mtDNA Variation in the Chibcha Amerindian Huetar from Costa Rica

MARÍA SANTOS,[1] R.H. WARD,[2] AND RAMIRO BARRANTES[1]

Abstract   The genetic variation in a Chibcha-speaking Amerindian tribe from lower Central America, the Huetar, was analyzed using nucleotide sequences of the hypervariable segments of the mitochondrial DNA (mtDNA) control region, the frequencies of 10 Amerindian-specific mtDNA haplotypes, and the regional distribution of private protein polymorphisms. The sequencing of 713 base pairs (bp) in the control regions of 27 individuals revealed 11 distinct lineages. These were defined by 24 variable sites and a 6-bp deletion between nucleotide pairs (np) 106 and 111. The 6-bp deletion is a new mtDNA marker that will be valuable for Amerindian taxonomic research. Control region sequences and mtDNA haplotype analyses reveal that Huetar mtDNAs are distributed in "Amerindian clusters" A, B, and D. A maximum-likelihood phylogenetic tree suggests a single origin for the 6-bp Huetar deletion in the sample. mtDNA haplotype analysis and the presence of previously characterized private protein variants (*PEPA*F*, *TF*DCHI*, and the absence of *DI*A*) show that the Huetar harbor polymorphisms of considerable antiquity, suggesting an early divergence from the regional founder gene pool for this population. The data also reflect a drastic constriction in population size, an evolutionary event with a proposed central effect on Huetar genetic structure.

The genetic singularity of Amerindian groups has been the subject of recent studies at the protein (Salzano and Callegari-Jacques 1988), nuclear (Kidd et al. 1991), and mtDNA levels (Ward et al. 1991; Torroni et al. 1992). In this context analysis of genetic variation of Chibcha-speaking tribes from lower Central America has revealed the presence of five rare variants and six private polymorphisms at the protein level that characterize the tribes as a distinctive stock (Barrantes et al. 1990). One of these groups not studied previously is the Huetar, whose genetic

affinities with other local tribes are uncertain. The Huetar were decimated almost to extinction during the sixteenth and seventeenth centuries and now exist as two remnant populations in Costa Rica. This study aims to further elucidate the interrelations of the Huetar with other Chibchan tribes by analyzing the genetic variability at their mtDNA control regions and restriction site haplotypes and by examining the presence of regional and private polymorphisms of considerable antiquity at the protein level.

Materials and Methods

Population Sample.   Although the Huetar (or Güetar) language is actually extinct, historical evidence suggests that Huetar communities could be descended biologically and culturally from a population that occupied most of the Costa Rican Central Valley during the sixteenth century and could represent the most numerous (probably around 40,000) and influential group. At contact times the Huetar inhabited an area between the Talamanca and Guatuso tribes [see Barrantes et al. (1990)]. Probable contacts with neighboring groups include the "Chorotegas" (Ibarra 1988), the Guatuso (Johnson 1948), and the Cabecares (Quesada 1990) as well as white and black groups. An estimate of genetic admixture based on genetic markers gives a value of 4–30% (R. Barrantes, unpublished data, 1991). Decimated to a dwindling population size of approximately 855 (Tenorio 1988), the Huetar fissioned and now occupy land in the Quitirrisí (present study) and Zapatón reserves. Quitirrisí (9°50'6" N, 84°15'10" W) is 32 km from the capital city of San José and has an estimated population of 642.

Blood samples were collected during two field trips to Quitirrisí in February 1989 and March 1990 and were separated for protein and DNA analyses. To minimize the effects of genetic infiltration, we removed from the sample all individuals possessing alleles from the ABO and Rh systems that could be attributed to admixture. Based on demographic and genealogical studies, we were able to identify 27 maternally nonrelated individuals for mtDNA studies.

Cell Protein, Blood Groups, and Plasma Protein Systems.   Ninety individuals were analyzed for the following polymorphic systems containing Chibchan private alleles [as described by Barrantes et al. (1990)]: acid phosphatase (ACP1), esterase A (ESA), glutamate-oxaloacetate transaminase (GOT), glucose-6-phosphate dehydrogenase (G6PD), lactate dehydrogenase (LDH), peptidase A (PEPA), triosephosphate isomerase (TPI), Diego (DI), and transferrin (TF).

