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Asian Affinities and Continental Radiation of the Four Founding Native American mtDNAs

Antonio Torroni,* Theodore G. Schurr,*,† Margaret F. Cabell,* Michael D. Brown,* James V. Neel,‡ Merethe Larsen,*,[1] David G. Smith,§ Carlos M. Vullo,‖ and Douglas C. Wallace*,†

Departments of *Genetics and Molecular Medicine and †Anthropology, Emory University, Atlanta; ‡Department of Human Genetics, University of Michigan Medical School, Ann Arbor; §Department of Anthropology, University of California, Davis; and ‖Hospital Nacional de Clinicas, Facultad de Ciencias Medicas, Universidad Nacional de Cordoba, Cordoba, Argentina

Summary

The mtDNA variation of 321 individuals from 17 Native American populations was examined by high-resolution restriction endonuclease analysis. All mtDNAs were amplified from a variety of sources by using PCR. The mtDNA of a subset of 38 of these individuals was also analyzed by D-loop sequencing. The resulting data were combined with previous mtDNA data from five other Native American tribes, as well as with data from a variety of Asian populations, and were used to deduce the phylogenetic relationships between mtDNAs and to estimate sequence divergences. This analysis revealed the presence of four haplotype groups (haplogroups A, B, C, and D) in the Amerind, but only one haplogroup (A) in the Na-Dene, and confirmed the independent origins of the Amerinds and the Na-Dene. Further, each haplogroup appeared to have been founded by a single mtDNA haplotype, a result which is consistent with a hypothesized founder effect. Most of the variation within haplogroups was tribal specific, that is, it occurred as tribal private polymorphisms. These observations suggest that the process of tribalization began early in the history of the Amerinds, with relatively little intertribal genetic exchange occurring subsequently. The sequencing of 341 nucleotides in the mtDNA D-loop revealed that the D-loop sequence variation correlated strongly with the four haplogroups defined by restriction analysis, and it indicated that the D-loop variation, like the haplotype variation, arose predominantly after the migration of the ancestral Amerinds across the Bering land bridge.

Introduction

Several independent lines of evidence suggest that humans entered the Americas in more than one, temporally distinct, population movement. According to one still controversial model, the colonization of the New World occurred in a tripartite fashion, with three separate genetic and linguistic stocks contributing, in order of their entry, to the Paleo-Indians (ancestral Amerind speakers), the Na-Dene, and the Eskimo-Aleuts (Greenberg et al. 1986; Greenberg 1987). Many Americanists do not agree with Greenberg's language classification and use the classification of Campbell and Mithun (1979).

Previous studies of Native American mtDNA restriction site polymorphisms revealed that Native Americans derive from four independent mtDNA haplotypes, suggesting that these populations were derived from a limited number of migrations (Wallace et al. 1985). Moreover, the mtDNA sequence diversity of the Amerind and Na-Dene mtDNAs suggested that these originated from separate migrations (Shields et al. 1992; Torroni et al. 1992), with the Amerinds arriving in the Americas much earlier than the Na-Dene. Using the published range of estimates for mtDNA divergence rate and the extent of diversity found in Amerinds and Na-Dene, we concluded that the Amerind mtDNA di-

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Address for correspondence and reprints: Dr. Douglas C. Wallace, Department of Genetics and Molecular Medicine, Emory University, Atlanta, GA 30322.

1. Present address: Department of Medical Genetics, Tromsö, Norway.

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versity was compatible with a pre-Clovis colonization of the New World (Schurr et al. 1990; Torroni et al. 1992; Wallace and Torroni 1992).

The mtDNA data reported by Schurr et al. (1990) have been used for a theoretical analysis (Chakraborty and Weiss 1991) which concluded that mtDNA haplotypes were in a mutation-drift equilibrium. The existence of such an equilibrium would make it impossible to determine whether a given mtDNA haplotype represented a founder or a new mutation or was lost because of drift. However, the results of more detailed experimental analyses of the same population samples did not appear to support the existence of such an equilibrium (Torroni et al. 1992).

In 1991, Ward and collaborators reported the D-loop sequences (360 nucleotides) of 63 Nuu-Chah-Nulth (Nootka) mtDNAs. This study revealed 28 different D-loop sequences defined by 26 variable nucleotides. In contrast to our results, these authors concluded that the extent of sequence variation that had accumulated in Native American mtDNAs was not compatible with a limited number of founder mtDNA lineages and that the overall diversity of Native American mtDNAs was similar to that found throughout Asia.

To further address the question of the number and nature of Native American migrations, we have analyzed the restriction site variation of an additional 15 Amerind and 2 Na-Dene tribes. The same four groups of related haplotypes (haplogroups) were again found in this much larger sample, and the distinct nature of the Amerind and Na-Dene populations was confirmed. Moreover, the D-loops of representative mtDNAs from each haplogroup were sequenced and found to also cluster into these same four groups. Since our D-loop sequences encompass all the variation detected by Ward et al. (1991), it appears that they did not perceive the underlying population substructure and that they thus overestimated the sequence diversity and age of Native American mtDNA lineages.

The data on Native American mtDNA variation presented in this paper are complemented by the data presented in the companion paper on mtDNA variation of aboriginal Siberian populations. The Siberian paper reveals that the Siberian and Native American populations were derived from common founder mtDNA haplotypes but that the genetic radiation from these haplotypes occurred independently in each continent (Torroni et al. 1993). This indicates that the haplogroup divergence of the Native American populations occurred since they arrived in the Americas, which supports previous observations that the marked intertribal mtDNA divergence emerged since the arrival of the ancestral Amerinds (Neel 1980). Using published mtDNA evolutionary rates and the continent-specific mtDNA diversity, we have concluded that the first Americans arrived before the Clovis lithic culture but much later than the mtDNA divergence time estimated by Ward et al. (1991).

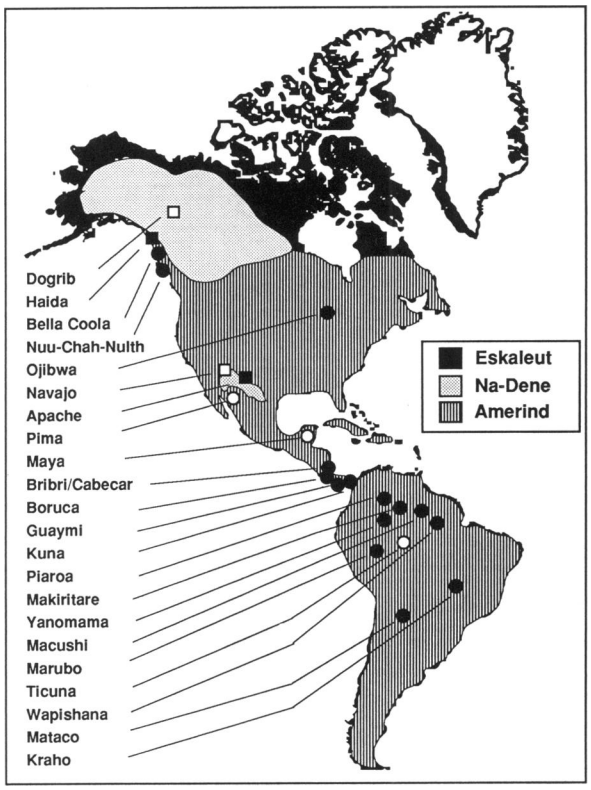


Figure 1 Geographic locations of Native American populations analyzed for mtDNA variation. The linguistic subdivisions are according to Greenberg (1987). Circles and squares indicate Amerind and Na-Dene tribes, respectively. Unblackened symbols indicate previously studied tribes (Torroni et al. 1992).

Subjects and Methods

Populations Sampled

The 361 samples of blood cells analyzed were a random selection from a much larger collection of Native American samples. They were obtained from 311 Amerinds and 50 Na-Dene. The locations of the 17 Amerind and 2 Na-Dene tribes analyzed in this study are presented in figure 1, along with those of the 3 Amerind and 2 Na-Dene tribes which were previously analyzed (Torroni et al. 1992). A list of all of the tribes

Table 1

Tribal Samples Analyzed for mtDNA Variation

Tribe	*N*	Linguistic Affiliation (first subaffiliation)[a]	Key Genetic References[b]	References for mtDNA Data
Amerinds:				
North America:				
Bella Coola	25	Northern Amerind (Almosan-Keresiouan)	Gofton et al. 1975; Field et al. 1988	Present study
Nuu-Chah-Nulth	15	Northern Amerind (Almosan-Keresiouan)	Ward et al. 1991	Ward et al. 1991; present study
Ojibwa	43	Northern Amerind (Almosan-Keresiouan)	Szathmary et al. 1974	Present study
Pima	30	Central Amerind (Uto-Aztecan)	Knowler et al. 1978	Wallace et al. 1985; Schurr et al. 1990; Torroni et al. 1992
Central America:				
Maya	27	Northern Amerind (Penutian)	Schurr et al. 1990	Schurr et al. 1990; Torroni et al. 1992
Boruca	14	Chibcha-Paezan (Chibchan)	Barrantes et al. 1990	Present study
Kuna	16	Chibcha-Paezan (Chibchan)	Barrantes et al. 1990	Present study
Guaymi	16	Chibcha-Paezan (Chibchan)	Spielman et al. 1979	Present study
Bribri/Cabecar	24	Chibcha-Paezan (Chibchan)	Barrantes et al. 1990	Present study
South America:				
Yanomama	24	Chibcha-Paezan (Chibchan)	Neel 1978*b*	Present study
Piaroa	10	Equatorial-Tucanoan (Equatorial)	Tanis et al. 1973; Ward et al. 1975	Present study
Makiritare	10	Ge-Pano-Carib (Macro-Carib)	Chagnon et al. 1970; Gershowitz et al. 1970	Present study
Macushi	10	Ge-Pano-Carib (Macro-Carib)	Neel et al. 1977*a*, 1977*b*	Present study
Wapishana	12	Equatorial-Tucanoan (Equatorial)	Neel et al. 1977*a*, 1977*b*	Present study
Ticuna	28	Equatorial-Tucanoan (Almosan-Keresiouan)	Neel et al. 1980	Schurr et al. 1990; Torroni et al. 1992
Kraho	14	Ge-Pano-Carib (Macro-Ge)	Salzano et al. 1977	Present study
Marubo	10	Ge-Pano-Carib (Macro-Panoan)	Mohrenweiser et al. 1979	Present study
Mataco	28	Ge-Pano-Carib (Macro-Panoan)	Vullo et al. 1984	Present study
Total	356			
Na-Dene:				
Dogrib	30	Na-Dene (Continental)	Szathmary 1983; Szathmary et al. 1987	Torroni et al. 1992
Navajo	48	Na-Dene (Continental)	Williams et al. 1981; Troup et al. 1982	Torroni et al. 1992
Haida	25	Na-Dene (Haida)	Gofton et al. 1975	Present study
Apache	25	Na-Dene (Continental)	Schell et al. 1978; Williams et al. 1985	Present study
Total	128			

[a] Linguistic affiliation and first subaffiliation follow the proposal of Greenberg (1987). For an alternative classification, see Campbell and Mithun (1979).

[b] Major genetic studies in which these populations were analyzed. These references usually also provide germane anthropological data of the tribes.

sampled and the references describing them are presented in table 1. Although controversial (Nichols 1990), the stated linguistic affiliations follow the terminology of Greenberg (1987). (For an alternative linguistic classification, see Campbell and Mithun [1979].) Documentation for the unreferenced statements in the present study which concern these tribes can be found in these previous publications. The tribal samples not previously described in any of our publications are as follows.

Bella Coola (Amerind).—The Bella Coola live along the coast of southern British Columbia. On the basis of

Gm haplotype frequencies, Field et al. (1988) estimated Caucasian admixture at 12%. The 25 samples used in this study were selected from a random sample of 132 subjects from the village of Bella Coola (790 residents), with each subject representing 1 of the 132 families living there.

