Mitochondrial DNA "clock" for the Amerinds and its implications for timing their entry into North America

(Amerind migrations/Chibcha time depth/Amerind mtDNA evolution)

ANTONIO TORRONI*†, JAMES V. NEEL‡, RAMIRO BARRANTES§, THEODORE G. SCHURR*¶, AND DOUGLAS C. WALLACE*

Departments of *Genetics and Molecular Medicine and ¶Anthropology, Emory University, Atlanta, GA 30322; ‡Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI 48109-0618; and §Escuela de Biologia, Universidad de Costa Rica, San José, Costa Rica

ABSTRACT   Students of the time of entry of the ancestors of the Amerinds into the New World are divided into two camps, one favoring an "early" entry [more than approximately 30,000 years before the present (YBP)], the other favoring a "late" entry (less than approximately 13,000 YBP). An "intermediate" date is unlikely for geological reasons. The correlation of the appropriate data on mtDNA variation in Amerinds with linguistic, archaeological, and genetic data offers the possibility of establishing a time frame for mtDNA evolution in Amerinds. In this paper, we estimate that the separation of the Chibcha-speaking tribes of Central America from other linguistic groups/nascent tribes began approximately 8000–10,000 YBP. Characterization of the mtDNA of 110 Chibcha speakers with 14 restriction enzymes leads on the basis of their time depth to an estimated mtDNA nucleotide substitution rate for Amerinds of 0.022–0.029% per 10,000 years. As a first application of this rate, we consider the mtDNA variation observed in 18 Amerind tribes widely dispersed throughout the Americas and studied by ourselves with the same techniques, and we estimate that if the Amerinds entered the New World as a single group, that entry occurred approximately 22,000–29,000 YBP. This estimate carries a large but indeterminate error. The mtDNA data are thus at present equivocal with respect to the most likely times of entry of the Amerind into the New World mentioned above but favor the "early" entry hypothesis.

The American Indians present a remarkable case study in human evolution. They belong to one of the few extant human groups whose ancestors entered a vast uninhabited area over a relatively short interval and then apparently remained isolated from other human contacts for a considerable period of time. Although there is consensus that their provenance was Eastern Siberia, the diversity of opinions on the exact time or times of the earliest human entry into the Americas has often been accompanied by acrimonious debate. As a broad generalization, the discussants of the "entry problem" favor either an "early" arrival [more than ≈30,000 years before present (YBP)] (e.g., refs. 1–3) or a "late" arrival (less than ≈13,000 YBP) (e.g., ref. 4).

Studies of variation in mitochondrial DNA (mtDNA) offer a new approach to this long-standing question. Within the past 8 years, we have described mtDNA variation in 16 Amerind tribes (5–8). [We reserve the term Amerind for the descendants of the first wave or waves of immigrants to the New World, accepting for now that there was a later wave or waves of immigration, the ancestors of the Na-dene speakers and the North American Eskimos (7, 9–12).] These studies revealed that the mtDNAs of modern Am-

erinds are defined primarily by four sets of specific mutations that cluster in four haplotype groups (haplogroups), termed A, B, C, and D. Moreover, the observation that each of these haplogroups was apparently founded by a single haplotype present in Asia permitted a quantification of the mtDNA variation that had accumulated within each of those haplogroups from the time of the first human arrival in the Americas (13).

We have also recently developed, from archeological, linguistic, and genetic criteria, an estimate of the times of divergence of the various Chibcha-speaking tribes of Central America (14). Samples from five of these Chibcha-speaking tribes were included in the previously mentioned studies of Amerind mtDNA. In this paper, we present data on the mtDNA of two additional Chibcha tribes. We then undertake to develop an mtDNA evolutionary time clock based on the Chibcha data, a time clock which for various reasons should have greater accuracy for humans than those in current usage. Finally, applying this clock to the data on mtDNA variation described in our previous studies of Amerind mtDNAs, and introducing other pertinent data, we will attempt to decide whether the weight of the evidence favors an early or a later arrival date for the first Amerinds.

MATERIAL AND METHODS

The Tribes. The locations of 16 of the 18 Amerind tribes that will enter in to these calculations, references to the present state of these 16 tribes, and the manner of sample collection from representatives of these groups have been reported by Torroni *et al.* (8). An important consideration in the present context is that the tribes represent a broad geographic sampling throughout the Americas. For the purpose of the present study, we have added an investigation of the mtDNAs of two additional tribes of Chibcha speakers to the data bank.

