

## Reduced mtDNA Diversity in the Ngöbé Amerinds of Panamá

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### ABSTRACT

Mitochondrial DNA (mtDNA) haplotype diversity was determined for 46 Ngöbé Amerinds sampled widely across their geographic range in western Panamá. The Ngöbé data were compared with mtDNA control region I sequences from two additional Amerind groups located at the northern and southern extremes of Amerind distribution, the Nuu-Chah-Nulth of the Pacific Northwest and the Chilean Mapuche and from one Na-Dene group, the Haida of the Pacific Northwest. The Ngöbé exhibit the lowest mtDNA control region sequence diversity yet reported for an Amerind group. Moreover, they carry only two of the four Amerind founding lineages first described by Wallace and coworkers. We posit that the Ngöbé passed through a population bottleneck caused by ethnogenesis from a small founding population and/or European conquest and colonization. Dating of the Ngöbé population expansion using the HARPENDING *et al.* approach to the analysis of pairwise genetic differences indicates a Ngöbé expansion at roughly 6800 years before present (range: 1850–14,000 years before present), a date more consistent with a bottleneck at Chibcha ethnogenesis than a conquest-based event.

IT is clear that humans occupied the New World more recently than any other continental landmass. However, archaeologists, geneticists, anthropologists and linguists disagree strongly over the timing, direction and number of colonization events (MELTZER 1993). One of the most firmly defended occupation scenarios (GREENBERG *et al.* 1986) proposed three distinct “waves” of migration that brought the Paleoindians (purportedly ancestral to all modern Amerinds), Na-Dene and Eskimo-Aleut to the New World. Recent support for the three wave hypothesis has come from mitochondrial DNA (mtDNA)-based studies, which suggest that mtDNA diversity differences between Amerind and Na-Dene indicate two distinct migrations for these groups (TORRONI *et al.* 1992, 1993). Of course, diversity-dependent arguments need first to establish empirically the nature of the diversity and, at present, only a small number of New World indigenous populations have been assayed for mtDNA sequence polymorphism. Herein we present mtDNA sequence data on the Chibcha-speaking Ngöbé Amerinds (sometimes known as Guaymí or Ngawbe), which we determined to carry low levels of mtDNA diversity. Smaller population samples of eight Chibchan groups (including the Ngöbé) have been previously demonstrated to exhibit low numbers of mtDNA haplogroups based primarily on restriction fragment length polymorphism (RFLP) analysis (TORRONI *et al.* 1994a; SANTOS *et al.* 1994).

Residents of western Panamá, the Ngöbé speak a lan-

guage assigned to the Chibchan stock (Paya-Chibchan phylum) currently spoken by indigenous groups distributed from Nicaragua to Colombia (CONSTENLA-UMAÑA 1991). Linguistic, standard genetic marker, ethnohistoric and archaeological data suggest that the Ngöbé descend from populations that have resided in or near their current location for several millennia (YOUNG 1971; LINARES and RANERE 1980; BARRANTES *et al.* 1990; CONSTENLA-UMAÑA 1991; COOKE and RANERE 1992). Their identification in historic documents is confounded by the fact that the term Guaymí applies to both the Ngöbé and their neighbors, the Buglé (or Bokotá), who speak a related, but mutually unintelligible language. A Spanish document of 1709 indicates that the Guaymí numbered 8000 at this time (FERNÁNDEZ 1976). In the 20th century, the Ngöbé population has expanded rapidly, from 20,000–40,000 in the 1940s (GJORDING 1991) to 125,000 individuals in 1990 (Panamanian government census) establishing the Ngöbé as the largest Chibchan group both in terms of numerical size and geographic range.

