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Amerindian Mitochondrial DNAs Have Rare Asian Mutations at High Frequencies, Suggesting They Derived from Four Primary Maternal Lineages

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Summary

The mitochondrial DNA (mtDNA) sequence variation of the South American Ticuna, the Central American Maya, and the North American Pima was analyzed by restriction-endonuclease digestion and oligonucleotide hybridization. The analysis revealed that Amerindian populations have high frequencies of mtDNAs containing the rare Asian RFLP *Hin*cII morph 6, a rare *Hae*III site gain, and a unique *Alu*I site gain. In addition, the Asian-specific deletion between the cytochrome c oxidase subunit II (COII) and $tRNA^{Lys}$ genes was also prevalent in both the Pima and the Maya. These data suggest that Amerindian mtDNAs derived from at least four primary maternal lineages, that new tribal-specific variants accumulated as these mtDNAs became distributed throughout the Americas, and that some genetic variation may have been lost when the progenitors of the Ticuna separated from the North and Central American populations.

Introduction

The nature and timing of the colonization of the New World by Asiatic peoples has been much debated. Some investigators have interpreted age estimates of human skeletal material from North America as indicating that all Amerindian tribes descended from a small group of Siberian hunter-gatherers who crossed the Bering land bridge in a single migration about 12,000–15,000 years ago (Bada et al. 1984; Owen 1984; Taylor et al. 1985; Nelson et al. 1986), although data from archaeological sites (Dillehay et al. 1982; Adovasio et al. 1983; Guidon and Delibrias 1986) suggest earlier occupation times. Others believe that dental morphology, linguistic associations, and blood protein variation indicate that Amerindians originated from three sequential migrations of northeast Asians (Turner 1983; Williams et al. 1985; Greenberg et al. 1986). According to this hypothesis, the first migration to the Americas took place 15,000–30,000 years ago and gave rise to the Paleo-Indians who, within several millenia, occupied all of South and Central America and most of North America (Griffin 1979; Hopkins 1979). Subsequent migrations gave rise to the Na-Dené Indians (≈10,000–15,000 years ago) and the Aluet-Eskimo (≈6,000–9,000 years ago), although the temporal separation of the movements is disputed (Szathmary 1985; Laughlin 1988).

We have investigated the Paleo-Indian migration by analyzing mtDNA variation in the Pima (the Pima and Tohono-O'odham [Papago] tribes) of the southwestern

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United States (Wallace et al. 1985). Mitochondrial DNA has proven useful for elucidating the genetic affinity of human populations. Its rapid evolutionary rate (Brown et al. 1979; Miyata et al. 1982; Wallace et al. 1987) has resulted in numerous genetic differences detected as RFLPs, or morphs (Brown and Goodman 1979; Brown 1980), which can be used to discriminate between even closely related groups. Because mtDNA is maternally inherited (Giles et al. 1980, Case et al. 1981), these differences can be traced through radiating female lineages in populations. Moreover, many mtDNA morphs correlate with the ethnic and geographic origin of the samples (Denaro et al. 1981; Blanc et al. 1983; Bonné-Tamir et al. 1986; Brega et al. 1986*a*, 1986*b*; Santachiara-Benerecetti et al. 1988).

In our initial Amerindian study, we demonstrated that 40.5% of the Pima individuals had *Hin*cII morph 6, while 55% of the individuals had *Hin*cII morph 2 (Wallace et al. 1985). In Asia, less than 2% of the mtDNAs are *Hin*cII morph 6 and approximately 80% are morph 2 (Blanc et al. 1983), the most common Eurasian mtDNA restriction pattern (Johnson et al. 1983). Morph 6 differs from morph 2 by the loss of the restriction site at nucleotide pair (np) 13259. Since *Hin*cII morph 6 is essentially Asian-specific, these data confirm the Asian origin of Amerindian mtDNAs. Moreover, the 20-fold increase in the frequency of morph 6 suggests that the ancestral population of the Paleo-Indians was composed of a small number of maternal lineages.