Teribe. The first recorded evidence of the Teribe is in 17th century documents, wherein they are described as living in the Talamanca region of Panama, between the Sixaola and Changuinola Rivers, and on Tojar Island (now Isla Colon) in Bocas del Toro Province, Panama (14). About 1700, part of the Teribe population was relocated by the Spanish colonists to southeastern Costa Rica, and the remaining Panamanian group began a retreat into the mountains toward the headwaters of the Teribe River. The Costa Rican Teribe have now largely merged with the Bribri, Cabecar, and Boruca tribes, but the Panamanian population remains relatively intact, spread along the Teribe, San-San, and Changuinola Rivers (14). The samples analyzed for this study were collected in early 1987 in the upper Teribe River region.

Abbreviations: YBP, years before present; haplogroups, haplotype groups; MP, maximum parsimony.
†To whom reprint requests should be addressed.

Guatuso. This tribe historically inhabited the plains of northern Costa Rica but now has been reduced to three small enclaves in three localities (Margarita, Tonjibe, and El Sol) in the upper drainage of the Rio Frio in the Alajuela Province of Costa Rica. The ethnographic and linguistic affiliations of the Guatuso are somewhat uncertain, but the consensus treats them as Chibcha. Our phylogenetic reconstruction of the relationships of 10 Chibcha-speaking groups places the Teribe and Guatuso on a branch well separated from the remaining tribes (14). The present samples were collected in 1986 in Margarita and Tonjibe.

Sample Preparation and Molecular Analysis. DNA from the Teribe and Guatuso samples was extracted from 50–250 μl of sera by the procedures described elsewhere (7). The entire mtDNA was then amplified with the polymerase chain reaction (PCR) (15) in nine partially overlapping segments. The oligonucleotide primers and PCR conditions used for these amplifications are described elsewhere (7). Each PCR segment was subsequently digested with the following 14 restriction enzymes: *Alu* I, *Ava* II, *Bam*HI, *Dde* I, *Hae* II, *Hae* III, *Hha* I, *Hinf*I, *Hinc*II, *Hpa* I, *Hpa* II/*Msp* I, *Mbo* I, *Rsa* I, and *Taq* I. These endonucleases permit the screening for variation of $\approx$15–20% of the mtDNA sequence per individual (about 2900 nucleotides). The resulting restriction fragments were resolved by electrophoresis in 1.0–2.5% NuSieve plus 1.0% SeaKem agarose (FMC) gels, visualized by ethidium bromide staining, and mapped by the sequence comparison method (16, 17).

Phylogenetic and Sequence Divergence Analyses. The evolutionary relationships among the Teribe and Guatuso haplotypes and the other previously reported (8) Amerind haplotypes were inferred by phylogenetic analysis using PAUP (18). Maximum parsimony (MP) trees were generated through random addition of sequences by using the Tree Bisection and Reconnection (TBR) branch-swapping algorithm. Because of the large number of terminal taxa, thousands of MP trees could be obtained. We terminated our search at 3000 trees after 1344 replications, with no more than 10 MP trees saved for each replication. About 2600 of the 3000 trees were obtained in the first 1000 replications. In most of the remaining replications, the MP trees were discarded,

since they were identical to those already saved, suggesting that the 3000 trees that were generated could represent a large portion of the existing MP trees. However, the possibility that shorter trees could exist cannot be excluded. The dendrograms were rooted by using an African haplotype as an outgroup (8). Intrahaplogroup sequence divergence estimations from restriction analysis data were calculated with the maximum likelihood procedure of Nei and Tajima (19) by using the program DREST (provided by L. Jin). The methodology of this program has been described (7).

