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Y-Chromosome Variability in Four Native American Populations from Panama

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Abstract The allele and haplotype frequencies for 13 Y-chromosome short tandem repeats (STRs) [nine STR loci of the minimal Y-chromosome haplotype (*DYS19*-*DYS385a*-*DYS385b*-*DYS389I*-*DYS389II*-*DYS390*-*DYS391*-*DYS392*-*DYS393*) plus four additional loci (*DYS388*, *DYS426*, *DYS439*, *DXYS156*)] were determined in 99 males from 4 Panamanian native American populations, including the Chibcha-speaking Ngöbé and Kuna and the Chocó-speaking Emberá and Wounan. Fifty haplotypes were identified, of which 48 (96%) were specific to a single population and 29 (63%) were found in only a single individual. Gene diversity per locus per population ranged from 0 to 0.814, with the highest gene diversity present at the *DYS389II* locus in the Emberá. The haplotypic discrimination capacity was low, ranging from 42.3% in the Kuna to 63.1% in the Wounan. The four tribes showed a high degree of differentiation both at the Y chromosome and in the mitochondrial genome, highlighting the importance of genetic structure even in geographically proximate and linguistically related populations.

Many studies have pointed out the importance of language, cultural differences, and geographic distance as barriers to gene flow between neighboring populations. In particular, the sex-specific modes of inheritance of the mitochondrial genome (maternal lineage) and the Y chromosome (paternal lineage) allow the description of female and male demographic patterns, respectively, which may be affected by different behaviors such as marriage practice. Seventy percent of human populations practice patrilocality customs, in which newly married women move into the natal household of their husbands (Murdock 1981). On the other hand, in matrilocal populations the women stay in their birthplace and the men move. In comparisons of mitochondrial and Y-chromosome data, some investigators have

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observed low differentiation at the mitochondrial DNA (mtDNA) level and high differentiation for the Y chromosome, suggesting that there is genetic evidence for a higher global female than male migration rate in humans through patrilocality (Seielstad et al. 1998). Other studies have proposed that patrilocality effects are evident only on the local and regional scale (Stoneking 1998; Hammer et al. 2001; Oota et al. 2001).

Over the last 20 years, the evolutionary history of New World peoples has been the subject of considerable research to understand the colonization of the Americas (Wallace et al. 1985; Schurr et al. 1990; Schurr and Sherry 2004; Torroni et al. 1993; Kolman et al. 1996). The presence of four major founder American mitochondrial DNA haplogroups (A, B, C, and D) was originally interpreted as indicating more than one migratory wave during the initial colonization of the Americas (Horai et al. 1993; Torroni et al. 1993). However, other mtDNA studies have proposed a single migration to the continent (Kolman et al. 1996; Merriwether and Ferrell 1996; Bonatto and Salzano 1997; Silva et al. 2002). In the last 10 years, Y-chromosome evidence has supported the occurrence of one (Pena et al. 1995; Santos et al. 1996; Underhill et al. 1996; Zegura et al. 2004) or two major male migrations (Karafet et al. 1999; Lell et al. 2002; Bortolini et al. 2003). Two groups of investigators have evaluated sex-biased migration patterns in some native American groups (Mesa et al. 2000; Bortolini et al. 2002). Neither group found a different migration rate between sexes in the native American populations analyzed, but a north to south gradient of increasing genetic drift in the Americas has been suggested by other investigators (Cavalli-Sforza et al. 1994).

Because of Panama's unique geographic position, as the land bridge between North and South America, several investigators have suggested that this area was a dynamic migration corridor through which Paleo-Indians traveled repeatedly during colonization of the New World (Bartlett and Barghoorn 1973; Linares 1977; Piperno 1988). These studies led to the hypothesis that native Americans from lower Central America would exhibit high genetic diversity. Chibcha-speaking tribes are distributed along lower Central America, extending from eastern Honduras to northern South America, reaching east of Lake Maracaibo in Venezuela (Hoopes and Fonseca 2003). Genetic studies using both protein polymorphisms (Barrantes et al. 1990; Thompson et al. 1992) and mtDNA evidence (Batista et al. 1995; Kolman et al. 1995; Kolman and Bermingham 1997; Melton et al. 2007) have found that the Chibcha present low genetic diversity and a high level of differentiation, reflecting an isolated long-term presence in lower Central America. However, based on the analysis of five Y-chromosome markers, Kolman and Bermingham (1997) did not find a significant genetic differentiation at the Y-chromosome level when comparing four tribes from Panama. Recently, a study of five Chibchan tribes, four from Costa Rica and one from Panama, indicated a genetic diversity structure on the basis of nine markers on the Y chromosome (Ruiz-Narváez et al. 2005).

