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Peopling of the Americas as inferred through the analysis of mitochondrial DNA

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ABSTRACT

MtDNA has been extensively employed to trace the origins and migration patterns of Amerindians. The advantage of mtDNA over nuclear DNA is that it accumulates base changes at an average rate 5-10 times faster than single-copy nuclear DNA does, which makes it suitable for the analysis of DNA differences among human populations. Moreover, its maternal inheritance and lack of recombination allow determination of phylogenetic divergence among lineages, without the ambiguities caused by the meiotic shuffling and mixing undergone by nuclear genes.

Thus far, the most useful mtDNA traits for Amerindian population studies have been the polymorphism of restriction sites, the variation in length of a region V non-coding segment containing a short repeat, and the base substitutions occurring in the D-loop region.

By analyzing mtDNA polymorphisms, several groups came to the conclusion that Amerindians could be clustered into four founder mitochondrial haplotypes. We reanalyzed the published data and studied a total of 673 Amerindians belonging to 23 different South American tribes. Our results showed that the four haplotypes proposed can be subdivided into several subsets to give rise to no less than 13 possible founder haplotypes. The number of founder Amerindian haplotypes is a problem at the center of an unsolved dispute. Some investigators support the idea that the colonization from Asia into the American continent was accompanied by a severe bottleneck that markedly restricted the number of maternal lineages entering the New World. Other groups hold an opposite view. Our findings of at least 13 founder haplotypes in Amerindians lend support to the latter position.

Estimations of the earliest date of colonization of the American continent are based on the construction of parsimonious trees. However, the trees so far reported have too many taxa and relatively too few phylogenetically informative sites to allow complete resolution of the phylogeny by parsimony. Additional uncertainties result from the inaccuracies in the estimated rate of mtDNA mutation used to calibrate the trees which varies from 2.2%/MYR (million years) to 33%/MYR according to different authors. Due to the above difficulties, the proposed dates of the New World colonization range between 14,000 and 29,000 years before present.

Studies of mtDNA have provided interesting, but preliminary, responses to some of the questions regarding the origin and evolution of Amerindians. Further clarification of these questions will require an understanding of the exact rates of mutation in different regions of the mitochondrial molecule, and to bear in mind that the answering of anthropological questions will always require a multidisciplinary approach in which the contribution of mtDNA analysis will be equivalent to the contribution provided by other methodologies.

When we were created we were given our ground to live on and from this time these were our rights. This is all true. We were put here by the Creator - I was not brought from a foreign country and did not come here. I was put here by the Creator.

Chief Wemnock, Yakima, 1915

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Background

Most Amerindians share the view of Chief Wemnock: they did not come to America from any other place. They were put into this continent by the Creator. Yet, this mythical view of the colonization of America is nowadays not shared by scientists. Thanks to the combined contribution of anthropologists, archaeologists, linguists, geneticists and molecular biologists, we know that the first Americans entered the continent from Asia. It is worth mentioning here that contemporary investigations more or less confirmed the view of the prescient Jesuit missionary José de Acosta, who in 1589 suggested that a small group of hunters, driven from their Asiatic homeland by starvation or warfare, might have followed now-extinct beasts across Asia into America, millenia before the Spaniards arrived in the Caribbean. Acosta remarked that such a journey would require "only short stretches of navigation" - an extraordinary premise if we take into account that Europeans would not discover the Bering strait until 139 years later (Thomas, 1993).

There is no reasonable doubt that the first Americans entered the continent sometime during the last Ice Age. Fossil bones from archaic human ancestors - such as Neanderthals - are entirely absent from this continent, suggesting that they were anatomically modern humans who first populated America. Archaeologists working in Asia have found that humans did not arrive in Siberia until about 35,000 to 40,000 years ago. This means that the first humans entered America some time after that date (Thomas, 1993; Morell, 1995).

Anthropological, archaeological, linguistic, dental, and nuclear genetic data have been used to propose that the peopling of the American continent took place by three successive migrations originating in northeast Asia and entering America via Beringia, with the first colonization giving rise to Amerindians (Paleo-Indians) and the following ones to Nadene and Aleut-Eskimo populations, respectively (Greenberg *et al.*, 1986; Cavalli-Sforza *et al.*, 1988). The number and timing of the colonizations into America are still matters of debate, and the proposed dates of migrations vary considerably, depending on the method used to estimate them (see reviews in Meltzer, 1993; Szathmary, 1993).