RESULTS

The Data. Table 1 presents the results of the new typings of the Teribe and Guatuso with the results of the previous typings of five other Chibcha-speaking tribes. The Bribri and Cabecar subjects were grouped together because the samples were collected from locations where the two groups have admixed. In 110 subjects analyzed, 15 haplotypes were observed. Of these 9 belong to haplogroup A, 5 to haplogroup B, 1 to haplogroup D, and none to haplogroup C. Four of these haplotypes (AM1, AM9, AM13, and AM44) were previously observed in non-Chibcha tribes (7, 8). All of the others have been encountered only in the Chibcha speakers.
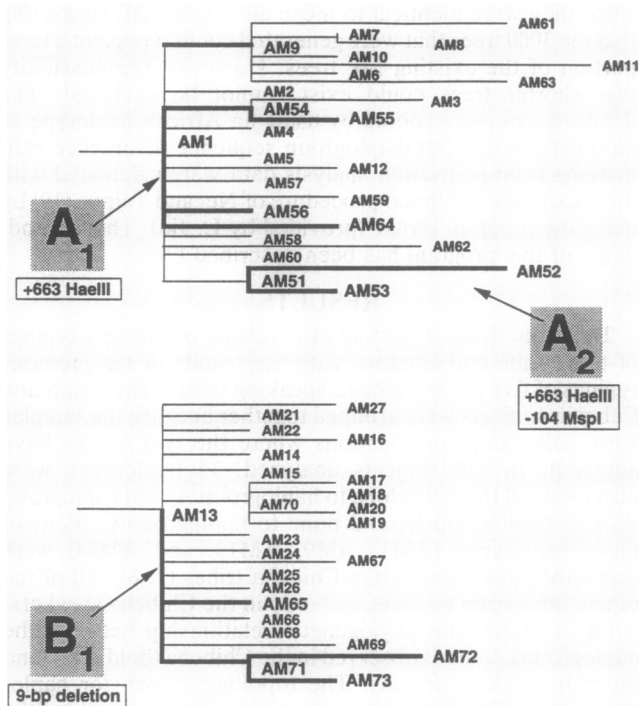
Fig. 1 shows the phylogenetic relationship between the haplogroups A and B observed in the Chibcha (bold lines) and those in other Amerinds. The topology shown for haplogroups A and B is that represented in the large majority of the 3000 MP trees that were generated. The MP trees for the haplotypes observed in Amerinds (AM1–AM96) were 140 mutational steps long with consistency and retention indices of 0.596 and 0.909, respectively. Fig. 1 *Inset* shows the strict consensus of the 3000 MP trees. (A strict consensus tree contains only those groups appearing in all MP trees.) This dendrogram is 172 steps long with consistency and retention indices of 0.382 and 0.783, respectively. With the exception of AM44, which is a group D haplotype, all other Chibcha haplotypes can be grouped into two subgroups of haplogroup A, termed A_1 and A_2, and one subgroup of haplogroup B, termed B_1 (see below).

The Inferences. *The Chibcha reference point.* These Chibcha-speaking tribes provide a potential reference point for the

Table 1. mtDNA haplotype distribution in the seven Chibcha-speaking tribes

Haplotype	Haplogroup	Teribe	Guatuso	Boruca	Kuna	Guaymi	Bribri/Cabecar	Total
AM1	A	—	16	—	1	1	—	18
AM9	A	—	—	—	—	5	—	5
AM51	A	16	—	2	—	2	8	28
AM52	A	—	—	1	—	—	—	1
AM53	A	—	—	—	15	—	—	15
AM54	A	—	—	—	—	2	—	2
AM55	A	—	—	—	—	1	—	1
AM56	A	—	—	—	—	—	5	5
AM64	A	—	1	—	—	—	—	1
AM13	B	—	—	10	—	5	6	21
AM65	B	—	—	—	—	—	5	5
AM71	B	—	2	—	—	—	—	2
AM72	B	4	—	—	—	—	—	4
AM73	B	—	1	—	—	—	—	1
AM44	D	—	—	1	—	—	—	1
Total		20	20	14	16	16	24	110