We compare Ngöbé mtDNA sequence data with similarly sized samples obtained from two other Amerind groups located near the northern- and southern-most extremes of Amerind geographic distribution, 63 Nuu-Chah-Nulth of the Pacific Northwest (WARD *et al.* 1991) and 38 Chilean Mapuche from the Chilean coast (GINTHER *et al.* 1993), and with one Na-Dene group represented by 40 Haida of the Pacific Northwest (WARD *et al.* 1993). Recent tribal history, including cultural admixture and the dramatic demographic impact

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FIGURE 1.—Geographic distribution of Ngöbé Amerinds and location of collection sites. The five collection sites are represented by letters: A, Bocas del Toro coastal sites ( $n = 15$ ); B, Río Cricamola ( $n = 5$ ); C, Soloy ( $n = 7$ ); D, Hato Chamí ( $n = 9$ ); and E, Tolé ( $n = 10$ ). The geographic range of modern Ngöbé is indicated by the line encircling the letters.

of European conquest, caution against overzealous cross-cultural comparisons between the sampled groups. Nevertheless, the increasing number of mtDNA studies of modern native peoples on both sides of the Bering Strait provide a powerful incentive for examining the advantages and limitations of mtDNA as a tool for exploring the historical processes at work in human populations.

#### MATERIALS AND METHODS

**Population samples:** Blood samples were collected from individuals in five population clusters located at widely separated locations across the area currently occupied by the Ngöbé in Panamá (Figure 1). Biographical information was recorded for each individual and included names, birthplaces, and languages spoken by their parents and grandparents. Forty-six individuals, unrelated in at least the fourth degree of consanguinity, were chosen for mtDNA sequence analysis based on family histories suggesting no obvious, *i.e.*, recent, admixture with other Amerind or non-Amerind populations.

**Isolation, amplification, and sequencing of DNA:** Blood (20–30 ml) was collected into Vacutainer tubes (Becton Dickinson) containing ACD buffer (acid citrate/dextrose). DNA was isolated from leucocytes in one of two ways. In the first method, the leucocytes were pelleted and treated with proteinase K followed by organic extraction and precipitation of the DNA. The second method of DNA isolation involved lysis of the erythrocytes followed by pelleting of the leucocytes.

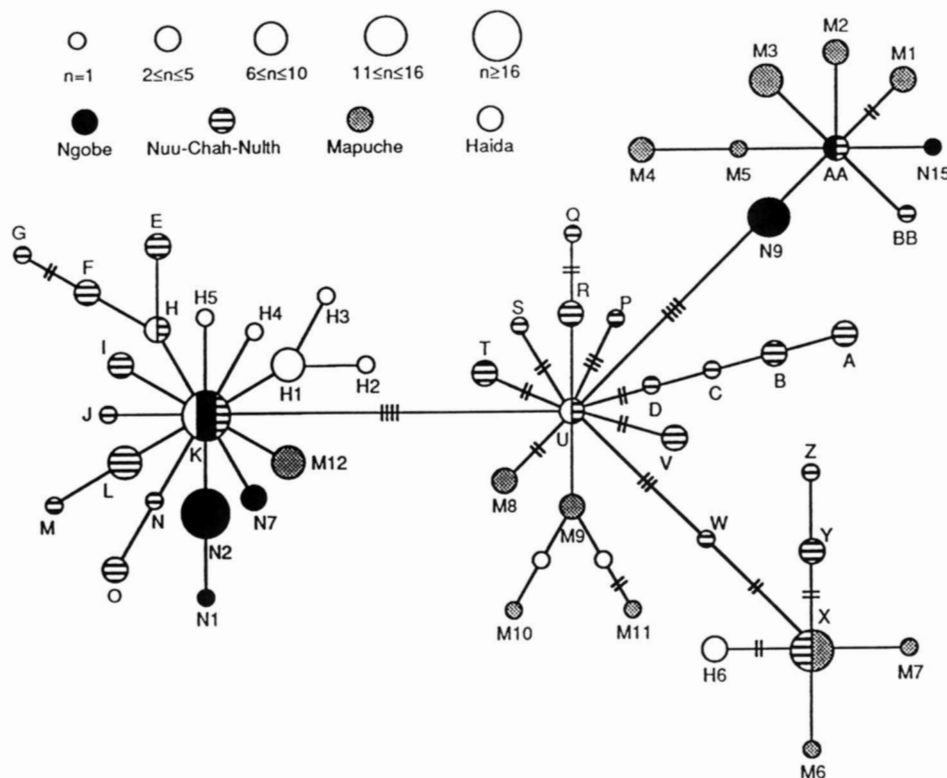
Primers L15997:H16401 (WARD *et al.* 1991) and L00029 (5'-GGTCTATCACCTATTAACCAC-3'):H00408 (5'-CTGTTAAAGTGCATACCGCCA-3') were used to amplify regions I and II of the control region, respectively, (nomenclature from VIGILANT *et al.* 1989) for each individual. An initial, balanced amplification reaction was carried out for 25 cycles using the following reaction conditions: 67 mM Tris-HCl, pH 8.8, 2 mM MgCl<sub>2</sub>, 250  $\mu$ M each of dATP, dCTP, dTTP and dGTP, 250  $\mu$ g/ml bovine serum albumin, 0.4  $\mu$ M of each primer and 0.625 units *Thermus aquaticus* DNA polymerase (USB). The amplification product was agarose-purified and used to seed a second, asymmetric amplification that was carried out for 35 cycles in the reaction conditions described above except that one primer was reduced in concentration by a factor of 50. (Thermal cycler conditions: 94° for 45 s, 55° for 1 min, 72° for 2 min). The final amplification products were purified through Centricon-30 microconcentrators and sequenced using Sequenase Version 2.0 (USB). Due to a T-to-C mutation at position 16189, which caused the *Taq* polymerase to stutter,