Because most of the Amerindians analyzed in the previous study descended from a single group, the Hohokam (Matson et al. 1968; Haury 1976), it was possible that the limited number of mtDNA types observed was the product of a recent (perhaps within the past 1,500–2,000 years) founder effect confined to these North American tribes. To determine whether the prevalence of *Hin*cII morph 6 was a more general characteristic of Paleo-Indian-derived populations, we examined mtDNAs from two other Amerindian tribes, the Central American Maya and the South American Ticuna.

Material and Methods

Subjects

The Ticuna are a linguistically distinct and geographically isolated tribe from the western Amazon rain forest of Brazil (Mestriner et al. 1980; Neel et al. 1980). An analysis of blood-group markers indicated that the tribe was essentially free of genetic admixture (2.2%) with other nonnative populations (Neel et al. 1980). HLA data suggested that recent population growth and migration from jungle to river settlements resulted in the partial genetic homogenization of Ticuna tribes (Lawrence et al. 1980; Neel et al. 1980; Salzano et al. 1980). Blood samples were collected from three villages along the Rio Solimões (Lawrence et al. 1980).

The lowland Maya occupy parts of Mexico, Guatemala and Honduras, and are known to have inhabited the area continuously for at least 5,000 years (MacNeish 1983). Blood samples were taken from a remote Mexican population in the Yucatan Peninsula that spoke Yucatec, one of the many Maya languages belonging to the Mexican Penutian subgroup (Greenberg 1987). Analysis of blood types and serum proteins revealed about 10% European ancestry in this tribe (unpublished data).

As described previously (Matson et al. 1968; Haury 1976), the Pima and Tohono-O'odham (Papago) descended from the Hohokam of northwestern Mexico, and now live in the Gila River valley of southwestern Arizona. Their languages are affiliated linguistically with the Uto-Aztecan group (Matson et al. 1968). In a study of Gm allotypes, Caucasian admixture was estimated at ≈1% (Williams et al. 1985, 1986). Blood samples were obtained in a study of chronic diseases (Knowler et al. 1978).

Methods

Frozen lymphocytes representing 31 and 37 independent maternal pedigrees (unrelated through at least one generation) from the Ticuna and Maya, respectively, were thawed and transformed into lymphoblastoid cell lines using Epstein-Barr virus (for Ticuna, see Bird et al. 1981; Novotny et al. 1987; for Maya, see Anderson and Gusella 1984). DNA was prepared from cultured cell pellets and buffy coats (Hirt 1967; Kan and Dozy 1978). Pima DNA had been extracted from platelets or lymphoblastoid cell pellets and buffy coats (Wallace et al. 1985). Thirty-one of the 74 independent Pima pedigrees examined in that study were selected for further analysis.

The mtDNAs were digested with the restriction endonucleases *Hpa*I, *Bam*HI, *Hae*II, *Msp*I, *Ava*II and *Hin*cII, which have proven informative in our work (see, e.g., Blanc et al. 1983; Johnson et al. 1983) and that of others (Scozzari et al. 1988; Vilkki et al. 1988). The fragments were electrophoresed in agarose (SeaKem™, FMC BioProducts) gels (0.8% for *Ava*II and *Hpa*I; 1.0% for *Hae*II and *Bam*HI; 1.4% for *Hin*cII; 1.8% for *Msp*I), and Southern (Southern 1975) or vacuum (VacuBlot™,

ABN) blotted to nylon (BioTrans™; ICN) filters. Each filter was hybridized to ^{32}P-labeled HeLa mtDNA (Feinberg and Vogelstein 1983 [primer extension method]; Overhauser et al. 1987 [hybridization method]), then exposed to Kodak X-OMAT film at −80°C for autoradiography. The deduced morphs were compared with those observed in the Pima and other Asian populations.