In the present study, we describe the genetic variability at 13 Y-chromosome STR loci in four native American populations from Panama: the Chibcha-speaking

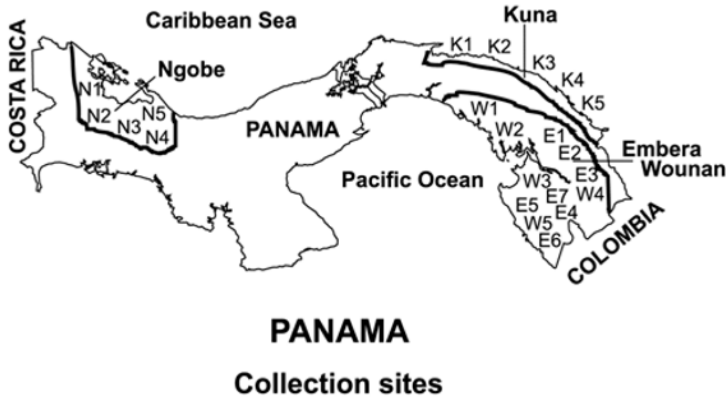

Figure 1. Distributions in Panama of the four native American tribes analyzed. The geographic range of each population is indicated by lines encircling the letters, and letters represent collection sites. N, Ngöbé; K, Kuna; E, Emberá; W, Wounan.

Ngöbé and Kuna and the Chocó-speaking Emberá and Wounan. The Y-chromosome STR loci analyzed in this study consist of the nine STR loci of the minimal Y-chromosome haplotype defined by the Y Chromosome Haplotype Reference Database (available at http://www.yhrd.org/) (*DYS19-DYS385a-DYS385b-DYS389I-DYS389II-DYS390-DYS391-DYS392-DYS393*) plus four additional loci (*DYS388*, *DYS426*, *DYS439*, *DXYS156*). The new Y-chromosome data, which include a larger number of individuals and more Y-chromosome markers than in previous publications (Kolman and Bermingham 1997; Karafet et al. 1999; Zegura et al. 2004), allow us to evaluate the genetic differentiation among these Panamanian populations by comparing Y-chromosome and previously reported mitochondrial data. We also incorporate previously published Y-chromosome data of other native American populations for a comprehensive study of the lower Central American and northern South American region in terms of male genetic structure. We observed different patterns of genetic differentiation, highlighting the importance of generating a regional Y-chromosome database for evolutionary and forensic purposes.

Materials and Methods

Samples. Blood samples were collected from individuals from four Amerindian groups of Panama: Ngöbé and Kuna, belonging to the Chibcha linguistic family; and Emberá and Wounan, representing the Chocó linguistic family (Figure 1). DNA was isolated from blood samples using proteinase K digestion of leukocytes followed by organic extraction and ethanol precipitation (Kolman and Bermingham 1997). Ninety-nine males were included in the Y-chromosome

microsatellite analysis: 32 Ngöbé, 26 Kuna, 22 Emberá, and 19 Wounan. Previously published mitochondrial control region I sequences from 46 Ngöbé, 63 Kuna, 44 Emberá, and 31 Wounan (Batista et al. 1995; Kolman et al. 1995; Kolman and Bermingham 1997) were incorporated into the study. RFLP analysis was performed on the sequenced individuals to define mtDNA haplogroups following the haplogroup definition by Torroni et al. (1993). Six sets of primers were used in balanced PCR reactions to screen for seven polymorphic sites located outside mtDNA control region I, including *Hae*III (bp 663), *Alu*I (bp 5176), COII/tRNA^{Lys} deletion (bps 8272–8289), *Dde*I (bp 10394) and *Alu*I (bp 10397), *Alu*I (bp 13262), and *Hae*III (bp 16517) (Batista et al. 1995; Kolman et al. 1995; Kolman and Bermingham 1997).