Nuu-Chah-Nulth (Amerind).—The Nuu-Chah-Nulth inhabit Vancouver Island, British Columbia. An analysis of Nuu-Chah-Nulth blood group markers indicated less than 5% Caucasian admixture (Ward et al. 1991). The mtDNAs analyzed in the present study represent a random sample of 15 unrelated, allegedly full-blooded individuals.

Ojibwa (Amerind).—The Ojibwa (called "Chippewa" in the United States) inhabit the North American regions around the Great Lakes. The analyses of blood group markers indicated that some Ojibwa groups have almost 30% Caucasian admixture (Szathmary et al. 1974; Szathmary 1984). Twenty-eight of the Ojibwa analyzed were randomly chosen from a sample of 231 subjects provided by numerous nursing stations and clinics (Angling Lake, Eagle Lake, Frenchman's Head, Fort Hope, Kejicka, Landsdowne House, Summer Hill, Sachigo Lake, etc.) located in the northwest region of northern Ontario. Eight samples represented southwestern Ojibwa and were randomly chosen from 95 specimens collected at the Lac Courte Oreilles Community Health Center in Hayward, WI. Seven samples were Salteaux Ojibwa from the Turtle Mountain Reservation and were randomly chosen from 189 specimens collected at the Public Health Service Indian Hospital in Belcourt, ND. All samples were taken from patients who declared themselves to be full blooded and who were not known to be related.

Mataco (Amerind).—The Mataco inhabit the central part of the Chaquense region of northern Argentina. In the past, they lived in nomadic bands of 50–60 individuals, in which bands group endogamy was prevalent. At present, the Mataco have become more sedentary and number 14,000–16,000 individuals. The subjects analyzed in this study were randomly chosen from a larger number of specimens collected in a village called El Sauzalito (approximately 700 people) on the southern bank of the Bermejo River, El Chaco province. Because of the high degree of inbreeding, it was not possible to select completely unrelated individuals. However, siblings and first cousins were excluded from the sampling (Vullo et al. 1984).

Haida (Na-Dene).—The Haida live on the Queen Charlotte Island, British Columbia. On the basis of linguistic evidence, the Haida are postulated to belong to the Na-Dene and to be related to the Athapaskans (Campbell and Mithun 1979; Greenberg 1987), although their association with Na-Dene speakers is controversial (Krauss 1964; Levine 1979). On the basis of Gm haplotype frequencies in this group, Field et al. (1988) estimated Caucasian admixture at 20%. These samples were collected for biomedical studies, from two communities (Gofton et al. 1975). The 25 subjects analyzed in this study were randomly chosen from 238 samples initially collected after a solicitation of all community members over the age of 30 years.

Apache (Na-Dene).—The Apache are a group of Southern Na-Dene tribes (Jicarilla, Lipan, Kiowa Apache, Western Apache, Mescalero, and Chiricahua) which are linguistically closely related to the Northern Na-Dene of Alaska and Canada (Spencer et al. 1977). Together with the Navajo, they originated from peoples who migrated from northwestern Canada to the southwestern United States after A.D. 1000 (Haskell 1987). The occurrence of genetic admixture with surrounding Amerinds (mainly Pimas and Pueblos) has been confirmed by the presence of Gm haplotypes (Williams et al. 1985) and albumin variants, such as Albumin Mexico, which are specific to southwestern Amerinds but absent in the Northern Na-Dene (Schell and Blumberg 1988). In 1977, the Apache numbered some 15,000 individuals (Spencer et al. 1977). These Apache samples were collected in the San Carlos reservation from members of the San Carlos group, which is a division of the Western Apache tribe. Our collection is a random sample from 569 specimens drawn in 1973 from all consenting adults of the tribe over age 35 years.

Sample Preparation

The mtDNAs from the Bella Coola, the Nuu-Chah-Nulth, the Haida, and the San Carlos Apaches and from 15 (8 from Wisconsin and 7 from North Dakota) of the 43 Ojibwa were extracted from platelets present in small aliquots (20–50 μl) of plasma. Total DNAs of all Central and South American Amerinds except the Mataco were extracted from contaminant white cells present in washed red cell pellets (0.5–1.0 ml). These blood samples were collected between 15 and 25 years ago and stored in liquid nitrogen. Total DNAs from the Mataco and the 28 Ojibwa from northern Ontario were extracted from buffy coats. DNAs were extracted by using the procedures described by Torroni et al. (1992).

Molecular Genetic Techniques

The entire mtDNAs of 256 Amerinds (28 Ojibwa from northern Ontario and 228 Central and South

American Indian samples) were PCR amplified (Saiki et al. 1985) in nine partially overlapping segments. The oligonucleotide primers and PCR conditions used for these amplifications are described by Torroni et al. (1992). 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, *Hin*fI, *Hin*cII, *Hpa*I, *Hpa*II/*Msp*I, *Mbo*I, *Rsa*I, and *Taq*I. These endonucleases permit the screening of approximately 15%–20% of the mtDNA sequence per individual. The resulting restriction fragments were resolved by electrophoresis in 1.0%–2.5% NuSieve plus 1.0% SeaKem agarose (FMC BioProducts) gels, visualized by ethidium bromide staining, and mapped by the sequence comparison method (Johnson et al. 1983; Cann et al. 1984). This screening with 14 endonucleases generated complete haplotypes for each mtDNA (table 2 and Appendix).

Because of the poor quality of the mtDNA extracted from plasma specimens, it was not possible to amplify large PCR segments and define the complete mtDNA haplotypes of 105 subjects (the Bella Coola, the Nuu-Chah-Nulth, the Haida, the Apache, and the 15 Ojibwa from Wisconsin and North Dakota). However, because PCR segments of 200–400 bp could be routinely amplified from these samples, they were screened for the following eight mutations characterizing Native American mtDNAs (Schurr et al. 1990; Torroni et al. 1992): +*Hae*III np 663; −*Alu*I np 5176; 9-bp COII-tRNA^{Lys} deletion; +*Dde*I np 10394; +*Alu*I np 10397; −*Hin*cII np 13259/+*Alu*I np 13262; −*Rsa*I np 16329; and +*Hae*III np 16517.

For each suboptimal sample, six small segments encompassing the characteristic mutations were PCR amplified and digested with the appropriate endonucleases. The oligonucleotide primers and PCR conditions used for these amplifications are given in table 3. After the endonuclease digestion, the resulting restriction fragments were resolved through electrophoresis in NuSieve plus SeaKem agarose gels and were visualized by UV-induced fluorescence. This screening generated partial haplotypes for each mtDNA on the basis of the eight polymorphic restriction sites (table 4).

The dideoxy sequencing of a 341-np segment (np 16030–16370) of the mitochondrial control region (D-loop) of 38 Native American and 11 East Asian mtDNAs was also performed using previously described direct sequencing protocols (Shoffner et al. 1990; Brown et al. 1992) (table 5). A double-stranded PCR segment was generated from primers positioned at light strand np 15723 (L15723) and heavy strand np 221 (H221) at a T_H of 47°C, with all primers numbered according to the published mtDNA sequence (Anderson et al. 1981). Asymmetric PCRs were generated from this segment by using the following primer pairs and annealing temperatures, with the first primer listed being the limiting primer: (1) L15723 and H16344 (T_H = 49°C), (2) L15813 and H16527 (T_H = 51°C), and (3) L15997 and H16527 (T_H = 55°C). The following primers were then used to sequence the single-stranded mtDNA generated by the asymmetric PCR: (1) L15997, (2) L16048, (3) L16128, (4) H16242, (5) H16344, and (6) H16401.

The Native American samples which were subjected to the additional step of D-loop sequencing were randomly selected from each of the haplogroups found in that population. To maximize the extent of detectable sequence variation, tribes which were the most broadly distributed geographically were chosen. One or two mtDNAs from each of the haplogroups existing in the selected tribes were then selected at random. The sequenced East Asian samples were selected from those mtDNAs which belonged to the same haplogroups (A, B, C, and D) as observed in Native Americans (Ballinger et al. 1992).

Phylogenetic Analysis

The evolutionary relationships among the Native American haplotypes (fig. 2), and between Native American and Asian haplotypes (fig. 3), were inferred by parsimony analysis using PAUP (version 3.0s; Swofford 1992). For each analysis, maximum parsimony (MP) trees were generated through random addition of sequences by using both the Tree Bisection and Reconnection (TBR) and Nearest-Neighbor Interchange (NNI) branch-swapping algorithms. Because of the large number of terminal taxa, thousands of MP trees could be obtained with both branch-swapping methods. We terminated our searches at 3,000 trees after about 1,350 replications, with no more than 10 MP trees saved for each replication. About 2,600 of the 3,000 trees were obtained in the first 1,000 replications. In most of the remaining replications, the MP trees were discarded, since they were identical to those already saved, suggesting that the 3,000 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. In all cases, the MP trees generated by the TBR and NNI algorithms were of the same length. Strict consensus trees of 3,000 MP trees generated by the TBR and NNI algorithms were

Table 2

mDNA Haplotype Distribution in Native American Tribes

								Amerinds		
	North America		Central America							
Haplotype	Ojibwa	Pima	Maya	Boruca	Kuna	Guaymi	Bribri-Cabecar	Yanomama	Piaroa	Makiritare
Haplogroup A:										
AM 1	...	...	5	...	1	1	...	...	3	1
AM 2	...	2	...	...	...	...	...	...	...	...
AM 3	...	...	2	...	...	...	...	...	...	...
AM 4	...	...	2	...	...	...	...	...	...	...
AM 5	...	...	...	...	...	...	...	...	...	...
AM 6	...	...	...	...	...	...	...	...	...	...
AM 7	...	...	...	...	...	...	...	...	...	...
AM 8	...	...	...	...	...	...	...	...	...	...
AM 9	...	...	2	...	...	5	...	...	...	...
AM 10	...	...	1	...	...	...	...	...	...	...
AM 11	...	...	1	...	...	...	...	...	...	...
AM 12	...	...	1	...	...	...	...	...	...	...
AM 51	...	...	...	2	...	2	8	...	...	...
AM 52	...	...	...	1	...	...	...	...	...	...
AM 53	...	...	...	...	15	...	...	...	...	...
AM 54	...	...	...	...	...	2	...	...	...	...
AM 55	...	...	...	...	...	1	...	...	...	...
AM 56	...	...	...	...	...	...	5	...	...	...
AM 57	...	...	...	...	...	...	...	...	2	...
AM 58	...	...	...	...	...	...	...	...	...	1
AM 59	...	...	...	...	...	...	...	...	...	...
AM 60	...	...	...	...	...	...	...	...	...	...
AM 61	4	...	...	...	...	...	...	...	...	...
AM 62	10	...	...	...	...	...	...	...	...	...
AM 63	4	...	...	...	...	...	...	...	...	...
Subtotal	18	2	14	3	16	11	13	...	5	2
Haplogroup B:										
AM 13	...	5	5	10	...	5	6	4	...	...
AM 14	...	...	...	...	...	...	...	...	...	...
AM 15	...	...	...	...	...	...	...	...	...	...
AM 16	...	...	...	...	...	...	...	...	...	...
AM 17	...	...	...	...	...	...	...	...	...	...
AM 18	...	...	...	...	...	...	...	...	...	...
AM 19	...	1	...	...	...	...	...	...	...	...
AM 20	...	1	...	...	...	...	...	...	...	...
AM 21	...	...	1	...	...	...	...	...	...	...
AM 22	...	1	...	...	...	...	...	...	...	...
AM 23	...	2	...	...	...	...	...	...	...	...
AM 24	...	1	...	...	...	...	...	...	...	...
AM 25	...	1	...	...	...	...	...	...	...	...
AM 26	...	3	...	...	...	...	...	...	...	...
AM 65	...	...	...	...	...	...	5	...	...	...
AM 66	...	...	...	...	...	...	...	...	...	...
AM 67	...	...	...	...	...	...	...	...	...	...
AM 68	..	...	...	...	...	...	...	...	...	...
AM 69	...	...	...	...	...	...	...	...	...	...
AM 70	1	...	...	...	...	...	...	...	...	...
Subtotal	1	15	6	10	...	5	11	4	...	...
Others:										
AM 28	...	...	1	...	...	...	...	...	...	...
AM 29	1	...	...	...	...	...	...	...	...	...
AM 74	1	...	...	...	...	...	...	...	...	...
AM 75	3	...	...	...	...	...	...	...	...	...
AM 76	2	...	...	...	...	...	...	...	...	...
Subtotal	7	...	1	...	...	...	...	...	...	...