All samples were also surveyed for previously reported Asian-specific RFLPs (Cann 1982; Cann et al. 1984; Horai et al. 1984; Harihara et al. 1986, 1988; Horai and Matsunaga 1986; Stoneking et al. 1986). A set of fragments covering the mtDNA was amplified by the polymerase chain reaction (PCR) with *Taq* DNA polymerase (Perkin Elmer-Cetus) using the conditions described by Saiki et al. (1985) and 30–35 cycles of denaturation at 93°–94°C for 1 min, primer annealing at the lowest T_H of the primer pair for 1 min, and extension of 72°C for 1 min, in a DNA Thermal Cycler (Perkin Elmer-Cetus). Oligonucleotide primers used in the amplification were synthesized on an ABI 380A DNA synthesizer (Microchemical Facility, Emory University) according to the Cambridge sequence (Anderson et al. 1981) (table 1). The size and and quantity of the PCR products were confirmed by the electrophoresis of 5 μl of amplified DNA on 1.5% agarose gels containing 0.001 mg/ml ethidium bromide in Tris borate–EDTA buffer and visualization using UV fluorescence. For each PCR fragment, 100 ng of amplified mtDNA was digested with the restriction enzyme and electrophoresed in 2.0%–4.0% NuSieve® agarose (FMC BioProducts) gels containing ethidium bromide. The resulting restriction fragments were visualized under UV

Table 1

PCR Fragments and Restriction Enzymes Used to Screen Amerindian mtDNAs for Asian-specific Polymorphisms

5′→3′ Coordinates (Forward Primer, Reverse Primer)	T_H (°C)	Asian-specific Polymorphisms	Restriction Enzymes Used
16453-16527, 724-706	59	**207h, 64i, 712i**	a,b,e,f,h,i
534-553, 1696-1677	61	**1403a,†** **748b, 663e,†** 1463e, **1536f, 712i**	a,b,e,f,h,i
3281-3298, 4508-4489	47	3537a, **4411a,†** 3315e, **3842e**	a,e,f,k,l
4831-4847, 5917-5898	51	5176a,† 5261e, 5742i, 5269l	a,e,f,i,l
5971-5988, 7608-7588	55	6931g, **64091,** 74611	a,e,f,g,l
7392-7410, 8628-8608	61	**8165e, 8250e,†** 8391e, **7617f,** 8150i,† 7859j, 8592j, 7461l	a,e,f,i,j,k,l
8829-8845, 10107-10088	51	**9009a, 10028a,** 9380f, 9746k, 9751l	a,e,f,k,l
9911-9932, 10728-10712	47	10364e, 10689e, **10725e**	e
11673-11691, 12576-12557	59	**12026h,** 12407h	a,h,k
12861-12877, 13950-13932	49	**12990a, 13068a,** 13031g,† 13103g,† 13268g, **13004j, 13096k**	a,e,g,j,k
13914-13930, 15865-15845	47	14015a, **14322a, 14509a,** 15606a, **14567i, 14279j,** 14869j,† **15195j**	a,e,g,i,j
15553-15569, 16547-16527	45	**16254a,** 16390b, 15883e,† **16398e,†** 16517e,† 16000g, **16490g,** 15925i, 16049k, **16089k,** 16208k, **16718l**	a,b,e,g,i,j,k,l

NOTE.—PCR fragments are indicated by the nucleotide coordinates of the forward (value before comma) and reverse (value after comma) primers according to Anderson et al. (1981). The annealing temperature for each PCR fragment is the lowest T_H of the primer pair calculated from the nucleotide sequence of each primer, $T_H = 4(C + G) + 2(T + A) - 5$°C. Asian-specific restriction-site polymorphisms are indicated by their beginning nucleotide and a single letter code denoting the enzyme (Cann et al. 1984; Cann et al. 1987): a, *Alu*I; b, *Ava*II; c, *Dde*I; d, *Fnu*DII; e, *Hae*III; f, *Hha*I; g, *Hin*fI; h, *Hpa*I; i, *Hpa*II; j, *Mbo*I; k, *Rsa*I; l, *Taq*I; boldface type represents site gains, standard type site losses, and a dagger (†) denotes sites that are not Asian-specific but were checked in the analysis. The restriction enzymes used for the analysis of PCR fragments are lettered as above.

fluorescence, and the patterns compared to the published sequence (Anderson et al. 1981).