DNA Extraction.   Venous blood samples were collected in tubes containing ACD and digested in a solution of 10 mM Tris (pH 8.0), 2 mM

EDTA, and 10 m*M* NaCl containing 100 μg/ml proteinase K (Promega). This solution was incubated overnight at 37°C and extracted twice with water-saturated phenol (pH 8.0) and once with chloroform/isoamyl-alcohol (24:1). The samples were then precipitated with ethanol and re-suspended in TE buffer.

PCR Amplification and Direct Sequencing.   Target sequences were amplified in a 30-μl reaction mixture containing 0.6 μg genomic DNA, 20 nmol of each deoxynucleotide (dATP, dCTP, dGTP, dTTP; Phar-macia), 100 pmol of each amplification primer, 2 units (U) of *Taq* DNA polymerase, and the enzyme buffer (10×) for 30 cycles. Each cycle consisted of denaturation at 93°C for 45 s, annealing at 55°C for 1 min, and extension at 74°C for 3 min. Primers L15997:H16401 (Ward et al. 1991) and L29 (5′-GGTCTATCACCCTATTAACCAC-3′):H376 (5′-TGAAATCTGGTTAGGCTGGT-3′) were used to amplify the 5′ and 3′ ends of the control region, respectively. Three microliters of the poly-merase chain reaction (PCR) product were used to seed two asymmetric amplifications using a 1:50 ratio of two internal primers. After purifi-cation of the single-stranded DNA by Centricon-30 microconcentrators, 7 μl of the retentate were dideoxy-sequenced using the Sequenase kit (United States Biochemical). Reaction products were separated by elec-trophoresis through 6% polyacrylamide gels containing 7 *M* urea. Gels were fixed in 5% glacial acetic acid/5% methanol for 30–60 min, dried, and exposed to Kodak XAR film for 12–48 hr [as described by Ward et al. (1991)].

Restriction Endonuclease Digestion.   Ten microliters of the ampli-fication product were digested with 2 U of the corresponding endonu-clease according to the amplified sequence and 2 μl of the appropriate buffer. This solution was incubated for 2–4 hr at 37°C. The presence of the restriction site was determined by electrophoresis in agarose or poly-acrylamide gels run at 35 mA for 4–5 hr. The primers used were L8215:H8297 and L7773:H8001 (Ward et al. 1991) and those described in Table 1.

Data Analysis.   Sequences were read into the computer, aligned, and compared using the ESSE computer program (Cabot 1988). The pairwise sequence differences presented here are derived from direct sequence comparisons. Phylogenetic trees based on a 713-nucleotide sequence from hypervariable parts of the mtDNA control region were constructed using the maximum-likelihood algorithm of the PHYLIP (Felsenstein 1989) and PAUP (Swofford 1989) computer programs and assuming a 1:30 trans-version to transition ratio.

Table 1. Oligonucleotide Primers Used for PCR Amplifications

Primer	Sequence
L13197	5'-GCGCTATCACCACTCTGTTC-3'
H13366	5'-GGTTGTGGATGATGGACCCG-3'
L00762	5'-CACGCAGCAATGCAGCTCA-3'
H01139	5'-TAAGCTGTGGCTCGTAGTGT-3'
L00602	5'-GTAGCTTACCTCCTCAAAGC-3'
H00805	5'-ATCACTGCTGTTTCCCGTGG-3'
L13925	5'-CTAGCATCACACACCGCACA-3'
H14446	5'-CTACAGCGATGGCTATTGAG-3'

Results

mtDNA Analysis

Sequence Polymorphisms. The complete sequence of a 390-bp seg-
ment [5' region between positions 16003 and 16392 according to the
published sequence (Anderson et al. 1981)] and a 323-bp segment (3'
region between positions 48 and 370) of the mtDNA control region was
determined for 27 individuals. Polymorphisms were caused by transi-
tions and length mutations. Most of the transitions (91.7%) at the 5'
region (which contains 55.8% pyrimidines and 44.2% purines) involve
pyrimidines, with C → T as the most common change. For the 3' region
(which contains 54.2% pyrimidines and 45.2% purines), purine transi-
tions were more frequent (54.5%), with A → G being the most common
change. The variability was not randomly distributed for either region
(Poisson, $p \leq 1\%$ and $\leq 5\%$, respectively).