Haplotypes AM1, AM9, AM13, and AM44 have also been reported in non-Chibcha tribes (8). The polymorphic restriction sites of the six haplotypes observed in the Teribe and the Guatuso are the following: −104i [AM51]; +255f [AM64]; +663e [AM1, AM51, AM64]; −3849e [AM73]; +8872e [AM71–AM73]; −9553e [AM72]; +9589b [AM64]; +16389m/+16390j/−16390b [AM72]; +16517e [AM71–AM73]. Restriction sites are numbered from the first nucleotide of the recognition sequence, with a plus indicating a site gain and a minus indicating a site loss with respect to the published sequence (20). Each site is accompanied by a single-letter code indicating which of the 14 enzymes used in the analysis detected the sequence variant: a, *Alu* I; b, *Ava* II; c, *Dde* I; e, *Hae* III; f, *Hha* I; g, *Hinf*I; h, *Hpa* I; i, *Hpa* II/*Msp* I, j, *Mbo* I; k, *Rsa* I; l, *Taq* I; m, *Bam*HI; n, *Hae* II; o, *Hinc*II. Diagonal lines separating restriction sites indicate that a single mutation alters the recognition sequence of more than one enzyme; these sites are considered to be a single-site polymorphism in the statistical and phylogenetic analyses. Numbers in brackets correspond to the mtDNA haplotypes in which the site changes appeared. All samples have been shown to differ from the published sequence for the following restriction sites: −4769a, +7025a, +8858f, −13702e, −14199o, +14268g, and −14368g.
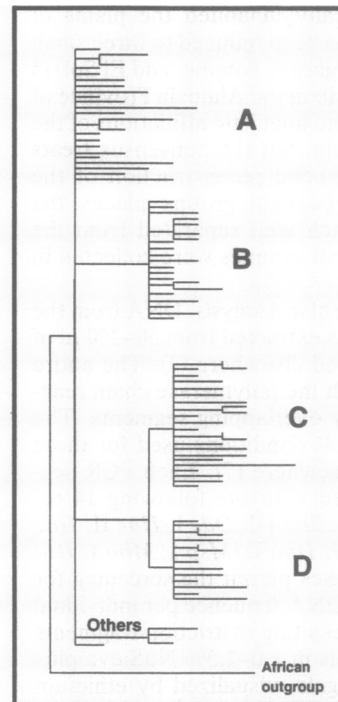
FIG. 1. Phylogenetic relationship of haplogroups A and B observed in the Chibcha (bold lines) and other Native Americans (thin lines). The Chibcha haplotypes form the three sub-haplogroups, A_1, A_2, and B_1. (*Inset*) Strict consensus of 3000 most parsimonious trees obtained by phylogenetic analysis (PAUP) using parsimony when the four new haplotypes detected in the Teribe and Guatuso tribes are integrated and analyzed together with those previously reported by Torroni *et al.* (8).

rate of divergence of mtDNA in Amerinds. On the basis of archeological, linguistic, and genetic (nuclear genes) studies of eight of these tribes, we have suggested a phylogenetic reconstruction of tribal origins in which the first bifurcation in the dendrogram of relationship occurs 7000 YBP (14). Data on mtDNA haplotypes for seven of these tribes now permit a direct estimate of the rate of evolution of mtDNA within these tribes. However, although the derivation of dendrograms results in the appearance of a clear-cut dichotomous branching procedure, in fact, the evolution of one tribal population into two must usually be a more gradual process preceded by a period of tribal growth, with incipient isolation between the two groups well prior to the split identified by the dendrogram. Likewise, prior to the events leading up to the spin-off of a new tribe from a mother population, there would be a period in which the tribe was developing an internal heterogeneity (microdifferentiation), which we have shown to be striking in some tribes (21, 22). For instance, in the Yanomama a private polymorphism of albumin, which attains allele frequencies of 0.3 to 0.4 in the inhabitants of some villages, is totally absent in other villages (23). An offshoot of such an internally differentiated tribe—i.e., a potentially new tribe—might not possess this albumin variant. Thompson (24) has estimated the most likely age of this variant at about 168 generations.

Accordingly, the initiation of the mtDNA diversity upon which an mtDNA evolutionary rate is calculated must precede the time of the first designated split in a dendrogram by a considerable period. Estimation of the duration of this period presents many difficulties. We suggest that prior to the root of the dendrogram at 7000 years, there was a period of 1000–3000 years during which an interbreeding group destined to evolve into the Chibcha tribes was accumulating unique genetic variation. The time depth for the accumulation of the unique variation in mtDNA encountered in these seven tribes is thus estimated at 8000–10,000 YBP.

This estimate gains some support from archeological evidence of continuous occupation of the lower Central American region for as long as 10,000 years (25). However, there is, of course, no assurance that these early inhabitants spoke Chibcha. On the other hand, Greenberg (ref. 10, p. 335) finds that, on the basis of glottochronological evidence, a date of separation 10,000 or 11,000 YBP for representative Macro-

Panoan and Chibcha-Paezan "does not seem unreasonable." This estimate of 8000–10,000 years is not the coalescence time for all of the variants present in this group but is the time at which the variants distinguishing these tribal groups began to accumulate—i.e., the time at which free exchange of the evolving Chibcha with other nascent tribal groups decreased to the point at which a new mutation had a high probability of remaining within the tribe.