Mitochondrial DNA (mtDNA) has been extensively employed to trace the origins and the migration patterns of modern humans (Cann *et al.*, 1987). The advantage of mtDNA over nuclear DNA is that it accumulates base changes at an average rate 5-10 times faster than single-copy nuclear DNA does (Brown *et al.*, 1979), which makes it suitable for the analysis of DNA differences among human populations. Moreover, its maternal inheritance and lack of recombination (Giles *et al.*, 1980) allow determination of phylogenetic divergence among lineages, without the ambiguities caused by the meiotic shuffling and mixing undergone by nuclear genes.

Thus far, the most useful mtDNA traits for human population studies have been the polymorphism of restriction sites (Cann *et al.*, 1987; Schurr *et al.*, 1990; Torroni *et al.*, 1992,1993a), the variation in length of a region V non-coding segment containing a short repeat (Wrischnik *et al.*, 1987; Hertzberg *et al.*, 1989), and the base substitutions occurring in the D-loop region (Vigilant *et al.*, 1989; Ward *et al.*, 1991; Horai *et al.*, 1993). Data on mtDNA are consistent in confirming the Asian origin of Amerindians. However, for estimating the time of the first colonization into the Americas, the mtDNA clock has not provided much better resolution than archaeological data have, as different authors have proposed dates that range from 11,500 to 29,000 years ago (Ward *et al.*, 1991; Torroni *et al.*, 1992,1993a; Horai *et al.*, 1993; Shields *et al.*, 1993). Conversely, the mitochondrial genome has been more informative on other aspects of the evolutionary history of Amerindians.

Founder mitochondrial haplotypes in Amerindians

According to Torroni *et al.* (1993a) founding Amerindian haplotypes should be: (a) both widespread within Amerindians and shared among tribes, (b) central to the branching of their haplogroups in parsimony trees, and (c) still present in Asiatic populations. Haplotypes meeting the criterium of Torroni *et al.* (1993a) very likely are founder haplogroups; yet, there may exist founder haplotypes that fail to meet some of the above premises. Thus, for instance, if a founder Amerindian haplotype exists at a low frequency in Asiatics, its discovery in these populations will depend on the size of the sample studied. Furthermore, the haplotype may have existed in the original Asiatic population that colonized America and may have become lost in extant Asiatic populations (Bailliet *et al.*, 1994). Moreover, some authors have proposed that mtDNA is in mutation-drift equilibrium and that it may be difficult to identify ancestral lineages in Amerindians (Chakraborty and Weiss, 1991).

Torroni *et al.* (1993a) analyzed the restriction- site polymorphisms in the mtDNAs of 356 Amerindians belonging to 18 different tribes and reported that most individuals surveyed had one of four mitochondrial haplotypes that could be traced to Asian populations; these four haplotypes were assumed to be the founder Amerindian lineages. The ancestral haplotype hypothesis is further supported by the findings of three other groups of investigators (Ward *et al.*, 1991; Ginther *et al.*, 1993; Horai *et al.*, 1993) who studied the frequency of base substitution in equivalent segments of the D-loop region and also came to the conclusion that Amerindians could be clustered into four founder mitochondrial haplotypes. The four basic ancestral lineages proposed by Torroni *et al.* (1992) as fulfilling the above premises are depicted in Table I.

Table I - Basic founder haplotypes in Amerindians*.

Mt markers	Haplotypes			
	A	B	C	D
*Hae*III bp 663	+	-	-	-
*Alu*I bp 5176	+	+	+	-
*Hinc*II bp 13259	+	+	-	+
Region V repeat**	2	1	2	2

* According to Torroni *et al.* (1993a).

