Am. J. Hum. Genet. 59:213–225, 1996

mtDNA Variation in the Yanomami: Evidence for Additional New World Founding Lineages

Ruth D. Easton,[1] D. Andrew Merriwether,[1] Douglas E. Crews,[2] and Robert E. Ferrell[1]

[1]Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh; and [2]Department of Anthropology and School of Public Health, Ohio State University, Columbus

Summary

Native Americans have been classified into four founding haplogroups with as many as seven founding lineages based on mtDNA RFLPs and DNA sequence data. mtDNA analysis was completed for 83 Yanomami from eight villages in the Surucucu and Catrimani Plateau regions of Roraima in northwestern Brazil. Samples were typed for 15 polymorphic mtDNA sites (14 RFLP sites and 1 deletion site), and a subset was sequenced for both hypervariable regions of the mitochondrial D-loop. Substantial mitochondrial diversity was detected among the Yanomami, five of seven accepted founding haplotypes and three others were observed. Of the 83 samples, 4 (4.8%) were lineage B1, 1 (1.2%) was lineage B2, 31 (37.4%) were lineage C1, 29 (34.9%) were lineage C2, 2 (2.4%) were lineage D1, 6 (7.2 %) were lineage D2, 7 (8.4%) were a haplotype we designated "X6," and 3 (3.6%) were a haplotype we designated "X7." Sequence analysis found 43 haplotypes in 50 samples. B2, X6, and X7 are previously unrecognized mitochondrial founding lineage types of Native Americans. The widespread distribution of these haplotypes in the New World and Asia provides support for declaring these lineages to be New World founding types.

Introduction

Modern *Homo sapiens* did not arise in the New World. Anthropological evidence shows that American Indians came from Asia across the Bering Straits when the Beringia land bridge was exposed during periods of glaciation (Zegura 1985). The timing of the first migration is intensely debated. Archaeological evidence for human habitation of 33,000 years before present (ybp) have been claimed, still the most widely agreed upon North American sites date to 12,000–15,000 ybp (Wormington 1983; Lynch 1991). Ancient sites are scarce, and no human remains have been identified >18,000 ybp of age.

The number and nature of migrations also is unknown. Linguistic evidence has been interpreted to support three migrations corresponding to three major language groups (Amerind, NaDene, and Eskaleut). Amerinds are postulated to have arrived 11,000–14,000 ybp, with the Nadene and the Eskaleut following later (Turner 1985; Zegura 1985; Greenberg et al. 1986; Greenberg 1987; Szathmary 1993). Turner (1988) later suggested that the NaDene entered last. However, language groups included as Amerind are diverse, and their common origin is disputed. It also is conceivable that American Indian linguistic and genetic diversity occurred indigenously, when deglaciation inundated the Bering Strait, again separating the New World and Asia.

Once humans reached the New World, they quickly occupied both continents. While Monte Verde in Chile may have been settled as long as 33,000 years ago (Dillehay 1989; Marshall 1990), no strong evidence of human habitation in the New World exists prior to 18,000 ybp, leading many to question human presence in the New World prior to 18,000 ybp. Humans were certainly in many parts of South America by 11,000–14,000 ybp (Salzano and Callegari-Jacques 1988). Again, the major migration routes and relationships between groups are speculative. Using molecular genetic techniques, anthropologists, archaeologists, and geneticists are attempting to trace these ancient migratory routes and determine when contemporary populations separated from each other.

Johnson et al. (1983) first demonstrated that human mtDNA RFLP haplotypes clustered according to geographical regions and that relationships between populations could be established by examining mtDNA RFLP patterns. Wallace et al. (1985), studying mtDNA RFLP variation in three Native American populations, detected mtDNA types also found in Asians. However, the frequencies of the mtDNA haplotypes observed in Amerindians differed markedly from those found in Asia, which is indicative of a founder effect for New World populations. Schurr et al. (1990) suggested that all Native American haplotypes clustered into four

Received October 31, 1995; accepted for publication April 17, 1996.

Address for correspondence and reprints: Dr. Robert E. Ferrell, Department of Human Genetics, 130 DeSoto Street, Pittsburgh, PA 15261.

© 1996 by The American Society of Human Genetics. All rights reserved.

0002-9297/96/5901-0028$02.00

Table 1

RFLP Sites That Define the Major Haplogroups Observed in American Indian Populations

Type	*Hae*III 663	9-bp Deletion	*Hinc*II 13259	*Alu*I 5176	*Dde*I 10394	*Alu*I 10397	*Hae*III 16517	*Rsa*I 16329	*Hae*III 16398	*Rsa*I 16049	*Hinc*II 12406	*Rsa*I 16303	*Eco*RV 16274	*Sau*96I 16516
A1	+	N	+	+	−	−	+	+	−	+	...	...	...	...
A2	+	N	+	+	−	−	−	+	−	+	...	...	...	...
B1	−	D	+	+	−	−	+	+	−	+	...	...	...	...
B2	−	D	+	+	−	−	−	+	−	+	...	...	...	...
C1	−	N	−	+	+	+	+	+	−	+	...	...	...	...
C2	−	N	−	+	+	+	−	+	−	+	...	...	...	...
D1	−	N	+	−	+	+	+	+	−	+	...	...	...	...
D2	−	N	+	−	+	+	−	+	−	+	...	...	...	...
"E"	−	N	+	+	−	−	...	...	...	...	...	...	...	...
X6	−	N	+	+	+	+	−	+	−	+	+	+	+ or −	+
X7	−	N	+	+	+	+	+	+	−	+	+	+	+ or −	+

haplogroups. These four haplogroups, designated "A," "B," "C," and "D," were defined by Wallace and Torroni (1992) and the specific markers defining each haplotype are given in table 1. The A, C, and D haplogroups have been further subdivided by the presence (A1, C1, and D1) or absence (A2, C2, and D2) of an *Hae*III site at nt 16517 (Bailliet et al. 1994). In American Indians, haplotype B has always been found in association with the *Hae*III site at nt 297. These specific markers are found primarily in Amerindians and other populations of Asian descent. Native American populations in all parts of North and South America were found to have haplotypes from all four haplogroups, which indicates that all were descended from one ancestral stock (Merriwether et al. 1991; Ballinger et al. 1992; Torroni et al. 1993*a*).

In addition, Bailliet et al. (1994) proposed a lineage E made up of haplotypes identified as "others" (not A, B, C, or D) by Torroni et al. (1993*a*). These consist of haplotypes AM28, AM29, and AM74 to AM76. Torroni et al. (1993*a*) postulated that these were due to European admixture. Bailliet et al. (1994) also include haplotype AM83 (which Torroni et al. [1993*a*] include as a lineage C haplotype) in their lineage E. Haplotype AM83 lacks the *Hinc*II 13259 site loss and would not typically be assigned to haplogroup C. AM83 lacks the *Hae*III 663 site gain (lineage A), the 9-bp deletion (Lineage B), and the *Alu*I 5176 site loss (lineage D) (see table 1), and thus does not fit criteria for any of the founding haplogroups. Bailliet et al. (1994) noted that unlike the other "lineage E" haplotypes, AM83 showed *Dde*I and *Alu*I site gains at nts 10394 and 10,397, respectively.

DNA sequence analysis of the mitochondrial D-loop has revealed a high level of diversity in Amerindian mtDNA. D-loop sequence haplotypes cluster in four groups (Ward et al. 1991, 1993; Ginther et al. 1993; Horai et al. 1993; Merriwether et al. 1993; Torroni et al. 1993*a*), and RFLP haplogroups A, B, and C segregate into analogous D-loop groups due to group-specific mutations. Haplogroup A sequences have a "T" at nt 16290 and an "A" at nt 16319. Haplogroup B sequences usually have "Cs" at nts 16189 and 16217. Lineage C sequences have a "C" at nt 16298 and a "T" at nt 16327. Haplogroup D sequences do not cluster tightly, because they lacked distinguishing polymorphisms. Comparison of these sequences with Asian D-loop sequences found that these group-specific mutations are shared between the two geographic regions, while other mutations seen in the D-loop are not. This indicates that the group-specific mutations probably arose in Asia while the other mutations occurred after Native Americans separated from Asian populations (Torroni et al. 1993*a*).

We have used a combination of RFLP haplotype and D-loop sequence analysis to characterize mtDNA variation in an isolated Yanomami Indian population from Brazil to examine the occurrence and frequency of mtDNA haplotypes in an isolated Amerindian sample. Our results suggest there were at least three additional mtDNA lineages in the New World founder population.