We must now estimate which of the haplotypes currently detected in these seven tribes were already present 8000–10,000 YBP, the date we have set for the beginning of the divergence of these Chibcha linguistic groups from other groups. Referring to Table 1, we note the presence of AM1 (haplogroup A) in the Guatuso, the Kuna, and the Guaymi, and AM13 (haplogroup B) in the Boruca, Guaymi, and Bribri/Cabecar. Elsewhere we have developed evidence that these were among the founding mtDNA genotypes for the Amerinds, so there is a high probability they were in the Chibcha at the outset of tribal divergence (8). From AM1 a set of haplotypes can be derived that constitute a subgroup of haplogroup A, designated A_1. Haplotype AM13 and its Chibcha derivatives form a subgroup of haplogroup B that we termed B_1 (Fig. 1). Haplotype AM51, which can be derived from AM1 by a single mutational event, also meets several criteria to be considered ancestral to the Chibcha radiation. It is found in five of the tribes (Boruca, Guaymi, Bribri/Cabecar, and Teribe) in relatively high numbers, and its derivative, AM53, is almost fixed in the Kuna. Haplotype AM51 and its derivatives AM52 and AM53 are defined by a unique mutation, an *Msp* I site loss at nucleotide position 104, which is a Chibcha private polymorphism, and together these define a subgroup termed A_2. Because of these characteristics, AM51 may have been present at the outset of the tribalization process, although the possibility of a later origin cannot be rigorously excluded.

Ten of the remaining 12 haplotypes encountered in these tribes can be derived from these three stem haplotypes by mutation, as shown in the dendrograms of Fig. 1, and to date have been observed only in the Chibcha speakers. We are left to account for two haplotypes, AM44, present in one Boruca, and AM9, present in five Guaymi. AM44 is the only group D haplotype observed in the Chibcha and has also been ob-

Genetics: Torroni *et al.*

Proc. Natl. Acad. Sci. USA 91 (1994)   1161

Table 2. The possible surviving founding haplotypes and mtDNA haplotype subgroups for the Chibcha

Haplotype	Subhaplogroup	n	N	Sequence divergence, %
AM1	A$_1$	5	27	0.020
AM13	B$_1$	5	33	0.033
AM51	A$_2$	3	44	0.018
AM9*	—	1	5	0.000

n, Number of haplotypes; *N*, number of subjects.
*Haplotype AM9 has no derivatives in the Chibcha (Fig. 1). Therefore, it is not the source of any subgroup of haplogroup A, and the sequence divergence is equal to zero.

served in several non-Chibcha tribes, including the Yanomama; AM9 is a group A haplotype also observed in other Amerind linguistic groupings (8). Because of its presence in tribes located around the Chibcha, we will assume the presence of AM44 results from admixture. However, AM9 could be a founding genotype of the Chibcha but is equally likely to be a haplotype acquired by genetic admixture or to have originated from mutation at the hypervariable *Hae* III site at nucleotide position 16517 on different AM1 haplotypes (haplotypes AM1 and AM9 differ only for this site).

Table 2 presents the sequence divergence accumulated in the haplotype subgroups originating from the four haplotypes postulated to be present when the Chibcha became a distinct breeding population. Table 3 presents two almost identical estimates of average sequence divergences and mtDNA rates of evolution, the two estimates differing because of the inclusion of the AM9 haplotype in one. We will work with the extreme values that emerge from these estimates (0.022–0.029% per 10,000 years). Most previous estimates of mtDNA evolutionary rates have been based on the mtDNA differences between higher primates, such as humans, chimpanzees, gorillas, orangutans, and lar gibbons, and depend heavily on assumptions as to the date of the ancestral divergence time. This approach has yielded nucleotide divergence estimates per million years of 0.5–1% (26), 2% (27), 0.7% (28), and 0.8% (29). Our estimate, equivalent to 2.3–2.9% per million years, is thus higher than these estimates. However, estimates based on such extreme time depths have been criticized severely (30, 31); we suggest that our use of a more restricted time depth in the present calculation yields an estimate more appropriate to the present situation.