Only two mutations, a transition at position 263 and a C insertion
at position 311, were found in all sequences. All variable sites at the 5'
region have been detected in other populations, although sites 89, 97,
106–111, 235, and 237 (at the 3' region) have not been detected as
variables before.

Hypervariable Domains. Horai and Hayasaka (1990) previously de-
scribed a hypervariable domain between np 16180 and np 16193. When-
ever a T → C transition occurs at np 16189, the DNA polymerase ap-
parently has difficulty reading through the resulting 10 C homopolymer,
hindering further sequencing beyond this site [polymerase stuttering, a
condition also observed in other Chibchan sequences (M. Santos, un-
published results, 1991)]. According to Horai and Hayasaka (1990), the
T → C mutation has independently occurred several times in different
lineages, including but not exclusive to those carrying the Asian 9-bp
deletion observed in the present study and in 32% of a pooled Amer-

Table 2. mtDNA Sequences in Huetar Hypervariable Domains

Lineage	Sequence	Frequency (%)
5' region		
1 (reference)	AAAACCCCCTCCCC	59.3
2[a]	AA??????CCCCCC	29.6
3	AAAACCCTCTCCCC	11.1
3' region		
1 (reference)	AAA--CCCCCCCT-CCCCC	0
2	AAA--CCCCCCCTCCCCCC	33.3
3	AAA-CCCCCCCCTCCCCCC	18.5
4	AAACCCCCCCCCTCCCCCC	48.2

a. Stutters.

indian sample (Horai et al. 1993). The Huetar show three sequences based on the varying nucleotide composition of this homopolymer (Table 2). The most frequent lineage in the Huetar and in Horai et al.'s (1993) Amerindian study is identical to the reference sequence (Anderson et al. 1981). Because of polymerase stuttering, sequence 2 cannot be compared with other published sequences. Sequence 3 was observed in 15% of Native Americans (Horai et al. 1993) and in 3% of Japanese (Horai and Hayasaka 1990).

   Another hypervariable domain [which was not described by Horai and Hayasaka (1990)], encompassing np 303–315, was observed in the 3' segment of the control region. In this region a 12 C homopolymer sequence is interrupted by a T at np 310. The Huetar exhibit three different sequences in this region, including two sequences previously observed in the !Kung (Table 2) (Vigilant et al. 1989).

Characterization of the 6-bp Huetar Deletion.   A unique 6-bp deletion was discovered among Huetar control region sequences (Figure 1). This marker, the 6-bp Huetar deletion, occurs between np 106 and 111 (Santos 1992). It shares sequence identity with the first 5 bp of the preceding 6-bp sequence. In this respect it resembles the second part of a tandem duplication, with one copy being lost in the creation of the Huetar 6-bp deletion. The deletion also shares symmetric elements flanking the repeated region, suggesting that this length polymorphism was generated through slipped misreplication (Krawczak and Cooper 1991):

5'-CC∗GGAGCC∗GGAGCA∗CC-3'

normal sequence: 5'-GGAGCCGGAGCA-3'

deleted sequence: 5'-GGAGCC-3'