Material and Methods

The Study Sample

The Yanomami were, until recently, an isolated contemporary culture living in the lowland, tropical rain forest along the border of Brazil and Venezuela (Chagnon 1977, 1992; Good and Chanoff 1991). During January and February, 1990, Dr. Jairo J. Mancilha-Carvalho and a Brazilian Ministry of Health team visited eight villages of the Surucucu and Catrimani Plateau regions of the Território de Roraima in northwestern Brazil. Blood was drawn from an average of 12 to 13 adult volunteers in each village, giving a total of 61 men

and 39 women. Most of these individuals were not first-degree relatives, although more distantly related individuals were included. No pedigree or village-of-origin data were obtained as part of this survey. The 83 samples used in this study are from this survey (Crews et al. 1993; Crews and Manchilha-Carvalho 1993).

Laboratory Methods

DNA was extracted following a modification of the method of Boom et al. (1990) described by Merriwether et al (1994) and amplified by PCR (Saiki et al. 1988). All samples were amplified using primer sets encompassing the following regions: nts 577–743, 5099–5333, 8215–8297, 10284–10489, 13232–13393, 16201–16547, and 16453–00048. In addition, the "X" types were amplified using an additional primer set encompassing nts 11994–12701. All primers were 20- to 27-mer oligonucleotides that precisely match the published sequence (Anderson et al. 1981). RFLP typing was performed by amplifying the mtDNA with primers flanking the desired site. The DNA was digested in a cocktail of 1 μl BSA (50 mg/ml), 2 μl manufacturer's 10× enzyme buffer, 5 U restriction enzyme, and dH_20 to 10 μl total volume added to each 10-μl sample of DNA. The digest was incubated at 37°C overnight. The resulting fragments were electrophoresed in 2% agarose gels and visualized by ethidium bromide (EtBr) staining.

Samples to be sequenced were amplified by PCR in 100-μl reactions using primers encompassing nts 15908–00048 and nts 16555–00430. Amplification was verified on 2% agarose/EtBr gels. Those that amplified were, in the case of nts 16555–00430, extracted with 10 vol of equilibrated phenol (pH 7.9, Amresco) and 10 vol of 24:1 chloroform:isoamyl alcohol to aid in resolving long runs of cytosines. The resulting samples were concentrated using Micron 100 microconcentrators (Amicon). Samples amplified for nts 15908–00048 were not phenol-chloroform extracted prior to concentration.

Seven microliters of each purified sample was then reamplified in 20 μl total volume reactions using nested sequencing primers encompassing nts 15975–16420 and 00008–00430 with the Dydeoxy™ terminator cycle sequencing kit (Applied Biosystems). Unincorporated label was removed with Princeton Separation Centrisep™ Columns. The samples were analyzed on an Applied Biosystems 373A automated sequencer.

Analytical Methods

Sequences were aligned in Seq.ed 1.03, using the published Cambridge sequence (Anderson et al. 1981) for comparison, and edited and checked in MacClade 3.01 (Maddison and Maddison 1992). Kimura 2 parameter distance matrices (Kimura 1980) were generated in PHYLIP 3.5 (Felsenstein 1993) DNADist. Neighbor-joining trees (Saitou and Nei 1987) were generated in Phylip 3.5's Neighbor program. A parsimony tree was generated using Phylogenetic Analysis Using Parsimony (PAUP) version 3.0s (Swofford 1989). One thousand replicates of heuristic searches, saving 20 trees of the shortest length from each replicate, were run on the 50 samples that were merged for both hypervariable regions. A total of 16,200 equally parsimonious trees were saved, and 50% majority-rule consensus trees were calculated from the 16,200 trees.

Table 2

Summary of Yanomami Haplogroups

Type	No.	%
A	0	0
B1	4	4.8
B2	1	1.2
C1	31	37.5
C2	29	34.9
D1	2	2.4
D2	6	7.2
X6	7	8.4
X7	3	3.6
Total	83	100.0

Results

RFLP Data

All 83 Yanomami samples were typed for Wallace and Torroni's (1992) diagnostic markers for the four founding lineages and the *Hae*III site at nt 16517, which further defines these lineages (table 1) (Bailliet et al. 1994). The *Hae*III site at nt 16455 was also surveyed, but this site is monomorphic in humans and was excluded. Samples that did not have the markers defining the four founding haplogroups were typed for four additional RFLP sites in an attempt to further define these "other" haplotypes. A total of ≤15 polymorphic mtDNA markers (14 RFLP sites and the 9-bp deletion site) were analyzed. The results are summarized in table 2.

Wallace and Torroni's (1992) four founding lineage types made up 88% of this sample. Lineage C was the most frequent haplotype (C1 = 37.5%; C2 = 34.9%). Lineage D2 made up 7.2% and D1 made up 2.4% of the sample. Lineage B1 represented 4.8% and B2 represented 1.2% of the sample. No lineage A haplotypes were observed.

The remainder of the samples were of two haplotypes denoted as "X6" and "X7." These haplotypes are defined by the lack of the diagnostic markers for lineages A, B, C, or D. X6 and X7 have lost the *Hae*III site at nt 663, are nondeleted, and have the *Hinc*II site at nt

Table 3

Yanomami Haplotypes, by Region, Sex, and Age

Region and Haplotype	No. of Males (%)	No. of Females (%)	Age (years) <33 (%)	Age (years) >33 (%)	Total No. (%)
Surucucu:					
B1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
B2	1 (2.4)	0 (0)	0 (0)	1 (2.4)	1 (2.4)
C1	11 (26.8)	9 (21.9)	11 (26.8)	9 (21.9)	20 (48.8)
C2	3 (7.3)	0 (0)	1 (2.4)	2 (4.9)	3 (7.3)
D1	1 (2.4)	1 (2.4)	2 (4.9)	0 (0)	2 (4.9)
D2	3 (7.3)	2 (4.9)	3 (7.3)	2 (4.9)	5 (12.2)
X6	6 (14.6)	1 (2.4)	5 (12.2)	2 (4.9)	7 (17.1)
X7	1 (2.4)	2 (4.9)	1 (2.4)	2 (4.9)	3 (7.3)
Subtotal	26 (63.4)	15 (36.6)	23 (56.1)	18 (43.9)	41 (100.0)
Catrimani:					
B1	2 (4.8)	2 (4.8)	2 (4.8)	2 (4.8)	4 (9.5)
B2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
C1	6 (14.9)	5 (11.9)	6 (14.9)	5 (11.9)	11 (26.2)
C2	18 (42.9)	8 (19.0)	10 (23.8)	16 (38.1)	26 (61.9)
D1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
D2	0 (0)	1 (2.3)	0 (0)	1 (2.4)	1 (2.4)
X6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
X7	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Subtotal	25 (59.5)	17 (40.5)	18 (42.9)	24 (57.1)	42 (100.0)
Total	52 (62.7)	31 (37.3)	41 (49.4)	42 (50.6)	83 (100.0)

13259 and the *Alu*I site at nt 5176. They also have the *Dde*I site at nt 10394 and the *Alu*I site at nt 10397. In addition, they have a *Hinc*II site at nt 12406, a *Rsa*I site at nt 16303, and a *Sau*961 site at nt 16516. They are differentiated by the *Hae*III site at nt 16517: the X7 haplotype has that site, while the X6 haplotype does not. X6 made up 8.4% of the sample, and X7 made up 3.6% of the sample. No other haplotypes were observed.

The 83 individuals were subdivided by sex, age, and region of origin. The sample consisted of 52 males (62.7%) and 31 females (37.4%). The ages of the sampled individuals ranged from 15 to 63 years, with a mean of 33.6 years. The population was divided into two groups: those <33.6 years of age ($N = 41$; 49.4%) and those >33.6 years of age ($N = 42$; 50.6%). Samples were divided by region of origin: Surucucu and Catrimani. Results of comparisons of these subgroups by haplotype are given in table 3.

Previous studies that utilized RFLP typing of Native American, Asian, and Caucasian populations (Ballinger et al. 1992; Torroni et al. 1993*a*, 1993*b*, 1994*a*, 1994*b*, 1994*c;* Merriwether 1994) were reanalyzed for comparison with the Yanomami results. Several other studies (Cann et al. 1987; Stoneking et al. 1990; Stone and Stoneking 1993; Bailliet et al. 1994; Monsalve et al. 1994) were examined, but, because these studies did not survey for all of the requisite RFLP sites, they were excluded from this analysis. The results of this analysis are found in table 4.