we could only confidently read sequence in each direction until the location of the transition for the 15 individuals carrying this mutation. In all other cases (the remaining 31 individuals for region I, all 46 individuals for region II) the sequences analyzed were completely verified through full overlap of light and heavy strands.

**Restriction fragment length polymorphism analysis:** Limited RFLP analysis was performed on the sequenced individuals to permit comparison of our data to the four major mtDNA restriction site classes defined by SCHURR *et al.* (1990) and TORRONI *et al.* (1992, 1993); mtDNA restriction site classes are henceforth referred to as mtDNA haplogroups following the nomenclature used in TORRONI *et al.* 1993). Three sets of primers were used in balanced PCR reactions (run for 29 cycles) to screen for presence of the *Hae*III site at bp 663 (haplogroup A), the COII/tRNA<sup>Leu</sup> intergenic deletion (haplogroup B), and the *Aha*I site at bp 13262 (haplogroup C); haplogroup D was defined by absence of the above three sites. The polymorphic sites we used to identify the four mtDNA haplogroups are different than those used in TORRONI *et al.* (1992, 1993), but are supported by data presented in this paper. The primer pairs were L577 (5'-GTTTATGTAGCTTACCTCCTC-3'):H743 (5'-GATCGTGGTGATTAGAGGGTG-3'), L8215:H8297 (WARD *et al.* 1991) and L13232:H13393 (WARD *et al.* 1991). Primers were annealed at temperatures of 55, 55 and 49°C, respectively and, except for the one noted change in annealing temperature, reaction and thermal cycler conditions were as described above. PCR products were electrophoresed through 15% polyacrylamide gels.