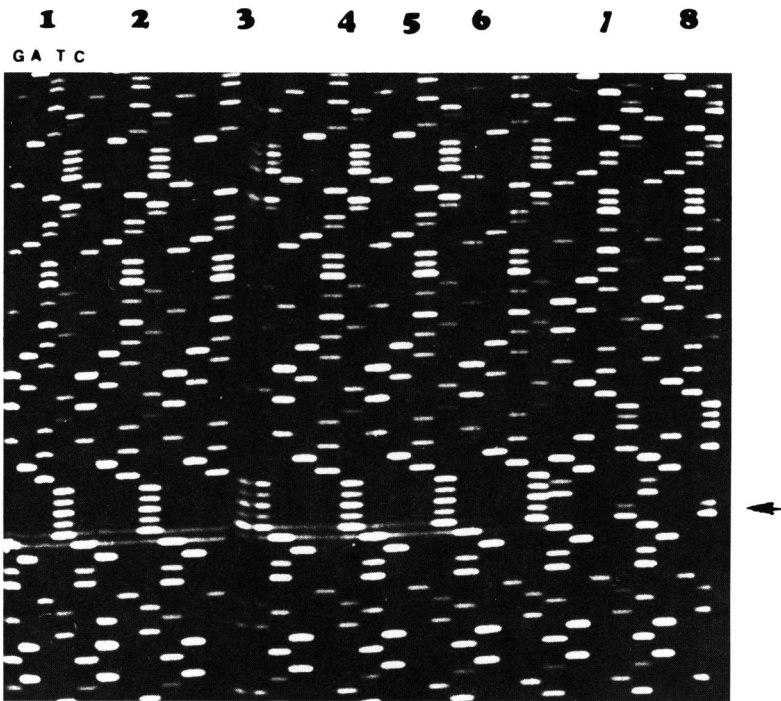   Six Huetar control region lineages were found to contain the 6-bp

Figure 1. Autoradiogram of a 6% polyacrylamide gel revealing the 6-bp Huetar deletion characterized here (samples 1–6). Samples 7 and 8 are from individuals not carrying the deletion.

deletion. Overall, the deletion was observed in 16 of 27 individuals (59%). Based on phylogenetic analysis, these lineages are grouped in a cluster of related sequences of relatively recent occurrence (see Figure 3).

Sequence Diversity. The 5′ region sequence data allow the identification of seven lineages; the 3′ region sequence data identify 9 lineages. This shows a higher genetic diversity in the Huetar in the last segment, although the effect of polymerase stuttering, which hinders other 5′ end lineages from being identified, cannot be ignored. Merging the sequences for both segments reveals 11 lineages (Figure 2), encompassing 24 variable sites. Three lineages (H1, H3, and H10) represent 63% of the sample.

A comparison of Huetar lineages with !Kung lineages (Vigilant et al. 1989) results in no lineages in common, as expected. Because no analogous sequences for the 3′ region from other Amerindian populations have been published [Horai et al. (1993) included only np 1–36], the populations can be compared only for the 5′ region. The comparison
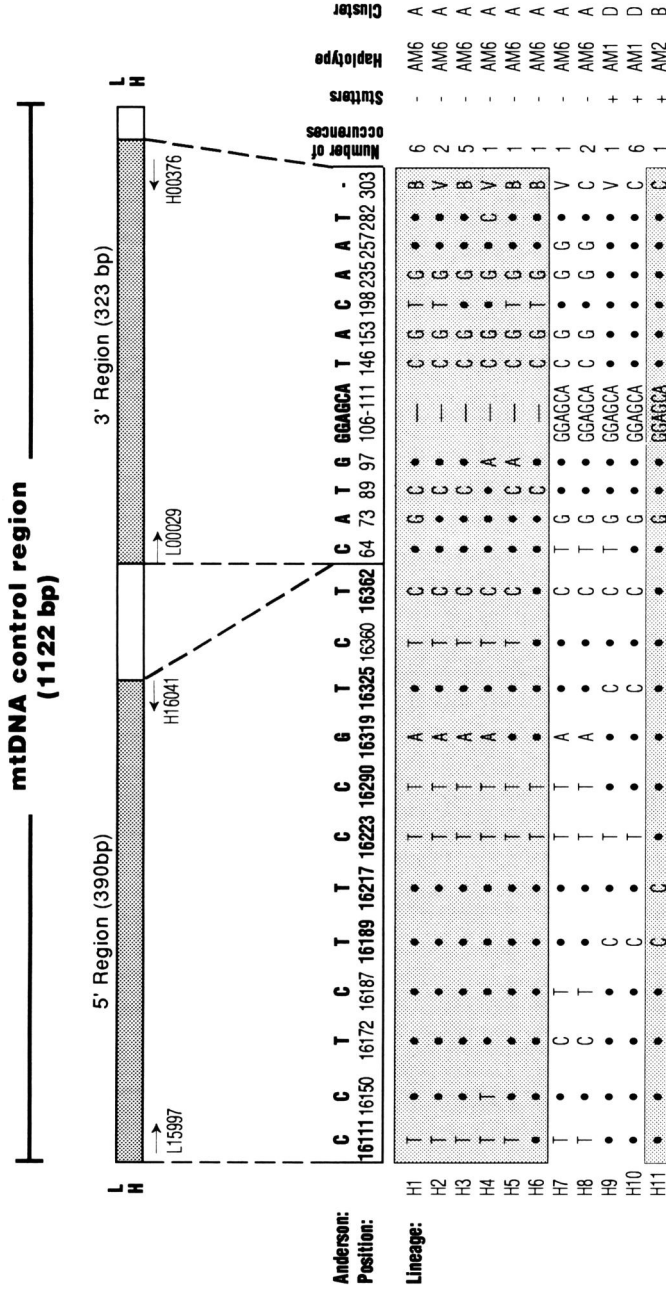
Figure 2. Variable sites in the mtDNA control region defining 11 lineages found in the sample. Dots indicate identity with the published sequence (Anderson et al. 1981); dashes represent deletions; B and V indicate the insertion of 2 and 1 C's, respectively. The two shaded regions highlight the lineages carrying the 6-bp Huetar deletion (lineages H1–H6) and the Asian deletion (lineage H11). The number of individuals carrying each lineage, their respective haplotypes, and the stutter lineages are indicated on the right-hand side of the figure.