Sequencing Data

Fifty sequences from the first (nts 16003–16393) and second (nts 00030–00396) hypervariable regions of the mitchondrial D-loop were obtained. The two regions were combined into one composite sequence for an analysis of 765 nt (from a total of 1,121 bp in the D-loop). This number is 7 nt larger than the Cambridge reference sequence, because of gaps added at nts 16366.1, 00309.1–00309.4, and 00315.1–00315.2, to allow for insertions seen in the Yanomami samples.

Analysis of the 50 merged sequences identified 43 mitochondrial haplotypes defined by 53 polymorphic sites. Thirty-four polymorphic positions were observed in the nts 16003–16393 region and 19 in the nt 00030–00396 region (table 5). Only those positions that differ from the Cambridge reference sequence (Anderson et al. 1981) are included. Dots signify that the haplotype sequence was identical to the published Cambridge sequence (shown in row 1) at that position. One haplotype was observed in three individuals (Yan 12, 65, and 76), five haplotypes each occurred in two individuals (Yan 2 and 9, Yan 21 and 89, Yan 24 and 36, Yan 31 and 63, and Yan 72 and 94), and 37 haplotypes were each seen in only one individual. Table 5 includes RFLP hap-

lotype designations of the samples. All sequence haplotypes consisting of more than one sample contained only one RFLP haplotype except one, which consisted of an X6 and a D2 RFLP haplotype. There were 2 B1 haplotypes, 20 C1 haplotypes, 7 C2 haplotypes, 2 D1 haplotypes, 3 D2 haplotypes, 1 combined D2-X6 haplotype, 5 X6 haplotypes, and 3 X7 haplotypes.

Figure 1 is a phylogenetic tree generated by the PHYLIP 3.5 Seqboot program (Felsenstein 1993) from 100 bootstrap resamplings of the 50 Yanomani sequences, along with the Cambridge reference sequence, four African sequences, and a chimp sequence used as outgroups. The Africans and the chimp were taken from studies by Redd et al. (1995), Vigilantet al. (1991), and Vigilant et al. (1989, 1991). Biaka Pygmy (45) and Mbuti Pygmy (43) are region V 9-bp deleted individuals. The other Africans and the chimp are nondeleted. PHYLIP 3.5's DNADist and Neighbor programs were used to generate 100 replicate trees, and Consense was used to create the consensus tree.

Figure 2 is a 50% majority-rule parsimony phylogenetic tree generated by PAUP version 3.1s (Swofford 1989) from the 50 sequences, plus the Cambridge sequence and the four Africans and the chimp sequence mentioned above, which were merged for both hypervariable regions. Figure 2 was generated by conducting 1,000 replications of heuristic search using the tree bisection and reconnection method and saving the 20 shortest trees from each replication. A total of 16,200 equally parsimonious trees were generated (all with a tree length of 208 steps).

Discussion

RFLP Data

Analysis of the RFLP data from this study shows that the Yanomami have a substantial level of mtDNA diversity (see table 2). Three of the four founding haplogroups were identified in this sample; haplogroup A was absent. This is consistent with other South American Indian populations, since the frequency of lineage A is generally much lower in South American Amerinds than in North American Amerinds (Merriwether et al. 1994). Lineages C1 (37.5%) and C2 (34.9%) were the most common, as they are in several nearby populations: the Yanomama in neighboring Venezuela had 25.2% C1 and 29.17% C2, the Marubo had 60.0% C2 (Torroni et al. 1993*a*), and the Trapa Trapa Pehuenche had 36.5% C2 (Merriwether et al. 1994) (see table 1). The other lineage haplotypes were found in lower frequencies in our sample of Yanomami: 4.8% were B1, 1.2% were D1, and 7.2% were D2.

One unexpected finding was a sample identified as haplotype B2. This sample had all the markers of the B haplotype (the 9-bp deletion, loss of the *Hae*III site at nt 663, the *Hinc*II site gain at nt 13259, the *Alu*I site gain at nt 5176, loss of the *Dde*I site at nt 10394, and loss of the *Alu*I site at nt 10397) but had lost the *Hae*III site at nt 16517 (see table 1). The loss of this *Hae*III site distinguishes A2 from A1, C2 from C1, and D2 from D1. As previously noted, Bailliet et al. (1994) have stated that, in 297 Amerindians typed as lineage B, haplotype B was always found in association with the *Hae*III site at nt 16517. Bailliet et al. (1994) state that the loss of the *Hae*III site at nt 16517 would be four times more frequent than a gain, because it would be caused by a mutation in any of the 4 nts forming the *Hae*III site, versus the single transition required to produce the site gain. The presence of haplotype B2 in 5% of Taiwanese Han (haplotype 61 in Ballinger et al. 1992) suggests haplotype B2 could be an additional founding lineage.

Perhaps the most intriguing aspect of this study is the finding, in a significant percentage of the samples, of two haplotypes (designated X6 and X7) that have not previously been recognized as founding lineages. These haplotypes have none of the defining markers of lineages A through D but have the *Dde*I site gain at nt 10394 and the *Alu*I site gain at nt 10397 (see table 1). Haplotypes X6 and X7 are identical except for the *Hae*III site at nt 16517: X7 has this site and X6 has a site loss at this position.

Haplotype X6 is the same as the haplotype that Torroni et al. (1993*a*) identified in one Makiritare individual and designated as "AM83," except that the AM83 sample has a *Rsa*I site loss at nt 16049, and our X6 samples have the *Rsa*I site at this position. Torroni and Wallace (1995) have stated that, among Native Americans, variants with the *Rsa*I site loss at nt 16049 have been found associated with haplotype C in populations that are linguistically related and geographically close to the Makiritare. They believe that the lack of the haplotype C marker (loss of the *Hinc*II site at nt 13259) "is most likely due to a reversion that has recently occurred in a South American-specific subset of haplogroup C mtDNAs" (Torroni and Wallace 1995, p. 1235). Bailliet et al. (1994) included haplotype AM83 in their putative lineage E, noting that the other lineage E haplotypes had losses of the *Dde*I site at nt 10394 and the *Alu*I site at nt 10397, while AM83 had these sites. AM83 is, therefore, distinct from "lineage E." The findings of this study suggest that haplotype AM83 or X6 is a possible new founding lineage. W. W. Hauswirth (personal communication) has identified both lineages X6 and X7 at high frequencies in 90 well-preserved 8,000-year-old Florida Windover bog burials (Doran et al. 1986; Hauswirth et al. 1994), which indicates that these lineages predate European colonization of the New World.

Torroni et al. (1993*a*) define the criteria for a founding lineage: (1) a founding haplotype should be widespread in the Amerindian populations, because it would