South America							Na-Dene		
Macushi	Wapishana	Ticuna	Kraho	Marubo	Mataco	Total No. of Amerinds	Dogrib	Navajo	Total No. of Na-Dene
...	...	...	...	1	3	15	19	11	30
...	...	...	...	...	...	2	...	...	...
...	...	...	...	...	...	2	...	...	...
...	...	...	...	...	...	2	...	...	...
...	...	...	...	...	...	...	7	13	20
...	...	...	...	...	...	...	1	...	1
...	...	2	...	...	...	2	...	...	...
...	...	1	...	...	...	1	...	...	...
...	...	2	...	...	...	9	3	4	7
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	12	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	15	...	...	...
...	...	...	...	...	...	2	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	5	...	...	...
...	...	...	...	...	...	2	...	...	...
...	...	...	...	...	...	1	...	...	...
1	...	...	...	...	...	1	...	...	...
...	...	...	4	...	...	4	...	...	...
...	...	...	...	...	...	4	...	...	...
...	...	...	...	...	...	10	...	...	...
...	...	...	...	...	...	4	...	...	...
1	...	5	4	1	3	98	30	28	58
1	3	...	3	...	10	52	...	11	11
...	...	...	...	...	...	...	...	2	2
...	...	...	...	...	...	...	...	1	1
...	...	...	...	...	...	...	...	2	2
...	...	...	...	...	...	...	...	1	1
...	...	...	...	...	...	...	...	1	1
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	2	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	3	...	...	...
...	...	...	...	...	...	5	...	...	...
1	...	...	...	...	...	1	...	...	...
...	...	...	1	...	...	1	...	...	...
...	...	...	1	...	...	1	...	...	...
...	...	...	3	...	...	3	...	...	...
2	3	...	8	...	10	75	...	18	18
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	1	...	2	2
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	3	...	...	...
...	...	...	...	...	...	2	...	...	...
...	...	...	...	...	...	8	...	2	2

(*continued*)

Table 2 (continued)

								Amerinds		
	North America		Central America							
Haplotype	Ojibwa	Pima	Maya	Boruca	Kuna	Guaymi	Bribri-Cabecar	Yanomama	Piaroa	Makiritare
Haplogroup C:										
AM 30	...	...	1	...	...	...	...	...	...	...
AM 31	...	...	1	...	...	...	...	...	...	...
AM 32	...	1	...	...	...	...	...	...	1	5
AM 33	...	1	...	...	...	...	...	...	...	...
AM 34	...	1	...	...	...	...	...	...	...	...
AM 35	...	5	...	...	...	...	...	...	...	...
AM 36	...	...	...	...	...	...	...	...	...	...
AM 37	...	3	...	...	...	...	...	...	...	...
AM 38	...	1	...	...	...	...	...	...	...	...
AM 39	...	1	...	...	...	...	...	...	...	...
AM 40	...	...	...	...	...	...	...	...	...	...
AM 41	...	...	...	...	...	...	...	...	...	...
AM 42	...	...	...	...	...	...	...	...	...	...
AM 43	...	...	2	...	...	...	...	...	...	...
AM 77	...	...	...	...	...	...	...	6	...	...
AM 78	...	...	...	...	...	...	...	1	...	...
AM 79	...	...	...	...	...	...	...	6	...	...
AM 80	...	...	...	...	...	...	...	...	...	...
AM 81	...	...	...	...	...	...	...	...	...	...
AM 82	...	...	...	...	...	...	...	...	...	1
AM 82	...	...	...	...	...	...	...	...	...	1
AM 84	...	...	...	...	...	...	...	...	...	...
AM 85	...	...	...	...	...	...	...	...	...	...
AM 86	1	...	...	...	...	...	...	...	...	...
AM 87	1	...	...	...	...	...	...	...	...	...
Subtotal	2	13	4	...	...	...	...	13	1	7
Haplogroup D:										
AM 44	...	...	...	1	...	...	...	2	...	...
AM 45	...	...	...	...	...	...	...	...	...	...
AM 46	...	...	...	...	...	...	...	...	...	...
AM 47	...	...	...	...	...	...	...	...	...	...
AM 48	...	...	...	...	...	...	...	...	...	...
AM 49	...	...	1	...	...	...	...	...	...	...
AM 50	...	...	1	...	...	...	...	...	...	...
AM 88	...	...	...	...	...	...	...	4	...	...
AM 89	...	...	...	...	...	...	...	...	...	...
AM 90	...	...	...	...	...	...	...	...	...	...
AM 91	...	...	...	...	...	...	...	...	...	1
AM 92	...	...	...	...	...	...	...	1	...	...
AM 93	...	...	...	...	...	...	...	...	...	...
AM 94	...	...	...	...	...	...	...	...	...	...
AM 95	...	...	...	...	...	...	...	...	2	...
AM 96	...	...	...	...	...	...	...	...	2	...
Subtotal	...	...	2	1	...	...	...	7	4	1
Total	28	30	27	14	16	16	24	24	10	10

Note.—Different studies have used different terminologies to indicate haplotypes and haplogroups, D-loop sequences, and groups of related D-loop sequences. The term "haplotype" is equivalent to the term "type" used by Cann et al. (1987) and Stoneking et al. (1990). The term "haplogroup" is equivalent to the term "clans of types" used by Stoneking et al. (1990) and to the term "haplotype clusters" used by Torroni et al. (1992). Individual "D-loop sequences" correspond to the "lineages" of Ward et al. (1991).

South America							Na-Dene		
Macushi	Wapishana	Ticuna	Kraho	Marubo	Mataco	Total No. of Amerinds	Dogrib	Navajo	Total No. of Na-Dene
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	1	...	...	...
...	1	...	...	1	...	9	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	5	...	...	...
...	...	1	...	...	...	1	...	...	...
...	...	...	...	...	...	3	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	3	...	...	...	3	...	...	...
...	...	2	...	...	...	2	...	...	...
...	...	3	...	...	...	3	...	...	...
1	...	...	...	...	...	3	...	...	...
...	...	...	...	...	...	6	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	1	...	...	7	...	...	...
1	...	...	...	...	...	1	...	...	...
...	...	...	...	1	...	1	...	...	...
1	...	...	...	...	...	2	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	1	...	...	1	...	...	...
...	...	...	...	4	...	4	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	1	...	...	...
3	1	9	2	6	...	61	...	...	...
...	...	4	...	3	...	10	...	...	...
...	...	1	...	...	...	1	...	...	...
...	...	4	...	...	...	4	...	...	...
...	...	2	...	...	...	2	...	...	...
...	...	3	...	...	...	3	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	...	1	...	...	...
...	...	...	...	...	15	19	...	...	...
...	7	...	...	...	...	7	...	...	...
1	...	...	...	...	...	1	...	...	...
...	1	...	...	...	...	2	...	...	...
...	...	...	...	...	...	1	...	...	...
1	...	...	...	...	...	1	...	...	...
2	...	...	...	...	...	2	...	...	...
...	...	...	...	...	...	2	...	...	...
...	...	...	...	...	...	2	...	...	...
4	8	14	...	3	15	59	...	...	...
10	12	28	14	10	28	301	30	48	78

Table 3

PCR Primers Used for Amplification of Suboptimal DNA Samples

Primer Coordinates[a]	T_H[b] (°C)	Size (bp)	Polymorphic Sites
534–553,[c] 725–706[d]	51	211	+*Hae*III 663
5151–5170, 5481–5464	55	330	−*Alu*I 5176
8150–8166, 8366–8345	51	216	9-bp deletion
10235–10254, 10569–10550	43	334	+*Dde*I 10394 +*Alu*I 10397
13197–13213, 13403–13384	45	206	−*Hin*cII 13259 +*Alu*I 13262
16287–16306, 16547–16527[e]	55	280	−*Rsa*I 16329 +*Hae*III 16517

NOTE.—"Suboptimal" DNA samples refer to those that were of poor quality and that were screened only for the presence of eight characteristic Native American mutations.

[a] Numbered according to Anderson et al. (1981).

[b] Calculated from the primer sequence without the tail sequence added.

[c] Has 5′-CCACCTGCAG tail

[d] Has 5′-CCACAAGCTT tail.

[e] Has 5′-CCACAAGCTT tail.

also obtained. Consensus trees are hierarchical summaries of the information common to a set of MP trees. A strict consensus tree contains only those groups appearing in all MP trees (Sokal and Rohlf 1981). Of the consensus trees, those generated by the TBR method were always several steps shorter than those produced by the NNI method, and, consequently, showed a higher consistency index (CI) and retention index (RI) than the latter. Nevertheless, the overall topology of the TBR and NNI consensus trees was always very similar. The CI for all characters (mutations) on a tree is the minimum possible tree length divided by the observed tree length. The CI would be 1 if the characters have no homoplasy, .5 if there are twice as many steps as needed, and so on. Higher values of the CI are thus desirable. The RI for all characters on a tree is calculated as (maximum possible tree length − actual tree length)/(maximum possible tree length − minimum possible tree length). If the characters in the data are perfectly congruent with each other and the tree, RI will have a value of 1. If the data are maximally homoplastic on the tree, RI will have a value of 0 (Maddison and Maddison 1992).

All dendrograms were rooted by using an African haplotype as an outgroup. The African outgroup haplotype was obtained from a Senegalese mtDNA analyzed in our laboratory and is characterized by the presence of an *Hpa*I site at np 3592 (Appendix). This site defines haplotypes which are African specific and observed in 70%–100% of the sub-Saharan Africans but are completely absent in Asians and Europeans (*Hpa*I morph-3; Denaro et al. 1981; Cann et al. 1987; Scozzari et al. 1988).

The evolutionary relationships between Native American and East Asian D-loop sequences were similarly determined through parsimony analysis (fig. 4). These dendrograms were generated through random addition using the TBR algorithm and were rooted from an African D-loop sequence obtained from a !Kung (subject 1; Vigilant et al. 1989). As for haplotypes, although no shorter trees were obtained, they could exist, and a large number of MP trees were obtained.

Sequence Divergence Estimations

Intragroup sequence divergence estimations from restriction analysis data were calculated with the maximum likelihood procedure of Nei and Tajima (1983) by using the program DREST (provided by L. Jin). This program considers the ratio of shared restriction sites between two haplotypes, as well as the mean length of the restriction enzyme recognition sequences, to calculate an initial estimate of π (the probability that two mtDNAs have different nucleotides at a given nucleotide position). When this initial value is used, π is solved iteratively by using equation 28, and the sequence divergence (σ) is estimated by using equation 21 (Nei and Tajima 1983). When the divergence times of the haplogroups are calculated, a mtDNA evolution rate of 2%–4%/million years (MYR) was used (Stoneking et al. 1986; Cann et al. 1987; Wallace et al. 1987). This standard rate of human mtDNA evolution does not incorporate any variance components. Therefore, a 95% confidence interval for the rate would be considerably broader.