Each sample was screened for small insertion-deletion mutations in the cytochrome c oxidase subunit II (COII)/tRNA^{Lys} region (Cann 1982; Cann and Wilson 1983 [region V]) by differential oligonucleotide hybridization. PCR fragments amplified from np 7706–8628 were bound to nylon filters by the alkaline dot blot method (Farr et al. 1988) using a HYBRI-DOT® manifold (BRL), hybridized to end-labeled oligonucleotides (Maxam and Gilbert 1980) and subjected to autoradiography under conditions described above. The probe for the 6-bp addition, COII-LONG (5′-CACCCCCCC-CCCCTACCCC-3′), was hybridized at 65°C; for the normal sequence, COII-NORM (5′-CACCCCCTCT-ACCCCCTCT-3′), at 59°C; and for the 9-bp deletion, COII-DEL2 [5′-AGCACCCCCTCTAGAGCCC-3′], at 59°C. All dot blots were washed in 3 × SSC for 1 h at 23°C. Filters were repeatedly hybridized by stripwashing in 0.1 M NaOH at 23°C for 1 h, rinsing briefly in 3 × SSC, then incubating with the next probe.

Results

In corroboration of the Pima results, 11% of Maya mtDNAs and 42% of the Ticuna mtDNAs were *Hin*cII morph 6, with remaining samples in both tribes being morph 2 (table 2). The high frequency of *Hin*cII morph 6 in all three tribes suggests that this polymorphism was characteristic of the founding Paleo-Indian population and not simply a tribal-specific marker.

As seen in the Pima, all mtDNAs from the Maya and Ticuna were morph 1 for *Bam*HI and morph 2 for *Hpa*I. Most Maya and Ticuna mtDNAs were morph 1 for *Hae*II, but three Ticuna individuals were morph 7 (Johnson et al. 1983). Likewise, most were morph 1 for *Ava*II, but four Maya individuals were morph 3 (Johnson et al. 1983). Finally, most were morph 1 for *Msp*I, but

Table 2

Mitochondrial DNA RFLPs in Amerinidian Tribes and Comparative Asian Frequencies

Morph[a]	Pima[b]	Maya	Ticuna	Asia (Reference)[c]
*Hin*cIII:				
1	1.4 (0)	0	0	5.6 (1,2,3,4,5,6,9)
2	55.4 (48.4)	89.2	58.1	83.4 (1,2,4,5,6,9)
6	40.5 (45.2)	10.8	41.9	.4 (1,2,4,5,6,9)
9	2.7 (6.5)	0	0	0 (1,2,4,5,6,9)
*Hae*II:				
1	92.0 (96.8)	100.0	90.3	77.8 (2,6,8)
2	2.7 (0)	0	0	4.9 (2,6,8)
3	4.1 (3.2)	0	0	0 (2,6,8)
4	1.4 (0)	0	0	.4 (2,6,8)
7	0 (0)	0	9.7	0 (2,6,8)
*Msp*I:				
1	100.0 (100)	91.9	100.0	84.2 (2,5,7,8,9)
14	0 (0)	8.1	0	0 (2,5,7,8,9)
*Ava*II:				
1	100.0 (100)	89.2	100.0	91.4 (2,4,5,7,8)
3	0 (0)	10.8	0	2.2 (2,4,5,7,8)
*Hpa*I:				
1	1.4 (0)	0	0	5.6 (1,2,3,4,5,6,9)
2	98.6 (100)	100.0	100.0	79.2 (2,3,4,5,9)
*Bam*HI:				
1	100.0 (100)	100.0	100.0	100.0 (2,4,5,6,8,9)

[a] Frequencies of each morph are given as percentages of the total number of independent maternal pedigrees surveyed.

[b] The Pima data for 74 independent maternal pedigrees (71 Pima and three non-Pima) were previously published as table 1 in Wallace et al. (1985). The frequencies of morphs in the 31 samples chosen for further analysis are presented in parentheses.

[c] The frequency of each morph in Asia is the percentage of the total samples surveyed for the RFLPs in previous mtDNA studies: 1 = Blanc et al. (1983); 2 = Brega et al. (1986); 3 = Denaro et al. (1981); 4 = Harihara et al. (1986); 5 = Harihara et al. (1988); 6 = Horai et al. (1984); 7 = Horai and Matsunaga (1986); 8 = Johnson et al. (1983); 9 = Yu et al. (1988). The morph 9 described in Horai et al. (1984) is identical to morph 6. Morph 6 was originally reported as having lost restriction sites at both np 7853 (site d) and 13259 (site e) (Blanc et al. 1983), but subsequent analysis found the site at np 7853 to be present.

three Maya individuals had the new morph 14 resulting from a site loss at np 931 (table 2). These data confirmed that mtDNAs from the three tribes share Amerindian characteristics, while showing that private polymorphisms had accumulated since their separation. Most Amerindian tribes have also shown a number of private polymorphisms in their nuclear DNAs (Neel 1973, 1978; Neel et al. 1977; Salzano et al. 1977, 1978; Tanis et al. 1977).

