Genetic Variation of the Y Chromosome in Chibcha-Speaking Amerindians of Costa Rica and Panama

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Abstract   Genetic variation of the Y chromosome in five Chibchan tribes (Bribri, Cabecar, Guaymi, Huetar, and Teribe) of Costa Rica and Panama was analyzed using six microsatellite loci (DYS19, DYS389A, DYS389B, DYS390, DYS391, and DYS393), the Y-chromosome-specific alphoid system (αh), the Y-chromosome Alu polymorphism (YAP), and a specific pre-Columbian transition (C → T) (M3 marker) in the DYS199 locus that defines the Q-M3 haplogroup. Thirty-nine haplotypes were found, resulting in a haplotype diversity of 0.937. The Huetar were the most diverse tribe, probably because of their high levels of interethnic admixture. A candidate founder Y-chromosome haplotype was identified (15.1% of Chibchan chromosomes), with the following constitution: YAP−, *DYS199*T*, αh-II, *DYS19*13*, *DYS389A*17*, *DYS389B*10*, *DYS390*24*, *DYS391*10*, and *DYS393*13*. This haplotype is the same as the one described previously as one of the most frequent founder paternal lineages in native American populations. Analysis of molecular variance indicated that the between-population variation was smaller than the within-population variation, and the comparison with mtDNA restriction data showed no evidence of differential structuring between maternally and paternally inherited genes in the Chibchan populations. The mismatch-distribution approach indicated estimated coalescence times of the Y chromosomes of the Q-M3 haplogroup of 3,113 and 13,243 years before present; for the mtDNA-restriction haplotypes the estimated coalescence time was between 7,452 and 9,834 years before present. These results are compatible with the suggested time for the origin of the Chibchan group based on archeological, linguistic, and genetic evidence.

The Y chromosome is the male counterpart of mtDNA, and as such is a tool for evolutionary studies. Its nonrecombining portion, known as NRY, is haploid and

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is inherited patrilineally. The NRY embraces all of the Y chromosome except the pseudoautosomal regions, which are homologous to and undergo normal recombination with the X chromosome (Müller and Schempp 1982; Freije et al. 1992). Therefore the NRY depicts, to a good extent, the evolution of a genomic region affected mainly by mutation pressure and male-limited mechanisms of population structure, thus allowing researchers to estimate divergence times and relative contributions of males and females (mtDNA) to gene pools and to detect some demographic events molding contemporary male population structures (Jobling and Tyler-Smith 1995). The first studies of variation of the Y chromosome in Amerindians showed a major paternal haplotype (Pena et al. 1995; F. R. Santos et al. 1995a, 1996; Underhill et al. 1996) possibly related to a major migration into the Americas from central Siberia (F. R. Santos et al. 1999; Karafet et al. 1999).

The Chibcha-speaking groups inhabit most of the Intermediate Zone (lower Central America and northern South America), and they include most of the languages (24 out of 32) of this area (Constenla-Umaña 1991). There is convincing evidence of continuous occupation of the lower Central American region for as long as 10,000 years (Cooke and Ranere 1992). Chipped-stone spear points used by hunters of the Pleistocene megafauna 10,000–12,000 years ago have been discovered in paleo-Indian sites in the Atlantic Highlands of Costa Rica and in Madden Lake near the Panama Canal (Abel 1981), and evidence of early phases of sedentary horticulture in central Panama can be traced to 5,000 B.C. (Cooke and Ranere 1992). However, the relationship between the original colonizers of Central America and the people who resided in this region at the time of European contact (approximately A.D. 1,500) is unclear. Linguistic reconstruction, using the theory of linguistic affinity decay, shows that Central American Chibchan languages, both those still extant and those extinct since the Colonial Period, can be traced to a common linguistic ancestor, perhaps dating to 6,000–8,000 years ago (Constenla-Umaña 1991). Because it is customary to scale linguistic affinity based on the calibrated decay rate for written European languages (Greenberg 1987), some questions remain about the accuracy of linguistic dating for these purely verbal languages. On the other hand, genetic studies using classical autosomal markers have suggested a range of 7,000–10,000 years before present for the origin of the Chibchan group (Barrantes et al. 1990).

In this study we characterize Y-chromosome variation in Chibchan populations, considering the process of microdifferentiation among the screened tribes. Because the demographic histories of males and females may be different, we compare the results with earlier findings based on mtDNA data (M. Santos et al. 1994; Torroni et al. 1994) and estimate coalescence times for both genetic systems.

Materials and Methods

Study Population.   Blood samples from individuals belonging to five Chibchan tribal groups of lower Central America were collected at seven localities
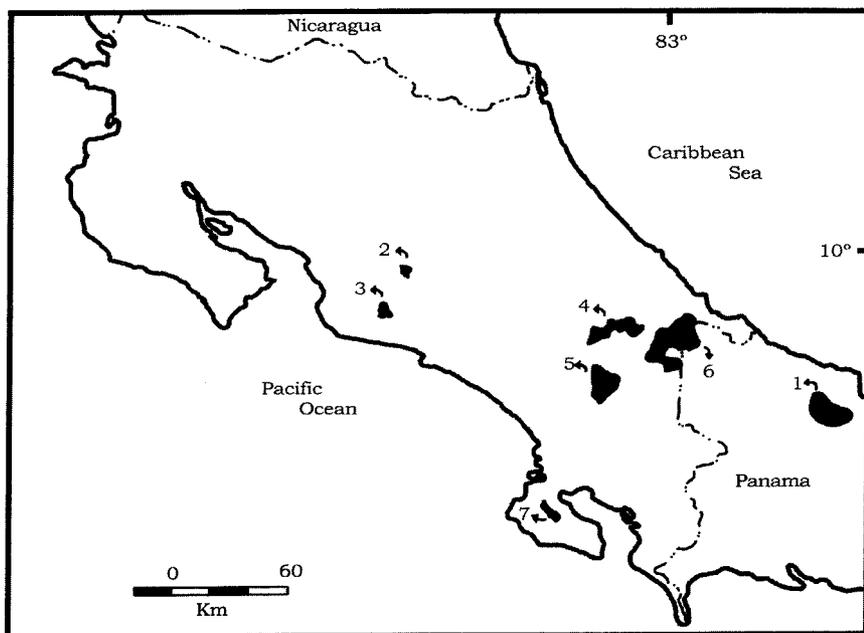
Figure 1. Geographic location in Costa Rica and Panama of the populations or tribes screened: (1) Sieyik Teribe, (2) Quitirrisi Huetar, (3) Zapaton Huetar, (4) Alto Telire Cabecar, (5) Ujarras Cabecar, (6) Mojoncito Bribri, (7) Osa Guaymi.