Results

Among the 216 Amerinds whose mtDNAs were characterized with the complete set of 14 endonucleases, 49 different mtDNA haplotypes were observed. Fifty haplotypes were previously observed in the analysis of 167 Na-Dene and Amerind samples (Torroni et al. 1992). Because 7 haplotypes observed in both studies were identical, the total number of complete Native American mtDNA haplotypes identified in 383 Native Ameri-

Table 4

Partial Haplotype Distribution of Suboptimal Native American Samples

	Na-Dene		Amerinds		
Partial Haplotypes[a]	Haida (N = 25)	Apache (N = 25)	Bella Coola (N = 25)	Nuu-Chah-Nulth (N = 15)	Ojibwa (N = 15)
Haplogroup A:					
+663e	18	9	11	5	2
+663e, −16329k	0	7	0	0	0
+663e, +16517e	6	0	4	1	2
Total	24	16	15	6	4
Haplogroup B:					
9-bp deletion, +16517e	0	4	2	1	2
Haplogroup C:					
−13259o, +13262a, +10394c, +10397a	0	3	0	0	2
−13259o, +13262a, +10394c, +10397a, +16517e	0	0	2	2	3
Total	0	3	2	2	5
Haplogroup D:					
−5176a, +10394c, +10397a	1	2	5	3	0
−5176a, +10394c, +10397a, +16517e	0	0	0	1	0
Total	1	2	5	4	0
Others:					
+16517e	0	0	1	2	4

[a] Enzyme letter code is the same as that defined in the Appendix.

cans is 92 (AM1–AM63, AM65–AM70, and AM74–AM96) (table 2 and Appendix). A total of 109 polymorphic restriction sites and the 9-bp COII/tRNA^{Lys} intergenic deletion (Cann and Wilson 1983; Horai and Matsunaga 1986; Wrischnik et al. 1987; Hertzberg et al. 1989; Schurr et al. 1990) define these haplotypes.

Amerind mtDNA Haplogroups

The analysis of Amerind mtDNAs confirmed that they belonged to four well-defined haplogroups, A, B, C, and D, each defined by specific sets of associated polymorphisms (table 2). The genetic relationships between these haplotypes are illustrated in figure 2. The inset of figure 2 shows one of the MP trees which was generated with both TBR and NNI branch-swapping algorithms. MP trees obtained with both algorithms were 134 mutational steps in length with CI and RI of .593 and .911, respectively. Strict consensus trees of 3,000 of the MP trees were also generated. The consensus tree illustrated in figure 2 was obtained with the TBR method and is 166 steps in length, with CI and RI of .372 and .780, respectively. Despite some unresolved relationships between group A haplotypes in the consensus dendrogram, the overall structure of the MP and

Table 5

D-Loop Sequences from Amerinds, Na-Dene, and East Asians

Sample	Haplotype	Polymorphic Nucleotide Positions[a]
		1111111111111111111111111111111111111111111111111111111111111111
		6666666666666666666666666666666666666666666666666666666666666666
		0000000011111111111111112222222222222222222222222233333333333333
		3455899901222445577789901112333444666777899999901112233456 6
		9219623541469080412492832473235089036148801345801495715460 2
Cambridge[b]		GGAATTTCCCTTGTCCTATCTCTAACTCCAAACTCTCTGCTCCACCTATAGTCAACTCT
Haplogroup A:		
1 Bella Coola	Partial	---------T--A----------T----------T--------A-------C
2 Ojibwa	Partial	----------CA----------T----------T--------A-------C
3 Pima	AM2	---------T--A----------T----------T--------A-------C
4 Maya	AM1	---------T----------C------T----------T--------A-------C
5 Boruca	AM52	---------TC----------T----------T--------A------TC
6 Makiritare	AM58	---------T----------T---G---------T--------A-------C
7 Kraho	AM60	----------C----------T----------T-T--------A-------C
8 Ticuna	AM9	---------T----------T-T----------T--------A-------C
9 Mataco	AM1	--G------T----------T----------T--------A-------C
10 Haida	Partial	---------T----------T----------T--------A-------C
11 Dogrib	AM1	---------T----------T----------T--------A-------C
12 Dogrib	AM5	---------T----------T-----T-G----------T--------A--G----C
13 Tlingit	AM1	---------T----------T----------T--------A-------C
14 Tlingit	AM5	---------T----------G--T-G----------T--------A--G----C
15 Navajo	AM1	---------T----------T----------T--------A-------C
16 Navajo	AM5	---------T----------T-----T-G----------T--------A--G----C
17 Han (Taiwan)	AS56	----------T----T--------T-----G--A-------C
18 Han (Taiwan)	AS28	----------C----------T--G---------T--------A--------
19 Korean	AS103	----------C----------T--G---------T--------A--------
Haplogroup B:		
20 Ojibwa	Partial	---------T----------C-----C-------T----------C----------
21 Bella Coola	Partial	---------T----------C-----C----------
22 Pima	AM23	---------T----------C----------T----------
23 Maya	AM13	----------C-----C----------
24 Guaymi	AM13	----------C-----C----------C----------
25 Yanomama	AM13	----------C-----C----------
26 Kraho	AM67	----------C----------
27 Mataco	AM13	----------C-C---C----------
28 Han (Taiwan)	AS54	----------C------C-----C----------A----------G----
29 Han (Taiwan)	AS58	----------C----------A----------C
30 Korean	AS101	----------C-C-----C----------
Haplogroup C:		
31 Bella Coola	Partial	----C----------T------T----------C----CT------
32 Ojibwa	Partial	----------G---T----------C-----T-----C
33 Pima	AM34	---G----------T----------C--G-CT------
34 Maya	AM31	------C----------T----------C----CT------
35 Yanomama	AM78	----------T----------C----CT------
36 Kraho	AM79	----------C----------T----------C----CT------
37 Ticuna	AM42	--G----------T----------C----CT------
38 Han (Taiwan)	AS65	----------A-T----------T----------C-----T------
39 Han (Tibet)	Partial	----------T----------C-----T-----

(continued)

Table 5 (continued)

Sample	Haplotype	Polymorphic Nucleotide Positions[a]
		1111111111111111111111111111111111111111111111111111111111111111
		6666666666666666666666666666666666666666666666666666666666666666
		0000000011111111111111112222222222222222222222222233333333333333
		3455899901222445577789901112333444666777899999901112233456 6
		9219623541469080412492832473235089036148801345801495715460 2
Haplogroup D:		
40 Bella Coola	Partial	-----C----------T----------C-------
41 Apache	Partial	-----C------A----------T---------C----------C
42 Maya.....	AM50	----------T----------C-------
43 Boruca.....	AM44	----------T----------C------C
44 Yanomama	AM88	--------A----------T----------C
45 Ticuna.....	AM46	----------T----------C------C
46 Mataco	AM88	----------T-------T-------C----------C------C
47 Han (Taiwan) ..	AS25	----------T----------C
48 Korean	AS97	----------T-------T----------C
49 Korean	AS25	----------T----------G----------C

[a] Boxes indicate the nucleotide variants specific to each D-loop group.
[b] From Anderson et al. (1981).

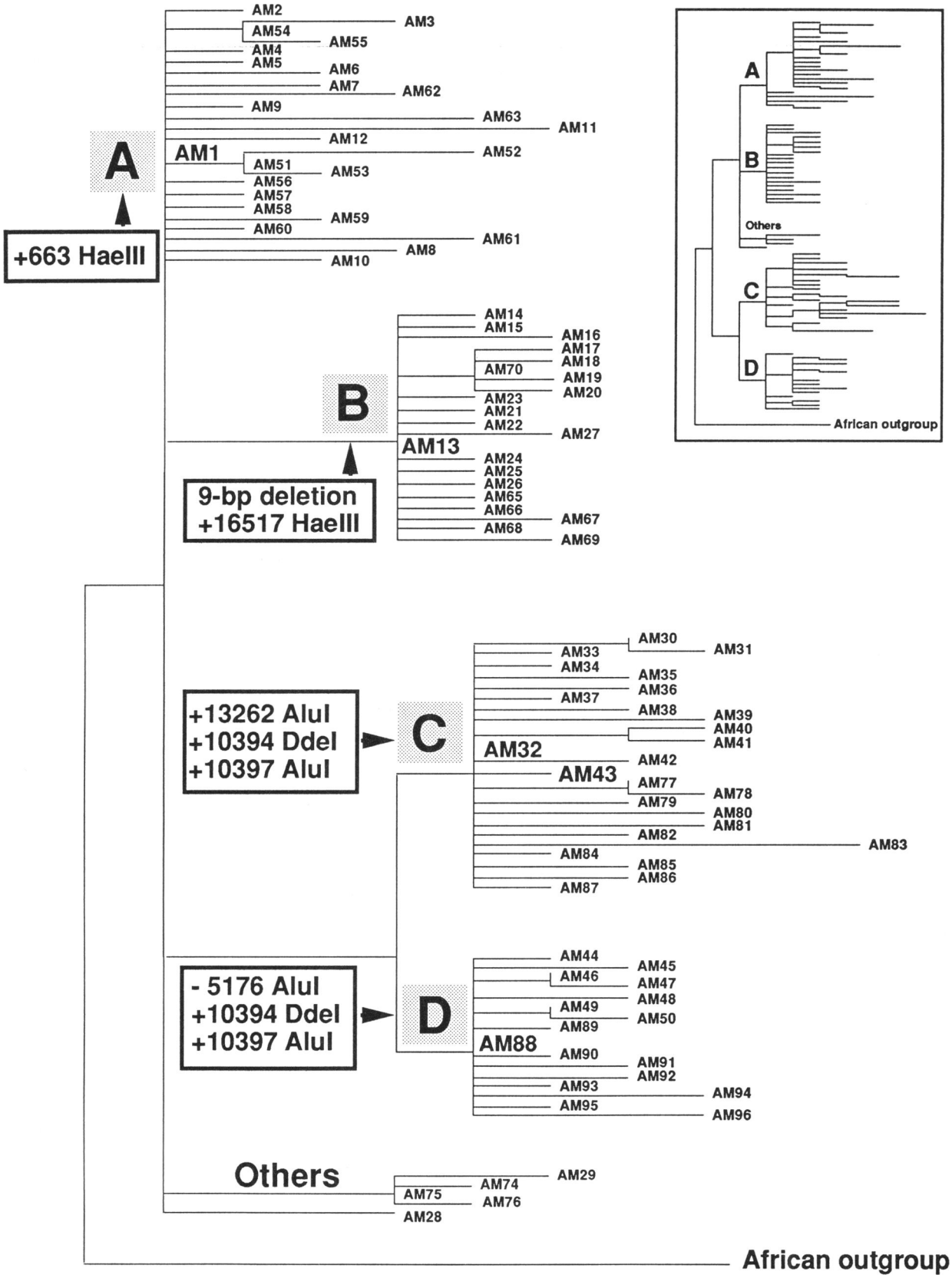
consensus trees remains similar and is defined by the presence of the four haplogroups.

All Native American group A haplotypes (AM1–AM12 and AM51–AM63) are defined by an A-to-G transition at np 663 which creates an *Hae*III site gain at that position. Group B haplotypes (AM13–AM27 and AM65–AM70) are distinguished by the 9-bp deletion occurring between np 8272–8289. The deletion is always found in association with the *Hae*III site gain at np 16517. Amerind group C haplotypes (AM30–AM43 and AM77–AM87) are characterized by an A-to-G transition at np 13263 which simultaneously eliminates a *Hin*cII site at np 13259 and creates an *Alu*I site at np 13262. This mutation is almost always found in association with two linked nucleotide changes which are found exclusively in Asian or Asian-derived populations—an A-to-G transition at np 10398 which creates a *Dde*I site gain at 10394 and a C-to-T transition at np 10400 which produces an *Alu*I site gain at np 10397 (Ballinger et al. 1992). Finally, Group D haplotypes (AM44–AM50, AM88–AM96) are defined by a C-to-A transversion at np 5178 which eliminates an *Alu*I site at np 5176. Like group C haplotypes, these haplotypes exhibit the *Dde*I site gain at np 10394 and the *Alu*I site gain at np 10397.

