that the isthmian region acted as an effective barrier against movement between distant cultures and that apparent cultural transitions in lower Central America were the result of cultural adaptation by endogenous populations rather than large-scale population infiltration or replacement. In other words, it appears that isthmian populations acquired specific cultural components, such as tool technologies, exogenous plant use, and certain words (BARTLETT and BARGHOORN 1973; LINARES 1977; ICHON 1980; PIPERNO 1988) from neighboring populations without significant levels of genetic contact and exchange.

Origin of New World indigenous groups: The data presented here can also be used to address the issue of Kuna ancestry, a question that remains unresolved by current evidence. The fact that linguists have noted several Chocó-like words in the Kuna language has led some scientists to postulate non-Chibchan origins for the Kuna. Furthermore, the Kuna currently practice a mixture of Chibchan and Chocoan cultural traditions. In response to these conflicting associations, it has been suggested that the Kuna may not represent a distinct Amerind group but may instead encompass an agglomeration of remnant Amerind groups decimated during the Spanish conquest (HOWE 1978; STIER 1979). Alternatively, some historians have suggested that the Kuna are descendants of the "Cueva," a group of Amerind populations of unknown linguistic and cultural affiliation who inhabited the Pacific coast of Panamá in the 1500s and were thought to have been extirpated by the Spanish. Yet another theory proposed that slaving practices of the Chocó brought Kuna into Chocoan populations.

The lack of haplogroups C and D in the Kuna, Ngöbé, and other Chibchan groups stands in sharp contrast to the significant frequencies of these haplogroups in neighboring Chocoan groups and suggests that, by a mitochondrial measure, the Kuna are Chibcha, not Chocó. As measured by *F* statistics, the closeness of Emberá and Wounan populations is not mirrored in the Kuna-Chocó distances, suggesting there is no evidence for a strong nuclear association between the Kuna and Chocoan groups either. Thus, it would appear that any words or cultural traditions shared between the Kuna and Chocó are evidence of the movement of ideas rather than genes between these groups. Furthermore, since language evolves faster than genetic differences, it seems unlikely that both words and genes could have been exchanged between the two groups, yet only evidence of the shared words has been preserved.

The mtDNA data, specifically the lack of haplogroups C and D and attendant reduced genetic diversity, support the proposed population bottleneck at Chibchan ethnogenesis. However, the same evidence, in fact lack of the same haplogroups, has been interpreted in Na-Dene groups as evidence of an independent migration to the New World by the Na-Dene relative to Amerind groups (TORRONI *et al.* 1993). We have argued that this is an incorrect interpretation and that proto-Na-Dene populations, like the proto-Chibcha, were simply a smaller sampling of the migration from Asia and that the Na-Dene do not represent an independent migration event (BATISTA *et al.* 1995; KOLMAN *et al.* 1995). Now that we have extended our study of Chibchan groups to nuclear loci, we can ask how a similar analysis might shed light on questions of New World colonization. Levels of diversity at autosomal and *Y* loci are sufficiently high that one could reasonably expect to detect differences between New World groups that might indicate different genetic origins in Asia or arrival in the New World as separate waves of migration. For example, reported autosomal heterozygosities for Asian populations were 0.80 and for New World groups ranged from 0.51 to 0.61 (DEKA *et al.* 1995b). *Y* heterozygosities for the Japanese were 0.89 (HAMMER and HORAI 1995) and for Amerinds were 0.53 (this study). Our results indicate that a joint mtDNA/nDNA study of groups representing the different proposed migrations to the New World could be instructive in determining the number, time depth, and genetic origin of migrations to the New World.

Conclusion: Our study of four Panamanian popula-

tions from two linguistically distinct but geographically proximal groups provides evidence of a strong barrier to gene flow between these neighboring populations as measured by both mtDNA and nDNA. In contrast to the archaeological record, the genetic data indicate that ideas have flowed across the isthmian region more successfully than genes. Our nuclear results provide qualitative support for our previous mtDNA-based hypothesis of a mild population bottleneck at Chibchan ethnogenesis. Furthermore, our results suggest that, due to its smaller effective population size, mtDNA may provide an enhanced signal over nDNA for defining distinct human populations. In terms of applicability to anthropological questions, our study argues against several theories regarding Kuna ancestry and demonstrates the potential of comparable studies to address questions of New World colonization.

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