Table 3. Control Region Variable Sites Defining the Four Amerindian mtDNA Clusters [after Horai et al. (1993)]

	Torroni et al. (1992)	Horai et al. (1993)	Anderson et al. (1981) Reference Sequence											
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
			C	T	T	T	C	C	T	G	T	C	T	T
Cluster	A	III	T	·	·	·	T	T	·	A	·	·	C	(a)
Cluster	B	I	·	·	C	C	·	·	·	·	·	·	·	C
Cluster	C	IV	·	·	·	·	T	·	C	·	C	T	·	(a)
Cluster	D	II	·	T	·	·	T	·	·	·	C	·	C	(a)

1 = 16111; 2 = 16187; 3 = 16189; 4 = 16217; 5 = 16223; 6 = 16290; 7 = 16298; 8 = 16319; 9 = 16325; 10 = 16327; 11 = 16362; 12 = 16519.

a. Each of these lineages may have either a T or a C at np 16519.

of the Huetar lineages with the Nuu-Chah-Nulth lineages from North America (Ward et al. 1991) resulted in only one lineage in common (lineages H11 and 27) with frequencies of 4% and 2%, respectively. A further comparison (between np 16129 and np 16392) with a sample of 72 Amerindians (Horai et al. 1993) results in three lineages (H9 and H10, which are identical for the 5′ region, and again H11) that differ from lineages 3 and 13, respectively, by a unique mutation positioned in the 5′ end of the hypervariable domain.

A number of particularly informative sites (Table 3) and two additional polymorphisms detected in other Amerindian populations (Horai et al. 1993) but not among Huetar lineages are highlighted in Figure 2; the two additional polymorphisms are at np 16298 and np 16327. Collectively, these polymorphisms characterize the four clusters of haplotypes that define Amerindian mtDNAs through restriction fragment length polymorphism (RFLP) analysis (Torroni et al. 1992) and through control region sequencing [detected by Ward et al. (1991) and Horai et al. (1993)]. Table 3 reveals that specific mutations identify each lineage, and some of these distinctive mutations are shared between lineages, indicating that they may have a deep genetic root in Asia. The absence of polymorphic sites exclusive of cluster C haplotypes (np 16298 and 16327) in the Huetar control region sequences is consistent with the restriction site data presented in this study.