** 1 and 2 indicate number of copies of the 9 bp repeat.

We reanalyzed the data from Torroni *et al.* (1992, 1993a,b) and studied 673 Amerindians belonging to 23 different South American tribes (Bailliet *et al.*, 1994, and this report). The results are summarized in Table II. In addition to the four founder haplotypes we detected a low frequency of compound haplotypes (coexistence of two or more haplotypes A-D in an individual), which could also be found in Asiatics (Bailliet *et al.*, 1994). Since mtDNA does not undergo recombination, compound haplotypes must have arisen by parallel mutations that occurred independently in the Asian and American continents. Alternatively, it is also possible that compound haplotypes originated by parallel mutations in Asia and entered into America during the colonization of this continent. In this latter case, their low frequency in Amerindians may indicate that they are in the process of becoming lost because of genetic drift.

We identify every individual not belonging to haplogroups A-D and not showing a compound haplotype as carrying an E haplotype (this haplogroup is named "others" in Torroni *et al.*, 1992, 1993a). This haplotype was detected in 3.8% of Amerindians (Table III). The variant E has been assumed to be a marker of Caucasian admixture on the basis that haplotype E is very frequent in Europeans (Cann *et al.*, 1987) and has been detected mainly in Amerindian populations with history of miscegenation. However, haplotype E is also very frequent (75%) in Asiatics (Cann *et*

Table II - Variants and frequencies of Mt-haplotypes in Amerindians.

Populations	Haplotypes[a] (%)									
	N	A	B	C	D	E	A/B	A/C	B/D	Reference[b]
Mapuche	63	6.4	34.9	19	27	9.5	0	0	3.2	1
Atacama	13	23.1	69.2	7.7	0	0	0	0	0	1
Huilliches	38	5.3	28.9	18.4	47.4	0	0	0	0	1
Bella Coola	25	60	8	8	20	4	0	0	0	2
Nuu-Chah-Nulth	15	40	6.6	13.3	26.6	13.3	0	0	0	2
Ojibwa	43	51.2	7	16.3	0	25.6	0	0	0	2
Pima	30	0	50	43.3	0	0	0	6.7	0	3,4
Maya	28	50	21.4	14.3	7.1	3.6	3.6	0	0	3,4
Boruca	14	26.6	66.7	0	6.6	0	0	0	0	2
Kuna	16	100	0	0	0	0	0	0	0	2
Guaymi	16	68.7	31.3	0	0	0	0	0	0	2
Bribri-Cabecar	24	54.2	45.8	0	0	0	0	0	0	2
Yanomama	24	0	16.7	54.2	29.1	0	0	0	0	2
Piaroa	10	50	0	10	40	0	0	0	0	2
Makiritare	10	20	0	10	60	10	0	0	0	2
Macushi	10	10	20	30	40	0	0	0	0	2
Wapishana	12	0	25	8.3	66.7	0	0	0	0	2
Ticuna	28	17.9	0	32.1	50	0	0	0	0	2
Kraho	14	28.6	57.1	14.3	0	0	0	0	0	2
Marubo	10	10	0	60	30	0	0	0	0	2
Mataco	28	10.7	35.7	0	53.6	0	0	0	0	2
Mataco	57	7	63.2	3.5	22.8	3.5	0	0	0	1
Toba	5	0	40	0	60	0	0	0	0	1
Chorote	14	14.3	50	14.3	21.4	0	0	0	0	1
Mixteca Alta	15	73.4	13.3	13.3	0	0	0	0	0	5
Mixteca Baja	14	71.4	7.2	0	0	0	21.4	0	0	5
Zapoteca	15	33.3	33.3	33.3	0	0	0	0	0	5
Mixe	16	62.5	31.3	6.2	0	0	0	0	0	5
Teribe	20	80	20	0	0	0	0	0	0	6
Guatuso	20	85	15	0	0	0	0	0	0	6
Huetar	27	70	4	0	26	0	0	0	0	7
Overall		33.2	28.3	15.3	18.4	3.6	0.6	0.3	0.3	
	(674)	(224)	(191)	(103)	(124)	(24)	(4)	(2)	(2)	

[a] Data in parentheses (in "Overall" row) are no. of haplotypes.

[b] 1 = Present study; 2 = Torroni *et al.* (1993a); 3 = Torroni *et al.* (1992); 4 = Schurr *et al.* (1990); 5 = Torroni *et al.* (1994b); 6 = Torroni *et al.* (1994a); 7 = Santos *et al.* (1994).