Estimation of arrival time of Amerind ancestors. We will now apply the mtDNA evolutionary rates derived from the Chibcha data to the question of when the ancestors of the Amerinds entered North America. Thus far, the mtDNA genotypes of the 325 Amerinds examined can be organized into four haplogroups, termed A, B, C, and D (8). Each of these presumably is rooted in one or several haplotypes represented in the founding populations. The assumption that

Table 3. Estimates of mtDNA evolutionary rate from the Chibcha

Scenario	Founding haplotypes	Average sequence divergence,* %	mtDNA evolution rate,[†] %/10,000 years
1	AM1, AM13, AM51	0.023	0.029–0.023
2	AM1, AM9, AM13, AM51	0.022	0.028–0.022

The two estimates differ only in the inclusion of AM9 in the second estimate.
*The average divergence is weighted by the number of subjects within each of the haplotype subgroups that arose from the indicated founding haplotypes (Table 2).
[†]The evolutionary rate is estimated on the basis of an accumulation time of between 8000 and 10,000 years as described in the text.

Table 4. Time of arrival of the Amerind to the New World calculated from sequence divergence accumulated in four haplogroups

Haplogroup	n	N	Sequence divergence, %	Arrival time (0.029–0.022% per 10,000 years)
A	24	131	0.075	25,862–34,091
B	19	83	0.034	11,724–15,456
C	25	61	0.096	33,103–43,636
D	16	60	0.053	18,276–24,091
Average*	84	335	0.065	22,414–29,545

The arrival time was calculated by multiplying the intra-group sequence divergences by the mtDNA evolution rate of 0.029–0.022% per 10,000 years, which was estimated from the divergence of the Chibcha haplotype sub-groups (Table 3). *n*, Number of haplotypes; *N*, number of subjects.
*The average was weighted by the number of subjects within each haplogroup.

for each haplogroup there may have been a single founding haplotype is favored by the demonstration that the haplotypes AM43 and AM88, which are the point of departure for the Amerind haplogroups C and D, are the only haplotypes shared between the Siberians and Amerinds and also are the founding haplotypes for the Siberian portions of haplogroups C and D (13). However, the possibility of more than one founding haplotype for each haplogroup cannot be completely excluded. In addition, there may have been present in the population(s) reaching the New World mtDNA variants not falling within these four haplogroups that were subsequently lost through drift from the presumably small founder population, as well as variants within the four haplogroups lost through drift.

In Table 4, we present time-of-arrival estimates for each of these four haplogroups. The divergence percents are those reported in Torroni *et al.* (8), modified by the inclusion of the data obtained by the analysis of the Teribe and Guatuso, and the arrival times have been calculated from the mtDNA nucleotide divergence rates of 0.022–0.029% per 10,000 years developed in Table 3. These calculations are based on the assumption of a single founder haplotype for each haplogroup, but since we have identified a total of 89 variant genotypes in addition to the 4 presumed to have arrived from Siberia, the possibility of several additional founding haplotypes for a haplogroup would introduce only a small error into the calculation. The four estimates range from ≈12,000–44,000 YBP. The average of these four estimates is 22,414 YBP when the time depth assigned to the Chibcha is 8000 years and 29,545 YBP when the time depth is placed at 10,000 years.

DISCUSSION

In the introduction, we emphasized that our intent is not to use mtDNA data to fix upon an exact arrival time in the New World for the Amerind but to inquire whether the estimates concerning their arrival are more compatible with an "early" or a "late" arrival date. The averaged estimate for the arrival of the Amerinds resulting from the data now available is between 22,414 and 29,545 YBP on the basis of dating the onset of Chibcha diversification at 8000–10,000 YBP. For a variety of reasons thoroughly discussed by Templeton (31), the 95% probability limits for both the haplogroup-specific and the averaged estimate cannot be calculated at the present time. The reasons for this inability range from lack of knowledge concerning certain parameters basic to such a calculation (such as the nucleotide mutation rate per generation) to the fallibility of the assumption that the mutants that have been encountered are neutral in their phenotypic effects. The fluctuations in numbers characteristic of the Amerindian populations under consideration introduce further

complications into the calculation of an error term. Some of the fluctuations are well documented (32), while others are conjectural. However, for theoretical reasons (31), the 95% confidence interval for this estimate must be very broad relative to the actual estimate and probably would include a date of arrival as recent as 13,000 YBP. Furthermore, in calculating an error term, allowance cannot be made for the uncertainty created by possible departures from the biological assumptions that entered into the derivation of the "Chibcha yardstick." Finally, in developing "point" estimates for the arrival date of the ancestors of the Amerinds, we recognize that the migration across Beringia, whether early or late, may well have extended over a period of 1000 years or more, during which time mtDNA differentiation was also occurring.