A number of additional Asian-specific RFLPs have been reported (table 1), but few of these were observed in Amerindian mtDNAs. The *Hae*III site gain at np 663 observed in two Chicanos and one Chinese (Cann 1982) was observed in 6.7% of the Pima, 66.7% of the Maya, and 40.0% of the Ticuna mtDNAs having *Hin*cII morph 2. This polymorphism was also found in one *Hin*cII morph 6 mtDNA in the Pima. The *Rsa*I site loss at np 16049 seen in one Australian aborigine (Cann 1982) and one Japanese (Horai and Matsunaga 1986) was found in 91% of Ticuna mtDNAs having *Hin*cII morph 6. In addition, the *Alu*I site loss at np 14015 observed in one Australian aborigine (Cann 1982) was found in one Maya mtDNA. All site losses and gains observed in Amerindian mtDNAs are contained within the haplotype data in table 3.

New mutations were also detected in the Amerindian populations. An *Alu*I site gain at np 13245 occurred in nearly all *Hin*cII morph 6 mtDNAs from the three tribes. An *Alu*I site loss at np 14304 was also found in 26.7% of the Pima mtDNAs that were *Hin*cII morph 2. These mutations had not previously been reported in Asian populations, and have not been observed in

Table 3

Amerindian mtDNA Haplotype Polymorphisms and Tribal Frequencies

Haplotype No.	Length Polymorphism[a]	Restriction Site[b]											Tribal Frequency[c]			Type[d]
		A	B	C	D	E	F	G	H	I	J	K	Pima	Maya	Ticuna	
AM 1	N	+	+	−	−	+	+	+	+	+	+	+	0	2.7	38.7	1–2
AM 2	D	+	+	−	−	+	+	+	+	+	+	+	32.3	18.9	0	1–2
AM 3	D	+	+	−	−	+	+	+	−	+	+	+	12.9	0	0	1–2
AM 4	D	+	+	−	+	+	+	+	+	+	+	+	0	2.7	0	1–2
AM 5	N	+	+	−	−	+	+	+	+	+	−	+	0	8.1	0	95–2
AM 6	N	+	+	−	+	+	+	+	+	+	+	+	3.2	43.2	25.8	1–2
AM 7	N	−	+	−	+	+	+	+	+	+	+	+	3.2	0	0	1–6
AM 8	N	+	+	−	+	+	+	+	+	+	+	−	0	10.8	0	47–2
AM 9	N	+	+	−	+	+	+	+	+	−	+	+	0	2.7	0	1–2
AM X[e]	N	−	+	−	−	+	+	+	+	+	+	+	0	0	0	1–6
AM 10	N	−	+	+	−	+	+	+	+	+	+	+	38.7	10.8	3.2	1–6
AM 11	N	−	+	+	−	+	−	+	+	+	+	+	3.2	0	0	46–6
AM 12	N	−	−	+	−	+	+	+	+	+	+	+	6.5	0	0	1–9
AM 13	N	−	+	+	−	−	+	+	+	+	+	+	0	0	25.8	1–6
AM 14	N	−	+	+	−	−	+	−	+	+	+	+	0	0	6.5	64–6
Total													100.0	100.0	100.0	

[a] Denotes polymorphisms located in the COII/tRNA^{Lys} intergenic region, with D indicating the deleted and N indicating the normal sequence.

[b] All polymorphic restriction sites reported in this study are lettered, each site being numbered according to its beginning nucleotide position in the published sequence (Anderson et al. 1981). Site gains relative to the reference sequence are shown in bold type, site losses in standard type: A = *Hin*cII 13259; B = *Hin*cII 13634; C = *Alu*I 13245; D = *Hae*III 663; E = *Rsa*I 16049; F = *Hae*II 4529; G = *Hae*II 14858; H = *Alu*I 14304; I = *Alu*I 14015; J = *Msp*I 931; K = *Ava*II 16390; the presence of a site is denoted by a plus sign (+), and the absence by a minus sign (−).