Table III - Frequency of haplotype subsets.

Haplotype subsets[a]	No. (% of haplotype)
A_1	43 (21)
A_2	162 (79)
Total	205 (100)
C_1	40 (38.8)
C_2	63 (61.2)
Total	103 (100)
D_1	23 (19.8)
D_2	93 (80.2)
Total	116 (100)

Source.- Torroni *et al.* (1992,1993a,1994a,b) and this report.
[a]A_1, C_1 and D_1 are characterized by a *Hae*III gain at bp 16517.

al., 1987; Ballinger *et al.*, 1992) and Siberians (27%) (Torroni *et al.*, 1993b). Thus, the possibility of an Asian ancestry for some of the Amerindian haplotypes E cannot be ruled out. In fact, the use of one additional polymorphism may help to differentiate between Amerindian and Caucasian E haplotypes. Roughly 50% of Amerindian individuals exhibit the gain of an *Alu*I site at the 10397 bp position of mtDNA. This *Alu* gain has been observed in 0.7% of Africans and, thus far, it has not been detected in Caucasians (Cann *et al.*, 1987; Marzuki *et al.*, 1991; Chen *et al.*, 1995). Thus, the presence of this site in combination with an E mt-variant strongly suggests an Amerindian origin for the haplotype. Conversely, the combination of an E haplogroup with the lack of *Alu*I 10397 in most - though not in all - cases, would indicate miscegenation.

All Amerindians bearing a haplotype B also have a *Hae*III gain at bp 16517 (Torroni 1992, 1993a; Bailliet *et al.*, 1994). On the other hand, haplotypes A, C and D may or may not exhibit the *Hae*III 16517 gain. We have identified as A_1, C_1 and D_1 the subset of haplotypes having the *Hae*III gain, while A_2, C_2 and D_2 define the subsets lacking the *Hae*III site (Bailliet *et al.*, 1994). Table III depicts the frequencies of each subgroup of haplotypes in Amerindians. Torroni *et al.* (1993b) propose that A_2, C_1 and D_2 are founder haplotypes because they are found at a high frequency in Siberians. Conversely, A_1, C_2 and D_1 are assumed to result from mutations occurring after the colonization of the American continent (Torroni *et al.*, 1993b). A_1, C_2 and D_1 haplotypes represent approximately 33% of the total group of Amerindians having haplotypes A, C and D.

If we assume both a D-loop mutation rate of 30%/1 million years (Ward *et al.*, 1991) and a pre-Clovis colonization time of 30,000 years ago, it can be estimated that $1x10^{-3}$ is the probability of mutation of a given nucleotide during the 30,000 years of colonization; thus, it is unlikely that 23% of the haplotypes could have arisen by parallel nucleotide substitutions (lower estimates of the mutation rate in the D-loop region and shorter estimates of the period of colonization of America would decrease further the probability of parallel nucleotide substitutions). A loss of the *Hae*III bp 16517 site with reversion to the original state is theoretically four times more frequent than a gain, because it results from a mutation in any of the four nucleotides forming the *Hae*III site, *versus* the single transition required to produce the gain of the site. Thus far, neither in Asiatics (Ballinger *et al.*, 1992) nor in Amerindians has a single case of haplotype B with lack of the *Hae*III site at bp 16517 been reported. Therefore, if we assume that C_2 cases arise by reverse mutations, we have to explain why these mutations do not occur in haplotypes B. From the above considerations it seems that the alternative that best accounts for the subsets found in groups A-C is the assumption that A_1, A_2, C_1, C_2, D_1 and D_2 are all founder maternal lineages.

D-loop sequencing

The determination of the nucleotide composition of the D-loop has been used to characterize the haplotypes of Amerindians. Four groups of investigators have employed this approach and have sequenced equivalent regions of the D-loop, allowing us to compare the results obtained by each group (Ward *et al.*, 1991; Ginther *et al.*, 1993; Horai *et al.*, 1993; Torroni *et al.*, 1993a). There is agreement among these investigators in proposing that most Amerindian lineages can be grouped into three or four clusters, each cluster being characterized by one or two specific transitions. Table IV details the mutations that identify the different D-loop mitochondrial haplogroups. The numbering used in the table (first column) corresponds to that of Horai *et al.* (1993); in the last column of the table the equivalences with the haplotype identification used by other authors are indicated.

