Ann. Hum. Genet. (2001), **65**, 137–151
Printed in Great Britain

MtDNA from extinct Tainos and the peopling of the Caribbean

C. LALUEZA-FOX[1], F. LUNA CALDERÓN[2], F. CALAFELL[3],
B MORERA[3] AND J. BERTRANPETIT[3]

[1] *Secció Antropologia, Dept. Biologia Animal, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain*
[2] *Departamento de Antropología Física, Museo del Hombre Dominicano, Santo Domingo, República Dominicana; Universidad Nacional Pedro Henríquez Ureña, República Dominicana*
[3] *Unitat de Biologia Evolutiva, Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, Barcelona, Spain*

(Received 10.7.00. Accepted 30.11.00)

SUMMARY

Tainos and Caribs were the inhabitants of the Caribbean when Columbus reached the Americas; both human groups became extinct soon after contact, decimated by the Spaniards and the diseases they brought. Samples belonging to pre-Columbian Taino Indians from the La Caleta site (Dominican Republic) have been analyzed, in order to ascertain the genetic affinities of these groups in relation to present-day Amerinds, and to reconstruct the genetic and demographic events that took place during the peopling of the Caribbean.

Twenty-seven bone samples were extracted and analyzed for mtDNA variation. The four major Amerindian mtDNA lineages were screened through amplification of the specific marker regions and restriction enzymatic digestion, when needed. The HVRI of the control region was amplified with four sets of overlapping primers and sequenced in 19 of the samples. Both restriction enzyme and sequencing results suggest that only two (C and D) of the major mtDNA lineages were present in the sample: 18 individuals (75%) belonged to the C haplogroup, and 6 (25%) to the D haplogroup. Sequences display specific substitutions that are known to correlate with each haplogroup, a fact that helped to reject the possibility of European DNA contamination. A low rate of *Taq* misincorporations due to template damage was estimated from the cloning and sequencing of different PCR products of one of the samples. High frequencies of C and D haplogroups are more common in South American populations, a fact that points to that sub-continent as the homeland of the Taino ancestors, as previously suggested by linguistic and archaeological evidence. Sequence and haplogroup data show that the Tainos had a substantially reduced mtDNA diversity, which is indicative of an important founder effect during the colonization of the Caribbean Islands, assumed to have been a linear migratory movement from mainland South America following the chain configuration of the Antilles.

INTRODUCTION

When Christopher Colombus reached two of the Greater Antilles (Bahamas and Hispaniola) during his first discovery voyage, in 1492, he was greeted by indigenous people who called themselves Tainos. At that time, Columbus was convinced of having arrived in either Japan or China; later he changed his mind, and, believing he had reached India, called the aborigines 'Indians', a misleading name for the Native Americans that has remained in use to this day. Thus, the wrong and biased perceptions

Correspondence: Jaume Bertranpetit, Unitat de Biologia Evolutiva, Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, C. Dr. Aiguader 80, 08003 Barcelona, Spain. Tel: (+3493) 542 28 40; Fax (+3493) 542 28 02.
E-mail: jaume.bertranpetit@cexs.upf.es

of Westerners about Caribbean aborigines date back to the very first moment both cultures collided. However, we don't really know what the Tainos thought about the Spaniards, since they were extinguished in just one or two generations after this first contact, decimated by the harsh treatment of the Spaniards and the diseases they brought with them. It is difficult to know how many people were killed during this process of extinction; according to different authors they could have numbered between 2 and 7 million throughout the Caribbean (Ubelaker, 1992; Crawford, 1992). At the beginning of the 16th century, to replace the decreasing Tainos as agricultural and mining labour, the Spaniards brought African slaves (Kiple, 1984), who came to constitute the major present-day human substratum in the Caribbean. Despite claims of Taino heritage survival in some rural communities in the east of Cuba, it must be concluded that, after 500 years of cultural and genetic disruption, the original Caribbean people have disappeared forever as a distinct human group. The study of the so-called Black Caribs from Belize (Monsalve & Hagelberg, 1997), a population which is presumed to derive from the admixture of Island Caribs with West African slaves, illustrates the limitations of working with the highly admixtured modern Caribbean populations, since at least 16 of the 17 sequences found were clearly of African origin. Therefore, we need to rely on ancient DNA analysis if we want to know the genetic affinities of these groups in relation to the other peoples of the Americas.