between 1986 and 1998. All these individuals have been typed for classical markers (Barrantes et al. 1990; M. Santos et al. 1994), and those bearing alleles that could be clearly attributed to admixture with non-Amerindians were removed from the sample. Not all non-Amerindian ancestry is detectable in this fashion; therefore we also removed all individuals who admitted European or African ancestry, regardless of whether it was genetically evident. Although this strategy of sample selection does not completely remove the effects of more ancient gene flow from non-Amerindian groups, we think that we have probably attained a high degree of depuration, given the fact that admixture seems to have increased in the 20th century.

Ninety-three unrelated samples were obtained and classified according to demographic and genealogical studies, as follows (Figure 1): Bribri ($n = 14$), from Mojoncito in the lowlands of the Atlantic side of the Talamanca Cordillera (Talamanca); Cabecar ($n = 15$), from Alto Telire in the highlands of Talamanca; 13 males from Ujarras, on the Pacific side of Talamanca; Guaymi ($n = 8$), from Osa; Huetar ($n = 13$), from Quitirrisi; 15 males from Zapaton; and Teribe ($n = 15$), from Sieyik (Panama).

DNA was extracted from peripheral whole blood either with phenol-chloroform (Hermann and Frischauf 1987) or with NaCl (Miller et al. 1988).

Y-Chromosome Polymorphisms. Nine polymorphic markers were screened. The Y-chromosome Alu polymorphic element (YAP) was typed according to the method of Hammer and Horai (1995). The polymorphic C → T transition (M3 marker) in the DYS199 locus (Underhill et al. 1996), which defines the Q-M3 haplogroup, was typed using a PCR RFLP method using the *Mfe*I restriction enzyme (F. R. Santos et al. 1999). The Y-chromosome-specific αh system was typed according to the method of F. R. Santos et al. (1995b). The tetranucleotide microsatellites DYS19, DYS389A, DYS389B, DYS390, DYS391, and DYS393 were scored and designated by the number of repeats according to the method of Kayser et al. (1997). Naming of the Y-chromosome haplogroups was based on the Y Chromosome Consortium (2002) nomenclature.

Statistical Analysis The intrapopulation variability, measured through gene and haplotype frequencies and diversity indexes, and the interpopulation genetic differentiation index among the Chibchan tribes were calculated using the Arlequin software (Schneider et al. 1997). For the interpopulation genetic differentiation index, two methods were used: (1) the exact test of population differentiation (Raymond and Rousset 1995), to determine whether significant differences in allele frequencies exist among the Central Amerindian groups, and (2) the analysis of molecular variance (AMOVA) (Excoffier et al. 1992), which takes into account the information on the allelic content of haplotypes and their frequencies. To evaluate a possible difference in the genetic structure at the paternal and maternal levels, we compared mtDNA restriction data to the Y-chromosome data for the following groups: Boruca ($n = 14$), Bribri/Cabecar ($n = 20$), Guatuso ($n = 18$), Guaymi ($n = 16$), Teribe ($n = 20$), and Kuna ($n = 16$) (Torroni et al. 1993, 1994).

Two estimates of fixation indexes that are especially suitable for studies with microsatellites (Slatkin 1995) were computed: F_{ST} (which counts the number of differences between two haplotypes) and R_{ST} (which counts the sum of the squared number of repeat differences between two haplotypes). The statistical significance of these values was evaluated using a random permutation test (Excoffier et al. 1992).

We also estimated genetic distances between tribes using the squared number of repeat differences between haplotypes, corrected by the average intrapopulation distance among haplotypes. To have a broader perspective of the place of the Chibcha-speaking populations in the context of other native tribes, we used published microsatellite data of the Cayapa (tropical forests of Ecuador), Tayacaja (Peruvian Andes), Arequipa (Peruvian Andes), Gaviao (Brazilian Amazonia), Ticuna (Brazilian Amazonia) (Tarazona-Santos et al. 2001), and Inuit (southwestern Greenland) (Kayser et al. 2001) to draw a neighbor-joining tree

(Saitou and Nei 1987) using the Neighbor software of the PHYLIP package (Felsenstein 1995).

The mismatch-distribution approach (Rogers and Harpending 1992; Rogers 1995) was used to estimate the coalescence time of the Y chromosomes of the Q-M3 haplogroup in the Chibchan tribes. Mismatch distribution refers to the pairwise count of mutations between all pairs of individuals within a population, and it is one-wave shaped when the coalescence events are concentrated in a narrow interval of time (e.g., a demographic expansion or fixation of a haplotype). This distribution is defined for the parameter $\tau = 2\mu_t t$, which measures the time of coalescence in units of $1/2\mu_t$ generations, where μ_t is the total mutation rate within the studied genomic region (Rogers 1995). This method was originally developed based on the model of infinite sites (Kimura and Crow 1964), an assumption that is not suitable for microsatellite loci. Because the number of mutations in microsatellites seems to follow a stepwise mutation model described by Ohta and Kimura (1973), we used an approximation according to Chakraborty and Nei (1976):

$$\delta_d = \frac{(4N_e\mu)^2}{1 + 8N_e\mu} + \frac{d(1 + 4N_e\mu)}{(1 + 8N_e\mu)^{1/2}}, \tag{1}$$

where δ_d is the expected number of mutations between two alleles that differ for d repeats. The parameter $4N_e\mu$ (four times the effective population size times the mutation rate) for each microsatellite was estimated using a likelihood approach and the MISAT software (Nielsen 1997) with 1,000,000 runs. Two different mutation rates were used: (1) 3.17×10^{-3} per locus per generation, estimated using 4,999 male germ-line transmission events (Kayser et al. 2000), and (2) an effective mutation rate of 6.90×10^{-4} per locus per generation, estimated using microsatellite variation within Y-chromosome haplogroups in populations with known short-term histories (Zhivotovsky et al. 2004).