Ginther *et al.* (1993) and Horai *et al.* (1993) are in complete agreement in the detection of the four D-loop haplogroups (Table IV). Torroni *et al.* (1993a) recognized haplotypes I, III, and IV of Horai *et al.* (1993) and identified them as "B", "A", and "C", respectively (Table IV), because there is coincidence among the polymorphisms characterizing haplotypes A-C and the transitions characterizing the D-loop haplotypes. Ward *et al.* (1991) have determined that the 28 lineages identified in Nuu-Chah-Nulth individuals can also be grouped into four clusters. Three of the Ward *et al.* clusters correspond to clusters I-III in Table IV; however, one of these four clusters finds no correspondence with any of the four clusters of Horai *et al.*; therefore, we identify it as haplotype V (Table IV). Ward *et al.* (1991), Ginther *et al.* (1993), and Horai *et al.* (1993) have also studied the correspondence between haplotype B (9 bp deletion) and the D-loop haplotypes. Ginther *et al.* and Horai *et al.* agree with Torroni *et al.* in finding correspondence between the D-loop haplotype I and the 9 bp deletion in region V (haplotype B). Ward *et al.*, on the other hand, found that the Nuu-Chah-Nulth individuals showing haplotype I

Table IV - Founding Amerindian haplotypes identified by sequencing of a D-loop segment*.

Haplotypes	Transitions	Position	Haplotype equivalences
I	T → C	16189 bp	I Horai *et al.* 1993
	T → C	16217 bp	I Ginther *et al.* 1993
			IV Ward *et al.* 1991
			B Torroni *et al.* 1993a
II	C → T	16187 bp	II Horai *et al.* 1993
	T → C	16325 bp	III Ginther *et al.* 1993
			III Ward *et al.* 1991
III	C → T	16290 bp	III Horai *et al.* 1993
	G → A	16319 bp	IV Ginther *et al.* 1993
			II Ward *et al.* 1991
			A Torroni *et al.* 1993a
IV	T → C	16298 bp	IV Horai *et al.* 1993
	C → T	16327 bp	II Ginther *et al.* 1993
			C Torroni *et al.* 1993a
V	C → T	16278 bp	I Ward *et al.* 1991

* Nucleotide numbering corresponds to Anderson *et al.* (1981).

from Table IV have two copies of the 9 bp repeat. Hence, there are two subsets of haplogroup I:I/B (deletion of the 9 bp repeat) and of I (two copies of the 9 bp repeat).

Although all authors sequencing the D-loop detected three or four haplogroups, the comparative analysis indicates the presence of five haplogroups and two subsets of haplogroup I. All the transitions listed in Table III are also present in Asiatics (Table V). On the other hand, the combination of transitions that define each one of haplotypes I-V is found in Asiatics, for all haplotypes except haplotype II (Table V). When the low frequency of haplotypes I and IV in Asiatics is taken into account, it seems logical to assume that the lack of haplotype II in this ethnic group is due to a combination of the low frequency of the haplotype and the small size of the sample analyzed (101 individuals).

Table VI summarizes the polymorphisms that characterize each one of the founder mt-haplotypes proposed by our group.

The use of mtDNA to trace the origin of Amerindians

The number of founder Amerindian haplotypes is a problem at the center of an unsolved dispute. According to Torroni *et al.* (1993b), the colonization from Asia into the American continent was accompanied by a severe bottleneck that markedly restricted the number of maternal lineages entering the New World. Ward *et al.* (1991) and Horai *et al.* (1993) propose an opposite view. The genetic diversity detected in Amerindians is, according to these investigators, too extensive, and consequently, does not support the hypothesis of the genetic bottleneck. Our findings indicating the presence of at least 13 founder haplotypes in Amerindians lend additional support to the positions of Horai *et al.* and Ward *et al.*