By the time of Columbus, and according to the Spanish chroniclers, there were two main human groups in the Caribbean, the Tainos, and the Caribs (whose name is the source of the region's name). The Tainos inhabited la Hispaniola, Puerto Rico, the east of Cuba, and probably Jamaica, the Bahamas, and the Turks and Caicos Islands, while the Caribs inhabited the Windward Islands and Guadeloupe (Rouse, 1986, 1993). The latter group – sometimes called Island Caribs – was culturally related to some mainland American groups (called Mainland Caribs), that were established mainly in Venezuela. The Tainos consisted of hierarchical societies organized into chiefdoms; they had advanced agricultural techniques that allowed them to establish some settlements of thousands of inhabitants, with ceremonial squares and ball game courts. In contrast, the Caribs were ferocious nomadic hunters that raided the Taino villages, expanding from the South through the Lesser Antilles. In addition to Tainos and Caribs, there were other groups at Columbus' times: the so-called Arawaks, inhabitants of Trinidad and the Guianas, and the Guanajuatabeys, inhabitants of West Cuba.

The names of the Caribbean groups and the languages they spoke are a source of debate among scholars; it seems that both Tainos and Island Caribs spoke Arawakan languages that belong to the Equatorial sub-family, in the Equatorial-Tucanoan family (Ruhlen, 1991). In contrast, the Mainland Caribs spoke Caribbean languages, which are classified into the Macro-Carib subfamily, within the Ge-Pano-Carib family (Greenberg, 1987; Ruhlen, 1991). The existence of some words with clear Caribbean origin in the language of the Island Caribs points to a close relationship with the Mainland Caribs. The original homeland of the Taino groups in mainland South America is more controversial. Archaeological evidence shows that the Caribbean area was already settled by 5000 B.C.; however, it has been suggested that the direct ancestors of the Tainos might have come from populations that migrated from the Lower Orinoco Valley, the Guianas or Trinidad and Tobago, around 1000 B.C. Thereafter, they undertook a long series of voyages, from one island to another, progressing from the mainland to the Lesser Antilles and from there to the Greater Antilles, eventually mixing with or pushing west the pre-existing populations, like the Guanajuatabeys. The islands are so close to one another that, with three exceptions, it is possible to see the next island in the migratory chain. If this hypothesis is correct, the peopling of the Caribbean had to take place as a linear migratory movement from South East to North

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520137.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

West, following the chain configuration of the Antilles Islands.

Therefore, whether or not the Caribbean was peopled from South-America is a hypothesis that can be reliably explored with ancient DNA analysis. The vast majority of ancient DNA studies have been based on the analysis of mitochondrial DNA (mtDNA). This cytoplasmic genome has a better chance of recovery, since a cell with a single copy of the nuclear genome can contain several thousand copies of the mtDNA genome. MtDNA has been widely used as a molecular tool for reconstructing the history of present-day human populations, by virtue of its special evolutionary properties, such as a rapid mutation rate relative to nuclear DNA, lack of recombination and maternal inheritance (Avise, 1986; Stoneking, 1993).

In the Americas, many studies have shown that most of the mtDNA of Amerindian populations falls into four major lineages (named 'A', 'B', 'C', 'D'), primarily defined by specific mtDNA markers (Schurr *et al.* 1990; Torroni *et al.* 1992, 1993*b*, 1994; Horai *et al.* 1993). Haplogroup A is defined by an *Hae*III site at np 663, haplogroup B by a COII/tRNA^{Lys} intergenic 9bp deletion, haplogroup C by an *Alu*I site at np 13262 and haplogroup D by the absence of the *Alu*I site at np 5176. Sequence data show a correlation between these lineages and particular mutations in the Control Region I of the mtDNA genome (Torroni *et al.* 1993*a*). An additional residual fifth founding haplogroup, named 'X', has been recently described (Bandelt *et al.* 1995). This lineage, ancestrally related to the lineage X found in some European populations, is characterized, at its basal level, by some RFLP and control region markers, such as −1715 *Dde*I, +16517 *Hae*III, and the 16223T-16278T substitutions; in the Americas, it has only been found in populations from North America.