**Diversity, phylogenetic, and statistical analyses:** Haplotype diversity,  $h$  (NEI and ROYCHOUDHURY 1974) and two measures of nucleotide diversity,  $\pi$  (NEI and TAJIMA 1981) and  $E(\nu)$  (WATTERSON 1975) were calculated. Sequence differences between mtDNA haplotypes were measured using Kimura's two-parameter model. These genetic distance data were summarized by the neighbor-joining (NJ) algorithm in NTSYS (ROHLF 1993). Phylogenetic trees were constructed with the Phylogenetic Analysis Using Parsimony package (PAUP; SWOFFORD 1989), although computational limitations prevented us from analyzing all possible trees. Minimum-spanning trees (MSTs) were constructed by hand following the recommendations of EXCOFFIER and colleagues (1992) and used in the analysis of molecular variance (AMOVA) program provided by L. EXCOFFIER. The AMOVA analyses were performed using a squared Euclidean distance matrix based on the number of nucleotide differences between pairs of mtDNA haplotypes and a distance matrix calculated from the evolutionarily parsimonious network shown in Figure 2. Significance of variance terms was tested by permuting the original data 1000 times. Pairwise genetic difference analyses were performed using the programs described in ROGERS and HARPENDING (1992) and HARPENDING *et al.* (1993) and population expansion estimates were based on the mutation rate for mtDNA control region I determined by HARPENDING *et al.* (1993).

**Data analyzed:** In analyses specific to the Ngöbé, we compared 701 bases across the 46 individuals analyzed: bp 16040–16400 (region I) and bp 39–380 (region II). In analyses across cultural groups, we used all 63 Nuu-Chah-Nulth mtDNA sequences determined by WARD *et al.* (1991), 38 Mapuche mtDNA sequences (GINTHER *et al.* 1993; the fourth haplotype of pattern 3 was not used because of missing sequence data at position 16362) and 40 Haida mtDNA haplotypes (WARD *et al.* 1993; sequence 35 was eliminated because of suspected non-Native American origin). The 45 Chilean Amerind mtDNA sequences determined by HORAI *et al.* (1993) gave levels of diversity similar to the Mapuche but, because no information on the ethnic identity of the samples was available, those results are not presented here. To compare haplo-