Since founder Amerindian haplotypes were detected at variable frequencies in Asiatic populations (Schurr *et al.*, 1990; Bailliet *et al.*, 1994), it has been concluded that mtDNA polymorphisms are an undisputed proof of the Asiatic origin of early Americans (Schurr *et al.*, 1990; Torroni *et al.*, 1993a,b). In fact, the deletion of 9 bp in region V has also been reported in a small number of Africans (Merriwether *et al.*, 1994; Torroni and Wallace, 1995) and the haplotypes A and D have been detected in Caucasians (Cann *et al.*, 1987). Moreover, since mutations originating Amerindian founder haplogroups are very likely not exclusive to Asiatics or Amerindians, the finding of most putative ancestral Amerindian variants in every population studied is only a matter of sample size.

A matter of hot dispute is the number and timing of colonizations into America. Hoffecker *et al.* (1993) have reviewed the geological, archaeological and anthropological data thus far available, and proposed that Beringia was settled 11,000 to 12,000 years before present and that at about the same time the first colonizations of the New World took place. Conversely, investigators using mtDNA propose much earlier times for the migrations into America. A critical appraisal of the hypothesis put forward by Hoffecker *et al.* (1993) exceeds the province of this review. On the other hand, the reconstruction of the evolutionary history of Amerindians by using mtDNA is a central issue of our presentation.

Estimations of the earliest date of colonization of the American continent are based on the construction of parsimonious trees (Horai *et al.*, 1993; Torroni *et al.*, 1994a).

For these trees to be accurate, it is essential to have enough informative characters. However, the trees so far reported have too many taxa and relatively too few phylogenetically informative sites to allow complete resolution of the phylogeny by parsimony (Stewart, 1993; Bianchi and Bailliet, 1993); indeed, numerous equally parsimonious trees can be constructed with the data published (Horai *et al.*, 1993; Torroni *et al.*, 1993a,1994a). Additional uncertainties result

Table V - D-loop haplotypes and transitions. Frequency (%) in Amerindians and Asiatics*.

Haplotypes	Asiatics	Amerindians	Transitions	Amerindians	Asiatics
I	5.9	17	T- > C 16189 bp	21.6	33.6
			T- > C 16217 bp	21.6	5.9
II	0	28.4	C- > T 16187 bp	30.2	10.9
			T- > C 16325 bp	36	2
III	3.9	28.4	C- > T 16290 bp	32.4	2
			G- > A 16319 bp	34.5	13.9
IV	0.1	19.3	T- > C 16298 bp	23.7	9.9
			C- > T 16327 bp	24.5	2
V	12.8	13.9	C- > T 16278 bp	3.9	12.8
Unclassified . .	77.8	2.9			

Note - Total no. of Amerindians analyzed: 176; total no. of Asiatics analyzed: 101
*Source - Horai and Hayasaka (1990); Horai *et al.* (1993); Ward *et al.* (1991).

Table VI - Extended Amerindian haplotypes*,**.

Mt markers	A_1	A_2	B	B/I	C_1	C_2	D_1	D_2	V	E	A/B	A/C	B/D
*Hae*III bp 663	+	+	-	-	-	-	-	-	0	-	+	+	+
*Alu*I bp 5176	+	+	+	+	+	+	-	-	0	+	+	+	-
*Hinc*II bp 13259	+	+	+	+	-	-	+	+	0	+	+	-	+
Region V repeat***	2	2	1	1	2	2	2	2	0	2	1	2	1
*Hae*III bp 16517	+	-	+	+	+	-	+	-	0	+/-	0	0	0
*Alu*I bp 10397	-	-	-	-	+	+	+	+	0	+	-	0	0
T → C bp 16189	-	-	-	+	-	-	-	-	0	-	0	0	0
T → C bp 16217	-	-	-	+	-	-	-	-	0	-	0	0	0
C → T bp 16278	0	0	0	0	0	0	0	0	+	0	0	0	0

*Bailliet *et al.* (1994).
** - = absence; + = presence; 0 = not studied.
***1 and 2 indicate number of copies of the 9 bp repeat.