Greenberg *et al.* (1986) postulated that three different migrations (Amerind, Na-Dene and Eskimo-Aleut speakers) from Asia across the Bering Straits peopled the Americas. However, the first sequence data (Ward *et al.* 1991) showed a rather high mtDNA diversity in one single tribe, suggesting a much more complex scenario than that expected from the three-migration model. Subsequent genetic studies (Horai *et al.* 1993; Torroni *et al.* 1993*a*, 1993*b*) demonstrated that the Native American mtDNAs clustered in few, but relatively deep, lineages that were widespread along the continent and not restricted to any particular ethnic group or linguistic family. The ubiquity of the Native American mtDNAs in Asia suggested that a single initial migration into America, instead of successive migration waves, was a more plausible scenario (Merriwether *et al.* 1995; Merriwether & Ferrell, 1996). From that common mitochondrial founding pool, different demographic events would have produced the differences observed among present-day Native American populations, thus complicating the interpretation of both genetic and ethnohistorical data (Forster *et al.* 1996).

The purpose of this study is to recover mtDNA from pre-Columbian Taino remains from Hispaniola (Dominican Republic) to ascertain the genetic affinities of these groups in relation to present-day mainland Amerinds and to reconstruct the process of peopling of the Caribbean Islands, along with the possible existence of demographic events during that process, such as genetic drift or bottlenecks. The future aim of this project is to analyse the genetic composition of the pre-Columbian remains from other Caribbean Islands, to provide a clear picture of the whole migration process; if successful, this can constitute a case study on ancient human migrations similar to that of Polynesia, although on a smaller scale.

MATERIALS AND METHODS

DNA extraction and amplification

Twenty-seven bone samples from the pre-Columbian site of La Caleta (República Dominicana) were analyzed. The site is located 25 km east of Santo Domingo city, and is one of the most important Taino necropolises in the island; the bodies are buried with Boca Chica style ceramics, ornaments and tools (unpublished

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520137.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

data). Samples were chosen from well preserved post-cranial bones, and belong to different individuals; the radiocarbon dating of several individuals has yielded dates from 670 ± 70 A.D. to 1680 ± 100 A.D.; however, most of the dates are pre-Columbian.

Extraction was undertaken with strict procedures to minimize the potential for contamination, in a positive-air pressure room separated from the main laboratory. Sterile gloves, face masks, sterile reagents, pipette filter tips and frequent bleaching of the working surfaces were some of the precautions adopted during the process. In addition, the laboratory where the analysis was done is totally new, and no extraction of DNA from Amerindians had ever been performed there. The external surface of the bone samples (± 1 mm) was removed with a sterile surgical blade. Between 1 and 2 g of the bone samples were powdered in a coffee grinder; between each extraction, the grinder was washed with bleach. The powder was washed overnight, with shaking, in 10 ml of 0.5 M EDTA pH 8.0 at 37 °C; after centrifugation, the supernatant was removed, and the remaining sample was incubated overnight at 37 °C with 8.5 ml of water, 1 ml 5% SDS, 0.5 ml 1 M Tris-HCl pH 8.0 and 50 μl of 1 mg/ml proteinase K. After incubation, the digests were extracted three times, first with phenol, second with phenol-chloroform and third with chloroform, and the aqueous phase was concentrated by dialysis centrifugation using Centricon-30 microconcentrators (Amicon) to a 100–200 μl volume.

One microlitre of template was subjected to 35 cycles of amplification in 25 μl-reaction volume containing 1 unit of *Taq* polymerase (Ecogen, Madrid, Spain), 10 × reaction buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 12 mg/ml of BSA and 20 pmoles of each pair of primer. Each cycle consisted of 1 min steps, with denaturation at 94 °C, annealing at 55 °C and extension at 72 °C. Negative controls (extraction blanks and PCR blanks) were undertaken along with the ancient samples, to monitor against contamination; no positive controls were used. PCR products were electrophoresed in 0.8% low-melting agarose gels in TA buffer and visualized with ethidium bromide staining. Positive amplification bands were excised from the gels, melted at 65 °C for 20 min and eluted in 100–200 μl of sterile water, depending on the intensity of the band. The samples were subjected to a further 15 cycles of PCR, with limiting primers, annealing at 57 °C, one initial step at 94 °C for 5 min and one final step at 72 °C for 5 min. The PCR products were purified with the silica binding method (modified from Höss & Pääbo, 1993); 20 μl of reaction volume was mixed with 100 μl of 8.2 M NaI and 40 μl of silica suspension, and left for 5 min at room temperature. After a spin, the supernatant was removed and the silica pellet washed twice with 250 μl of 70% ethanol. The nucleic acids were eluted in 20–30 μl of water; 2–6 μl of these samples was used as the template for direct sequencing on an ABI 377™ automated DNA sequencer (Applied Biosystems, Foster City, CA, USA), according to the supplier's instructions.