An estimate of variation given by the diversity value h [Nei 1987, Eq. (8.5)] was 84% (excluding length mutations). The identity index indicates that identical individuals are more likely in the Huetar (0.19) than in the Nuu-Chah-Nulth (0.06) or hunter-gatherer groups from Africa (0.13) (Ward et al. 1991; Vigilant et al. 1989), whose populations are several orders of magnitude larger. An estimated nucleotide diversity value (c) [Nei 1987, Eq. (10.5)] of 0.0044 (excluding length mutations) cannot be compared with other populations because no analogous value

was found. In addition, the mitochondrial sequence difference in the Huetar was compared with the amount of mitochondrial diversity found in the Nuu-Chah-Nulth in terms of nucleotide differences (restricted for 360 bp from the 5′ end, common for the 35 lineages); sequence difference values of 0.55% and 1.47%, respectively, were found. The data indicate that the average number of sequence differences among the 27 Huetar lineages amounts to 37% of the sequence differences observed among the 63 Nuu-Chah-Nulth.

A similar comparison of direct pairwise 5′ region sequences among all the lineages (16) containing the deletion allows a tentative estimation of the age of the 6-bp deletion in the Huetar. From these sequences the average sequence difference is 0.07% (0.25 substitution) for a 360-bp segment, for which Ward et al. (1991) estimated an evolution rate of 33% divergence per million years. According to this value, the approximate age would be 2120 years. Taking into account that population statistics underestimate the time of molecular divergence and that this marker was detected in the Kuna (unpublished results, 1991), we suggest that this deletion most likely occurred 3000–5000 years ago.

Figure 3 depicts the phylogenetic tree obtained from the maximum-likelihood method (excluding length polymorphisms and restriction sites). The fact that the three haplotypes belonging to different clusters segregated into analogous groups of control region sequences reveals a strong correlation between both analyses.

Analysis of Sequence Variability Outside the Control Region

Asian 9-bp Deletion. The enzymatic amplification of region V revealed the presence (4%) of the Asian 9-bp deletion described by Wrischnik et al. (1987). This 9-bp deletion is carried by the unique lineage shared by Huetar (H11) and Nuu-Chah-Nulth (lineage 27) samples and is almost identical to one (lineage 3) observed in a pooled Amerindian study (Horai et al. 1993). This is in agreement with Wallace and Torroni's (1992) assertion that although this mutation was found to have occurred more than once, most of the lineages associated with it were derived from a single ancient event and are closely related.

Haplotype Analysis. The restriction isotyping technique was applied for screening seven sites outside the control region (*Hae*III np 663; *Msp*I np 931; *Alu*I np 13245, 13262, 14015, and 14304; and *Hinc*II np 7853), adding 26 sites to the analysis and summarizing 739 bp per sample.

By combining information extracted from 11 restriction sites plus the Asian 9-bp deletion for the Pima (Arizona), Maya (Honduras), and Ticuna (Brazil), Schurr et al. (1990) identified 10 Amerindian-specific haplotypes. When screening these sites in the Huetar, we found haplo-
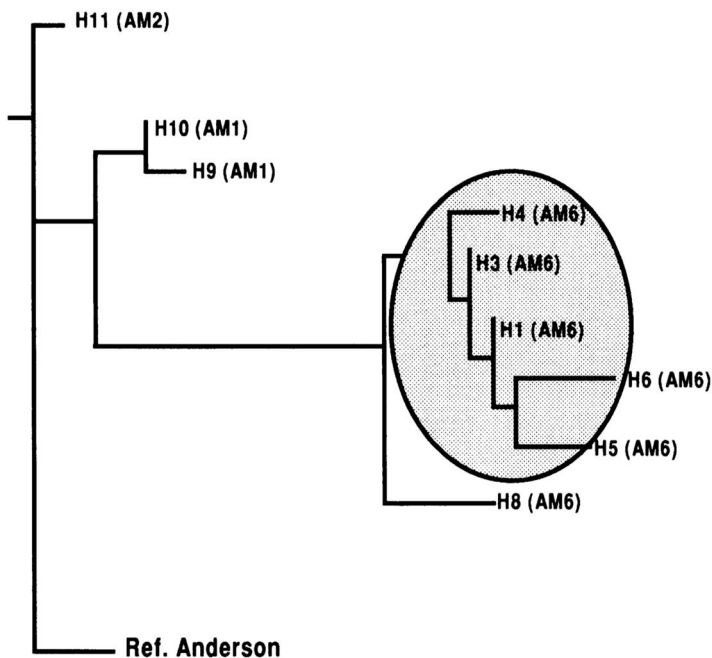
Figure 3. Phylogenetic tree for nine Huetar mtDNA lineages obtained from the maximum-likelihood method using the reference sequence (Anderson et al. 1981) as an outgroup to root the tree. Two lineages (H2 and H7) generated by length mutation at hypervariable domains were excluded from the analysis. The data were also analyzed by the maximum parsimony method, giving equivalent results. Restriction site haplotype segregation is indicated for each control region lineage.