from the inaccuracies in the estimated rate of mtDNA mutation used to calibrate the trees. The estimated rates of nucleotide substitution are: 8.4%/million years (MYR) (Vigilant *et al.*, 1989), 11.5-17.3%/MYR (Vigilant *et al.*, 1991), 33%/MYR (Ward *et al.*, 1991), 11.8%/MYR (Stoneking *et al.*, 1992) and 2.2-2.9%/MYR (Torroni *et al.*, 1994a). Due to the above difficulties, the proposed dates of the New World colonization range between 14,000 and 20,000 years before present for Horai *et al.* (1993) and between 22,000 and 29,000 years before present for Torroni *et al.* (1994a). In this regard, it is worthwhile to quote the last sentence of the abstract in Torroni *et al.* (1994a, p.1100): "The mt-DNA data are thus at present equivocal with respect to the most likely times of entry of the Amerindians into the New World mentioned above, but favor the early entry hypothesis".

From the above considerations we must conclude that, given the state of the art in the use of mtDNA, trying to draw conclusions that exceed either the possibilities of the method or the extent of our knowledge is unreasonable. Studies of mtDNA have provided interesting, but preliminary, responses to some of the questions regarding the origin and evolution of Amerindians. Further clarification of these questions will require an understanding of the exact rates of mutation in different regions of the mitochondrial molecule. Answering anthropological questions will require a multidisciplinary approach in which the contribution of mtDNA analysis will be equivalent to the contribution provided by other methodologies.

RESUMO

MtDNA tem sido muito usado para traçar as origens e os padrões de migração entre os ameríndios. A vantagem do mtDNA sobre o DNA nuclear é que o primeiro acumula mudanças de base em média 5-10 vezes mais rapidamente que o DNA nuclear, o que o faz mais apropriado para a análise das diferenças de DNA entre populações humanas. Além disso, sua herança materna e a falta de recombinação permitem a determinação de divergência filogenética entre linhagens sem as ambigüidades causadas pelo embaralhamento e mistura meióticos sofridos pelos genes nucleares.

Até agora, as características mais úteis do mtDNA para estudos da população ameríndia têm sido o polimorfismo de sítios de restrição, a variação do comprimento de um segmento não codificador da região V contendo uma repetição curta e a substituição de bases que ocorre na região D-loop.

Analisando os polimorfismos de mtDNA, diversos grupos chegaram à conclusão de que os ameríndios poderiam ser agrupados em quatro haplótipos mitocondriais fundadores. Reanalisamos os dados publicados e estudamos 673 ameríndios pertencentes a 23 diferentes tribos da américa do Sul. Nossos resultados mostraram que os quatro haplótipos propostos podem ser subdivididos em vários outros, dando origem a não menos que 13 possíveis haplótipos fundadores. O número de haplótipos ameríndios fundadores é o problema central de uma disputa não resolvida. Alguns investigadores defendem a idéia de que a colonização do continente americano a partir da Ásia caracterizou-se por um severo gargalo que restringiu o número de linhagens maternas no Novo Mundo. Outros grupos defendem um ponto de vista oposto. Nossa descoberta de no mínimo 13 haplótipos fundadores nos ameríndios reforça a última posição.

Estimativas da data mais remota da colonização do continente americano são baseadas na construção de árvores parcimoniosas. Entretanto, as árvores até agora relatadas têm grupos taxonômicos demais e relativamente poucos sítios filogeneticamente informativos para permitir uma resolução completa da filogenia por parcimonia. Incertezas adicionais resultam da imprecisão das taxas estimadas de mutação de mtDNA usadas para calibrar as árvores, que variam de 2,2% a 33% por milhão de anos, de acordo com diferentes autores. Devido às dificuldades citadas, as datas propostas para a colonização do Novo Mundo variam de 14000 a 29000 anos antes da presente data.

Estudos do mtDNA têm trazido respostas interessantes, mas preliminares, a algumas questões que dizem respeito à origem e evolução dos ameríndios. Esclarecimentos posteriores destas questões exigirão o conhecimento exato das taxas de mutação em diferentes regiões da molécula mitocondrial, e que se esteja atento ao fato de que as respostas às questões antropológicas exigirão uma abordagem multididicsplinar em que a contribuição da análise do mtDNA será equivalente à contribuição dada por outras metodologias.

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