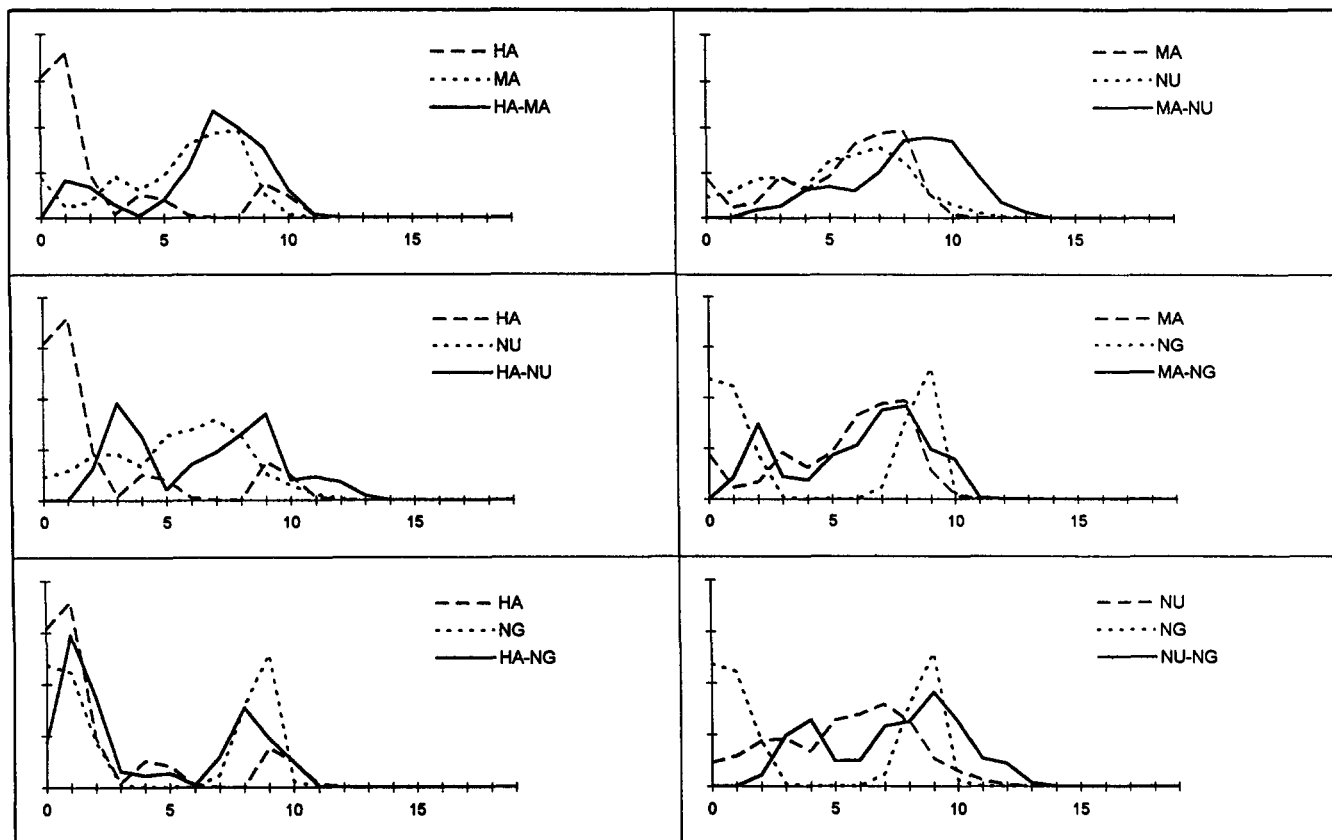


FIGURE 3.—Mismatch distributions from Ngöbé, Nuu-Chah-Nulth, Mapuche and Haida populations (abbreviated NG, NU, MA and HA, respectively, and depicted by the dotted and dashed lines) and intermatch distributions (solid lines) of all six population pairs. The X-axis represents pairwise differences in units of mutational difference (one unit is equivalent to 9300 years, HARPENDING *et al.* 1993) and the Y-axis represents the frequency of each pairwise difference from 0.0 to 0.4 in increments of 0.1.

Nuu Chah Nulth, 14%; Ngöbé/Mapuche, 21%; Ngöbé/Haida, 19%; Nuu Chah Nulth/Mapuche, 14%; Nuu Chah Nulth/Haida, 15%; and Mapuche/Haida, 40% ( $P < 0.001$  for all six analyses). However, when investigated at the broader linguistic level of Amerind (as represented by the Ngöbé, Nuu-Chah-Nulth and Mapuche) *vs.* Na-Dene (as represented by the single Haida sample), AMOVA failed to detect statistically significant differences between these two linguistic groups. Six percent of the variance was partitioned between Amerinds and Na-Dene ( $P = 0.25$ ), 16% among Amerind populations ( $P < 0.001$ ) and 78% within Amerind and Haida populations ( $P < 0.001$ ).

**Pairwise genetic difference distributions:** Mismatch distributions were calculated for the Ngöbé, Nuu-Chah-Nulth, Mapuche and Haida and intermatch distributions were calculated for all population pairs (Figure 3; ROGERS and HARPENDING 1992; HARPENDING *et al.* 1993). Mismatch distribution refers to the pairwise count of nucleotide differences within a population and intermatch distribution refers to the mismatch distributions of sequences from two different populations. All four groups shared a peak in their mismatch distributions at seven to nine units of mutational time suggesting a common expansion at 84,000–65,000 YBP.

A second major peak was revealed in the mismatch distributions of the Ngöbé and Haida suggesting a second, more recent expansion. This expansion occurred at 0.74 units of mutational time in the Ngöbé or roughly 6800 YBP. This expansion date was based on the estimate of 9300 years/unit of mutational time as calculated by HARPENDING *et al.* (1993). They utilized a divergence rate for mtDNA control region I of 30%/million years that was determined by WARD *et al.* (1991) using a human/chimpanzee divergence date of ~4 mya based on molecular genetic data. Paleontological data, however, support a human/chimp divergence closer to nine million years ago which would result in a mtDNA control region I divergence rate of 15%/million years (WARD *et al.* 1991) and an estimated Ngöbé expansion at 14,000 YBP. A more extreme divergence rate for mtDNA control region I based on a coalescent model has been calculated by LUNDSTROM *et al.* (1992) at 110%/million years and would result in a Ngöbé expansion date of ~1850 YBP.