Y-Chromosome STR Analysis. Eight Y-chromosome STR loci were amplified for the 99 Panamanian males using previously reported primer pairs (Kayser et al. 1997; Thomas et al. 1999; Bosch et al. 2002). Six STRs (*DYS19*, *DYS388*, *DYS390*, *DYS391*, *DYS392*, and *DYS393*) were amplified in one multiplex reaction, which was slightly modified from Thomas et al. (1999) following Bosch et al. (2002), and two STRs (*DYS389I* and *DYS389II*) were amplified separately following Bosch et al. (2002). PCR reactions were carried out in 10-μl volumes containing 200 μM each dNTP, 0.02 ng/ml BSA, 2.2 mM $MgCl_2$, 0.08 unit AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, California), 1× PCR Gold buffer, 2 μl of DNA template, and 1 μl of 10× mix primers. Amplifications were performed in a GeneAmp PCR System 9700 (Applied Biosystems) using the PCR parameters described by Bosch et al. (2002). Aliquots (0.5 μl) of the PCR products were run on a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, California) using the program Fragment 3 with default conditions. Allele sizes were determined automatically using the Fragment Analysis program of the CEQ 8000 Genetic Analysis software. Y-chromosome STR alleles were labeled according to the number of repeat units, which was established using reference DNA samples provided by Mark Thomas. Previously published data on five Y-chromosome markers (*DYS385*A*, *DYS385*B*, *DYS426*, *DYS439*, *DXYS156*; Kolman and Bermingham 1997; Karafet et al. 1999; Zegura et al. 2004), assayed in a subset of the samples analyzed (17 Ngöbé, 9 Kuna, 10 Emberá, and 14 Wounan), were also incorporated into the current study. Haplogroups were named according to the proposals of the Y Chromosome Consortium (2002).

Analysis of Data. Y-chromosome allele frequencies, number of polymorphic loci, and haplotypic diversity based on Nei (1987) were calculated using Arlequin, version 2.00 (Schneider et al. 2000). To determine the power of our sample sizes to detect differences in allele and haplotype frequencies, we followed Allendorf and Luikart (2007), using the product rule probability to evaluate the minimum sample size necessary to detect rare alleles or genotypes. The probability of not detecting an allele at frequency $p = 0.1$ in a sample size of x is $x(1 - p)$. Therefore the sample size required to have a 95% chance of sampling an allele with a frequency

of 0.10 is 29 haploid individuals. The Ngöbé presented an adequate sample size and the Kuna presented a borderline adequate sample size, but both the Emberá and Wounan did not, indicating the need for further studies including more samples from these populations to confirm the results.

We measured the distribution of Y-chromosome diversity using an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) based on the sum of squared differences (R_{ST}), as implemented in Arlequin, version 2.00 (Schneider et al. 2000). The statistical significance of this test was evaluated using 1,023 random permutations. Gene diversity based on Nei (1987), number of alleles sampled, and allele richness were calculated using FSTAT (Goudet 2001). Haplotype discrimination capacity was calculated as DC $= H/N$, where H is the total number of different haplotypes and N is the total number of individuals in the sample (Kayser et al. 1997). Y-chromosome haplotypes were compared with the worldwide Y Chromosome Haplotype Reference Database to evaluate European and African admixture. Comparative analysis of genetic structure of Y-chromosome diversity was performed using previously published data from native American populations from northern South America distributed in northern Colombia and Venezuela (Ruiz-Linares et al. 1999; Mesa et al. 2000; Bortolini et al. 2003). These groups were assigned to the following linguistic families, as described by Bortolini et al. (2003): the Chibcha-Paenzan, Bari ($n = 12$) and Warao ($n = 12$); the equatorial Tucano, Wayuu ($n = 15$); the Ge-Pano-Carib, Zenu ($n = 12$); and with unknown linguistic affiliation, Yukpa ($n = 11$) (Ruiz-Linares et al. 1999; Mesa et al. 2000; Bortolini et al. 2003).

Results and Discussion

We examined 13 Y-chromosome STR loci in four Native American populations from Panama: the Chibcha-speaking Ngöbé and Kuna and the Chocó-speaking Emberá and Wounan. Allele frequencies within populations ranged from 0.031 to 1 (data not shown). Five unique alleles were found in the Ngöbé, three in the Kuna, and two each in the Emberá and Wounan. The highest power of exclusion (gene diversity) was found at locus *DYS389II* in all populations (Table 1). *DYS388* and *DYS426* exhibited zero gene diversity in some populations, whereas *DYSX156-Y* was monomorphic in all individuals (Table 1). When pooling all populations, gene diversity values tended to increase relative to gene diversity in a single population, demonstrating the risk of overestimating the exclusion capacity when ethnic composition is not taken into consideration (Table 1).