All but five haplotypes segregated into haplogroups A, B, C, and D (fig. 2). Haplotypes AM28, AM29, and AM74–AM76 lacked characteristic Amerind mutations and probably represent European mtDNAs. For example, haplotype AM28 was found in one Maya and was characterized by an *Alu*I site loss at np 7025 and the *Hae*III site gain at np 16517. The same haplotype has been found in about 10% of Caucasian mtDNAs, and, if haplotypes deriving from AM28 are included, the frequency of this group of haplotypes in Caucasians increases to about 30% (Shoffner et al., in press).

Haplotypes AM29, AM74, AM75, and AM76 have been observed almost exclusively in the Ojibwa from northern Ontario in which they are present in 25.0% of the samples analyzed. The only exception is AM29, which is shared between Navajo and Ojibwa. All four of these haplotypes are defined by a *Dde*I site loss at np 1715 and the *Hae*III np 16517 site gain and cluster together in a specific group (designated "others") in the Native American dendrograms (fig. 2). Haplotypes having these same mutations have been reported at low frequencies in a sample of mixed Caucasians from the United States (Shoffner et al., in press) and are absent in aboriginal Siberians (Torroni et al. 1993).

Previous data obtained through nuclear gene analysis indicated that the degree of detectable European admixture in three Ojibwa communities ranges from 3% to 30% (Szathmary 1984). The most likely source of European gene flow in the Ojibwa would be either the


A
+663 HaeIII
AM1
AM2
AM3
AM54
AM55
AM4
AM5
AM6
AM7
AM62
AM9
AM63
AM11
AM12
AM52
AM51
AM53
AM56
AM57
AM58
AM59
AM60
AM61
AM8
AM10
B
9-bp deletion
+16517 HaeIII
AM13
AM14
AM15
AM16
AM17
AM18
AM70
AM19
AM20
AM23
AM21
AM22
AM27
AM24
AM25
AM26
AM65
AM66
AM67
AM68
AM69
C
+13262 AluI
+10394 DdeI
+10397 AluI
AM32
AM43
AM30
AM31
AM33
AM34
AM35
AM36
AM37
AM38
AM39
AM40
AM41
AM42
AM77
AM78
AM79
AM80
AM81
AM82
AM83
AM84
AM85
AM86
AM87
D
- 5176 AluI
+10394 DdeI
+10397 AluI
AM88
AM44
AM45
AM46
AM47
AM48
AM49
AM50
AM89
AM90
AM91
AM92
AM93
AM94
AM95
AM96
Others
AM29
AM74
AM75
AM76
AM28
African outgroup
A
B
Others
C
D
African outgroup

French or the British who settled in the region since the beginning of the European migration to the northeastern North American coast (Spencer et al. 1977). Our mtDNA analysis supports the concept that there was extensive European gene flow in some Ojibwa communities. However, the four Caucasian haplotypes observed in the Ojibwa are closely related to each other and are not frequent in samples of mixed Caucasians from the United States (1.6%; Shoffner et al., in press). This suggests that they were acquired by the Ojibwa from Caucasian females of a specific European subpopulation in which those haplotypes have a particularly high incidence. To clarify the origin of those haplotypes, analysis of additional Ojibwa groups and of Caucasian groups living in the region is obviously necessary.

The partial haplotype analysis of the Bella Coola, the Nuu-Chah-Nulth, and the 15 Ojibwa from Wisconsin and North Dakota further supports the existence of the four Amerind mtDNA haplogroups (table 4). In the Bella Coola and the Nuu-Chah-Nulth, mtDNAs from all four haplogroups were observed, while in the Ojibwa group D mtDNAs were absent. The frequency of "other" haplotypes, which lack characteristic Native American mutations, was also particularly high in the southern Ojibwa (26.7%), and these mtDNAs were present in 13.3% and 4.0% of the Nuu-Chah-Nulth and the Bella Coola, respectively. These frequencies appear to indicate that, among the population samples we analyzed, the extent of genetic admixture with Caucasians was much higher in the Northern Amerind tribes than in the more isolated and unacculturated tribes from Central and South America.

The coherence of the four Native American haplogroups was confirmed when the MP trees were derived from the 92 Native American haplotypes combined with 106 East Asian haplotypes (AS17–AS122) identified by others in 153 subjects from seven populations (Ballinger et al. 1992) (fig. 3). Figure 3 represents one MP tree generated by the TBR method and is 348 steps in length, with CI and RI of .460 and .814, respectively. The consensus tree from 3,000 MP trees is shown in the inset of figure 3. In the consensus tree, however, most of the relationships between haplotypes were unresolved, indicating that parsimony analysis is unable to resolve the deep branches of trees when the number of taxa and character states is particularly large (Hedges et al. 1991; Templeton 1991). In all of the MP trees, including figure 3, virtually all haplotypes of the four Native American haplogroups (A, B, C, and D) remained together and clustered with those Asian haplotypes which were characterized by the same sets of linked mutations. In figure 3, the exception is represented by haplotype AM83, a group C mtDNA observed in one Makiritare which no longer segregated into haplogroup C. The anomalous position of AM83 results from the reversion of the linked *Hin*cII np 13259 site loss and *Alu*I np 13262 site gain. However, this haplotype does share several mutations with group C haplotype AM82 which was also found in the Makiritare.

Na-Dene mtDNA Haplotypes

The results from a previous study of Native American mtDNA variation (Torroni et al. 1992) supported the hypothesis that the Na-Dene originated from a migration which occurred independently and much more recently than the one giving rise to the Amerinds (Greenberg et al. 1986). Further, these ancestral Na-Dene were proposed to have carried exclusively group A haplotypes from Siberia. If accurate, this hypothesis implies that the Canadian and Alaskan Na-Dene should be the most direct descendants of these migrants. In addition, it was proposed that an A-to-G transition at np 16331 creating an *Rsa*I np 16329 site loss (A-to-G transition at np 16331) was specific to the Na-Dene, being observed in 27.0% of the Navajo, 26.7% of the Dogrib, 1 of 2 Tlingit mtDNAs, and 1 of 22 Alaskan Athapaskan mtDNAs but in 0 of the Amerind mtDNAs (Shields et al. 1992; Torroni et al. 1992).

The analysis of mtDNAs from two additional Na-Dene tribes, the Haida and the Apache, provided further insight into the mtDNA variation of this group. The mtDNAs from both tribes were screened for the eight mutations which define Native American haplogroups (table 4). The resulting data supported the hypothesis that the Na-Dene comprise only group A mtDNAs. The first tribe analyzed, the Haida, showed 96.0% group A mtDNAs, a distribution similar to that of the Canadian Dogrib (Torroni et al. 1992). However, a previous study of the Gm and Km allotype frequencies in the Haida suggested that this tribe could have derived from an amalgam of Na-Dene and Amerinds (Field et al. 1988). To test this possibility, we compared

Figure 2 Strict consensus tree of 92 Native American mtDNA haplotypes. The capital letters A, B, C, and D in shaded boxes indicate the four haplogroups observed in Native Americans. The nos. at the end of each branch denote the different mtDNA haplotypes. The horizontal branch lengths are proportional to the no. of mutational events between haplotypes. The indicated polymorphic sites define each of the four haplogroups. This dendrogram is the consensus of 3,000 MP trees generated by the TBR branch-swapping method. The inset shows 1 of the 3,000 MP trees used to generate the consensus tree.

Figure 3 Phylogenetic tree of 92 Native American (AM) and 106 Asian (AS) mtDNA haplotypes. The Asian mtDNA data are from Ballinger et al. (1992). Thicker lines indicate Native American haplotypes. Haplogroup classification corresponds to that indicated in fig. 2. This is one of thousands of MP trees which were obtained with the TBR branch-swapping algorithm. The inset shows the strict consensus tree of

the Haida mtDNAs with those observed in two Amerind groups, the Nuu-Chah-Nulth and the Bella Coola. These two populations are geographically and culturally very close to the Haida (Spencer et al. 1977) and, together with other Amerind populations living in the same region, could have genetically admixed with this tribal group. This comparison showed that mtDNA haplotypes of groups B, C, and D represented only 4.0% of the Haida mtDNAs but were observed in 43.2% of the Nuu-Chah-Nulth and the Bella Coola (table 4). From these frequencies, the total amount of gene migration (*M*) from Amerinds into the Haida was estimated as 9.3% (Bernstein 1931). This result suggested that the Haida are a Na-Dene population which experienced limited genetic admixture with the Amerinds. Alternatively, such admixture could have been mediated primarily by Amerind males.

The Haida also differ from the other Na-Dene tribes in lacking the *Rsa*I np 16329 site loss in any of their group A mtDNAs. One explanation for this difference is that mtDNAs having the *Rsa*I site loss were present in the ancestral Na-Dene population but were lost by genetic drift as the Haida became a distinct tribe. Alternatively, the Na-Dene–specific *Rsa*I np 16329 site loss may have occurred after the ancestral Haida had become separated from the rest of the Na-Dene. The latter scenario appears the more likely because this mutation is absent in the mtDNAs of aboriginal Siberians (Shields et al. 1992; Torroni et al. 1993), indicating that the *Rsa*I site loss must have occurred in the Americas during the genetic differentiation of the Na-Dene tribes. Moreover, the Haida are the most linguistically divergent of the Na-Dene tribes (Krauss 1964; Levine 1979). Thus, they probably separated from the other Na-Dene before the occurrence of the *Rsa*I np 16329 site loss.

The second Na-Dene tribe characterized for mtDNA variation was the San Carlos Apache. The mtDNAs of this population show direct and unambiguous relationships with other Na-Dene speakers. Like the Navajo, the majority (64.0%) of the Apache mtDNAs belonged to haplogroup A, and 28.0% of the Apache group A mtDNAs have the *Rsa*I np 16329 mutation (table 4). The remaining 36.0% of Apache mtDNAs belong to Amerind haplogroups B, C, and D. This finding implies that, like the Navajo, the Apache have experienced admixture with the surrounding Amerind peoples. Previous mtDNA data (Torroni et al. 1992), as well as (*a*) the presence of Gm haplotypes (Williams et al. 1985) and albumin variants specific to Amerinds (Albumin Mexico; Schell and Blumberg 1988) in the Apache and (*b*) historical evidence for the raiding and capturing of women from neighboring Amerind tribes (Basso 1983), support this interpretation.

mtDNA Haplogroup Distribution in Amerinds and Na-Dene

The overall distribution of mtDNA haplogroups in the North, Central, and South American Amerinds, the Northern Na-Dene (Canada), and the Southern Na-Dene (southwestern United States) is shown in figure 5. While virtually all of the Northern Na-Dene mtDNAs belong to haplogroup A, the Southern Na-Dene showed significant frequencies of group B (30.0%), C (4.0%), and D (3.0%) haplotypes, a result consistent with the occurrence of Amerind genetic admixture. In contrast, the North, Central, and South American Amerinds are characterized by haplotypes belonging to all of the four haplogroups and show important frequency variations within the three population groupings. The Northern Amerinds also show a relatively high incidence (12.0%) of "other" haplotypes, primarily because of the high incidence of Caucasian mtDNAs in the Ojibwa samples.