#### DISCUSSION

The Ngöbé are the first Amerind group, surveyed at the mtDNA sequence level, to exhibit strikingly depau-

perate mtDNA haplotype diversity (Figure 2). Thus, they stand in vivid contrast to the North American Nuu-Chah-Nulth (WARD *et al.* 1991) and Chilean Mapuche (GINTHER *et al.* 1993), both of which carried extensive mtDNA diversity. The Ngöbé are represented by only seven of the 51 haplotypes pictured in the MST (Figure 2), fewer than those exhibited by either the Amerind or Na-Dene groups compared here; 28 haplotypes were recorded for the Nuu-Chah-Nulth, 13 for the Mapuche and nine for the Haida. Furthermore, the Ngöbé carried only two of the four major mtDNA haplogroups observed in the other two Amerind groups despite the fact that each group is represented by similar sample sizes.

The reduced mtDNA diversity noted for the Ngöbé was also apparent at the nucleotide level whether calculated as a current generation,  $\pi$ , or a long-term,  $E(\nu)$ , value (Table 2). EXCOFFIER and LANGANEY (1989) have suggested that, to speculate on the evolutionary history of a group, one should use an estimator of nucleotide diversity, such as  $E(\nu) = K/[0.577 + \log_e(n - 1)]$  ( $K$ , number of polymorphic sites;  $n$ , sample size), that is independent of haplotype frequencies and measures a long-term average rather than a current generation value. This consideration was particularly appropriate for the Ngöbé whose rapid population increase during the past 50–60 years (GJORDING 1991) might bias a current-generation measure of diversity. However, both nucleotide diversity values were reduced in the Ngöbé relative to their Amerind counterparts: the Ngöbé had a  $\pi$  value that was 81% that of the Nuu-Chah-Nulth and Mapuche and a  $E(\nu)$  value that was 49–54% that of the Nuu-Chah-Nulth and the Mapuche, respectively. Furthermore, although the Ngöbé displayed a  $\pi$  value almost twice as high as the Haida, Ngöbé nucleotide diversity calculated as a long-term average,  $E(\nu)$ , was only 71% that of the Haida.

The reduced mtDNA diversity of the Ngöbé has been found to extend to seven additional Chibchan groups distributed throughout Costa Rica and Panamá. Analogous to the mtDNA genetic diversity revealed in the Ngöbé, the Teribe, Kuna, Guatuso, Bribri and Cabécar have also been found to exhibit only group A and B haplotypes (TORRONI *et al.* 1994a,b; O. BATISTA, C. J. KOLMAN and E. BERMINGHAM, unpublished data). However, in addition to the presence of haplogroups A and B, a low frequency of group D haplotypes have been found in the Chibcha-speaking Huetar and Boruca (SANTOS *et al.* 1994; TORRONI *et al.* 1994a,b). These results suggest that the C haplogroup may have been lost at Chibchan ethnogenesis whereas loss of group D haplotypes may reflect genetic drift affecting each Chibchan group individually. Alternatively, haplogroup D may have been introduced into the Huetar and Boruca populations through admixture although this explanation seems unlikely given that no neighboring groups have been found to carry group D haplotypes. Further-

more, Caucasian and Negroid admixture levels in the Chibcha have been demonstrated to be among the lowest for Amerind groups studied thus far (BARRANTES 1993) making non-Amerind admixture an unlikely explanation for the presence of group D haplotypes in the Huetar and Boruca. In sum, the Chibcha appear to depict a linguistic family with extensive geographic and numeric representation who, as a group, carry reduced levels of mtDNA diversity.