Table 4

Frequencies of mtDNA Haplotypes in New World and Asian Populations

Map	Population	Reference	No.	A1 (%)	A2 (%)	B1 (%)	B2 (%)	C1 (%)	C2 (%)	D1 (%)	D2 (%)	X6 (%)	X7 (%)	Other (%)
1	Malays	1	14	0 (0)	0 (0)	2 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.1)	6 (42.9)	5 (35.7)
2	Sabah Aborigines	1	32	0 (0)	0 (0)	5 (15.6)	0 (0)	0 (0)	0 (0)	2 (6.3)	0 (0)	1 (3.1)	17 (53.1)	7 (21.9)
3	Malay Aborigines	1	32	0 (0)	0 (0)	1 (3.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (18.8)	7 (21.9)	18 (56.3)
4	Malaysian Chinese	1	14	1 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (14.3)	1 (7.1)	1 (7.1)	9 (64.3)
5	Vietnamese	1	28	0 (0)	0 (0)	2 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (21.4)	3 (10.7)	17 (60.7)
6	Taiwanese Han	1	20	1 (5.0)	1 (5.0)	3 (15.0)	1 (5.0)	1 (5.0)	0 (0)	0 (0)	0 (0)	4 (20.0)	0 (0)	9 (45.0)
7	Tibetans	2	54	5 (9.3)	1 (1.9)	3 (5.6)	0 (0)	0 (0)	2 (3.7)	4 (7.4)	5 (9.3)	16 (29.6)	6 (11.1)	12 (22.2)
8	Koreans	1	13	1 (7.7)	0 (0)	1 (7.7)	0 (0)	0 (0)	0 (0)	1 (7.7)	2 (15.4)	4 (30.8)	0 (0)	4 (30.8)
9	Mongolians	3	42	0 (0)	2 (4.8)	1 (2.4)	0 (0)	5 (11.9)	0 (0)	3 (7.1)	5 (11.9)	7 (16.7)	4 (9.5)	15 (35.7)
10	Udegeys	4	45	0 (0)	0 (0)	0 (0)	0 (0)	8 (17.8)	0 (0)	0 (0)	0 (0)	11 (24.4)	9 (20.0)	17 (37.8)
11	Nivkhs	4	57	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.8)	15 (26.3)	0 (0)	2 (3.5)	38 (66.7)
12	Sel'kups	4	20	0 (0)	0 (0)	0 (0)	0 (0)	7 (35.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13 (65.0)
13	Evenks	4	51	0 (0)	2 (3.9)	0 (0)	0 (0)	43 (84.3)	0 (0)	0 (0)	5 (9.8)	0 (0)	0 (0)	1 (2.0)
14	Nganasans	4	49	0 (0)	1 (2.0)	0 (0)	0 (0)	19 (38.8)	0 (0)	10 (20.4)	8 (16.3)	2 (4.1)	1 (2.0)	8 (16.3)
15	Koryaks	4	46	1 (2.2)	10 (21.8)	0 (0)	0 (0)	4 (8.7)	0 (0)	0 (0)	1 (2.2)	0 (0)	7 (15.2)	23 (50.0)
16	Evens	4	43	0 (0)	0 (0)	0 (0)	0 (0)	25 (58.1)	0 (0)	0 (0)	3 (7.0)	0 (0)	15 (34.9)	0 (0)
17	Yukagirs	4	27	0 (0)	0 (0)	0 (0)	0 (0)	16 (59.3)	0 (0)	0 (0)	2 (7.4)	0 (0)	2 (7.4)	7 (25.9)
18	Chukchi	4	24	1 (4.2)	8 (33.3)	0 (0)	0 (0)	2 (8.3)	0 (0)	0 (0)	4 (16.7)	0 (0)	2 (8.3)	7 (29.2)
19	Eskimos	4	50	0 (0)	40 (80.0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.0)	7 (14.0)	0 (0)	0 (0)	2 (4.0)
20	Savoonga Eskimos	3, 5	41	0 (0)	34 (82.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4.9)	0 (0)	0 (0)	5 (12.2)
21	Gambell Eskimos	3, 5	52	0 (0)	34 (65.4)	0 (0)	0 (0)	2 (3.9)	2 (3.9)	0 (0)	13 (25.0)	0 (0)	0 (0)	1 (1.9)
22	St. Paul Aleuts	3, 5	57	0 (0)	16 (28.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	38 (66.7)	2 (3.5)	1 (1.8)	0 (0)
23	Yupik Eskimos	3, 5	165	2 (1.2)	41 (24.9)	0 (0)	0 (0)	0 (0)	3 (1.8)	1 (.6)	105 (63.6)	0 (0)	0 (0)	13 (7.9)
24	Old Harbor Eskimos	3, 5	128	10 (7.8)	56 (43.8)	1 (.8)	2 (1.6)	0 (0)	0 (0)	6 (4.7)	50 (39.1)	1 (.8)	0 (0)	2 (1.6)
25	Ouzinkie Eskimos	3, 5	42	6 (14.3)	23 (54.8)	0 (0)	0 (0)	0 (0)	1 (2.4)	0 (0)	8 (19.1)	2 (4.8)	0 (0)	2 (4.8)
26	Dogrib	3, 5	143	28 (19.6)	112 (78.3)	0 (0)	0 (0)	1 (.7)	2 (1.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
27	Haida	6	25	6 (24.0)	18 (72.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.0)	0 (0)	0 (0)	0 (0)
28	Bella Coola	6	25	4 (16.0)	11 (44.0)	2 (8.0)	0 (0)	2 (8.0)	0 (0)	0 (0)	5 (20.0)	0 (0)	0 (0)	1 (4.0)
29	Nuu-Chah-Nulth	6	15	1 (6.7)	5 (33.3)	1 (6.7)	0 (0)	2 (13.3)	0 (0)	1 (6.7)	3 (20.0)	0 (0)	0 (0)	2 (13.3)
30	Ojibwa	6	43	10 (23.3)	12 (27.9)	3 (7.0)	0 (0)	4 (9.3)	3 (7.0)	0 (0)	0 (0)	0 (0)	0 (0)	11 (25.6)
31	Mohawk	3, 5	128	65 (50.8)	6 (4.7)	21 (16.4)	0 (0)	3 (2.3)	26 (20.3)	2 (1.6)	0 (0)	2 (1.6)	1 (.8)	2 (1.6)
32	French Canadian Caucasians	7	28	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	28 (100.0)

33	U.S. Caucasians	7	147	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	147 (100.0)
34	Pima	6	30	2 (6.7)	0 (0)	15 (50.0)	0 (0)	1 (3.3)	12 (40.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
35	Navajo	6	48	28 (58.3)	0 (0)	18 (37.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4.2)
36	Apache	6	25	0 (0)	16 (64.0)	4 (16.0)	0 (0)	0 (0)	3 (12.0)	0 (0)	2 (8.0)	0 (0)	0 (0)	0 (0)
37	Oklahoma Muskoke	3, 5	70	3 (4.3)	23 (32.9)	10 (14.3)	0 (0)	0 (0)	7 (10.0)	8 (11.4)	19 (27.1)	0 (0)	0 (0)	0 (0)
38	Mixtec (Alta)	8	15	1 (6.7)	10 (66.7)	2 (13.3)	0 (0)	1 (6.7)	1 (6.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
39	Mixtec (Baja)	8	14	3 (21.4)	10 (71.4)	1 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
41	Zapotec	8	15	2 (13.3)	3 (20.0)	5 (33.3)	0 (0)	0 (0)	5 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
42	Mixe	8	16	1 (6.3)	9 (56.3)	5 (31.3)	0 (0)	1 (6.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
43	Maya	6	27	13 (48.2)	1 (3.7)	6 (22.2)	0 (0)	2 (7.4)	0 (0)	0 (0)	1 (3.7)	0 (0)	0 (0)	4 (14.8)
44	Bribri-Cabecar	6	24	0 (0)	13 (54.2)	11 (45.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
45	Boruca	6	14	0 (0)	2 (14.3)	10 (71.4)	0 (0)	0 (0)	0 (0)	1 (7.1)	0 (0)	0 (0)	0 (0)	1 (7.1)
46	Guatuso	9	20	0 (0)	17 (85.0)	3 (15.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
47	Teribe	9	20	0 (0)	16 (80.0)	4 (20.0)	0 (0)	0 (0)	0 (0)	0 (0)	(0)	0 (0)	0 (0)	0 (0)
48	Guaymi	6	16	6 (37.5)	5 (31.3)	5 (31.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
49	Kuna	6	16	1 (6.3)	15 (93.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
50	Piaroa	6	10	3 (30.0)	2 (20.0)	0 (0)	0 (0)	0 (0)	1 (10.0)	2 (20.0)	2 (20.0)	0 (0)	0 (0)	0 (0)
51	Makiritare	6	10	1 (10.0)	1 (10.0)	0 (0)	0 (0)	0 (0)	6 (60.0)	1 (10.0)	0 (0)	1 (10.0)	0 (0)	0 (0)
52	Yanomamo	6	24	0 (0)	0 (0)	4 (16.7)	0 (0)	6 (25.0)	7 (29.2)	2 (8.3)	5 (20.8)	0 (0)	0 (0)	0 (0)
53	Yanomami	10	83	0 (0)	0 (0)	4 (4.8)	1 (1.2)	31 (37.5)	29 (34.9)	2 (2.4)	6 (7.2)	7 (8.4)	3 (3.6)	0 (0)
54	Wapishana	6	12	0 (0)	0 (0)	3 (25.0)	0 (0)	0 (0)	1 (8.3)	1 (8.3)	7 (58.3)	0 (0)	0 (0)	0 (0)
55	Macushi	6	10	0 (0)	1 (10.0)	2 (20.0)	0 (0)	2 (20.0)	1 (10.0)	2 (20.0)	2 (20.0)	0 (0)	0 (0)	0 (0)
56	Ticuna	6	28	2 (7.4)	0 (0)	0 (0)	0 (0)	4 (14.3)	5 (17.9)	5 (17.9)	9 (32.1)	0 (0)	0 (0)	3 (10.7)
57	Marubo	6	10	1 (10.0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (60.0)	3 (30.0)	0 (0)	0 (0)	0 (0)	0 (0)
58	Kraho	6	14	0 (0)	4 (28.6)	8 (57.1)	0 (0)	1 (7.1)	1 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
59	Quechua	3, 5	19	0 (0)	4 (21.1)	6 (31.6)	2 (10.5)	0 (0)	2 (10.5)	0 (0)	5 (26.3)	0 (0)	0 (0)	0 (0)
60	Aymara Groups	3, 5	66	0 (0)	0 (0)	49 (74.2)	3 (4.6)	0 (0)	5 (7.6)	5 (7.6)	3 (4.6)	1 (1.5)	0 (0)	0 (0)
61	Mataco	6	28	3 (10.7)	0 (0)	10 (35.7)	0 (0)	0 (0)	0 (0)	0 (0)	15 (53.6)	0 (0)	0 (0)	0 (0)
62	San Pedro Atacam.	3, 5	16	1 (6.3)	0 (0)	12 (75.0)	1 (6.3)	1 (6.3)	1 (6.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
63	Trapa Trapa Pehuenche	3, 5	74	0 (0)	2 (2.7)	1 (1.4)	8 (10.8)	0 (0)	27 (36.5)	6 (8.1)	30 (40.5)	0 (0)	0 (0)	0 (0)
63	Butalelbum Pehuenche	3, 5	25	0 (0)	0 (0)	0 (0)	1 (4.0)	1 (4.0)	6 (24.0)	5 (20.0)	12 (48.0)	0 (0.0)	0 (0)	0 (0)
64	Huilliche	3, 5	89	3 (3.4)	1 (1.1)	22 (24.7)	1 (1.1)	5 (5.6)	14 (15.7)	8 (9.0)	35 (39.3)	0 (0)	0 (0)	0 (0)
	Africans	11	140	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	140 (100.0)