Fifty haplotypes were identified, of which 48 (96%) were specific to a single population and 29 (63%) were found in only a single individual (Table 2). Only two haplotypes were shared, and they may reflect shared ancestry. The apparent absence of gene flow among these populations, as indicated by the low number of shared haplotypes, is striking, considering their geographic proximity, related languages, and shared cultural practices (Constenla-Umaña 1991). This lack of shared haplotypes is even stronger than the pattern observed among Chibchan

Table 1. Gene Diversity, Number of Alleles, and Allelic Richness per Locus in 13 Y-Chromosome Short Tandem Repeats (STRs) in Four Panamanian Native American Populations

Locus	*Measure*	*All* ($N = 99$)	*Ngöbé* ($N = 32$)	*Kuna* ($N = 26$)	*Emberá* ($N = 22$)	*Wounan* ($N = 19$)
DYS19	Gene diversity	0.333	0.063	0.551	0.091	0.281
	Number of alleles	3	2	3	2	2
	Allelic richness	2.577	1.839	2.931	1.984	2.000
DYS388	Gene diversity	0.060	0.063	0.157	0.000	0.000
	Number of alleles	3	2	3	1	1
	Allelic richness	1.932	1.839	2.892	1.000	1.000
DYS389I	Gene diversity	0.596	0.280	0.280	0.495	0.602
	Number of alleles	3	3	3	3	3
	Allelic richness	2.999	2.838	2.931	2.993	3.000
DYS389II	Gene diversity	0.809	0.778	0.638	0.814	0.743
	Number of alleles	9	7	5	5	5
	Allelic richness	6.426	6.516	4.919	5.000	5.000
DYS390	Gene diversity	0.576	0.466	0.618	0.455	0.105
	Number of alleles	4	2	4	2	2
	Allelic richness	3.570	2.000	3.928	2.000	2.000
DYS391	Gene diversity	0.241	0.417	0.080	0.173	0.199
	Number of alleles	3	3	2	2	2
	Allelic richness	2.863	2.997	1.946	2.000	2.000
DYS392	Gene diversity	0.592	0.458	0.630	0.255	0.444
	Number of alleles	6	4	4	3	3
	Allelic richness	4.403	3.815	3.944	2.984	3.000
DYS393	Gene diversity	0.241	0.333	0.077	0.000	0.509
	Number of alleles	4	3	2	1	3
	Allelic richness	3.408	2.994	1.931	1.000	3.000
		All ($N = 50$)	*Ngöbé* ($N = 17$)	*Kuna* ($N = 9$)	*Emberá* ($N = 10$)	*Wounan* ($N = 14$)
*DYS385*A*	Gene diversity	0.6048	0.6851	0.1975	0.4200	0.5612
	Number of alleles	5	5	2	2	3
	Allelic richness	3.909	4.533	2.000	2.000	2.881
*DYS385*B*	Gene diversity	0.6176	0.4567	0.4938	0.6400	0.6224
	Number of alleles	6	3	2	4	4
	Allelic richness	4.087	2.955	2.000	3.989	3.762
DYS426	Gene diversity	0.0392	0	0	0.1800	0
	Number of alleles	2	1	1	2	1
	Allelic richness	1.329	1.000	1.000	1.995	1.000
DYS439	Gene diversity	0.6216	0.5260	0.6420	0.5800	0.6122
	Number of alleles	3	3	3	3	3
	Allelic richness	2.981	2.786	3.000	2.995	2.990
DYSX156-Y	Gene diversity	0	0	0	0	0
	Number of alleles	1	1	1	1	1
	Allelic richness	1.000	1.000	1.000	1.000	1.000

tribes studied by Ruiz-Narváez et al. (2005), where from 39 Y-chromosome haplotypes, 6 were shared among the populations, although in this previous study only 9 markers were surveyed (Ruiz-Narváez et al. 2005).

Most of the Chibchan groups, including the Kuna, practice matrilocal marriage customs (the husband moves to the wife's home), whereas the Chocoan Emberá and Wounan and the Chibchan Ngöbé practice patrilocal marriage traditions. In terms of genetic diversity, in patrilocal groups one would expect increased mitochondrial diversity and decreased Y-chromosome variation. Y-chromosome haplotype diversity and Y-chromosome haplotypic discrimination capacity were lowest in the Kuna and slightly higher in the Ngöbé, Emberá, and Wounan (Table 3). However, when the standard deviations of the haplotype diversities were used to calculate confidence intervals, the Y-chromosome haplotype diversities were not significantly different among the populations (Figure 2). Mitochondrial DNA haplotype diversity was lowest in the matrilocal Kuna (0.59) and slightly higher in the patrilocal Ngöbé (0.76), Wounan (0.91), and Emberá (0.94). In contrast to the Y-chromosome diversity, when the standard errors of the diversity measures were taken into account, the mitochondrial haplotype diversity was significantly different between the Kuna and all other groups and between the Ngöbé and all other groups (Table 3; Figure 2). Among Panamanian groups, we could not detect a correlation between diversity values (Y chromosome and mitochondrial) and marriage traditions (matrilocal vs. patrilocal; Table 3). This may be a consequence of the historical demography of these groups, which included founder events associated with the colonization of the Americas as well as bottlenecks in relation to the tribes' ethnogenesis.