All of the four mtDNA haplogroups are represented in the North, Central, and South American populations (fig. 5) and imply their presence in the original migration. However, most of the individual tribes lack haplotypes from at least one of these groups (tables 2 and 3). For instance, group A is absent in the Yanomama, group B in the Ticuna, group C in the Mataco and all of the Chibcha speakers, and group D in the Ojibwa, the Pima, and the Bribri/Cabecar. While the limited sample size for many of the groups analyzed makes this observation preliminary, this trend was consistent for those tribes from which larger samples were studied. These observations confirm the expectation that the haploid nature and uniparental inheritance of mtDNA (Giles et al. 1980) allow genetic drift and founder events to play a more significant role in the stochastic extinction and fixation of mtDNA haplotypes in contrast to nuclear genes. This finding is supported by the high frequency

3,000 MP trees generated by the TBR method (for an explanation of the different topologies of MP and consensus trees, see Results). This tree is 638 steps in length, with CI and RI of .188 and .316, respectively. Some Asian mtDNA haplotypes (AS28, AS103, and AS111 in group A; AS61 in group B; and AS102 in group D) fall within the four Amerind haplogroups. This is due to parallel mutations of the hypervariable np 16517 *Hae*III site which is located in the D-loop region.

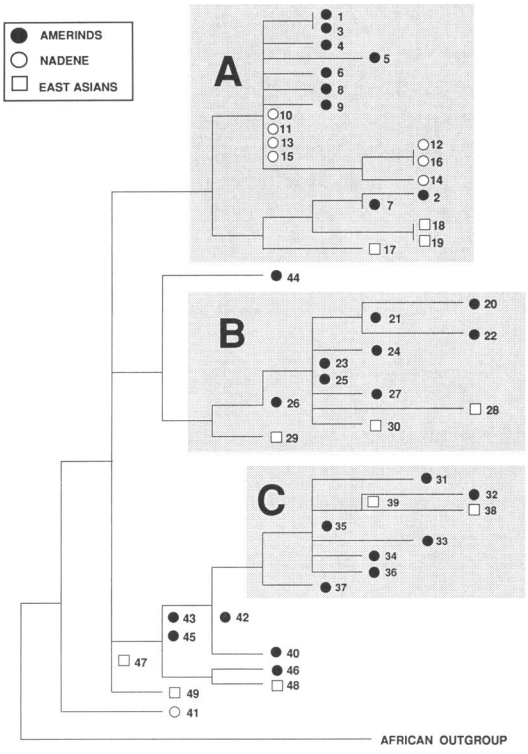


Figure 4 Dendrogram of the control-region sequences of Amerind, Na-Dene, and East Asian mtDNAs. The associations observed for the D-loop sequences completely overlap those of haplogroups A, B, and C as identified by restriction endonuclease analysis. The D-loop sequences of clade D are scattered throughout the dendrogram, since these D-loops lack distinctive sequence variants. This dendrogram is one of the large number of MP trees which can be generated by the TBR branch-swapping method. It is 72 mutational steps in length, with CI and RI of .545 and .868, respectively.

of "private haplotypes" ("private polymorphisms"; Neel 1978*a*) found in specific Amerind tribes. For example, haplotypes AM77 and AM79 each represent 25.0% of the Yanomama mtDNAs, haplotype AM62 represents 35.7% of the Ojibwa mtDNAs, haplotype AM89 constitutes 58.3% of the Wapishana mtDNAs, and haplotype AM53 was present in 15 of the 16 Kuna analyzed. This congruence of nuclear and mitochondrial genetic data reinforces a previous treatment of the role of early tribal isolation and founder effects leading to the divergence of tribal gene pools (Neel and Thompson 1978).

Multiple Origins of the 9-bp Deletion in Native American mtDNAs

The 9-bp deletion, in association with the *Hae*III np 16517 site gain, defines all group B haplotypes. With one exception, this length polymorphism was not observed in association with the mutations defining group A, C, and D haplotypes. The exception is represented by Boruca haplotype AM52, which has the 9-bp deletion in association with the *Hae*III np 663 site gain characterizing group A haplotypes and an *Msp*I np 104 site loss observed in most of the group A haplotypes of the Chibcha speakers. This unusual association indicates that the 9-bp deletion originated de novo through an independent mutational event in one of the group A haplotypes.

Further evidence in support of this conclusion was obtained through parsimony analysis, where haplotype AM52 clearly clustered with group A haplotypes (fig. 2). Moreover, the D-loop sequence analysis confirmed its association with group A mtDNAs (sequence 5; see below). Previous mtDNA studies have shown that the 9-bp deletion has occurred multiple times in both Asian and Siberian mtDNAs (Ballinger et al. 1992; Torroni et al. 1993). Hence, this is not an unlikely occurrence.

Correlation between Haplotype and D-Loop Sequencing Data

To clarify the issue of the number of founding D-loop sequences which colonized the Americas, we sequenced the D-loops from representatives of each of the four haplogroups. These included Amerind, Na-Dene, and East Asian samples. This analysis of D-loop sequences revealed 48 variable nucleotide positions of 341 nucleotides in 38 Native American and 11 East Asian mtDNAs and defined 42 different D-loop sequences (table 5).

The evolutionary relationships of these D-loop sequences are shown in figure 4. This tree is an MP tree generated by the TBR method and is 72 steps in length,

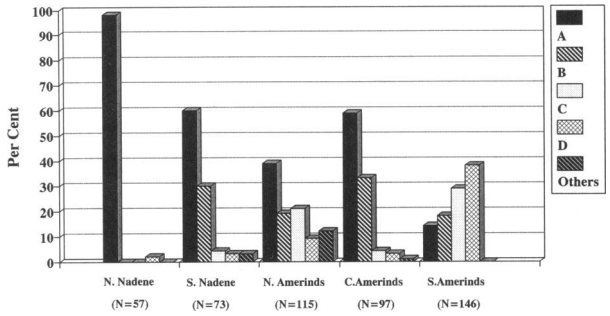


Figure 5 Distribution of mtDNA haplogroups in Northern Na-Dene (Canada), Southern Na-Dene (Southern Athapaskans), and North, Central, and South American Amerinds. "Others" indicates haplotypes of probable European origin.

with CI and RI of .545 and .868, respectively. All D-loop sequences obtained from mtDNAs of haplogroups A, B, and C segregated into analogous D-loop groups, which we now designate as D-loop "group A," "group B," and "group C." By contrast, D-loop sequences derived from mtDNAs belonging to haplogroup D did not cluster together tightly (fig. 4). The reason for this difference is that D-loop group A, B, and C sequences exhibited group-specific mutations, whereas D-loop group D sequences lack such distinguishing polymorphisms (table 5). D-loop group A sequences are defined by a T residue at np 16290 and an A residue at np 16319. D-loop group B sequences usually show two C residues at np 16189 and np 16217. D-loop group C sequences are characterized by a C residue at np 16298 and a T residue at np 16327. However, D-loop group D sequences had a T at np 16223 also found in sequences of D-loop groups A and C, as well as a C at np 16362 common with sequences of D-loop group A (table 5).

In addition, the comparison of Native American D-loop sequences with those from Asia revealed that the group-specific D-loop mutations were shared between Native American and Asian mtDNAs. Consequently, these mutations must have arisen in Asia and were carried to the Americas with the founding mtDNAs. The remainder of the other D-loop mutations were confined to either Americans or Asians and were individual specific. Therefore, Native American D-loop variation, like the restriction site variation, probably arose after Native Americans became separated from Asia.

The only exceptions to this generalization are represented by a T residue at np 16111 and a C residue at np 16325. The first is found in the Native American D-loop groups A and B, and the second is found in Native American D-loop groups C and D. However, these polymorphisms are not observed in the Asian or Siberian D-loop sequences belonging to the same D-loop groups (table 5) (Torroni et al. 1993). Whether these mutations imply additional Native American founding mtDNAs or parallel mutations which arose in the Americas remains to be resolved.

In addition, the D-loop sequencing analysis confirms previous evidence that the Na-Dene have much lower mtDNA heterogeneity than do the Amerinds (Torroni et al. 1992). Four of the seven Na-Dene sequences from D-loop group A had identical sequences (table 5 and fig. 4). Each of these four derived from subjects of different tribes (the Haida [10], the Tlingit [13], the Dogrib [11], and the Navajo [15]) which belong to three of the four major internal linguistic divisions of the Na-Dene (Haida, Tlingit, and Athapaskan) (Haskell 1987). Similarly, the D-loop sequences of the Dogrib, Tlingit, and Navajo mtDNAs, which were defined by the Na-Dene–specific *Rsa*I np 16329 site loss (haplotype AM5), clustered together (12, 14, and 16). These observations appear to add support to the hypothesized independent origin of the Na-Dene and the Amerinds.

In conclusion, the analysis of the D-loop sequence variation obtained from a limited number of mtDNAs belonging to diverse Native American tribes indicated a high degree of correspondence between the previously defined haplogroups and the newly defined D-loop groups. It also demonstrated that Native American mtDNAs can be more completely defined by the detailed restriction analysis reported in this paper than by D-loop sequencing. No evidence from the D-loop sequence analysis suggests that Native American mtDNAs derived from a large number of founding Asian mtDNAs. Hence, the existing mtDNA variation is still more compatible with the concept that mtDNAs of modern Amerind populations derived from only four original founding mtDNAs (Wallace et al. 1985; Schurr et al. 1990).

Discussion

Amerind Founding Haplotypes

The current study, together with previous studies (Wallace et al. 1985; Schurr et al. 1990; Torroni et al. 1992), confirms that all Native American mtDNAs fall into four distinct haplogroups (A–D). This result raises questions about which of the mtDNAs in each haplogroup represent the original founders. The attempt to identify ancestral founding haplotypes of Native Americans assumes that not enough time has elapsed since the colonization of the Americas for all founding haplotypes (in the Americas and remaining in Asia) to have been altered by mutation. Given the relatively short time span involved, this is probably a safe assumption. Several independent approaches have been used to investigate this issue. The concordance of the answers has permitted us to identify the founding haplotypes with substantial confidence.

To be considered a founding haplotype of the Amerinds, candidate mtDNAs have to meet several criteria. First, the founding haplotypes should be widespread within the Amerinds and should be shared between tribes because they preceded the Amerind tribal differentiation. Second, the founding haplotypes should be

Table 6

mtDNA Haplotypes Shared between Amerind Tribes

Tribe	Haplotype	Frequency (%)[a]
Haplogroup A:		
Maya, Kuna, Guaymi, Piaroa, Makiritare, Mataco	AM1	15.2
Maya, Guaymi, Ticuna	AM9	9.1
Boruca, Bribri/Cabecar, Guaymi	AM51	12.1
Haplogroup B:		
Pima, Maya, Boruca, Bribri/Cabecar, Guaymi, Yanomama, Macushi, Kraho, Wapishana, Mataco	AM13	69.3
Haplogroup C:		
Pima, Makiritare, Piaroa, Wapishana, Marubo	AM32	14.8
Maya, Macushi	AM43	4.9
Kraho, Yanomama	AM79	11.5
Macushi, Makiritare	AM82	3.3
Haplogroup D:		
Boruca, Yanomama, Ticuna, Marubo	AM44[b]	18.3
Mataco, Yanamama	AM88	26.7
Makiritare, Wapishana	AM91	3.3

[a] Percentage of mtDNAs with this haplotype found in the haplogroup.
[b] Also observed in the only Pomo (California) analyzed (Torroni et al. 1992).

central to the branching of their haplogroup in the phylogenetic analysis, because all new haplotypes would have originated from them. Third, it should still be possible to detect founding haplotypes in East Asian and Siberian populations, because they originated in Asia. By contrast, mtDNA haplotypes which derived from these founding haplotypes and which arose in the Americas should have a limited distribution, either being present only in single tribes or shared between tribes which are genetically, linguistically, or geographically close.

When these criteria were applied to the 85 Amerind mtDNA haplotypes, only 11 were found to be shared between tribes (table 6). The remaining haplotypes (private haplotypes) appeared at low frequencies in specific tribes. For haplogroup A, only haplotypes AM1, AM9, and AM51 are shared between tribes. Haplotype AM51 is confined to the Chibcha speakers of Central America and, thus, is unlikely to be a founding haplotype. However, haplotypes AM1 and AM9 are more broadly distributed throughout Amerind tribes and represent 15.2% and 9.1% of the Amerind group A haplotypes, respectively.