NOTE.—Map locations refer to figure 1 of Merriwether et al. (1996 [in this issue]).

Table 5

Polymorphic mtDNA D-Loop Sites in Both Hypervariable Regions of the D-loop in the Yanomami

	Site
	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	0 0 0 0 0 0 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 3 3 3 0 0 0 0 0 0 1 1 1 2 2 2 2 2 2 2 3 3 3
	0 4 4 6 8 9 0 2 2 7 8 8 1 2 3 4 4 5 5 8 9 9 0 2 2 3 3 4 5 6 6 7 8 9 3 6 7 7 8 8 4 5 5 1 5 6 6 7 8 8 1 3 7
	6 0 3 7 5 2 4 7 8 9 3 9 7 3 4 5 7 6 9 8 4 8 9 5 7 0 7 7 3 2 6 2 7 2 9 5 3 6 4 6 6 0 3 7 5 3 5 6 4 7 0 0 5
Cambridge sequence	A C A C C T C A C C A T T C C C A C C T C T A T C T C T C T - T A T C T A C A C T C A T G A T A A A T C C
Yan 61 B1	. . . . ? . . C C C . . . . . . C . . . . - . . . ? . G . . . . G . . . .
Yan 96 B1	. A . . T . . ? ? . C C C . . . . - . . . . . G . . . . G . . . . . A A
Yan 8 C1	. . . . T . . . . C . C T . . . . . - . . . . . ? A C G . . . . ? . . . C . . . .
Yan 10 C1	. . . . T . . . . C . C T . . . . . - . . . . . ? A C G C . . . . G . ? ? . . A .
Yan 11 C1	. G . . . . T . . ? . . C . C T G G G . G A . G C . . G A . G C . . . . G . . . . . A .
Yan 12 C1 + 65 C1 + 76 C1	. . . . T . . . . C . C T . . . . . - . . . . . G A . . . .
Yan 15 C1	. ? ? . . . . ? . . . . . T . . . . C . C T . . . . . - . . . ? . G A . . . .
Yan 19 C1	. . . . C . C ? . . . . . - . ? . . . G ? . G C . . . . G . . . . . A .
Yan 20 C1	. . . . T . . . . C . C T . . . . . - . . . . . G . . . .
Yan 22 C1	- . . . . . . ? . . . . . T . . . . ? . . C . C T . . . . . - . . . . . G . . . C . . . . G . . . .
Yan 33 C1	. . . . T . . . . C . C T . . . . . - . . . . . G A C G C . . . ? G . ? . . . . .
Yan 43 C1	. . . . T . . . . T . . C . C T . . . . . - . . . . . G A C G C . . . . G . . . . . . ?
Yan 44 C1	. . . . T . . . . T . . C . C T . . . . . - . . . . . G A C G C . . . . G . . . . A ? ?
Yan 47 C1	. . . . T . . . . C . C T . . . . . - . . . . . G A . . . . ? .
Yan 48 C1	. . . . T . . . . C . C T . . . . . - . . . . . G . . . . ? . . . . .
Yan 50 C1	. . . . T . . . . C . C T . . ? . . - . . . . . G . . . .
Yan 58 C1	. . . . T . . G . . . . C . C T . . . . . - . . . . . G ? . . . . T C ? . . .
Yan 59 C1	. . . . C . C ? . . . . . C A . ? . . G . . . .
Yan 60 C1	. . . . ? . . . . . T . . . . C . C T . . . . . - . . . . . G A . . C . . . . G . . . .
Yan 62 C1	. . . . T . . . . C . C T . . . . . - . . . . . G A . G . . . . ? . . . . .
Yan 69 C1	. . . . T . . . . C . C T . . . . . - . . . A . G ? . . . .
Yan 84 C1	. . . . ? . . ? . . . . . T . . . . C . C T . . . . . - . . . . . G A ? G . . . .
Yan 21 C2 + 89 C2	. . . . T . . . . C . C T . . . . . - . . . ? . G A . G C . . . . G . . . .
Yan 72 C2 + 94 C2	. . . . T . . . . T . . C . C T . . . . . - . . . A . G . . . C . . . . G . . . .
Yan 78 C2	. . . T . . . C . . . . . T . . . . C . C T . . . . . - . . . . . G A . G C . . . . G . . . .
Yan 80 C2	. . . ? . . . . G . . . . T . . . . C . C T . . . ? . - . . . . . G . . . C . . . . G . . . .
Yan 81 C2	. . . . . C . . . . T . . . . C . C T . . . . . - . . . . . G A . G . . G . . G . . . .
Yan 88 C2	. ? . . ? . . ? . . . . . T . . . . C . C T . . . . . - . . . . . G . . . C . . . . G . . . .
Yan 101 C2	. . . . T . . . . C . C T . . . . . - . . . A . G ? . ? C . . . . G . . . .
Yan 39 D1	. . . . A . . . T . . . T . . . . . C . . . . ? ? - . . . . . G . . . . G . . . .
Yan 42 D1	. ? . . . . . ? . A . . . T . . . T . . . . . C . . . . . ? - . . ? . A ? A C ? . . . . . G . . . .
Yan 1 D2	. . . . . . G . . . . T . . ? . . . . . ? - . . . . . G . . . . T . C . G . . . ? . . .
Yan 2 D2 + 9 D2	. . . . . . G . . . . T . . C . . . . . C - . . . . . G A . G . T . C . G . . . .
Yan 5 D2	. . . . . . ? . . . . T . . C . . . . . ? - . . . . . G A . G . T . C . G . . . .
Yan 31 X6 + 63 D2	. . . . . . G . . . . T . . C . . . . . ? - . . . . . G . . . . T . C . G . . . .
Yan 24 X6 + 36 X6	. . . . . C . . . . T T . . . . G C . . . . . ? - . . . . . G A . G . . . . . G . . . .
Yan 25 X6	T . G ? . . G ? . . . . T . . C . ? ? G . ? - . . ? . . G A . G . T . C . G . . . .
Yan 30 X6	. . . . . C . . . . T T . . . . G C . . . . . ? - . . . . . G . . . . T G A T C T . . .
Yan 32 X6	. . . . ? C . . . . T T . . ? . . ? . ? C . . . . . . - . . . . ? ? A C G . . . . . G . . . .
Yan 67 X6	. . . . . . A ? . . . . T . ? C . . . . . ? - . . . . . G . . ? . ? . . . G . . ? ? . . .
Yan 4 X7	. . . . T . . . . C . C . . . . . . - . . . . ? G A . . . . G . ? . ? . . .
Yan 46 X7	. . . . T . . . ? . C . . . . T C - . . . . . G A . . . . G . . . .
Yan 49 X7	. . . . T . . . ? . C . . . . T C - G . . . . G . . . . G . . . .

NOTE.—The Cambridge sequence refers to the published reference sequence (Anderson et al. 1981).