Two main Y-chromosome founding haplogroups have been identified for the Americas: haplogroups C and Q. In particular, founder lineages Q-M3 and Q-M242 are restricted to the Americas or Asia (Bortolini et al. 2003). Among the four Panamanian populations for which haplogroup classifications are available, all populations exhibit haplogroup Q-M3. In addition, the Ngöbé, Emberá, and Wounan present haplogroup Q-P36, and two Wounan men have haplogroup R-P25 (Table 2). Haplogroup R is also found in North and Central American indigenous populations, but it is thought to have been introduced by European populations, where it is the most frequent haplogroup (Bortolini et al. 2003; Zegura et al. 2004).

All haplotypes reported in Table 2 were compared with haplotypes reported in the Y Chromosome Haplotype Reference Database. The first search was conducted based on ethnic affiliation. The comparisons included 877 haplotypes from worldwide populations in a set of 14 populations. Only haplotypes H22 (Kuna), H24 (Kuna), and H36 (Emberá), which contain no data for loci *DYS385* and *DYS439*, present neighbor haplotypes (one allele difference in one locus) with European and Eurasian populations. Haplotype H22 matches three Eurasian haplotypes, H24 matches five Eurasian haplotypes and one Asian haplotype, and H36 matches seven Eurasian haplotypes. The same results were obtained when populations were defined by geographic affiliation (this comparison involved a worldwide population sample of 22,093 haplotypes) instead of ethnic affiliation.

Table 2. Y-Chromosome Haplotypes Identified by 12 STR Loci and Their Frequencies in Four Panamanian Native American Tribes

		Allele at														
H_t[a]	H_p[b]	*DYS19*	*DYS385*[c]	*DYS388*	*DYS389I*	*DYS389II*	*DYS390*	*DYS391*	*DYS392*	*DYS393*	*DYS426*	*DYS439*	*Ngöbé* ($N = 32$)	*Kuna* ($N = 26$)	*Emberá* ($N = 22$)	*Wounan* ($N = 19$)
H01	Q-P36	13	15–16	12	13	28	25	6	14	13	12	11	0.1250			
H02	Q-M3	13	14–17	12	13	30	24	11	15	13	12	12	0.0312			
H03	Q-M3	13	14–17	12	13	30	24	10	15	13	12	11	0.0625			
H04	Q-M3	13	14–17	12	13	30	24	10	15	13	12	12	0.0312		0.0454	0.0526
H05	Q-M3	13	16–19	12	13	30	24	10	17	14	12	11	0.0625			
H06	Q-P36	13	15–16	12	12	27	25	6	14	13	12	11	0.0312			
H07		13		12	14	32	25	10	15	12			0.0312			
H08	Q-M3	13	14–16	12	14	31	24	11	14	12	12	12	0.0312			
H09	Q-P36	15	13–19	12	14	31	24	10	10	14	12	12	0.0312			
H10		13		10	14	33	25	10	15	13			0.0312			
H11	**Q-M3**	**13**	**13–17**	**12**	**13**	**32**	**25**	**10**	**15**	**13**	**12**	**12**	**0.2500**			
H12	Q-M3	13	12–17	12	13	32	24	10	15	13	12	12	0.0312			
H13	Q-M3	13	14–17	12	13	31	24	10	15	13	12	12	0.0312			
H14	Q-M3	13	14–17	12	13	34	24	11	15	13	12	12	0.0312			
H15	Q-M3	13	13–17	12	13	31	25	10	15	13	12	12	0.0312			
H16		13		12	13	31	25	10	15	12			0.0312			
H17	Q-M3	13	13–17	12	13	32	25	10	15	13	12	11	0.0625			
H18	Q-M3	13	13–17	12	13	30	25	10	15	13	12	13	0.0625			
H19	**Q-M3**	**13**	**14–15**	**12**	**14**	**32**	**23**	**10**	**14**	**13**	**12**	**11**		**0.2690**		
H20	Q-M3	14	14–17	12	14	32	24	10	14	13	12	12		0.0384		
H21	Q-M3	14	15–17	12	14	31	24	10	15	13	12	13		0.1920		
H22		14		12	28	22	10	11	13				0.0770			
H23	Q-M3	13	14–15	12	14	31	23	10	14	13	12	11		0.0770		
H24		14		11	14		24			13				0.0384		
H25		14		12	14	32	24	10	15	13				0.1530		
H26	Q-M3	13	14–15	12	14	32	23	10	14	13	12	12		0.0384		
H27	Q-M3	14	14–17	12	14	31	24	10	15	13	12	13		0.0384		