The variance (S_t^2) of the estimated coalescence time was approximated as the variance of the ratio of two random variables (generation length and mutation rate), according to the following formula:

$$S_t^2 = \left(\frac{\tau}{12}\right)^2 \left(\frac{S_g^2}{\mu^2} + \frac{g^2 S_\mu^2}{\mu^4}\right), \tag{2}$$

where S_μ^2 is the variance of the mutation rate based on Kayser et al. (2000) and Zhivotovsky et al. (2004), g is the generation length in years, and S_g^2 is the estimated variance using data from Neel and Weiss (1975). Coalescence time was log-transformed to estimate 95% confidence intervals. If we define $h(t) = \log(t)$, then, based on the Delta method, a 95% confidence interval (CI) around t can be estimated as

$$95\% \text{ CI} = \exp[h(t) \pm 1.96h'(t)S_t], \tag{3}$$

where t is the estimated coalescence time.

Because the mismatch-distribution approach has not been applied to microsatellite haplotype data, we also estimated the coalescence time of the Y chromosomes of the Q-M3 haplogroup in Chibchan tribes, assuming that under stepwise mutations the total squared difference (Σd^2) (Goldstein et al. 1995) in allele size among all current chromosomes is an estimate of $2\mu_t t$.

The mismatch-distribution method was also applied to the mtDNA restriction data of haplogroup A ($n = 75$), because it includes almost 70% of the Chibchan mtDNA haplotypes (Torroni et al. 1994). The total mutation rate of the mtDNA restriction haplotypes was estimated using the formula

$$\mu_t = 2\mu k \tag{4}$$

(Nei and Tajima 1981), where 2μ is the divergence rate per nucleotide and k is the number of nucleotides embraced by the restriction sites. Torroni et al. (1994) estimated a divergence rate per nucleotide between 0.022% and 0.029% per 10,000 years. They studied 111 restriction sites that covered approximately 2,900 nucleotides. Therefore a total mutation rate, assuming 27 years per generation (Neel and Weiss 1975), between 1.72×10^{-3} and 2.27×10^{-3} per generation was calculated. Confidence intervals were estimated as previously explained.

Results

Table 1 shows the allele frequencies for each locus studied in the five screened Chibchan populations. All individuals sampled were YAP−. Four loci had the same allele as the modal in all the tribes: DYS199*T, αh-II, DYS19*13, and DYS389A*17. The allele frequency distributions of these four loci did not show statistical differences between the analyzed populations (exact test, $p > 0.05$). On the other hand, variation at the DYS389B, DYS390, DYS391, and DYS393 loci did not show a predominant allele in all the tribes, and their allele frequency distributions were statistically different between the populations (exact test, $p < 0.05$, applying a Bonferroni correction for multiple testing).

Table 2 shows the locus diversities for DYS19, DYS389A, DYS389B, DYS390, DYS391, and DYS393 in the sampled populations; they ranged from 0.322 ± 0.060 (DYS19) to 0.619 ± 0.041 (DYS390). The Huetar had the greatest average locus diversity (0.506 ± 0.046), whereas the Teribe showed the smallest (0.242 ± 0.078). The Chibchan average locus diversity was 0.434 ± 0.059.

The nine screened loci gave rise to 39 Y-chromosome haplotypes, grouped in two different haplogroups: the Q-M3 and the Y* (xYAP, M3) (Table 3). The microsatellite haplotype 13,17,11,23,10,13 of the Q-M3 haplogroup was the most frequent haplotype (0.183 ± 0.040), found mainly in the Cabecar (15 out of 17)

Table 1. Allele Frequencies at Eight Loci of the Y Chromosome in Five Chibchan Tribes of Lower Central America