Furthermore, a comparison of Chibchan genetic diversity with that of neighboring Panamanian groups representing a second linguistic family, the Chocó, also revealed reduced levels of mtDNA diversity in the Chibchan groups. Diversity calculations based on mtDNA sequence data were consistently lower for the Chibchan populations of Panamá (Ngöbé and Kuna) than the Chocoan groups (Emberá and Waunáan). For the Ngöbé, Kuna, Emberá and Waunáan, respectively, the haplotype diversities were 0.76, 0.65, 0.95 and 0.91 and the current-generation (and long-term) nucleotide diversity values were 0.013 (2.7), 0.009 (3.7), 0.017 (5.5) and 0.019 (7.0) (C. J. KOLMAN and E. BERMINGHAM, unpublished data). In addition, while the Chocoan speakers exhibited the four major Amerind mtDNA haplogroups, the Chibchan speakers did not.

The reduced mtDNA diversity of Chibchan groups may reflect passage through postconquest population bottlenecks. It is known that the Amerind populations of Central America crashed drastically after conquest with evidence for particular decimation of the Panamanian groups (NEWSON 1986; COOK 1992). Chocoan speakers, on the other hand, inhabited Colombia at the time of the conquest and appear to have survived European contact in larger numbers (WASSÉN 1963; BRAY 1984). An alternative, but not mutually exclusive, interpretation posits that the mtDNA diversity differences observed between the Chibcha and the Chocó predates the Spanish conquest of the Americas. Ethnogenesis of the Chibcha linguistic stock from a small founding population, compounded by reproductive isolation, agricultural subsistence lifestyle and strictly enforced endogamy rules, in contrast to the culturally diverse and far-reaching trading practices of the Chocó chiefdoms (BRAY 1984), could have resulted in the origin and maintenance of reduced diversity in Chibchan groups.

Although it is difficult to confirm or discredit either of the two scenarios, HARPENDING *et al.*'s (1993) pairwise genetic difference analysis may offer a means of dating the Ngöbé population expansion. Mismatch distributions of the Nuu-Chah-Nulth and Mapuche share a single major peak at seven to nine differences indicating an expansion at 84,000–65,000 YBP (presumably in Asia), which corresponds well with the first major expansion of human populations estimated at 80,000–30,000 YBP by HARPENDING *et al.* (1993). Mismatch distributions of the Ngöbé and Haida reveal an additional

major peak suggesting a second and much more recent expansion. A recent analysis of pairwise difference distributions by MARJORAM and DONNELLY (1994), while critical of the HARPENDING *et al.* (1993) approach, supported the population bottleneck "signature" of a recent, sharply defined growth peak as seen in the Ngöbé and Haida difference distributions. We have dated the Ngöbé expansion at ~6800 YBP. Although this estimate carries a large but indeterminate error, it is roughly consistent with the proposed time for the Chibchan linguistic stock ethnogenesis at 10,000–7000 YBP (BARRANTES *et al.* 1990; CONSTENLA-UMAÑA 1991; COOKE and RANERE 1992) and the first major population expansion in lower Central America estimated by archaeologists at approximately 4000–2000 YBP (LANGE 1992). The two more extreme estimates of the Ngöbé expansion presented in the results section (1850 and 14,000 YBP) are also inconsistent with a Spanish conquest-based population bottleneck and expansion occurring ~500 years ago.