Similarly, for haplogroup B, the only shared haplotype is AM13. It is widely distributed among Amerind tribes, occurring in 10 of the 19 populations studied and representing 69.3% of all Amerind group B mtDNAs analyzed.

For haplogroup C, four haplotypes are shared between Amerind tribes. One of them, AM82, shows a limited distribution, being shared between two geographically proximate tribes, the Makiritare and the Macushi, which belong to the same linguistic subdivision (table 1). A second one, AM79, is shared between the Yanomama and the Kraho, tribes which belong to two different linguistic subdivisions but live in relatively close geographic regions. The other two, AM32 and AM43, are widely distributed. The first is shared between five different populations and represents 14.8% of all group C mtDNAs, whereas the latter is shared between one Central American (Maya) and one South American (Macushi) tribe and represents 4.9% of all group C mtDNAs.

For haplogroup D, three haplotypes—AM44, AM88, and AM91—are shared among Amerind tribes. Haplotype AM91 is shared between only two geographically proximate populations, the Makiritare and the Wapishana. By contrast, AM44 and AM88 are more broadly represented. AM44 constitutes 18.3% of all group D haplotypes and is shared between five populations, whereas haplotype AM88 represents 26.7% of all group D mtDNAs and is shared between the Yanomama and the Mataco. Therefore, on the basis of only haplotype frequency and distribution, the number of putative founding haplotypes for the Amerind mtDNA haplogroups is reduced to seven: AM1 and AM9 for

Table 7

mtDNA Mutations Shared between Different Amerind Haplotypes within a Haplogroup

Tribe	Haplotypes	Mutation[a]
Haplogroup A:		
Kuna, Boruca, Guaymi, Bribri/Cabecar	AM51, AM52, AM53	−104i
Maya, Guaymi	AM3, AM54, AM55	+8569c/−8572e
Haplogroup B:		
Pima, Ojibwa, Navajo[b]	AM17, AM18, AM19, AM20, AM70	+10893l
Haplogroup C:		
Pima	AM37, AM38, AM39	+29c
Yanomama	AM77, AM78	+4051k
Yanomama	AM77, AM78	+8565j
Yanomama, Macushi, Kraho, Marubo	AM79, AM80, AM81	−7013k
Macushi, Makiritare, Ticuna, Marubo	AM40, AM41, AM42, AM82, AM83, AM85	−16049k
Haplogroup D:		
Maya	AM49, AM50	−931i
Ticuna	AM46, AM47	+3397k
Macushi	AM93, AM94	−4848e

[a] Mutations which are common to all members of each haplogroup (i.e., +663e [haplogroup A]; 9-bp deletion and +16517e [haplogroup B]; −13259o/+13262a, 10394c, and 10397a [haplogroup C]; and −5176a, +10394c, and +10397a [haplogroup D]) were not included. Enzyme letter code is the same as that defined in Appendix (table A1).

[b] Navajo (Na-Dene speakers) probably acquired haplogroup B haplotypes through recent genetic admixture with Amerinds.

group A, AM13 for group B, AM32 and AM43 for group C, and AM44 and AM88 for group D.

The second criterion used to identify the founding haplotypes is whether they appear central to the radiation of their haplogroup in phylogenetic analyses. This requirement is met only by AM1 of group A, AM13 of group B, AM32 of group C, and AM88 of group D (figs. 2 and 3). It is interesting that the three remaining putative founding haplotypes—AM9, AM43, and AM44—differ from the nodal haplotypes only by an *Hae*III np 16517 site change. This site is located in the hypervariable D-loop and is polymorphic in all human populations analyzed to date (Cann et al. 1987; Stoneking et al. 1990; Ballinger et al. 1992). Its particularly high rate of evolution makes it likely that this mutation has arisen repeatedly in different populations, thus creating the two similar haplotypes. Specific evidence for this assertion comes from two haplotypes, AM5 and AM6, present in the Canadian Dogrib. These share the *Rsa*I np 16329 site loss (A-to-G transition at np 16331) specific to the later Na-Dene radiation, but they differ by the presence of the *Hae*III np 16517 site. Similar situations are seen for the Macushi haplotypes AM89 and AM90 and the Ticuna haplotypes AM40 and AM42.

The comparison of Native American and Asian haplotypes provides a further support for the conclusion that the founding haplotypes were AM1 for group A, AM13 for group B, and AM88 for group D (fig. 3). These are the only haplotypes of their haplogroups which have been observed in Asians. Indeed, AM1 is identical to AS56 found in the Taiwanese Han; AM13 is the same as AS54 found in 5.0% of the Taiwanese Han, 7.1% of the Vietnamese, and 3.3% of the Sabah aborigines; and AM88 is identical to AS25 observed in 7.7% of the Koreans, 5.0% of the Taiwanese Han, and 14.3% of the Malaysian Chinese (Ballinger et al. 1992). However, contrary to the results of the parsimony analysis, the putative founding group C haplotype observed in East Asia was not AM32 but, instead, haplotype AM43, one which is identical to Asian haplotype AS65 found in 5.0% of the Taiwanese Han (Ballinger et al. 1992). Haplotypes AM32 and AM43 differ by only the hypervariable *Hae*III site at np 16517 located in the D-loop. This kind of hypervariable site could produce very "noisy" results in phylogenetic analysis (Stewart 1993). To clarify this ambiguity, group C haplotypes from aboriginal Siberian populations were analyzed. In that study, AM43 appeared at significant frequencies in

Siberians, whereas AM32 was not found (Torroni et al. 1993). This observation suggested that haplotype AM43 was the founding group C haplotype for Native Americans. In summary, the distribution and frequency of mtDNA haplotypes in the Americas, and their phylogenetic relationships to Asian and Siberian mtDNAs, appear to indicate that haplotypes AM1, AM13, AM43, and AM88 were the founding haplotypes for all modern Amerind mtDNAs.

The Tribalization Process

Having established that all Native American mtDNAs derived from four founding haplotypes, we tried to correlate our mtDNA data with previous models of the radiation of the various Native American tribes. Two alternative hypotheses about the origin and diversification of Native American tribes can be proposed. The first suggests that Native American populations have continued to branch into new tribal groups and exchange genetic information until relatively recent times. The second supposes that there was an early and rapid population radiation followed by tribal isolation and localized differentiation. The extent of nuclear DNA private polymorphisms and the potential antiquity of some of them tend to support the second hypothesis and also imply that tribal linguistic boundaries have been quite effective barriers to gene flow (Neel 1980). Our mtDNA data also appear to support this interpretation.

Evidence in favor of the second scenario can be seen in the pattern of mtDNA variation. On further scrutiny, Amerind mtDNA mutations can be divided into three classes. The first are those that define the haplogroups and are shared by all haplotypes of the same haplogroup. These mutations originated in Asia and are present in the mtDNA pool of modern Asians (Ballinger et al. 1992) and Siberians (Torroni et al. 1993).

The second class of mtDNA mutations (haplotype specific) are present in only single Native American haplotypes. These constitute the great majority of the mutations observed in Native American mtDNAs and most likely are "private mutations" which occurred in individual tribes after tribal differentiation. Haplotypes defined by these mutations usually occur at the terminal positions of the haplogroups (fig. 2).

The third class of mutations are shared by a subset of Amerind haplotypes of the same haplogroup. These probably occurred in situ in the Americas during an "intermediate" phase of the tribalization process. Only 11 of the 109 restriction site changes observed in Native Americans belong to this latter class of mutations, and they create the intermediate nodes in the haplogroups (fig. 2 and table 7). Six of them (*Dde*I np 29 site gain, *Rsa*I np 4051 site gain, *Mbo*I np 8565 site gain, *Msp*I np 931 site loss, *Rsa*I np 3403 site gain, and *Hae*III np 4848 site loss) are shared among haplotypes all found in the same tribe and most likely occurred since tribal differentiation. One of these, the *Msp*I np 104 site loss, was observed in four of the Chibcha-speaking tribes—the Boruca, the Bribri/Cabecar, the Guaymi, and the Kuna. This mutation has not been observed in any other population previously analyzed (Wallace et al. 1991; Ballinger et al. 1992) and, judging from its distribution, most likely arose in the ancestral group from which all modern Chibcha speakers derived. The remaining four mutations were shared between tribes belonging to different linguistic affiliations. The most interesting of them is the *Rsa*I np 16049 site loss. It is observed in six group C haplotypes belonging to the Yanomama, the Macushi, the Kraho, or the Marubo. All of these tribes have relatively close geographic locations (fig. 1), and three of them (the Macushi, the Kraho, and the Marubo) are affiliated with the same linguistic group (Ge-Pano-Carib; table 1).

Table 8

Sequence Divergence and Divergence Time of Amerind mtDNA Haplogroups

Haplogroup	n[a]	N[b]	Sequence Divergence (%)	Divergence Time[c] (years)
A	23	98	.091	22,750–45,500
B[d]	16	76	.024	6,000–12,000
C	25	61	.096	24,000–48,000
D	16	60	.053	13,250–26,500
Average:[e]				
A + B + C + D	80	295	.067	16,750–33,500
A + C + D	64	219	.082	20,500–41,000

[a] No. of haplotypes.

[b] No. of subjects.

[c] Divergence times were calculated by multiplying the intraclade sequence divergences by the mtDNA evolution rate of 2%–5%/MYR.

[d] If the Navajo group B haplotypes, thought to have been acquired through admixture with Amerinds, are included in haplogroup B, the corresponding values become $n = 21$; $N = 94$; sequence divergence = 0.027%; and divergence time = 6,750–13,500 years.

[e] Weighted by the no. of subjects within each haplogroup. The average sequence divergence of haplogroups A + C + D was calculated because of the possibility that haplogroup B arrived in the Americas as part of an independent migratory event (Torroni et al. 1993).

Therefore, this mutation either could have occurred before the tribal separation of these groups or could have been transferred between tribes through more recent intertribal gene flow. The *Rsa*I np 7013 site loss, the *Taq*I np 10893 site gain, and the *Dde*I np 8569 site gain are also shared among linguistic groups and could represent early mutations or intertribal gene flow (table 7). In conclusion, the high incidence of private mtDNA polymorphisms and the limited proportion and distribution of shared mtDNA mutations among Native American mtDNAs support the conclusion that the tribalization of the Amerinds was a relatively rapid process and that it was followed by extensive tribal isolation (Neel 1980).

Quantification of Native American mtDNA Variation and Entry Time into the Americas

To estimate the entry time of the first Americans into the New World, we calculated the sequence divergence values for each of the four mtDNA haplogroups (table 8). These calculations were based on both the sequence variation estimated from the restriction site haplotypes of all Amerind tribal groups and the consensus mtDNA sequence evolution rate of 2.0%–4.0%/MYR (Stoneking et al. 1986; Cann et al. 1987; Wallace et al. 1987).

The sequence divergence that has accumulated since the four Amerind haplogroups began to diverge from those of Asians and Siberians is 0.091% for group A, 0.024% for group B, 0.096% for group C, and 0.053% for group D. The average divergence value for all four haplogroups is 0.067% (table 8), which gives an overall divergence time of 16,750–33,500 years before present (YBP). As the Clovis culture is assumed to have begun in America about 13,500 YBP, the mtDNA data appear to support a pre-Clovis colonization of the New World.

Haplogroup B, the haplotype group associated with the 9-bp deletion, showed the lowest sequence divergence and, consequently, the lowest divergence time of 6,000–12,000 YBP. This difference in divergence time between haplogroups could simply reflect the large intrinsic error involved in these estimations. However, an extensive mtDNA analysis of aboriginal Siberian mtDNAs (Torroni et al. 1993) failed to reveal any group B mtDNAs, even though groups A, C, and D were widely dispersed in Siberia. This raises the possibility that group B mtDNAs came to the Americas through a different route and, therefore, possibly at a different time than that of the other haplogroups. If haplogroup B is removed from estimating the overall sequence divergence for Amerind mtDNAs, the average sequence divergence for the remaining haplogroups is 0.082% (table 8), and the time of divergence becomes 20,500–41,000 YBP. In either case, the mtDNA data provide divergence times which are consistent with a pre-Clovis origin of the first Americans.