Locus	Tribe[a]					
	Bribri (14)	Cabecar (28)	Guaymi (8)	Huetar (28)	Teribe (15)	Total (93)
αh						
I	0.214 ± 0.114	0.071 ± 0.050	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.054 ± 0.024
II	0.786 ± 0.114	0.893 ± 0.059	1.000 ± 0.000	0.857 ± 0.067	1.000 ± 0.000	0.892 ± 0.032
III	0.000 ± 0.000	0.036 ± 0.036	0.000 ± 0.000	0.107 ± 0.060	0.000 ± 0.000	0.043 ± 0.021
IV	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.036 ± 0.036	0.000 ± 0.000	0.011 ± 0.011
DYS199						
T	0.929 ± 0.071	0.821 ± 0.074	0.625 ± 0.183	0.643 ± 0.092	0.800 ± 0.107	0.763 ± 0.044
C	0.071 ± 0.071	0.179 ± 0.074	0.375 ± 0.183	0.357 ± 0.092	0.200 ± 0.107	0.237 ± 0.044
DYS19						
12	0.000 ± 0.000	0.036 ± 0.036	0.000 ± 0.000	0.036 ± 0.036	0.000 ± 0.000	0.022 ± 0.015
13	1.000 ± 0.000	0.750 ± 0.083	1.000 ± 0.000	0.679 ± 0.090	0.933 ± 0.067	0.817 ± 0.040
14	0.000 ± 0.000	0.107 ± 0.060	0.000 ± 0.000	0.214 ± 0.079	0.067 ± 0.067	0.108 ± 0.032
15	0.000 ± 0.000	0.107 ± 0.060	0.000 ± 0.000	0.036 ± 0.036	0.000 ± 0.000	0.043 ± 0.021
16	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.036 ± 0.036	0.000 ± 0.000	0.011 ± 0.011
DYS389A[b]						
15	0.071 ± 0.071	0.000 ± 0.000	0.375 ± 0.183	0.000 ± 0.000	0.000 ± 0.000	0.043 ± 0.021
16	0.000 ± 0.000	0.036 ± 0.036	0.000 ± 0.000	0.286 ± 0.087	0.133 ± 0.091	0.118 ± 0.034
17	0.714 ± 0.125	0.750 ± 0.083	0.500 ± 0.189	0.464 ± 0.096	0.600 ± 0.131	0.613 ± 0.051
18	0.143 ± 0.097	0.143 ± 0.067	0.125 ± 0.125	0.071 ± 0.050	0.200 ± 0.107	0.129 ± 0.035
19	0.071 ± 0.071	0.071 ± 0.050	0.000 ± 0.000	0.036 ± 0.036	0.067 ± 0.067	0.054 ± 0.024
20	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.143 ± 0.067	0.000 ± 0.000	0.043 ± 0.021
DYS389B[c]						
9	0.000 ± 0.000	0.071 ± 0.050	0.000 ± 0.000	0.107 ± 0.060	0.000 ± 0.000	0.054 ± 0.024
10	0.714 ± 0.125	0.357 ± 0.092	0.500 ± 0.189	0.464 ± 0.096	1.000 ± 0.000	0.559 ± 0.052
11	0.286 ± 0.125	0.571 ± 0.095	0.500 ± 0.189	0.429 ± 0.095	0.000 ± 0.000	0.387 ± 0.051

Table 1. (Continued)

Locus	Bribri (14)	Cabecar (28)	Guaymi (8)	Huetar (28)	Teribe (15)	Total (93)
			Tribe[a]			
DYS390[d]						
21	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.036 ± 0.036	0.000 ± 0.000	0.011 ± 0.011
22	0.000 ± 0.000	0.071 ± 0.050	0.000 ± 0.000	0.107 ± 0.060	0.000 ± 0.000	0.054 ± 0.024
23	0.286 ± 0.125	0.571 ± 0.095	0.000 ± 0.000	0.071 ± 0.050	0.000 ± 0.000	0.237 ± 0.044
24	0.571 ± 0.137	0.321 ± 0.090	0.500 ± 0.189	0.643 ± 0.092	0.867 ± 0.091	0.559 ± 0.052
25	0.071 ± 0.071	0.036 ± 0.036	0.500 ± 0.189	0.143 ± 0.067	0.133 ± 0.091	0.129 ± 0.035
26	0.071 ± 0.071	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.011 ± 0.011
DYS391[d]						
9	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.133 ± 0.091	0.022 ± 0.015
10	0.929 ± 0.071	0.893 ± 0.060	0.125 ± 0.125	0.714 ± 0.087	0.733 ± 0.118	0.753 ± 0.045
11	0.071 ± 0.071	0.036 ± 0.036	0.500 ± 0.189	0.214 ± 0.079	0.133 ± 0.091	0.151 ± 0.037
12	0.000 ± 0.000	0.071 ± 0.050	0.375 ± 0.183	0.071 ± 0.050	0.000 ± 0.000	0.075 ± 0.028
DYS393[c]						
11	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.036 ± 0.036	0.067 ± 0.067	0.022 ± 0.015
12	0.000 ± 0.000	0.071 ± 0.050	0.500 ± 0.189	0.000 ± 0.000	0.000 ± 0.000	0.065 ± 0.026
13	0.929 ± 0.071	0.857 ± 0.067	0.500 ± 0.189	0.679 ± 0.090	0.933 ± 0.067	0.796 ± 0.042
14	0.071 ± 0.071	0.036 ± 0.036	0.000 ± 0.000	0.286 ± 0.087	0.000 ± 0.000	0.108 ± 0.032
15	0.000 ± 0.000	0.036 ± 0.036	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.011 ± 0.011

a. Number of chromosomes for each tribe is given in parentheses.
b. $p < 0.05$ by exact test of population differentiation.
c. $p < 0.01$ by exact test of population differentiation.
d. $p < 0.001$ by exact test of population differentiation.

Table 2. Gene Diversities at Eight Loci of the Y Chromosome in Five Chibchan Tribes of Lower Central America

Locus	Bribri (14)	Cabecar (28)	Guaymi (8)	Huetar (28)	Teribe (15)	Total (93)
			Tribe[a]			
DYS199	0.143 ± 0.119	0.304 ± 0.094	0.536 ± 0.124	0.476 ± 0.056	0.343 ± 0.128	0.365 ± 0.047
αh	0.363 ± 0.130	0.203 ± 0.097	0.000 ± 0.000	0.257 ± 0.097	0.000 ± 0.000	0.201 ± 0.054
DYS19	0.000 ± 0.000	0.428 ± 0.107	0.000 ± 0.000	0.508 ± 0.096	0.134 ± 0.113	0.322 ± 0.060
DYS389A	0.494 ± 0.150	0.426 ± 0.107	0.679 ± 0.123	0.701 ± 0.060	0.619 ± 0.120	0.593 ± 0.053
DYS389B	0.439 ± 0.113	0.561 ± 0.060	0.571 ± 0.094	0.611 ± 0.046	0.000 ± 0.000	0.541 ± 0.028
DYS390	0.627 ± 0.110	0.585 ± 0.069	0.571 ± 0.094	0.568 ± 0.099	0.248 ± 0.131	0.619 ± 0.041
DYS391	0.143 ± 0.119	0.203 ± 0.098	0.679 ± 0.123	0.455 ± 0.094	0.458 ± 0.141	0.408 ± 0.057
DYS393	0.143 ± 0.119	0.268 ± 0.107	0.571 ± 0.094	0.474 ± 0.079	0.134 ± 0.113	0.354 ± 0.060
Average	0.294 ± 0.077	0.372 ± 0.053	0.451 ± 0.100	0.506 ± 0.046	0.242 ± 0.078	0.434 ± 0.059

a. Number of chromosomes for each tribe is given in parentheses.