types AM1 (25.9%), AM2 (3.7%), and AM6 (70.4%)—three of the four haplotypes designated as ancestral, that is, those most likely carried by the first Asian immigrants (Schurr et al. 1990). According to Torroni et al. (1992), three of the sites mentioned before—the *Hae*III np 663 site gain, the Asian 9-bp deletion, and the *Alu*I np 13262 (or *Hinc*II np 13259) site loss—have been found to define three of the four Amerindian mtDNA clusters (A, B, and C, respectively). In addition, Torroni et al. (1992) related Schurr et al.'s (1990) AM1, AM2, and AM6 haplotypes to clusters D, B, and A; respectively. The data reveal a higher frequency of cluster A haplotypes (70%), as detected by Horai et al. (1993), for Amerindian Maya and others originating from northern South America, supporting Horai's assertion that cluster A lineages are derived from an ancestral population that contributed greatly to the colonization of central and northern South America.

The Huetar turn out to be the less variable tribe, harboring only three haplotypes and an *h* value of 45%, compared with 75%, 77%, and 74% for the Pima, Ticuna, and Maya, respectively.

Private Polymorphism Screening at the Protein Level. Genetic screening for certain rare variants and private polymorphisms show the presence of *PEPA*F* (0.304) and *TF*D+CHI* (0.278) and the absence of *DI*A*. The other regional and private polymorphisms (*G6PD*C*, *TPI*3-BRI*, *ACP*GUA1*, *LDHB*GUA1*) were absent from the sample.

Discussion

The general properties of intraspecific sequence differences in the noncoding region of mtDNA observed here confirm several features originally reported by Greenberg et al. (1983). These properties include the predominance of transitions over transversions, the higher prevalence of transitions between pyrimidines (at least for the 5′ region), the presence of small insertions in hypervariable domains (Horai and Hayasaka 1990), and the nonrandom distribution of mutations, which suggests that some selective constraint may be operating at the control region of mtDNA (Horai 1991). Contrary to findings in a study of 15 African aborigines (Vigilant et al. 1989), which motivated many researchers of human mtDNA variation to rely solely on the 5′ region, the Huetar present more variability at the 3′ end. The characterization of another hypervariable domain (np 303–315) that contains mutations which could have originated by slipped mispairing and therefore most likely arose independently in different populations may be useful because it may obscure true phylogenetic relationships (Stewart 1993).

Several evolutionary events that result in a random loss of lineages may have contributed to the absence of divergent haplotypes in the sample, the loss of cluster C haplotypes, and the reduced genetic diversity of control region sequences among the Huetar. A random drift effect may have followed the separation of the ancestral proto-Chibchan populations from other Amerindian populations. Evidence based on blood markers suggests an analogous effect on nuclear DNA, resulting in a loss of genetic variation during the migration from North America to South America (Mourant et al. 1976). An example of this may be the loss of the gene responsible for the Di-A antigen in the Chibcha (Layriss and Wilbert 1961; Barrantes 1990).