[c] The tribal frequency of each haplotype is given as a percentage of the total number of independent maternal pedigrees analyzed; for the Pima, $N = 31$; for the Maya, $N = 37$; for the Ticuna, $N = 31$.

[d] Mitochondrial DNA types associated with each haplotype. Types are combinations of morphs for the six informative restriction endonucleases ordered *Hpa*I, *Bam*HI, *Hae*II, *Msp*I, *Ava*II, and *Hin*cII (Blanc et al. 1983; Johnson et al. 1983) hence, type 1-2 is represented as (2-1-1-1-1-2), type 95-2 as (2-1-1-1-14-2), type 1-6 as (2-1-1-1-1-6), type 47-2 as (2-1-1-1-3-2), type 46-6 as (2-1-3-1-1-6), type 1-9 as (2-1-1-1-1-9), and type 64-6 as (2-1-7-1-1-6). For each population sample, the sum of the frequencies of types corresponding to one restriction-enzyme RFLP (e.g., haplotypes AM7, AM10, AM11, AM12, AM13, and AM14 to *Hin*cII morph 6) equals the frequency of that morph shown in table 2.

[e] X represents the hypothesized ancestral haplotype AMX.

our Asian samples (unpublished data). Since most of the previously analyzed Asian mtDNAs came primarily from Southeast Asian populations, these polymorphisms may still be present in the mtDNAs of Siberian aboriginal groups to whom Amerindians are genetically most closely related. If absent, they probably occurred after the separation of the Paleo-Indian population from its Asiatic progenitors.

Another Asian-specific marker, the small (9-bp) intergenic deletion occurring between the COII and $tRNA^{Lys}$ genes, had been reported in the mtDNAs of Asians (Cann 1982; Cann and Wilson 1983; Horai and Matsunaga 1986), New Guineans (Wrischnik et al. 1987), Polynesians (Hertzberg et al. 1989) and one American black (Wrischnik et al. 1987), the latter case being attributed to admixture with Amerindians. To determine whether this polymorphism was a common Amerindian variant, mtDNAs from the three tribes were screened for the deletion by differential oligonucleotide hybridization (figure 1). Interestingly, the length mutation was found in 45.2% of the Pima and 21.6% of the Maya mtDNAs, all of which were *Hin*cII morph 2, whereas all Ticuna mtDNAs had the normal sequence.

The combination of the common and rare mtDNA variants generated 10 Amerindian-specific haplotypes. This grouping of polymorphisms into haplotypes and the frequencies of the haplotypes in each tribe are shown in table 3. All haplotypes were also arranged into a sequential mutation tree (figure 2). Because four of these (AM1, AM2, AM6, AM10) were observed in at least two of the three tribes and were present in more than 30% of the individuals of one tribe, they were designated founding mtDNAs. Since AM1 is one of two mtDNAs found on all continents and at the root of our world phylogeny (Johnson et al. 1983), it was placed at the center of the Amerindian tree.