The reduced mtDNA diversity in the Ngöbé and other Chibchan groups and in the Haida blurs the clear genetic distinction that has been drawn between Amerind and Na-Dene groups. Although it can be argued that the Haida are not a typical linguistic model of the Na-Dene, limited data suggest that they are representative of the Na-Dene from a mtDNA perspective (TORRONI *et al.* 1993), and it is this genetic evidence that has been used to support independent migrations of Na-Dene and Amerinds to the New World (TORRONI *et al.* 1993). When tested statistically using AMOVA, there is no support for a mtDNA-based distinction between the Amerind (Ngöbé, Nuu-Chah-Nulth and Mapuche) and Haida groups discussed here. Furthermore, when analyzed as population pairs, AMOVA fails to detect greater interpopulation variance in the Haida/Amerind comparisons than in the Amerind/Amerind comparisons as might be expected if the Haida and Amerinds arrived in the New World independently. The limited mtDNA sequence diversity of Na-Dene groups when compared with Amerinds has also been used in support of an independent Na-Dene migration (TORRONI *et al.* 1992, 1993). However, if we assume that diversity within the major mtDNA haplogroups has accrued since entry to the New World, the pairwise genetic difference results presented here reveal that the average distance among mtDNA haplotypes within the major haplogroups is equivalent between the Ngöbé and Haida. Although additional mtDNA sequence data is needed on Na-Dene groups to adequately test the hypothesis, these results raise the possibility that diversity differences among New World indigenous groups may reflect demographic processes unrelated to the issue of migration waves, such as founder effect and genetic drift.

**Comparing the mtDNA and anthropological/archaeological record:** The AMOVA results can be used to address the historical relationship between culture and

geography among Ngöbé populations. The central mountain range running lengthwise through Panamá has played a controversial role in terms of population subdivisions among Amerind groups. In an original description of the indigenous groups of Panamá, JOHNSON (1948) based his classifications on a barrier to communication between groups formed by the mountain range. However, careful ethnographic and linguistic research in the 1960s (YOUNG 1971) indicated that, contrary to JOHNSON's (1948) description, Ngöbé settlements were distributed on both sides of the cordillera. Subsequently, archaeologists have demonstrated that differential ecological conditions on either side of the mountains stimulated commercial and social contact between populations on the Pacific and Caribbean coasts (LINARES 1977; COOKE and RANERE 1992). The AMOVA results presented here support the current archaeological and anthropological view of long-term cross-cordillera contact by providing no evidence for population subdivision among the present-day Ngöbé.

The presence of the four Amerind mtDNA haplogroups north and south of Chibchan territory not only provides evidence that the Ngöbé samples were relatively free of maternal admixture with neighboring groups, but also refutes the idea that the isthmus was used as a migration corridor by cultures to the north and south; a conclusion also reached by BARRANTES *et al.* (1990) based on allozyme variation. Instead, it appears that the isthmus area acted more as a barrier against movement between distant cultures, especially from the south where all four haplogroups have been documented in the Chocó of eastern Panamá and Colombia (C. J. KOLMAN and E. BERMINGHAM, unpublished data). The unique distribution of haplogroups argues for the long-term presence of the Chibchan barricade; a result supported by previous genetic findings (BARRANTES *et al.* 1990). Archaeological evidence supports the stable geographic distribution of these indigenous groups as far back as 7000 YBP (LINARES and RANERE 1980; COOKE and RANERE 1992). As opposed to the idea of long-term interbreeding of Central and South American populations, the distinct distribution of lineages presented here supports the idea of a rapid *in situ* fragmentation of the ancestral population into distinct tribal groups. Archaeological evidence suggests that the fragmentation process was further accelerated and consolidated by the spread of specialized agriculture (COOKE and RANERE 1992).

A continuous presence by isthmian population may appear in conflict with certain archaeological evidence of cultural transitions or hiatuses in the lower Central American region. For example, an increase in archaeological sites associated with the introduction of several exogenous plants and the abandonment of a bifacial tool technology dating 7000 YBP (PIPERNO 1988; BARTLETT and BARGHOORN 1973) may be best explained as an adaptive response of the endogenous populations

as opposed to population replacement. Rather than choose between the archaeological record and the mtDNA results, it may be that different aspects of the same question are answered by the two types of data. While populations of lower Central America may have maintained a stable presence for thousands of years, different technologies and/or cultures may have passed through these populations without the introduction of new individuals and resultant change in genetic structure. More detailed sampling of isthmian populations will help confirm or refute this idea but the implication is, not surprisingly, that ideas may have flowed more freely than genes.

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