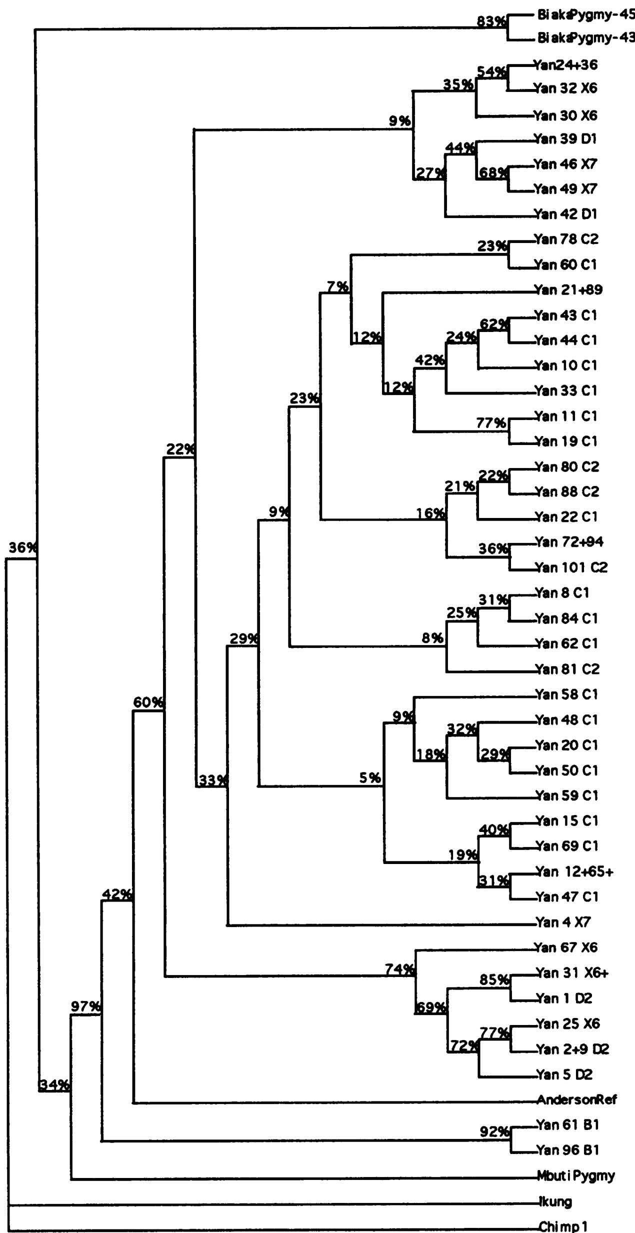

Figure 1 Bootstrap replicate neighbor-joining tree

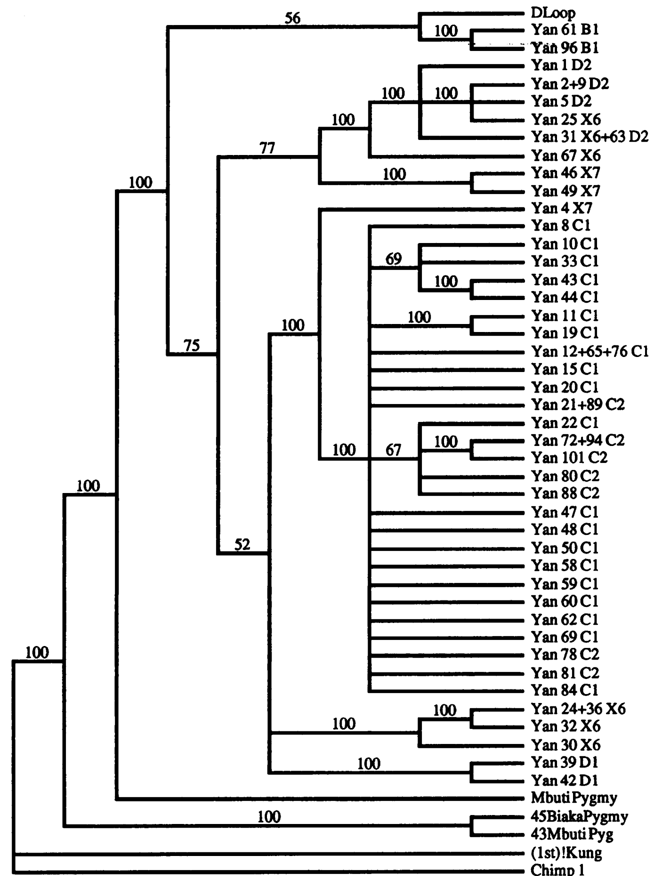

Figure 2 Fifty-percent majority-rule parsimony consensus tree

have preceded tribal differentiation; (2) a founding haplotype should be central to the branching of its haplogroup in the phylogenetic analysis, because new haplotypes would have originated from it; and (3) a founding haplotype should be present in east Asian and Siberian populations.

As can be seen in table 4, haplotypes X6 and X7 are widespread in Amerindian populations. These populations range from the far north of North America to well into South America, which is consistent with lineages X6 and X7 being founding lineages. X6 and X7 haplotypes are widespread in Asians and Siberians, as shown in table 4, and were the most frequent haplotypes found in some of these populations. Thus, they are unlikely to be the result of a recent reversion as proposed by Torroni and Wallace (1995). In addition, the *Dde*I site gain at nt 10394 in combination with the *Alu*I site gain at nt 10397 has never been detected in U.S. or French Canadian Caucasians (Torroni et al. 1994*b*). Chen et al. (1995) did detect the *Dde*I site at nt 10394 with the *Alu*I site at nt 10397 in one Senegalese (haplotype AF24). AF24 is identical to haplotype X6 except that AF24 has a *Hinc*II site loss at nt 12406, while haplotype X6 has this site. No other Africans have been found that have the *Dde*I site at nt 10394 in combination with the *Alu*I site at nt 10397 (Cann et al. 1987; Stoneking et al. 1990; Chen et al. 1995). It is, therefore, unlikely that these haplotypes are the result of admixture with non-Yanomami.

The Torroni et al. (1993*a*) study included 24 Yanomamo from neighboring Venezuela, but did not detect haplotype X6 or X7. It is possible that these haplotypes were present in the Yanomamo but not detected in the sample typed by Torroni et al. (1993*a*). A sample size of 24 should detect ~63% of lineages in a population with "limited" mtDNA diversity (Ward et al. 1993). If Torroni et al.'s (1993*a*) Yanomamo population pos-

sessed a similar level of diversity, they could have missed one third of the haplotypes present in the population.

A comparison of haplotypes by sex (see table 3) shows no significant differences in frequencies except in lineages C2 and X6. There is an excess of C2 males (18, or 42.9% of the sample) over females (8, or 19.0%) among the Catrimani and of X6 males (6, or 14.6%) over females (1, or 2.4%) among the Surucucu. In addition, taking into account that 62.7% of the sample is male and 37.4% of the sample is female, the C1 lineage frequency of almost equal distribution between males and females is not what would be expected. The other haplotypes are represented by too few individuals to detect any differences in frequency. These results may reflect the Yanomami practice of men marrying women from other villages and bringing the women to their home village to live, a practice that leads to the importation of new mitochondrial lineage types.

The sample was divided into two groups, those <33 years of age and those ⩾33 years of age, for the comparison of age versus haplotype (see table 3). Since the samples were collected in 1990, and permanent contact through missionaries began in the early 1960s, the individuals in the older group would have been born prior to sustained contact with outsiders. The proposed new founding haplotypes (B2, X6, and X7) are all represented in individuals born prior to sustained contact. Although prior transient contact is a possibility, it would have been most likely to involve males who do not contribute to the mitochondrial gene pool. These results are consistent with our argument that these haplotypes are not due to admixture.

We also examined the haplotype distribution by sample areas. Surucucu is a more remote area and has had less contact with outsiders (D. E. Crews, personal communication). We separated the samples by geography: Surucucu (Yan 1–57 and 67) and Catrimani (Yan 58–66 and 68–101). Table 3 shows the results of the comparison of area of origin versus haplotype. Nearly all of the haplotypes show variation in this comparison. Some of this variation is due to genetic drift in these small, isolated populations, but one difference that is apparent is that all of the proposed new founding haplotypes are confined to the more isolated Surucucu population. This result is further evidence that haplotypes B2, X6, and X7 are indigenous to the Yanomami and are new founding Amerindian lineages.