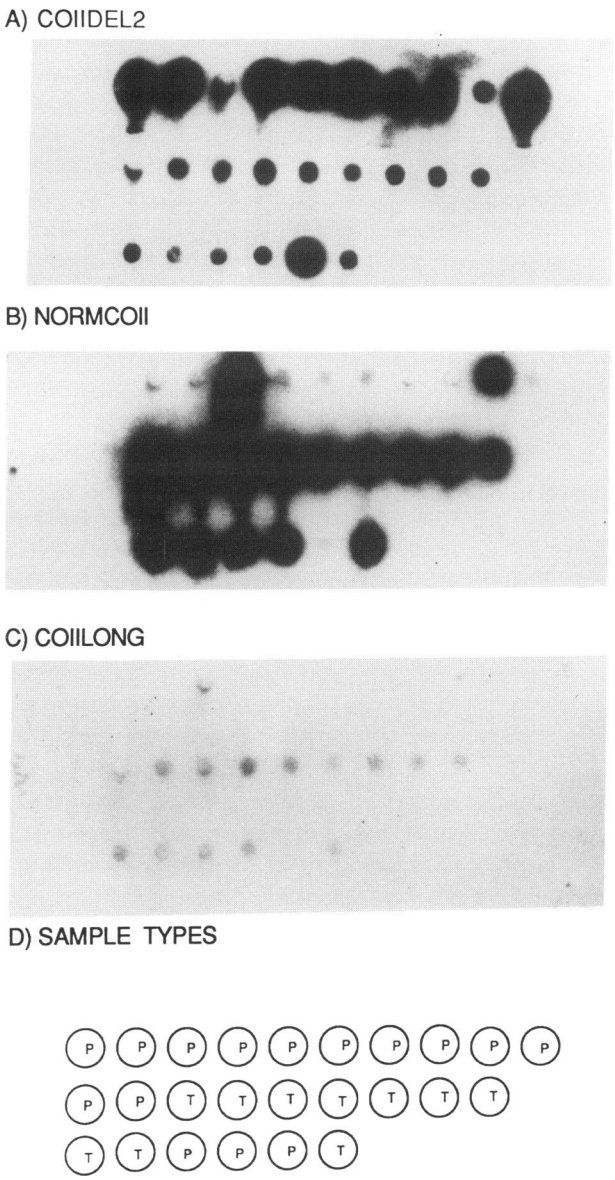

Figure 1 Length variants detected in the COII/$tRNA^{Lys}$ intergenic region (PCR fragment np 7706–8628) in Amerindian mtDNAs by differential oligonucleotide hybridization. For the sample types, *P* indicates Pima and *T* indicates Ticuna.

Discussion

The substantially higher frequency of *Hin*cII morph 6 in all Amerindian tribes compared to Asians and the high frequency of the 9-bp deletion in the Pima and the Maya provided further evidence that Amerindian mtDNAs derived from a small number of founders. This genetic restriction may have occurred in a northeast Asian progenitor population as small groups separated from more genetically variable tribes before migrating to the Americas, or it could have resulted from a population constriction occurring as the ancestral Paleo-Indians moved across the Bering land bridge and became isolated in the New World. In either case, these early immigrants carried with them at least four primary mtDNA haplotypes. Subsequently, as the founding population fragmented into smaller groups, new mutations accumulated and other rare polymorphisms became prevalent, thereby generating the ten tribal-specific haplotypes.

While most haplotypes can be derived from each other through the sequential accumulation of muta-

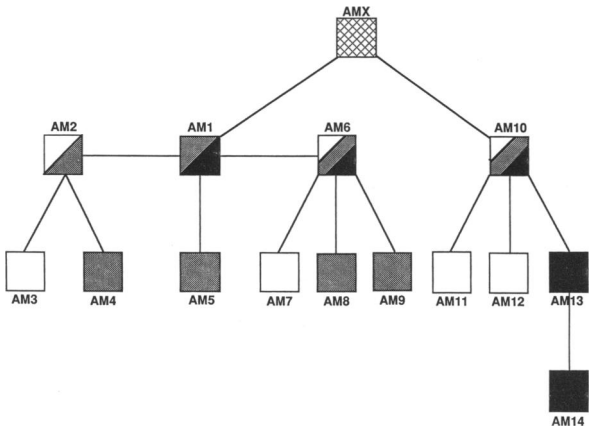

Figure 2 A sequential mutation tree relating Amerindian mtDNAs. All haplotypes are numbered according to table 3, and differ from adjacent ones by single mutations. Although not a phylogeny, the tree represents the most parsimonious organization of branching based on these differences. The tribal representation of mtDNAs is indicated by colors, with the Pima denoted by white, the Maya by grey, and the Ticuna by black. Haplotype AMX is the ancestral mtDNA to haplotype AM10. Presumably, AMX originated in Asia, as it was not observed in these Paleo-Indian-derived mtDNA samples.

tions, there are some exceptions. Haplotype AM10 is linked to AM1 through AMX by the loss of the *Hin*cII site at np 13259 and the gain of the *Alu*I site at np 13245. This hypothesized ancestral haplotype was not observed in the Amerindian tribes and, therefore, must have existed in the progenitor Asian population.

AM4 and AM7 are also anomalous. Haplotype AM4 shares the *Hae*III site gain at np 663 in the 12s rRNA region with the AM6 lineage but differs from it by having the 9-bp deletion found in the AM2 lineage. Similarly, AM7 shares the *Hin*cII site loss at np 13259 in the ND5 gene with the AM10 lineage but differs from it by having the *Hae*III site at np 663 and lacking the *Alu*I site at np 13245.