Four sets of overlapping primers (L16055-H16142, L16131-H16218, L16209-H16356, and L16347-H16410) published elsewere (Handt *et al.* 1996; Stone & Stoneking, 1998), were used to amplify 354 bp of the mtDNA control region I, between positions 16056 and 16409 (Anderson *et al.* 1981). All samples that yielded positive amplifications and sequences were extracted twice; additional sequences were randomly generated from the second extracts, using the shorter primer sets.

To obtain additional support for the attribution of the four primary mtDNA Amerindian lineages, small fragments of mtDNA containing the specific marker of each lineage were amplified in the same, previously sequenced, samples. Four sets of primers were used. For the haplogroup A, L635 and H709 (Handt *et al.* 1996); haplogroup B, L8215 and H8297 (Wrischnik *et al.* 1987); haplogroup C, L13257 and H13393 (Handt *et al.* 1996; Ward *et al.* 1991, respectively); haplogroup D, L5054 and H5190 (Stone & Stoneking, 1998; Handt *et al.* 1996, respectively). The PCR products were digested overnight at 37 °C with 0.5 μl of the appropriate restriction enzyme, and subsequently electrophoresed in 3% agarose gels,

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520137.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

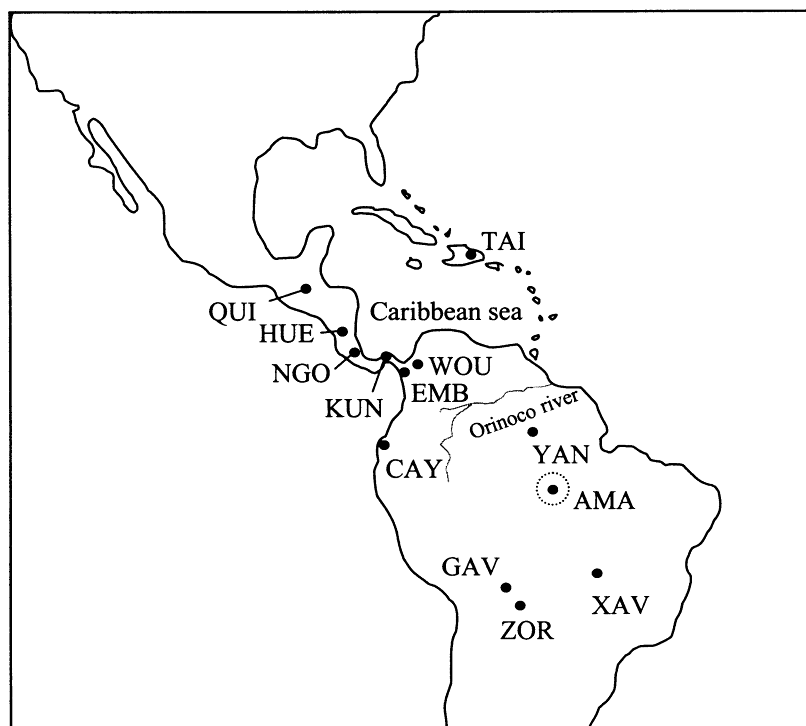

Fig. 1. Map of Central, South America and the Caribbean, with the populations included in this study. Abbreviations are: AMA, Amazonas, CAY, Cayapa, EMB, Embera, GAV, Gavião, HUE, Huetar, KUN, Kuna, NGO, Ngöbé, QUI, Quiche, TAI, Tainos, WOU, Wounan, XAV, Xavante, YAN, Yanomami, ZOR, Zoro.

except for the haplogroup B amplifications that were directly electrophoresed.

Cloning of PCR products

To estimate the rate of misincorporations due to the template damage or *Taq* errors in our sample, two different PCR amplifications (L16209-H16410 and L16209-H16356) from the same sample (# 163) were cloned and sequenced. Twelve microliters of the PCR product were treated with T4 polynucleotide kinase, purified by phenol-chloroform extraction and MicroSpin Column centrifugation and then ligated into a *Sma*I pUC18 plasmid vector, for 2 h at 16 °C, following the supplier's instructions (SureClone Ligation Kit-Pharmacia, Upssala, Sweden). Five microliters of the ligation product was transformed into 100 μl of competent cells and grown in 200 μl of LB medium for 1 h before plating on IPTG/X-gal agar plates. Colonies were left to grow overnight at 37 °C; white colonies were added to 50 μl PCR reactions for 25 cycles; inserts that yielded the expected size in a electrophoresed gel were excised, purified with silica and sequenced following the procedures described.