Table 3.   Y-Chromosome Haplotypes Found in Five Chibchan Tribes of Lower Central America

αh	DYS199[a]	DYS19	DYS389A	DYS389B	DYS390	DYS391	DYS393	Tribes[b]
II	T	12	16	10	24	10	12	Cabecar (1)
II	T	12	17	11	24	10	13	Huetar (1)
II	T	13	17	10	23	10	13	Huetar (2)
II	T	13	17	10	24	10	13	Bribri (4), Cabecar (2), Teribe (8)
I (L)	T	13	17	10	24	10	13	Bribri (3)
II	T	13	17	10	24	11	13	Teribe (1)
II	T	13	17	11	23	10	13	Bribri (2), Cabecar (15)
II	T	13	17	11	24	10	13	Bribri (1), Huetar (5)
II	T	13	17	11	24	11	12	Guaymi (4)
II	T	13	17	11	25	10	13	Huetar (3)
II	T	13	18	10	23	10	12	Cabecar (1)
II	T	13	18	10	23	10	13	Bribri (1)
II	T	13	18	10	24	10	13	Cabecar (1), Teribe (1)
II	T	13	18	10	24	10	14	Huetar (1)
II	T	13	18	10	25	10	13	Guaymi (1), Teribe (1)
II	T	13	18	11	24	10	13	Huetar (1)
II	T	13	18	11	25	11	14	Bribri (1)
II	T	13	19	10	24	10	13	Cabecar (1)
II	T	13	19	10	24	10	14	Huetar (1)
II	T	13	19	10	25	10	13	Cabecar (1), Teribe (1)

II	T	13	19	10	26	10	13	Bribri (1)
II	T	13	20	10	24	10	14	Huetar (4)
II	T	14	18	10	24	10	13	Cabecar (1)
II	C	13	15	10	23	10	13	Bribri (1)
II	C	13	15	10	25	12	13	Guaymi (3)
III	C	13	16	9	22	11	14	Huetar (1)
II	C	13	16	10	24	9	13	Teribe (2)
II	C	13	16	11	24	11	13	Huetar (1)
II	C	14	16	10	24	11	13	Huetar (2)
II	C	14	16	10	24	12	13	Huetar (2)
III	C	14	17	10	21	10	13	Huetar (1)
II	C	14	17	10	24	12	13	Cabecar (2)
II	C	14	17	11	25	11	13	Huetar (1)
II	C	14	18	10	24	11	11	Teribe (1)
III	C	15	16	9	22	10	11	Huetar (1)
I (L)	C	15	17	9	22	10	14	Cabecar (1)
I (L)	C	15	17	9	22	10	15	Cabecar (1)
III	C	15	18	11	24	11	13	Cabecar (1)
IV	C	16	16	9	22	11	14	Huetar (1)

a. Haplotypes with the *DYS199*T* allele belong to the Q-M3 haplogroup, and haplotypes with the *DYS199*C* allele belong to haplogroup Y* (xYAP, M3) (Y Chromosome Consortium 2002).

b. Number of individuals having each haplotype is given in parentheses.

Table 4. AMOVA of Eight Loci of the Y Chromosome in Five Chibchan Tribes of Lower Central America

Locus	Variance (%)	F_{ST}
DYS199		
Between populations	2.62	0.026
Within populations	97.38	
αh		
Between populations	2.80	0.028
Within populations	97.20	
DYS19		
Between populations	2.45	0.024
Within populations	97.55	
DYS389A		
Between populations	5.09	0.051[a]
Within populations	94.91	
DYS389B		
Between populations	16.28	0.163[c]
Within populations	83.72	
DYS390		
Between populations	17.52	0.175[c]
Within populations	82.48	
DYS391		
Between populations	17.98	0.180[c]
Within populations	82.02	
DYS393		
Between populations	13.94	0.139[b]
Within populations	86.06	

a. $p < 0.05$ by random permutation test.
b. $p < 0.01$ by random permutation test.
c. $p < 0.001$ by random permutation test.

and also in the Bribri (2 out of 17). Haplotype 13,17,10,24,10,13 of the Q-M3 haplogroup, with a frequency of 0.151 ± 0.037, was present in three tribes: Bribri, Cabecar, and Teribe. An exact test of differentiation indicated that the haplotypes were not randomly distributed among the tribes ($p < 0.001$). The estimated haplotype diversities were 0.890 ± 0.060 (Bribri), 0.717 ± 0.094 (Cabecar), 0.679 ± 0.122 (Guaymi), 0.942 ± 0.025 (Huetar), 0.724 ± 0.121 (Teribe), and 0.937 ± 0.015 (total).