These ambiguous haplotypes may simply represent parallel mutations occurring in different lineages, reflecting the more rapid evolution of tribal populations suggested by several reports on protein electrophoretic variants (Neel et al. 1986, and references therein). Alternatively, AM4 and AM7 could represent recombinational events between the AM2 and AM6 lineages and the AM6 and AM10 lineages, respectively. Recombination in mammalian mtDNAs has been postulated for the D-loop sequences encompassing the 7S DNA (Olivo et al. 1983), but has not been confirmed for mtDNAs in somatic cell hybrids (Wallace 1986). If recombination produced these haplotypes, AM7 must have been generated by an exchange between AM6 and the ancestral mtDNA AMX while AMX existed in the Paleo-Indian population or its progenitor population. This conclusion follows from the lack of the *Alu*I site at np 13245 in AM7, which is located only 14 bp away from the *Hin*cII site at np 13259 and would have been included in the recombinational exchange if this mtDNA were involved.

Amerindian individuals with the four mtDNA haplotypes have interbred since the ancestral Paleo-Indian population became isolated in the New World some 12,000–20,000 years ago, yet after over roughly 500–1,000 generations this population still maintains all four haplotypes at virtually complete linkage disequilibrium. Even a very low frequency of recombination would have begun to randomize the observed mtDNA haplotypes. Consequently, the Amerindian data suggest that if recombination does occur between separate mtDNA lineages it is a rare event.

Recently, mtDNA was obtained from 7,000–8,000-year-old Archaic Indian brains from Florida (Doran et al. 1986; Pääbo et al. 1988). DNA sequence analysis of the Little Salt Spring cranial mtDNA revealed that it was *Hin*cII morph 2 and lacked the deletion between the COII and $tRNA^{Lys}$ genes (Pääbo et al. 1988). This mtDNA also showed an *Hae*III site loss at np 8251 that had previously been observed at a low frequency in several Asian groups, and was proposed to be evidence of another founding mtDNA lineage. Our data suggest that either the polymorphism is a new parallel mutation that occurred in AM1 or that this "founding" haplotype may have been lost from or not represented in the three Amerindian tribes that we analyzed.

The founders of existing Amerindian tribes are thought to have become isolated long enough to genetically differentiate, and even in the presence of intertribal gene flow these tribes have maintained certain genetic identities (Neel 1980). Thus, the absence of founding mtDNA haplotypes AM1 from the Pima and AM2 from the Ticuna and the high frequencies of tribal-specific polymorphisms may reflect the effects of genetic drift in tribal populations. Because of the genetic microdifferentiation of aboriginal populations (Neel and Ward 1970), small sample sizes may not reveal the full extent of variation within each group. Consequently, it is difficult to ascertain whether the lack of a founding haplotype actually indicates its loss from a particular tribe. However, the absence of the 9-bp deletion in Ticuna mtDNAs supports blood marker data (Mourant et al. 1976); Lampl and Blumberg 1979) that suggest that a genetic restriction occurred between North and

South America. In addition, the greater variability of Maya mtDNAs (D. C. Wallace, unpublished data) tends to support evidence indicating appreciable levels of admixture with non-native populations in the tribe.

The shifting frequencies of founding haplotypes among tribes and the demonstration of the sequential accumulation of multiple new tribal mtDNA mutations indicate that reconstructing the migration patterns of Amerindians through their mtDNA phylogenies should be possible. These phylogenies could also be correlated with the linguistic affiliations, dental traits, and other genetic markers of Amerindian tribes. Such correlations, in conjunction with data we may now be able to collect from indigenous Siberian populations, should help clarify the relationships between the different Asian-derived populations in the Americas and define the progenitor populations in northeast Asia. As the extent of mtDNA variation is surveyed in Amerindian populations, it may also become possible to differentiate larger tribal groupings and thereby determine whether single or multiple migrations occurred. Hence, the current demonstration of unique mtDNA variants in tribal populations suggests that similar analyses may provide a powerful new approach for elucidating the origins of the Amerindians.

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