Thus far we have not considered certain external constraints on the peopling of the Americas. We accept that all significant human entry into the Americas was by way of Siberia during periods of glaciation, when a land bridge connected Siberia and the extreme northwest of the Americas. This being the case, the entry of humans into the New World cannot predate their entry into Siberia. Unfortunately, almost as much controversy surrounds the time of arrival of *Homo sapiens* in eastern Siberia as surrounds the time of his arrival in the New World (33). In this circumstance, it is important to consider the limits on time of arrival set by the need for the coexistence of a land bridge between Siberia and North America (Beringia) *and* a traversable inland passage permitting movement from Alaska to the Canadian plains. With respect to the inland passage, we accept the statement of Butzer (ref. 34; see also ref. 35) that "it would have been difficult to find and negotiate a both passable and productive route through the MacKenzie valley and along the front of the eastern Rocky Mountains at the height of the Wisconsian glacial, about 30,000–13,500 BP." (ref. 34, p. 138). Most authorities agree that Beringia was intact for several thousand years on either side of 30,000 YBP (33). Inasmuch as there is (somewhat controversial) evidence for human occupation of eastern Siberia some 30,000 YBP, there is no essential conflict between the requirements of the anthropological and geological dates. Considering these facts as well as the evidence cited earlier, we attempt to distinguish between an "early" and "late" arrival by working with a possible "early" arrival date of ≈30,000 YBP.

The desired outcome for this study would have been an estimated time of arrival that clearly coincided with (or slightly preceded or followed) either the "early" or "late" dates discussed earlier. This is obviously not the case. The errors to be attached to estimates of this type are so large that our estimated arrival time, of 22,414–29,545 YBP, is statistically consistent with either arrival time, although obviously favoring the earlier date. We note that one must recognize in principle the possibility of both an early *and* a later arrival date. From Table 4, we see that haplogroup B (whether or not accompanied by the other three haplogroups) is the obvious candidate for a later arrival date. Without this haplogroup, the average estimated arrival date for the remaining three haplogroups is between 25,707 and 33,939 YBP. However, the situation is complicated by the fact that haplogroup B has not yet been encountered in any of the ethnic groups in northeastern Siberia who are now considered the most probable source of the progenitors of the Amerindians (13).