Second, the Huetar experienced a significant diminution of their population size after sixteenth-century contact with the Spaniards, probably causing a drastic extinction of mitochondrial lineages. According to historical data, the Huetar were the most numerous of the Costa Rican Amerindian populations (Constenla 1984). Although the size of the Huetar population at the beginning of the sixteenth century is not known, estimates of 400,000 for the total Amerindian population at that time and of 10,000 at the beginning of the seventeenth century (Ibarra 1984) clearly

indicate a vertiginous decline in Costa Rican Amerindian populations between 1500 and 1600.

Furthermore, Ibarra (1984, 1990) describes the Huetar as having been organized in 10 *cacicazgos*, which were distributed through the Central Valley. These *cacicazgos* were significantly decimated by Spanish colonization, epidemics, etc. and were reduced to a single settlement near Ciudad Colón. This remnant population later fissioned into two smaller ones: Quitirrisí, from which the samples in this study were taken, and Zapatón. The fissioning could also have contributed to the loss of Huetar genetic variability. Lineage loss could also have come about through increased admixture with non-Indian peoples (admixed individuals were excluded from the sample) or through the partial sampling of the total mtDNA lineage representation among the Quitirrisí Huetar, thus preventing an accurate estimation of the real variability of the population.

Despite the linguistic diversity of the studied groups, the presence of ancestral haplotypes in the Huetar and in the other three groups located in North, Central, and South America suggests genetic homogeneity for Amerindian populations [as proposed by Schurr et al. (1990)]. According to shared haplotypes, the data reflect, as expected, a stronger similarity with neighboring groups, which is more pronounced to the north. Nevertheless, because of the small sample sizes, this observation does not necessarily imply a stronger genetic affinity. All the Huetar mtDNAs belong to three of the four described clusters of Amerindian mtDNAs, supporting Wallace and Torroni's (1992) assertion that mtDNAs from Amerindian populations come from only four original founding mtDNAs. The Huetar harbor haplotypes predominantly belonging to cluster A, for which Wallace and Torroni estimate a mean divergence time of 17,000–35,000 years. However, according to the phylogenetic analysis of the control region sequences, Huetar mtDNAs might be of more recent divergence. This is inferred from the fact that the Huetar deletion (with 60% prevalence in the sample) occurred later in some of the mtDNAs carrying the *Hae*III np 663 mutation that entered America, suggesting that Huetar mtDNAs are the product of a more recent evolution, which is not reflected by the haplotype analysis. This also points out the superior resolution produced by sequence analysis of hypervariable parts in the control region when studying closely related populations.

Because the proposed date for the 6-bp Huetar deletion is more recent than the generally accepted occupation of lower Central America [8000–10,000 years ago (Snarskis 1984)] and considering the apparent restricted distribution of the deletion, we suggest an in situ evolution for this marker. In addition, based on the estimated date, the deletion should occur in other regional groups, because the analyses from linguistic, geographic, and genetic data (from 48 loci at the protein level) indicate that the proto-Talamancan, proto-Guaymi, and proto-Kuna may have sepa-

rated 5000 years ago (Barrantes et al. 1990). In general, the results argue for an in situ evolution of around 60% of the mtDNA variability, supporting the view based on the presence of private genetic variants of a relatively isolated development of the lower Central American Chibcha.

The unexpected absence of *TPI*3-BRI*, present in the surrounding Guatuso and Talamancan tribes, may be due to the drastic diminution in population size and sampling effects. The occurrence of the protein variants considered older and those most probably present before the division of the major stocks in this region (Guatuso and their kin, proto-Talamancan, proto-Guaymi/Bokota, and proto-Kuna), which may have taken place about 7000 years ago [see Barrantes et al. (1990)], suggests that the Huetar diverged from the founder gene pool relatively early in time.

Taking into account the geographic distribution of the Huetar between the Talamancan and Guatuso tribes and the virtual extinction of this group, the protein results presented here confirm Thompson et al.'s (1992) considerations regarding the age and location of these variants and the possible effect of sampling. The conjunction of information provided by different genetic approaches, supported by linguistic and archeological studies, will help to elucidate the origins and affinities of Chibchan-speaking groups from Central America and will further our understanding of the main questions concerning Amerindian prehistory.

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