For each marker only the F_{ST} values were calculated, and they are shown in Table 4. The DYS199, αh, and DYS19 loci did not show F_{ST} values statistically different from 0. On the other hand, DYS389A, DYS389B, DYS390, DYS391, and DYS393 had a significant differentiation between the populations, with DYS391 having the greatest heterogeneity ($F_{ST} = 0.180$). Using only the microsatellite loci, the average F_{ST} value was 0.122, which reduces to 0.034 when corrected to take into account the smaller effective size of Y chromosomes compared to autosomes, according to Pérez-Lezaun et al. (1997). This value is half of the F_{ST} value (0.073) based on classical markers (Barrantes 1993a).
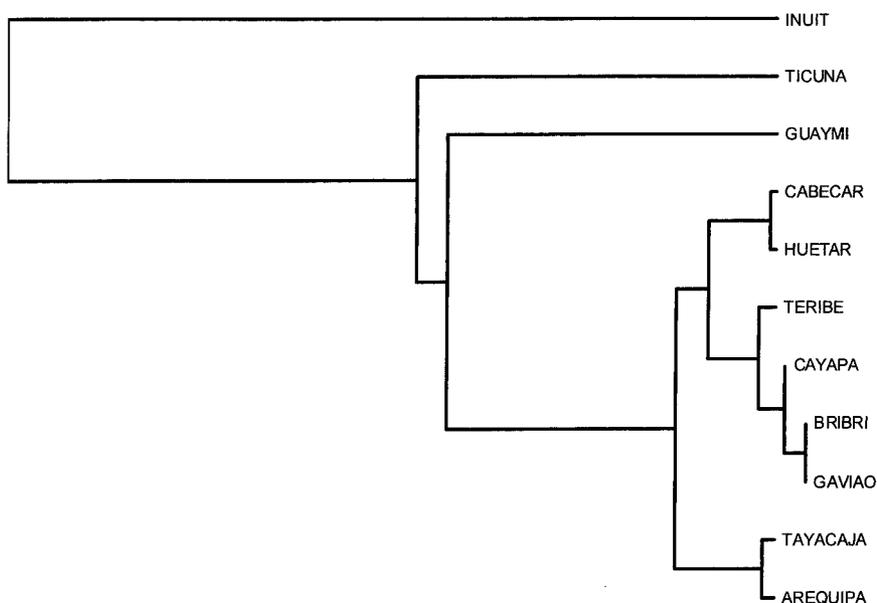
Figure 2. Neighbor-joining tree of five Chibchan tribes (Bribri, Cabecar, Guaymi, Huetar, and Teribe) of lower Central America, the Cayapa from Ecuador, the Tayacaja and Arequipa from the Peruvian Andes, the Gaviao and Ticuna from Brazilian Amazonia, and the Inuit from the Arctic. Squared number of repeat differences among microsatellite haplotypes was used as genetic distance between tribes.

Considering only the haplotype frequencies, we obtained an F_{ST} value of 0.183 (random permutation test, $p < 0.001$). Using the number of differences among haplotypes, we estimated an R_{ST} of 0.110 (random permutation test, $p < 0.001$). The F_{ST} value computed from mtDNA restriction data of six Chibchan tribes ($n = 108$) (Torroni et al. 1993, 1994) was 0.486. This estimate is almost three times the F_{ST} calculated from Y-chromosome haplotypes. When the AMOVA was performed using only those tribes for which mtDNA and Y-chromosome data can be matched (Bribri/Cabecar, Guaymi, and Teribe), we observed nonstatistically different F_{ST} values of 0.228 and 0.190, respectively.

Figure 2 shows a neighbor-joining tree, with squared number of repeat differences between haplotypes as genetic distance, of the five Chibchan populations, five Amerindian tribes of South America, and the Inuit from the Arctic. The neighbor-joining tree shows that all the Chibchan tribes, except the Guaymi, group together with the Cayapa from Ecuador and the Gaviao from Brazilian Amazonia. The two Andean populations (Tayacaja and Arequipa) formed a single group. The Inuit from the Arctic were the most divergent population compared to all the other tribes.

Figure 3 shows the mismatch distributions for the Y-chromosome and
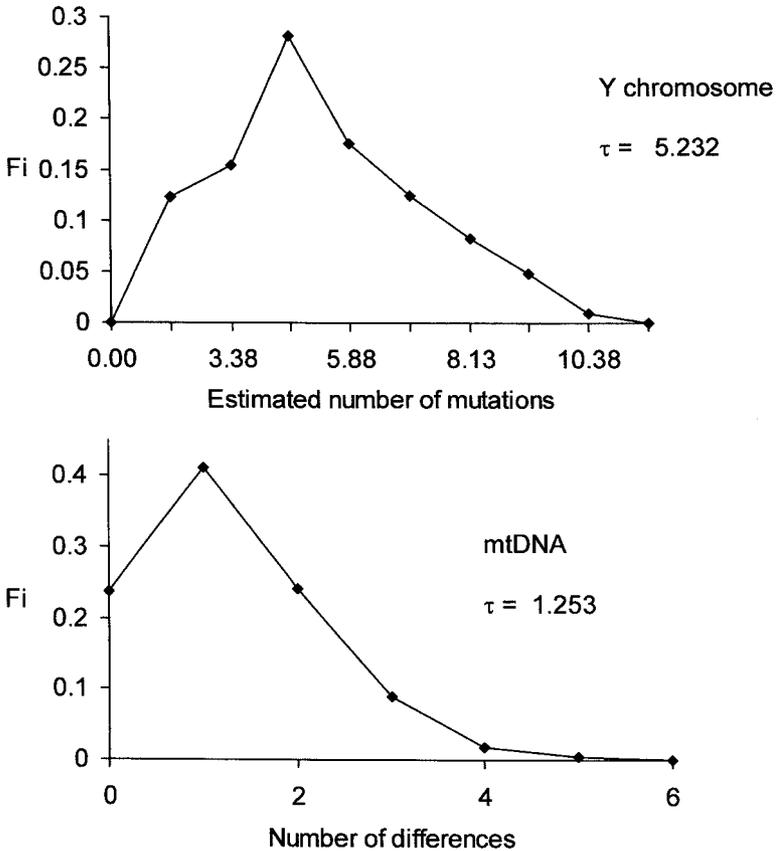
Figure 3. Mismatch distributions for the Y-chromosome microsatellites and mtDNA restriction haplotypes. F_i denotes the relative frequency of pairs of individuals that differ by i mutations or restriction sites.

mtDNA restriction data. Only the Y chromosomes of the Q-M3 haplogroup were used, because the M3 genetic marker (allele *DYS199*T*) is specific to native American populations (Underhill et al. 1996). The estimates of the parameter $4N_e\mu$ for each microsatellite were 0.341 for DYS19, 2.482 for DYS389A, 0.389 for DYS389B, 2.133 for DYS390, 0.300 for DYS391, and 0.955 for DYS393. An average of $4N_e\mu = 1.100$ was used to calculate the number of mutations between two individuals in the mismatch distribution.