1. Adovasio, J. M., Gunn, J. D., Donahue, J., Stuckenrath, R., Guilday, J. E. & Volman, K. (1980) *Am. Antiq.* **45**, 588–595.
2. Collins, M. B. & Dillehay, T. D. (1986) in *New Evidence for the Pleistocene Peopling of the Americas*, ed. Bryan, A. L. (Univ. of Maine, Orono), pp. 339–355.
3. Guidon, N. & Delibrias, G. (1986) *Nature (London)* **321**, 769–771.
4. Hoffecker, J. F., Powers, W. R. & Goebel, T. (1993) *Science* **259**, 46–53.
5. Wallace, D. C., Garrison, K. & Knowler, W. C. (1985) *Am. J. Phys. Anthropol.* **68**, 149–155.
6. Schurr, T. G., Ballinger, S. W., Gan, Y.-Y., Hodge, J. A., Merriwether, D. A., Lawrence, D. N., Knowler, W. C., Weiss, K. M. & Wallace, D. C. (1990) *Am. J. Hum. Genet.* **46**, 613–623.
7. Torroni, A., Schurr, T. G., Yang, C.-C., Szathmary, E. J. E., Williams, R. C., Schanfield, M. S., Troup, G. A., Knowler, W. C., Lawrence, D. N., Weiss, K. M. & Wallace, D. C. (1992) *Genetics* **130**, 153–162.
8. Torroni, A., Schurr, T. G., Cabell, M. F., Brown, M. D., Neel, J. V., Larsen, M., Smith, D. G., Vullo, C. M. & Wallace, D. C. (1993) *Am. J. Hum. Genet.* **53**, 563–590.
9. Williams, R. C., Steinberg, A. G., Gershowitz, H., Bennett, P. H., Knowler, W. C., Pettitt, D. J., Butler, W., Baird, R., Dowda-Rea, L., Burch, T. A., Morse, H. G. & Smith, C. G. (1985) *Am. J. Phys. Anthropol.* **66**, 1–29.
10. Greenberg, J. H. (1987) *Language in the Americas* (Stanford Univ. Press, Stanford, CA), pp. xvi and 438.
11. Szathmary, E. J. E. (1993) in *Genetics of Cellular, Individual, Family, and Population Variability*, eds. Sing, C. F. & Hanis, C. L. (Oxford Univ. Press, Oxford), pp. 213–238.
12. Callegari-Jacques, S. M., Salzano, F. M., Constans, J. & Maurieres, P. (1993) *Am. J. Phys. Anthropol.* **90**, 427–444.
13. Torroni, A., Sukernik, R. I., Schurr, T. G., Starikovskaya, Y. B., Cabell, M. F., Crawford, M. H., Comuzzie, A. G. & Wallace, D. C. (1993) *Am. J. Hum. Genet.* **53**, 591–608.
14. Barrantes, R., Smouse, P. E., Mohrenweiser, H. W., Gershowitz, H., Azofeifa, J., Arias, T. D. & Neel, J. V. (1990) *Am. J. Hum. Genet.* **46**, 63–84.
15. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230**, 1350–1354.
16. Johnson, M. J., Wallace, D. C., Ferris, S. D., Rattazzi, M. C. & Cavalli-Sforza, L. L. (1983) *J. Mol. Evol.* **19**, 225–271.
17. Cann, R. L., Brown, W. M. & Wilson, A. C. (1984) *Genetics* **104**, 699–711.
18. Swofford, D. (1992) *Phylogenetic Analysis Using Parsimony (PAUP), Version 3.0S.* (Illinois Natural History Survey, Champaign, IL).
19. Nei, M. & Tajima, F. (1983) *Genetics* **105**, 207–217.
20. Anderson, S., Bankier, A. T., Barrell, B. G., de Bruin, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, R. & Young, I. G. (1981) *Nature (London)* **290**, 457–465.
21. Spielman, R. S., Migliazza, E. C. & Neel, J. V. (1974) *Science* **184**, 637–644.
22. Neel, J. V. (1978) *Annu. Rev. Genet.* **12**, 365–413.
23. Tanis, R. E., Ferrell, R. E., Neel, J. V. & Morrow, M. (1974) *Ann. Hum. Genet.* **38**, 179–190.
24. Thompson, E. A. (1976) *Am. J. Hum. Genet.* **28**, 442–452.
25. Lange, F. & Stone, D. (1984) *The Archaeology of Lower Central America* (Univ. of New Mexico Press, Albuquerque), pp. xiv and 476.
26. Brown, W. M., George, M. & Wilson, A. C. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1967–1971.
27. Wilson, A. C., Cann, R. L., Carr, S. M., George, M., Gyllensten, U. B., Helm-Bychowski, K. M., Higuchi, R. G., Palumbi, W. S. R., Prager, E. M., Sage, R. D. & Stoneking, M. (1985) *Biol. J. Linn. Soc.* **26**, 375–400.
28. Nei, M. (1985) in *Population Genetics and Molecular Evolution*, eds. Ohta, T. & Aoki, K. (Jpn. Science Press, Tokyo), pp. 41–64.
29. Spuhler, J. N. (1989) *Am. J. Hum. Biol.* **1**, 509–528.
30. Frayer, D. W., Wolpott, M. H., Thorne, A. G., Smith, F. H. & Pope, G. G. (1993) *Am. Anthropol.* **95**, 14–50.
31. Templeton, A. R. (1993) *Am. Anthropol.* **95**, 51–72.
32. Thompson, E. A., Neel, J. V., Smouse, P. E. & Barrantes, R. (1992) *Am. J. Hum. Genet.* **51**, 609–626.
33. Fiedel, S. J. (1987) *Prehistory of the Americas* (Cambridge Univ. Press, New York), pp. x and 386.
34. Butzer, K. W. (1991) in *The First Americans: Search and Research*, eds. Dillehay, T. D. & Meltzer, D. J. (CRC, Boca Raton, FL), pp. 137–156.
35. Wright, H. E., Jr. (1991) in *The First Americans: Search and Research*, eds. Dillehay, T. D. & Meltzer, D. J. (CRC, Boca Raton, FL), pp. 113–135.