Sequence Data

Analysis of D-loop sequence data in the Yanomami shows a substantial level of mitochondrial DNA diversity (see table 5). Forty-three mitochondrial haplotypes defined by 53 variable positions were identified in 50 samples for nts 16003–16393 and nts 00030–00396 of the hypervariable regions in the mitochondrial D-loop. Thirty-four of the variable positions were in the nt 16003-16393 region, and 19 were in the nt 00030–00396 region. In other studies, Ward et al. (1991) found 28 sequence patterns in the nt 16024–16383 region in 63 members of the Nuu-Chah-Nulth tribe, Ginther et al. (1993) found 19 sequence haplotypes in the nt 16050–16400 and 00070–00370 regions in 60 Mapuche Indians, Horai et al. (1993) found 43 sequence haplotypes in the nt 16129-00036 region in 72 Amerindians from 16 populations, Ward et al. (1993) found 9 Amerindian sequence patterns in the nt 16024–16383 region in 41 Haida and 11 types in 40 Bella Coola, Shields et al. (1993) found 33 sequence haplotypes in the same region as the Ward et al. (1991, 1993) studies in 90 individuals from five circumpolar populations and compared them to 47 haplotypes found in 145 Bella Coola, Nuu-Chah-Nulth, and Yakima, and Torroni et al. (1993*a*) found 32 sequence haplotypes in the nt 16030–16370 region in 38 Native Americans from 17 populations.

Table 6 lists the D-loop point mutations associated with the RFLP haplotypes of the individuals in this study, including those mutations always seen in association with each Yanomami RFLP haplotype and those seen in more than one individual but not all individuals of each haplotype.

As has been observed by others, D-loop sequence haplotypes largely cluster into groups defined by RFLP haplogroups. For haplogroups B, C, and D, where a comparative sequence is available, the D-loop variation seen in the Yanomami largely overlaps that reported from other Amerindian populations.

Regarding the proposed new founding haplotypes, the five X6 sequencing haplotypes and the three X7 haplotypes all had 16325-T-C and 00263-A-G. They share 16325-T-C with the C and D haplotypes. Three of the five X6 haplotypes had four additional mutations. Two of the three X7 haplotypes had four addional mutations as well (see table 6). These X haplotypes have not been sequenced previously, so we had no other studies to use for comparison. Our data would suggest that X6 haplotypes are characterized by 16223-C-T, 16234-C-T, 16324-T-C, 00076-G-A, 00086-A-G, and 00263-A-G. Haplotypes X7 is more difficult to characterize, but may be defined by 16325-T-C and 00263-A-G and probably by 16256-C-T, 16353-C-T, 16362-T-C, and 00076-G-A.

The combined X6-D2 haplotype had a pattern with similarities to the characteristic D2 sequence haplotype pattern, sharing 16294-C-T, 00150-C-T, and 00217-T-C. This shared D2-X6 haplotype may indicate that haplotypes D2 and X6 are closely related, since both occurred on the same D-loop sequence background.

The shared polymorphisms in the sequence haplotypes caused them to cluster into groups in the neighbor-joining bootstrap (NJBS) tree (fig. 1) and the parsimony-

Table 6

Correlation Between RFLP Haplotype and D-Loop Sequence Haplotype

RFLP Haplotype	D-Loop Mutations Shared by All Yanomami of This Haplotype	D-Loop Mutations Shared by More Than One Yanomami of This Haplotype
B1	16183 A:C, 16189 T:C, 16217 A:G, 00263 A:G	Only two B1s present in this sample
C1	16298 T:C, 16325 T:C	16223 C:T, 00076 C:A, 00084 A:C; 00263 A:G, 00086 C:G, 16327 C:T, 00146 T:C
C2	16223 C:T, 16298 T:C, 16325 T:C, 16327 C:T, 16325 T:C, 00263 A:G	16223 C:T, 00076 C:A, 00084 A:C, 00263 A:G, 00086 C:G, 16327 C:T, 00146 T:C
D1	16179 C:A, 16223 C:T, 16256 C:T, 00263 A:G	Only two D1s present in this sample
D2	16294 C:T, 00150 C:T, 00217 T:C, 00263 A:G	16104 C:G, 16325 T:C, 00076 C:A, 00086 C:G
X6	16325 T:C, 00263 A:G,	16092 T:C, 16223 C:T, 16234 C:T, 00076 C:A, 00086 C:G
X7	16325 T:C, 00263 A:G,	16256 C:T, 16353 C:T, 16362 C:A, 00076 C:A

consensus (PC) tree (fig. 2). In both trees the African and chimp sequences acted as outgroups to the Yanomami sequences. The D haplotypes clustered with the X6 (and most of the X7 haplotypes) in both trees. The D1 haplotypes clustered closer to X7 haplotypes in the NJBS tree and closer to X6 haplotypes in the PC tree, and the D2 haplotypes were closer to X6 haplotypes in the NJBS tree and the X6 and X7 haplotypes in the PC tree. The C haplotypes clustered tightly, with some evidence of separation of the C2 from C1 haplotypes in the PC tree. One X7 haplotype (Yan 4) clustered closer to the C haplotypes in both trees. In general, haplotypes that cluster together are closely related, so this may indicate that the X6 and X7 haplotypes are more closely related to C and D haplotypes than to B1 haplotypes.

Phylogenetic trees of the sequenced samples show the RFLP types tend to cluster together. Because of the lack of depth in these trees, we were unable to determine the nodality of the X haplotypes in relation to the C and D haplotypes. We are, therefore, unable to address the issue of Torroni et al.'s (1993*a*) third criteria for a founding lineage: that it be central to the branching of its haplogroup in the phylogenetic analysis, because new haplotypes would have originated from it.

Conclusions

RFLP and sequence analysis of 83 Yanomami samples from the Surucucu and Catrimani regions of Roraima, Brazil, found a substantial level of mitochondrial diversity. Five of the seven previously identified Native American RFLP haplotypes and three new RFLP haplotypes, along with 43 sequencing haplotypes, were detected. Evidence is presented that the RFLP haplotypes B2 and especially X6 and X7 are previously unrecognized founding lineage types in the New World. Two of Torroni et al.'s (1993*a*) three criteria for a founding lineage are satisfied by these haplotypes: that a founding haplotype be widespread in Amerindian populations and that it should be detected in East Asian and Siberian populations. Haplotypes X6 and X7, and to a lesser extent haplotype B2, satisfy these conditions. In addition, all of these haplotypes were detected among older individuals (born before extensive outside contact) and only in the more isolated geographical area. We were unable to address the third Torroni et al. (1993*a*) criteria for a founding lineage, because of the shallowness of our phylogenetic trees. Our sequence analysis of both hypervariable regions found many shared mutations in samples of the same RFLP haplotype. These are delineated in the study. The mutations caused sequences to cluster by RFLP haplotype in neighbor-joining and 50% majority-rule parsimony trees. This was taken as evidence that our RFLP typing was valid.

Acknowledgments

This study was supported in part by a grant from the Wenner-Gren Foundation for Anthropological Research; the W. M. Keck Foundation for Advanced Training in Computational Biology at the University of Pittsburgh, Carnegie Mellon University, and the Pittsburgh Supercomputing Center; and by Pittsburgh Supercomputing Center grant P41 RR06009 from the National Institutes of Health. We wish to thank Dr. J. J. Mancilha-Carvalho for supplying the original samples for this study.

References

Anderson S, Bankier AT, Barrel BG, DeBulin MHL, Coulson AR, Drouin J, Eperon IC, et al (1981) Sequence and organi-