Table 5 shows estimated coalescence times of the Y chromosomes of the Q-M3 haplogroup and for mtDNA haplogroup A in Chibchan tribes. The τ estimator from the mismatch distribution gave older coalescence times compared to the Σd^2 estimator. An average coalescence time of 3,113 years before present

Table 5. Estimated Coalescence Times of the Y Chromosomes of the Q-M3 Haplogroup and mtDNA Haplotypes of Haplogroup A in Five Chibchan Tribes of Lower Central America

	Coalescence Time (Years) (95% CI)	
	$\mu_t = 1.90 \times 10^{-2}$ *per Generation*[a]	$\mu_t = 4.14 \times 10^{-3}$ *per Generation*[b]
Y chromosome		
$\tau = 5.232$[e]	3,713 (1,667–8,267)	15,797 (2,782–89,680)
$\sum d^2 = 3.540$[f]	2,513 (1,129–5,585)	10,668 (1,880–60,776)
Average	3,113 (1,400–6,919)	13,243 (2,326–75,358)
	$\mu_t = 2.27 \times 10^{-3}$ *per Generation*[c]	$\mu_t = 1.72 \times 10^{-3}$ *per Generation*[d]
mtDNA, $\tau = 1.253$	7,452 (3,102–17,890)	9,834 (3,805–25,438)

a. Total mutation rate estimated as 6 loci $\times$ (3.17×10^{-3} per locus per generation) based on Kayser et al. (2000).
b. Total mutation rate estimated as 6 loci $\times$ (6.90×10^{-4} per locus per generation) based on Zhivotovsky et al. (2004).
c. Total mutation rate equivalent to a divergence rate of 0.029% per nucleotide per 10,000 years (Torroni et al. 1994).
d. Total mutation rate equivalent to a divergence rate of 0.022% per nucleotide per 10,000 years (Torroni et al. 1994).
e. τ estimator from the mismatch distribution, which is equivalent to the expected number of mutations between two randomly sampled haplotypes.
f. Total squared difference in allele size between two randomly sampled haplotypes.

(95% CI = 1,400 to 6,919) was estimated using a mutation rate of 3.17×10^{-3} per locus per generation (Kayser et al. 2000); the average coalescence time was 13,243 years before present (95% CI = 2,326 to 75,358) when an effective mutation rate of 6.90×10^{-4} per locus per generation was used (Zhivotovsky et al. 2004). On the other hand, the mismatch distribution for mtDNA haplogroup A is also unimodal, which is compatible with coalescence times of 7,452 years before present (95% CI = 3,102 to 17,890) using a total mutation rate of 2.27×10^{-3} per generation and 9,834 years before present (95% CI = 3,805 to 25,438) using a total mutation rate of 1.72×10^{-3} per generation (Torroni et al. 1994).

Discussion

The survey of genetic variation along the Y chromosome in five Chibchan tribes of Costa Rica and Panama showed that, according to both gene and haplotype diversities, the Huetar are the most diverse group. The Huetar also have the highest value of European and African admixture among the Chibchan tribes of Costa Rica and Panama (Barrantes 1993a), and the greatest diversity of the Y chromosome is likely related to this fact, because most of the European contribution to the Costa Rican gene pool was male mediated. Although the samples

were selected to minimize the effects of the admixture with non-Amerindians, an absolute depuration cannot be guaranteed. Thus it is highly possible that a remnant of interethnic admixture was disclosed by the loci screened.

The AMOVA showed that the genetic variation of the Y chromosome within tribes was greater than the genetic variation between tribes. An average F_{ST} value of 0.122 based on the six microsatellite loci was obtained, which reduces to 0.034 when it is corrected to take into account the smaller effective size of Y chromosomes compared to autosomes (Pérez-Lezaun et al. 1997). This value is half the F_{ST} value of 0.073 based on classical autosomal markers (Barrantes 1993a). Although these results suggest that, after correction for effective size, the differentiation of Y chromosomes is smaller than the differentiation of classical autosomal markers among Chibchan tribes, some caution is needed. By restricting our analysis to the Q-M3 haplogroup, we estimated an F_{ST} value of 0.218, and after correction for the smaller effective size of Y chromosomes, the F_{ST} value obtained for the Q-M3 haplogroup became 0.065, in closer agreement with the F_{ST} value based on classical autosomal markers. A more plausible conclusion is to assert that Y-chromosome and autosomal markers have the same degree of structuring among Chibchan tribes. Although this conclusion differs from the observed worldwide structure, where Y chromosomes tend to show a higher stratification than autosomal markers do, the same pattern has been noted in at least two other human populations. Pérez-Lezaun et al. (1997) found the same degree of structure using either eight microsatellites in the Y chromosome ($F_{ST} = 0.0098$) or 20 autosomal microsatellites ($F_{ST} = 0.0100$) in Catalans and Basques from the Iberian Peninsula.