H28	Q-M3	13	14–17	12	13	29	25	10	15	14	12	12	0.0384		
H29		15		12	12	26	24	11	13	13			0.0384		
H30	Q-M3	13	14–15	12	13	31	25	10	14	13	12	13		0.1360	
H31		13		12	12	30	25	10	14	13				0.0910	
H32	**Q-P36**	**13**	**12–17**	**12**	**13**	**28**	**24**	**10**	**14**	**13**	**12**	**12**		**0.2730**	**0.1050**
H33	Q-M3	13	14–17	12	12	29	24	10	14	13	12	13		0.0910	
H34	**Q-M3**	**13**	**13–15**	**12**	**12**	**29**	**24**	**10**	**14**	**13**	**12**	**11**			**0.2110**
H35		13		12	12	29	24	11	14	13				0.0454	
H36		14		12	13	29	24	11	13	13				0.0454	
H37	Q-M3	13	14–15	12	13	32	25	10	14	13	13	13		0.0910	
H38		13		12	12	31	24	10	14	13				0.0454	
H39	Q-M3	13	14–20	12	13	29	24	10	15	13	12	12		0.0454	
H40	Q-M3	13	14–15	12	13	30	24	10	14	13	12	13		0.0454	
H41	Q-P36	13	14–16	12	14	30	24	10	14	13	12	11		0.0454	
H42	Q-M3	14	14–17	12	13	29	24	10	14	13	12	12			0.1050
H43		13		12	13	31	24	10	14	13					0.0526
H44	Q-M3	13	13–17	12	14	30	24	10	14	14	12	12			0.1050
H45		13		12	13	31	24	10	14	11					0.0526
H46		13		12	13	29	24	10	15	11					0.0526
H47	Q-M3	13	14–16	12	13	30	24	10	14	14	12	12			0.0526
H48	R-P25	14	14–15	12	13	30	24	11	13	13	12	11			0.1050
H49	Q-M3	13	14–14	12	14	32	23	10	14	13	12	13			0.0526
H50	Q-M3	13	13–15	12	12	30	24	10	14	13	12	11			0.0526

Empty cells denote that data are not available. The most frequent haplotype for each population is marked in boldface.

a. Haplotype defined as each distinct Y chromosome identified by Y-chromosome STRs.

b. Haplogroup definition based on biallelic markers as given by Kolman and Bermingham (1997), Karafet et al. (1999), and Zegura et al. (2004).

c. *DYS385* is characterized by the amplification of two fragments. In this study *DYS385* was amplified by a single pair of primers. In those cases, the Y Chromosome Consortium (2002) recommends the term *DYS385 loci* and that the observed fragments should be treated as genotypes with the alleles separated by a hyphen.

Table 3. Y-Chromosome STRs and Mitochondrial Genetic Diversity in Chibchan- and Chocó-Speaking Populations from Panama

Linguistic Family		*Chibcha-Speaking*		*Chocó-Speaking*	
Linguistic Family and Diversity Index	*All*	*Kuna (Matrilocal)*	*Ngöbé (Patrilocal)*	*Emberá (Patrilocal)*	*Wounan (Patrilocal)*
Y-chromosome STRs					
Sample size	99	26	32	22	19
Number of haplotypes	50	11	18	12	12
Discrimination capacity (%)	50.50	42.30	56.25	54.54	63.15
Number of unique haplotypes	48	10	17	10	9
Haplotype diversity[a]	0.96 ± 0	0.85 ± 0	0.90 ± 0	0.90 ± 0	0.96 ± 0
Number of polymorphic loci	12	11	11	10	10
Number of unique alleles	12	3	5	2	2
mtDNA					
Sample size	184	63	46	44	31
Number of haplotypes (A/B/C/D)[b]	56	7 (4/3/0/0)	15 (8/7/0/0)	20 (5/9/6/0)	14 (5/4/4/1)
Number of unique haplotypes	30	5	4	12	9
Haplotype diversity	NR	0.59 ± 0	0.76 ± 0	0.94 ± 0	0.91 ± 0
Nucleotide diversity (ϕ)	NR	0.009	0.012	0.017	0.019
Number of segregating sites	NR	10	12	23	29