Acknowledgments

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Appendix

Table A1

Polymorphic Restriction Sites Observed in 92 Distinct Native American mtDNA Haplotypes (AM1–AM63, AM65–AM70, and AM74–AM96)

	Status by Haplotype[b]
Site[a]	1111111111222222222233333333334444444444555555555566666666677777778888888889999999 1234567890123456789012345678901234567890123456789012345678901235678904567890123456789012345678901234567890123456
8e	0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000
29c	0000000000000000000000000000000000000000111000000000000000000000000000000000000000000000000000000000000000
64c	0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000001000000
104i	1111111111111111111111111111111111111111111111111111111000111111111111111111111111111111111111111111
663e	1111111111110000000000000000000000000000000000000000011111111111110000000000000000000000000000000000
717g	0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000010000000000000

(*continued*)

Table A1 (continued)

Site[a]	Status by Haplotype[b]
	1111111111222222222233333333334444444444555555555566666666677777778888888889999999
	1234567890123456789012345678901234567890123456789012345678901235678904567890123456789012345678901234567890123456
931i	1111111111111111111111111111111111111111111111111001111111111111111111111111111111111111111111111
951j	1101111111111111111111111111111101111111111111111111111111111111111111111111111111111111111111111
1004h	0000000000000000000000000000000000000000000000000000000000000010000000000000000000000000000000000
1413l	1111111111111111111111111101111111111111111111111111111111111111111111111111111111111111111111111
1622n/1623f	0000000000000000000000000000000000000000000000000000000000000000000000000000000010000000000000
1715c	1111111111111111111111111101111111111111111111111111111111111111111111000111111111111111111111
2113j	0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000010000
2247c/2250a	0000000000000000000000000000000000000000000000000000000010000000000000000000000000000000000000000
2636e	0000000000000000000000000000000000000000000000000000000000000010000000000000000000000000000000000
2849k	1111111111111111111111111111111111111111111111111111111111101111111111111111111111111111111111111
3192c	1111111111111111101111111111111111111111111111111111111111111111111111111111111111111111111111111
3315e	1111111111111111111111111111111111111111111111111111111111111101111111111111111111111111111111111
3337k	1111111111111111111111111111111111111111111101111111111111111111111111111111111111111111111111111
3371k	0000000000000000000000000000000000000010000000000000000000000000000000000000000000000000000000000
3388c/3391a	0000000000000000100000000000000000000000000000000000000000000000000000000000000010000000000000000
3397k	0000000000000000000000000000000000000000000011000000000000000000000000000000000000000000000000000
3412e	1111111111111111111111111111111111111111111111111111111111111111111111111111111101111111111111111
3534c	1111111111111111111111111111111111111111111111111111111111111111111111101111111111111111111111111
3846c/3849e	0000000000000000000000000000000000000000000000000000000000000010000000000000000000000000000000000
3981a	0000000000000000000000000000000000000011000000000000000000000000000000000000000000000000000000000
3987k	0000000000000000000000000000000000000000000000000000000000000010000000000000000000000000000000000
4051k	0000000000000000000000000000000000000000000000000000000000000011000000000000000000000000000000000
4310a	1111111111011111111111111111111111111111111111111111111111111111111111111111111111111111111111111
4546g	0000000100000000000000000000000000000000000000000000000000000000000000000000000000000000000000000
4769a[c]	0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000
4848e	1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111110011
5054k	1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111101111111111
5164k	0000000000000000000000000000000000000000000000000000000000000010000000000000000000000000000000000
5176a	1111111111111111111111111111111111111111100000011111111111111111111111111111111000000000
5351f	0000000000000000000000000000000000000000000000000000000000000010000000000000000000000000000000000
5584a	1111111111111111111111111111111101111111111111111111111111111111111111111111111111111111111111111
5983g	1111111111111111111011111111111111111111111111111111111111111111111111111111111111111111111111111
6204a	1111111111111111111111111111111111111111111101111111111111111111111111111111111111111111111111111
6618e	0000000000000000000000000000000000000000000000000000000000000010000000000000000000000000000000000
7013k	1111111111111111111111111111111111111111111111111111111111111111111111111111111100011111111111111
7025a	1111111111111111111111111011111111111111111111111111111111111111111111111111111111111111111111111
7497e	1101111111111111111111111111111111111111111111111111111111111111111111111101111111111111111111111
7607e	0000000000000000000000000000000000000000000000000000000010000000000000000000000000000000000000000
7750c	1111111111111111111111111111111111111111111111111111111111111111111111111111111101111111111
7853o	1111111111111111101111111111111111111111111111111111111111111111111111111111111111111111111111111
7859j	1111111111111111111111111111111101111111111111111111111111111111111111111111111111111111111111111
7897k	1111111111111111111111111111111111111111111111111111111111111101111111111111111111111111111111111
7979e	0000000000000000000000000000000000000000000000000000000000000010000000000000000000000000000000000
8150i	1111111111111111111111111111111111111111111111111111111111111111111111111101111111111111111111111
8269k	0000000000000000000000000000000000000000000000000010000000000000000000000000000000000000000000000
8565j	0000000000000000000000000000000000000000000000000000000000000000000000000000011000000000000000000
8569c/8572e	0010000000000000000000000000000000000000000000000000000110000000000000000000000000000000000000000
8774a	0000000000000000000000010000000000000000000000000000000000000000000000000000000000000000000000000
8858f[c]	1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111

(*continued*)

Table A1 (continued)

Site[a]	Status by Haplotype[b]
	1111111111222222222233333333334444444444555555555566666666677777778888888889999999
	1234567890123456789012345678901234567890123456789012345678901235678904567890123456789012345678901234567890123456
9052n/9053f	1111111111111111111111111011111111111111011111111111110111111111111111111111111111111111111111
9253e	0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000001000000000
9342e	1111111111111111111111111111111111111111111111111111111111111111011111111111111111111111111111111
9504a	0000000000000000000000000000000000000010000000000000000000000000000000000000000000000000000000000000
9714e	0000000000000001000000000000000000000000000000000000000000000000000000000000000000000000000000000000
9942j	0000000000000000010000000000000000000000000000000000000000000000000000000000000000000000000000000000
9953e	1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111011111
10097e	0000000000100000000000000000000000000000000000000000000000000000000000000000000000000000000000000000
10254j	1111111111111111111111111101111111111111111111111111111111111111111111111111111111111111111111111111
10394c	0000001100000000000000000000000011111111111111111100000000000000000000000011111111111111111111
10397a	0000000000000000000000000000001111111111111111110000000000000000000000011111111111111111111
10893l	0000000000000000111100000000000000000000000000000000000000000000000000010000000000000000000000
11074c	0000000000000000000000000000000000000000000000000000000000000000000000000000000000010000000000
11100a	0000000000000000000000000000000000000000000000010000000000000000000000000000000000000000000000
11321a	0000000000000000000010000000000000000000000000000000000000000000000000000000000000000000000000
11793c	0000000000000000000000000000000000010000000000000000000000000000000000000000000000000000000000
11892a	0000000000000000000000000000000000000000000000000010000000000000000000000000000000000000000000
11924l	0000000000000000000000000000001000000000000000000000000000000000000000000000000000000000000000
11974k	0000000000000000000000000000000000000000000000000000000000000000000000100000000000000000000000
12026h/o	0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000001
12406h/o	1111111111111111111111111011111111111111111111111111111111111111111111111111111111111111111111
12891c	1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111101111
13031g	1111111111111011111111111111111111111111111111111111111111111111111111111111111111111111111111
13259o	1011111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111
13259o/13262a	1111111111111111111111111100000000000001111111111111111111111111111000000100001111111111
13268g	1111111111111111111111111111111111111111111111111111111111111111111111111111111111111101111111
13633e/13634o	0000000000000000000000000000001000000000000000000000000000000000000000000000000000000000000000
13702e[c]	0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000
14015a	1111111110111111111111111111111111111111111111111111111111111111111111111111111111111111111111
14199o[c]	0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000
14268g[c]	1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111
14279e	0000000000000000000000000000000000000000000000000000000000000010000000000000000000000000000000
14304a	1111111111111111111111101111111111111111111111111111111111111111111111111111111111111111111111
14368g[c]	0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000
14858n/14859f	1111111111111111111111111111111111111101111111111111111111111111111111111111111111111111111111
15002n/15003f	1111111111111111111111111111111111111111111111111111111111111111111111111111111111111011111111
15073c	1111111111111111111111111111111111111111111111101111111110111111111111111111111111111111111111
15172e	1111111110110111111111111111111111111111111111111111111111111111111111111111111111111111111111
15245a	0000000000000000000000000000000000000000000000000000000000000000000000000000000001000000000000
15727c	0000000000000000000000000000000000000000000000000000000000000000000000000000000010000000000000
15776a	1111111111111111111111111111111111111111111111111111111111111111011111111111101111111111111111
16049k	1111111111111111111111111111111111111100011111111111111111111111111111111111001011111111111
16145e	0000000000000000000000000000000000001000000000000000000000000000000000000000000000000000000000
16145j	0000000000000001000000000000000000000000000000000000000000000000000000000000000000000000000001
16224l	0000000001000000000010000000000000000000000000000000000000000000000000000000000000000000000000
16226a	0000000000000000000000000000000000000000000000000000000000000000000000000000000010000000000000
16238l	0000000000000000000000000000000000000000000000000000000000010000000000000000000000000000000000
16240a	0000000000000000000000000000000000000000000000000000000000000000000000010000000000000000000000
16329k	1111001111111111111111111111111111111111111111111111111111111111111111111111111111111111111111

(*continued*)

Table A1 (continued)

Site[a]	Status by Haplotype[b]
	1111111111222222222233333333334444444444555555555566666666677777778888888889999999
	1234567890123456789012345678901234567890123456789012345678901235678904567890123456789012345678901234567890123456
16389g/16390b	0001000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000
16389m/16390j,b ..	0000000000010000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000
16398j	0000000000000000000000000010000000000000000000000000000000000000000000000000000000000000000000000000000000000000
16456e	1111111110111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111
16494i	0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000110
16517e	0000011111101111111111111111111100000001001111000000000000000010111111111001100000100001001010
9-bp deletion	0000000000001111111111111100000000000000000000000010000000000011111100000000000000000000000000

[a] Sites are numbered from the first nucleotide of the recognition sequence according to the published sequence (Anderson et al. 1981). The restriction enzymes used in the analysis are designated by the following single-letter code: a = *Alu*I; b = *Ava*II; c = *Dde*I; e = *Hae*III; f = *Hha*I; g = *Hin*fI; h = *Hpa*I; i = *Hpa*II; j = *Mbo*I; k = *Rsa*I; l = *Taq*I; m = *Bam*HI; n = *Hae*II; and o = *Hin*cII. Sites separated by a slash indicate either simultaneous site gains or site losses for two different enzymes or a site gain for one enzyme and a site loss for another because of a single inferred nucleotide substitution; these sites are considered to be only one restriction site polymorphism in the statistical analysis.

[b] A "1" indicates the presence of a site, and a "0" indicates the absence of a site, except for 9-bp deletion where a "1" indicates the presence of a 9-bp deletion between the COII and $tRNA^{Lys}$ genes, and a "0" indicates the absence of the deletion.

[c] Present or absent in all samples, contrary to the published sequence. The haplotype of the Senegalese used as an African outgroup was +907l, +2390j, −2758k, +3529h, +7025a, −7055a, +10394c, +10806g, +16517e.

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