zation of the human mitochondrial genome. Nature 290: 457–465
Bailliet G, Rothhammer F, Carnes FR, Bravi CM, Bianci NO (1994) Founder mitochondrial haplotypes in Amerindian populations. Am J Hum Genet 54:27–33
Ballinger SW, Schurr TG, Torroni A, Gan YY, Hodge JA, Hassan D, Chen K-H, et al (1992) Southeast Asian mitochondrial DNA analysis reveals genetic continuity of ancient Mongoloid migrations. Genetics 130:139–152
Boom R, Sol CJA, Salimans MMM, Jamsen CL, Dillen PMEW-V, Noordaa JVD (1990) Rapid and simple method for purification of nucleic acids. J Clin Microbiol 28:495–503
Cann RL (1994) mtDNA and Native Americans: a southern perspective. Am J Hum Genet 55:7–11
Cann RL, Stoneking M, Wilson AC (1987) Mitochondrial DNA and human evolution. Nature 325:31–36
Chagnon NA (1977) Yanomamo: the fierce people, 2d ed. Holt, Rinehart, Winston, NY
——— (1992) Yanomamo: the last days of Eden. Harcourt Brace Jovanovich, New York
Chen Y-S, Torroni A, Excoffier L, Santachiara-Benerecetti AS, Wallace DC (1995) Analysis of mtDNA variation in African populations reveals the most ancient of all human continent-specific haplogroups. Am J Hum Genet 57:133–149
Crews DE, Kamboh MI, Mancilha-Carvalho JJ, Kottke B (1993) Population genetics of apolipoprotein A-4, E, and H polymorphisms in Yanomami Indians of Northwestern Brazil: associations with lipids, lipoproteins, & carbohydrate metabolism. Hum Biol 65:211–223
Crews DE, Mancilha-Carvalho JJ (1993) Correlates of blood pressure in Yanomami Indians of Northwestern Brazil. Ethn Dis 3:362–371
Dillehay TD (1989) Monte Verde: a late Pleistocene settlement in Chile, vol 1. Smithsonian Institution Press, Washington
Doran GH, Dickel DN, Ballinger-Jr WE, Agee OF, Laipis PJ, Hauswirth WW (1986). Anatomical, cellular and molecular analysis of 8,000-year-old human brain tissue from the Windover archaeological site. Nature 323:803–806.
Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author, Department of Genetics, University of Washington, Seattle
Ginther C, Corach D, Penacino GA, Rey JA, Carnese FR, Hutz MH, Anderson A, et al (1993) Genetic variation among the Mapuche Indians from the Patagonian region of Argentina: mitochondrial DNA sequence variation and allele frequencies of several nuclear genes. In: Pena SDJ, Chakraborty R, Epplen JT, Jeffreys AJ (eds) DNA fingerprinting: state of the science. Birkhauser, Boston, pp 211–219
Good K, Chanoff D (1991) Into the heart: one man's pursuit of love and knowledge among the Yanomama. Simon and Schuster, New York
Greenberg JH (1987) Language in the Americas. Stanford University Press, Stanford
Greenberg JH, Turner CG II, Zegura SL (1986) The settlement of the Americas: a comparison of the linguistic, dental, and genetic evidence. Curr Anthropol 27:477–497
Hauswirth WW, Dickel CD, Lawlor DA (1994) DNA analysis of the Windover population. In: Herrmann B, Hummel S (eds). Ancient DNA. Springer, New York, pp 104–121
Horai S, Kondo R, Nakagawa-Hattori Y, Hayashi S, Sonoda S, Tajima K (1993) Peopling of the Americas, founded by four major lineages of mitochondrial DNA. Mol Biol Evol 10:23–47
Johnson MJ, Wallace DC, Ferris SC, Rattazzi MC, Cavalli-Sforza LL (1983) Radiation of human mitochondrial DNA types analyzed by restriction endonuclease cleavage patterns. J Mol Evol 19:255–271
Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120
Lynch TF (1991) The peopling of the Americas: a discussion. In: Dillehay TD, Meltzer DJ (eds) The first Americans: search and research. CRC Press, Boca Raton, pp 267–274
Maddison WP, Maddison DR (1992) MacClade 301: analysis of phylogeny and character evolution. Sinauer, Sunderland, MA
Marshall E (1990) Clovis counterrevolution. Science 249:738–741
Merriwether DA, Clark AG, Ballinger SW, Schurr TG, Soodyall H, Jenkins T, Sherry ST, et al (1991) The structure of human mitochondrial DNA variation. J Mol Evol 33:543–555
Merriwether DA, Hall WW, Vahlne A, Ferrell RE (1996) mtDNA variation indicates Mongolia may have been the source for the founding population for the new world. Am J Hum Genet 59:204–212 (in this issue)
Merriwether DA, Rothhammer F, Ferrell RE (1993) Mitochondrial DNA D-loop sequence variation in native South Americans. Am J Hum Genet Suppl 53:833
——— (1994) Genetic variation in the New World: ancient teeth, bone, and tissue as sources of DNA. Experientia 50: 592–601
Monsalve MV, Groot de Restrepo H, Espinel A, Correal G, Devine DV (1994) Evidence of mitochondrial DNA diversity in South American aboriginals. Ann Hum Genet 58:265–273
Redd AJ, Takezaki N, Sherry ST, McGarvey ST, Sofro ASM, Stoneking M (1995) Evolutionary history of the COII tRNALys intergenic 9 base pair deletion in human mitochondrial DNAs from the Pacific. Mol Biol Evol 12:604–615
Rothhammer F, Bianchi NO (1995) Origin and distribution of B mtDNA lineage in South America. Am J Hum Genet 56:1247–1248
Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491
Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
Salzano FM, Calegari-Jacques SM (1988) South American Indians: a case study in evolution. Clarendon, Oxford
Schurr TG, Ballinger SW, Gan Y-Y, Hodge JA, Merriwether DA, Lawrence DN, Knowler WC, et al (1990) Amerindian mitochondrial DNAs have rare Asian mutations at high frequencies, suggesting they derived from four primary maternal lineages. Am J Hum Genet 46:613–623
Shields GF, Schmiechen AM, Frazier BL, Redd A, Voevoda

MI, Reed JK, Ward RH (1993) mtNDA sequences suggest a recent evolutionary divergence for Beringian and northern North American populations. Am J Hum Genet 53:549–562

Stone AC, Stoneking M (1993) Ancient DNA from a Pre-Columbian Amerindian population. Am J Phys Anthropol 92:463–471

Stoneking M, Jorde LB, Bhatia K, Wilson AC (1990) Geographic variation in human mitochondrial DNA from Papua New Guinea. Genetics 124:717–733

Swofford DL (1989) PAUP: phylogenetic analysis using parsimony, version 3.0. Illinois Natural History Survey, Champaign

Szathmary EJE (1993) mtDNA and the peopling of the Americas. Am J Hum Genet 53:793–799

Torroni A, Chen Y-S, Semino O, Santachiara-Beneceretti AS, Scott CR, Lott MT, Winter M, et al (1994*a*) mtDNA and Y-chromosome polymorphisms in four Native American populations from southern Mexico. Am J Hum Genet 54: 303–318

Torroni A, Lott MT, Cabell MF, Chen Y-S, Lavergne L, Wallace DC (1994*b*) mtDNA and the origin of Caucasians: identification of ancient Caucasian-specific haplogroups, one of which is prone to a recurrent somatic duplication in the D-loop region. Am J Hum Genet 55:760–776

Torroni A, Miller JA, Moore LG, Zamudio S, Zhuang J, Droma T, Wallace DC (1994*c*) Mitochondrial DNA analysis in Tibet: implications for the origin of the Tibetan population and its adaptation to high altitude. Am J Phys Anthropol 93:189–199

Torroni A, Schurr TG, Cabell MF, Brown MD, Neel JV, Larsen M, Smith DG, et al (1993*a*) Asian affinities and continental radiation of the four founding Native American mitochondrial DNAs. Am J Hum Genet 53:563–590

Torroni A, Sukernik RI, Schurr TG, Starikovskaya YB, Cabell MJ, Crawford MH, Comuzzie AG, et al (1993*b*) mtDNA variation of aboriginal Siberians reveals distinct genetic affinities with Native Americans. Am J Hum Genet 53:591–608

Torroni A, Wallace DC (1995) mtDNA haplogroups in Native Americans. Am J Hum Genet 56:1234–1235

Turner CG II (1985) The dental search for Native American origins. In: Kirk R, Szathmary E (eds) Out of Asia: peopling the Americas and the Pacific. Journal of Pacific History, Canberra, pp 31–78

——— (1988) Teeth and prehistory in Asia. Sci Am 260:88–96

Vigilant L (1990) Control region sequences from African populations and the evolution of human mitochondrial DNA. PhD dissertation, University of California, Berkeley

Vigilant L, Pennington R, Harpending H, Kocher TD, Wilson AC (1989) Mitochondrial DNA sequences in single hairs from a Southern African population. Proc Natl Acad Sci USA 86:9350–9354

Vigilant L, Stoneking M, Harpending H, Hawkes K, Wilson AC (1991) African populations and the evolution of human mitochondrial. DNA. Science 253:1503–1507

Wallace DC, Garrison K, Knowler WC (1985) Dramatic founder effects in Amerindian mitochondrial DNAs. Am J Phys Anthropol 68:149–155

Wallace DC, Torroni A (1992) American Indian prehistory as written in the mitochondrial DNA: a review. Hum Biol 64: 403–416

Ward RH, Frazier BL, Dew-Jager K, Paabo S (1991) Extensive mitochondrial diversity within a single Amerindian tribe. Proc Natl Acad Sci USA 88:8720–8724

Ward RH, Redd A, Valencia D, Frazier B, Paabo S (1993) Genetic and linguistic differentiation in the Americas. Proc Natl Acad Sci USA 90:10663–10667

Wormington HM (1983) Early man in the New World: 1970–1980. In: Shutler R Jr (ed) Early man in the New World. Sage, Beverly Hills, pp 191–195

Zegura SL (1985) The initial peopling of the Americas: an overview. In: Kirk R, Szathmary E (eds) Out of Asia: peopling the Americas and the Pacific. Journal of Pacific History, Canberra, pp 1–18