The lack of differential structuring of the Chibchan populations, for maternally (mtDNA, $F_{ST} = 0.228$) and paternally (Y chromosome, $F_{ST} = 0.190$) inherited haplotypes, is noteworthy. This finding is remarkable because previous comparisons in other populations indicated that Y-chromosome haplotypes are more geographically clustered than mtDNA haplotypes (Ruiz-Linares et al. 1996; Pérez-Lezaun et al. 1999), perhaps because women move more often to find partners (Cavalli-Sforza 1998; Pérez-Lezaun et al. 1999). Because our samples were selected with the goal of minimizing the effects of European and African genetic infiltration over the past 500 years, it is possible that selection bias might affect our results. However, we think that bias resulting from sample selection cannot completely explain our data. First, an equal population structure of both mtDNA and Y chromosomes is supported by ethnological data, which show that estimated marital distances are small in these tribes, ranging between 2 km and 13 km. Also 76% of the unions happen within the same locality, and there are no differences between the migration rates of males and females in these populations (Barrantes 1993b). Last, the recent study by Bortolini et al. (2002) did not find differences in the degree of population structure between mtDNA RFLPs and Y-chromosome biallelic markers in Amerindians from North, Central, and South America, supporting our conclusion of equal migration rates of males and females among Chibchan populations.

Two Y-chromosome haplotypes of the Q-M3 haplogroup (13,17,11,23, 10,13 and 13,17,10,24,10,13) were found in one-third of the samples. The 13,17,10,24,10,13 haplotype was found in three of the five populations studied, and the 13,17,11,23,10,13 haplotype was found in only two of them. In addition, most of the individuals with the 13,17,11,23,10,13 haplotype (15 out of 17) were Cabecars from Alto Telire (see Figure 1). This population has a high inbreeding coefficient, and it was founded by only a few persons (J. Azofeifa, unpublished data, 1998). Therefore the 13,17,11,23,10,13 haplotype, despite its high frequency, is not a good candidate to be considered a Y-chromosome founder haplotype of the Chibcha. It is noteworthy that the 13,17,11,23,10,13 haplotype was not found in the survey of 95 Amerindian males of North and South America (Carvalho-Silva et al. 1999).

On the other hand, the high frequency and wider distribution of the 13,17,10,24,10,13 haplotype make it a better candidate. This haplotype is not specific to the Chibchan group, and it has also been found in other native American populations (Bianchi et al. 1998; Carvalho-Silva et al. 1999) and seems to be a major Y-chromosome founder haplotype of Amerindian populations, as postulated elsewhere (Pena et al. 1995; F. R. Santos et al. 1995a, 1996). Using only the Y chromosomes of the Q-M3 haplogroup, we found that the 13,17,10,24,10,13 haplotype has a frequency of 5.7% in a sample of linguistically diverse native Americans (Bianchi et al. 1998) and a frequency of 19.7% in the Chibchan populations (this study). This increment could be the joint result of genetic drift and a bottleneck at the Chibchan origin. It is noteworthy that the Chibcha show smaller mtDNA diversity than other Amerindian groups (M. Santos et al. 1994; Kolman et al. 1995), perhaps associated with a bottleneck at the separation of the ancestral proto-Chibcha from other Amerindian populations (Kolman et al. 1995).

The estimated coalescence times of the Y chromosome roughly agree with the proposed time of origin of the Chibchan group at 7,000–10,000 years before present (Barrantes et al. 1990; Constenla-Umaña 1991; Kolman et al. 1995). As expected, the estimated coalescence times are greatly dependent on the chosen mutation rates. A more than fourfold deviation (approximately 10,000 years) was observed between the average coalescence times by using two different estimates of the mutation rate of the Y-chromosome microsatellites. The effective mutation rate (Zhivotovsky et al. 2004) has several drawbacks that must be taken into account when interpreting estimated coalescence times. First, the effective mutation rate is based on current variation, and reverse mutations will tend to reduce the standing number of alleles, leading to an underestimate of the true mutation rate. Also, differential selection among haplotypes (Jobling and Tyler-Smith 2000) may bias the estimated coalescence times. Last, the high standard deviation across loci compared to the mean effective mutation rate produced very wide 95% confidence intervals of the estimated coalescence times, with an average upper limit of approximately 75,000 years before present. Because of these limitations, we think that the use of a mutation rate estimated from father-son pairs

(Kayser et al. 2000) is a more reliable approach; father-son pairs have the advantage of producing a tighter 95% confidence interval (1,400–6,919 years before present).

We used two different estimators of the coalescence time: the τ estimator from the mismatch distribution and the total squared differences in allele size (Σd^2 estimator). In general, the τ estimator produced older coalescence times relative to the squared differences estimator. The latter might underestimate the coalescence time because it does not take into account the effective size of the population. In our sample, only two haplotypes (13,17,10,24,10,13 and 13,17,11,23,10,13) add up to 43.7% of the Y chromosomes of the Q-M3 haplogroup. When the squared differences in allele size are calculated for all the Y-chromosome pairs, there are many pairs with 0 differences. Therefore the average squared difference underestimates the coalescence time of all the Y chromosomes of the sample. The use of the parameter $4N_e\mu$ corrected this bias, because within a population the estimated time of divergence between two chromosomes depends on the difference of allele size, the effective size of the population, and the mutation rate.

The approach used to estimate the number of mutations between two Y chromosomes supposes a steady-state distribution of the allele sizes; in other words, it assumes that the distribution of the allele sizes fits the expectation under a stepwise mutation model, and the frequency distribution of all six microsatellites fitted a single-step mutation model (data not shown). Estimated coalescence times from mtDNA data (7,452 and 9,834 years before present) roughly agree with the estimate from the Y-chromosome data (3,113 and 13,243 years before present). It might be argued that microsatellite loci and RFLP polymorphisms evolve at very different rates and thus that any comparison between Y-chromosome and mtDNA data would be meaningless. However, although the coalescence times are expressed in mutational units ($\tau/2\mu_t$), they are corrected according to the mutational rates of each genetic system. Last, it must be pointed out that all the estimates from both Y-chromosome and mtDNA data (see Table 5 for confidence intervals) are compatible with a Chibchan origin of approximately 6,000 years before present, which was estimated using classical markers (Barrantes et al. 1990), linguistic data (Constenla-Umaña 1991), and archeological data (Cooke and Ranere 1992).

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