NR, not reported.

a. Values of haplotype diversity were estimated including all the individuals and based on eight Y-chromosome STRs (*DYS19*, *DYS388*, *DYS389I*, *DYS389II*, *DYS390*, *DYS391*, *DYS392*, and *DYS393*).

b. A/B/C/D indicates the four mitochondrial founder lineages described for the Americas: lineages A, B, C, and D (Batista et al. 1995; Kolman et al. 1995; Kolman and Bermingham 1997).

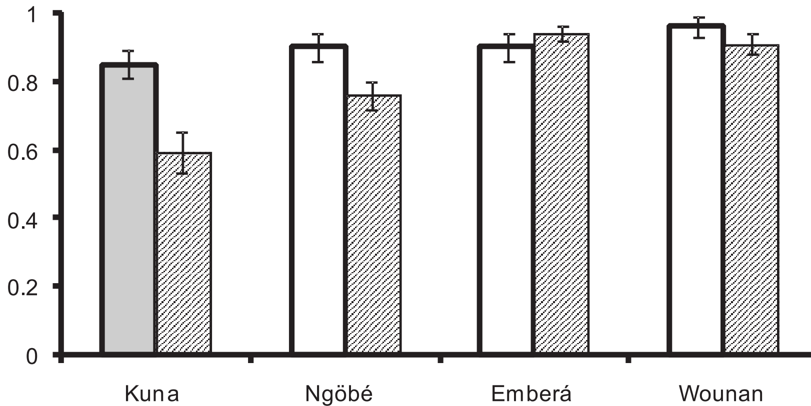

Figure 2. Mean and standard deviation for haplotype diversity of Y-chromosome STRs (open bars) and mitochondrial DNA (hatched bars) in the matrilocal Kuna population (gray bars) and the patrilocal tribes Ngöbé, Emberá, and Wounan.

Furthermore, H48 (Wounan), which belongs to haplogroup R-P25 and is characteristic of European populations, matches one haplotype from an admixed population from Colombia. In sum, these results suggest that the four Panamanian populations have experienced little European admixture.

The Y-chromosome AMOVA results indicate that the major component of variation corresponds to intrapopulation variation (80.19%) and that differences among populations also account for a significant amount of variation ($\phi_{ST} = 0.198$, $P < 0.00001$), indicating a high degree of differentiation among these populations. Using the sum of squared differences among Y-chromosome haplotypes, pairwise R_{ST} among populations indicate that the Emberá and Wounan populations are not significantly differentiated based on Y-chromosome data. Conversely, mitochondrial data indicate a significant level of differentiation (Kolman and Bermingham 1997). The pattern of high genetic structure at the Y-chromosome level has been found in other groups from Central America (Ruiz-Narváez et al. 2005) and South America (Mesa et al. 2000; Bortolini et al. 2002), suggesting that genetic drift has played a major role in the colonization of the Americas. One of the main observations in our study is the apparent absence of gene flow, as indicated by the low number of shared Y-chromosome haplotypes (only 2 haplotypes out of 50). This significant genetic differentiation both at the Y-chromosome and the mitochondrial level is more likely to be the result of fragmentation of ancestral populations into separate tribal groups in agreement with the idea of a continuous presence of Amerindian groups in the isthmian region since their arrival (Cooke 2005). In this scenario, cultural transitions in lower Central America were the result of cultural adaptation by endogenous populations rather than replacement

or introgression (Kolman and Bermingham 1997). Archeological records indicate that native Americans in lower Central America had strong social interactions among neighboring groups (Cooke 2005), suggesting that genetic barriers existed among these populations despite their geographic proximity, related languages, and social interactions.

To further evaluate the role of the Panama area during the colonization of South America, in terms of pattern of genetic diversity, we compared the Y-chromosome variability of populations of Panama and northern South America. These comparisons consisted of our Panamanian populations and previously analyzed Colombian and Venezuelan populations and included six STRs (*DYS19*, *DYS388*, *DYS390*, *DYS391*, *DYS392*, *DYS393*) (Ruiz-Linares et al. 1999; Mesa et al. 2000; Bortolini et al. 2003). *DYS389* (I and II) was not included because of a lack of information for populations from Colombia and Venezuela. *DYS389II* exhibits the highest level of gene diversity in Panamanians (see Table 1); thus some differentiation is lost with the absence of this marker. With this set of six STRs, 43 haplotypes were described among 161 males. Thirty-two haplotypes were present in only 1 tribe, whereas 11 were shared among tribes. Three haplotypes were shared among the Panamanian groups, four among the Colombian and Venezuelan populations, and four among populations from Panama, Colombia, and Venezuela (data not shown). The Y-chromosome AMOVA results indicate that the major component of variation corresponds to intrapopulation variation (75.32%) and that differences among populations also account for a significant amount of variation ($\phi_{ST} = 0.246$, $P < 0.00001$), indicating a high degree of differentiation among these populations. When groups are compared pairwise, among the Panamanian groups the Emberá and Wounan are not significantly differentiated as well as the Emberá and Kuna (Table 4). The loss of genetic differentiation between the Kuna and Emberá when only 6 STR loci are assayed points out the need to include more markers to further evaluate the Y-chromosome differentiation among these tribes. Among the populations from Colombia and Venezuela, only the Warao and Yukpa are not significantly differentiated (Table 4). Comparisons between Panama and Colombia/Venezuela show that the Wounan are not significantly differentiated from the Warao (Colombia) and that the Emberá are not significantly differentiated from the Zenu (Colombia), Warao (Venezuela), or Yukpa (Venezuela). One of the outstanding results is the constant differentiation of the Ngöbé group from the remaining tribes and to a certain degree the Kuna, both groups with the largest sample sizes. The uniqueness of these groups from Panama might be the result of a population bottleneck that was associated with Chibchan ethnogenesis (Kolman and Bermingham 1997). Thus genetic drift might have played a strong role in Chibchan groups because of particular demographic events.

Concluding Remarks. Our study provides new Y-chromosome data and includes a larger number of individuals than in previous studies (Kolman and Bermingham 1997; Karafet et al. 1999; Zegura et al. 2004). Panamanian Y-chromosome

Table 4. Population Pairwise R_{ST} (Below the Diagonal) and R_{ST} P Values (Above the Diagonal) (Significant at the 0.05 Level)[a]

	Panama				*Colombia and Venezuela*[a]				
	Ngöbé	*Kuna*	*Emberá*	*Wounan*	*Bari*	*Warao*	*Wayuu*	*Zenu*	*Yukpa*
Panama									
Ngöbé		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Kuna	0.31813		0.2612	0.0180	0.0000	0.0450	0.0000	0.0000	0.0450
Emberá	0.11659	**0.0185**		0.9909	0.0180	0.7567	0.0000	0.1712	0.2522
Wounan	0.24817	0.09608	**–0.04271**		0.0000	0.0991	0.0000	0.0000	0.0000
Colombia and Venezuela[b]									
Bari	0.56133	0.379	0.11134	0.44105		0.0000	0.0000	0.0000	0.0000
Warao	0.23593	0.11604	**–0.03463**	**0.08094**	0.34715		0.0270	0.0360	0.3513
Wayuu	0.59261	0.42725	0.20899	0.39122	0.22789	0.22251		0.0000	0.0090
Zenu	0.39992	0.34832	**0.07429**	0.40316	0.27028	0.19143	0.28009		0.0270
Yukpa	0.26233	0.13458	**0.01535**	0.20977	0.44998	**0.01446**	0.39934	0.13058	

Nonsignificant R_{ST} values are marked in bold.

a. Comparisons are based on data from six STRs (*DYS19*, *DYS388*, *DYS390*, *DYS391*, *DYS392*, *DYS393*) from groups from Panama and northern South America.

b. Haplotype data for Colombia and Venezuela were reported by Ruiz-Linares et al. (1999), Mesa et al. (2000), and Bortolini et al. (2003).

data are important for future evolutionary genetic studies of native Americans. The forensic utility of the assayed Y-chromosome markers is limited because of the low diversity in these populations, which is most likely a result of population bottlenecks associated with colonization of the Americas, ethnogenesis, and European contact (Kolman and Bermingham 1997). The four Panamanian native American populations show a high degree of differentiation both at the Y-chromosome and the mitochondrial level, highlighting the importance of population structure even in geographically proximate and linguistically related populations. It is significant that these populations show virtually no gene flow among themselves or with non-indigenous groups, suggesting that these populations have remained genetically isolated since their ethnogenesis.

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