Statistical analysis

Intrapopulation mtDNA variation in the Tainos was measured by two parameters. Nucleotide diversity (π) was computed as $\pi = (n/n-1)\ \Sigma l^{l}_{i=1}\ (1\text{-}x_i^{2})$, where n is the sample size, l the sequence length and x_i the frequency of each nucleotide at position i (Nei, 1987). Sequence diversity (h_s) was estimated as $h_s = (n/n-1)\Sigma^{k}_{i=1}\ (1-p_i^{2})$, where k is the number of different sequences and p_i the frequency of each sequence (Nei, 1987). The pairwise difference distribution (mismatch distribution) (Rogers & Harpending, 1992; Harpending *et al.* 1993) was also computed.

To provide a populational framework for testing the peopling of the Caribbean, all Meso and South American groups published, with ethnic attribution and large sample sizes, have been considered (Fig. 1). The populations used

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520137.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 1. *mtDNA haplogroup attribution from the amplification and enzymatic restriction of the specific markers in the Taino samples*

Samples (Sequenced)	nt663 HaeIII	COII/tRNALys 9 bp deletion	nt13262 AluI	nt(−)5176 AluI	HAPLOGROUP
166	−	−		+	D
196	−				D*
189	−	−	−	+	D
167	−	−	−	+	D
70	−	−	+	−	C
45	−	−		−	C
187	−	−		−	C
154	−		+	−	C
182	−		+		C
71	−	−		−	C*
48	−	−	+	−	C
191	−	−	+		C
162		−			C*
53	−		+	−	C
170	−		+	−	C
54	−	−	+	−	C
51	−			−	C*
58	−		+		C
163	−	−	+	−	C
(Not sequenced)					
50	−		?	?	?
175	−	−	+		C
164	−	−	+		C
197	−		+		C
72	−		?	?	?
40	−	−	−	+ ?	D
185	−	−	−	+	D
150			?	?	?

? accounts for unresolved enzymatic digestion due to low amplification efficiency. * attributions were confirmed by sequencing of the control region.

are the Cayapas (Rickards *et al.* 1999), Embera, Gavião (Ward *et al.* 1996), Huetar (Santos *et al.* 1994), Kuna (Batista *et al.* 1995), Mapuches (Ginther *et al.* 1993), Ngöbé (Kolman *et al.* 1995), Quiche (Boles *et al.* 1995), Wounan (Kolman & Bermingham, 1997), Xavante (Ward *et al.* 1996), Yanomami (Torroni *et al.* 1993*a*) and Zoro (Ward *et al.* 1996), as well as some individuals from related tribes (Yanomama, Wayampi, Kayapo, Arara, Katuena, Portujara, Awa-Guaja, and Tiriyo) grouped into 'Amazonas' (Santos *et al.* 1996). Analyses including these samples were made considering sequences between positions 16024 and 16383.

A distance matrix between populations was generated using the mismatch-intermatch distance. Principal coordinates analysis was performed on the distance matrix with the NTSYS programme, version 1.70 (Applied Biostatistics, Inc, Setanket, NY, USA). In order to understand the history of the sequences found in the Tainos, we have also performed a median network analysis (Bandelt *et al.* 1995) with 218 sequences from South American populations. To simplify the phylogeny obtained, we have repeated the network only with the C haplogroup sequences, which includes most of the Taino sequences. Analysis of Molecular Variance (AMOVA) (Excoffier *et al.* 1992) was carried out with the Arlequin 2000 package (Schneider *et al.* 2000).

RESULTS

The amplification results of the specific haplogroup markers are shown in Table 1, along with the putative haplogroup assignment. The amplification efficiency varied from one haplogroup to another, probably due to differences in the primer design and the length of the amplified

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520157.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 2. *Polymorphic sites of the sequences found in the Tainos*

Sample	HAPLOGROUP	16126	16129	16189	16223	16242	16254	16263	16265	16298	16311	16325	16327	16362	16400
166	D	·	·	·	T	·	·	·	·	·	·	**C**	·	**C**	·
196	D	·	·	·	T	·	·	·	·	·	·	**C**	·	**C**	·
189	D	·	A	·	T	·	·	·	·	·	·	**C**	·	**C**	·
167	D	·	·	·	T	T	·	·	·	·	C	**C**	·	**C**	T
70	C	·	·	·	T	·	·	·	·	**C**	C	C	**T**	·	·
45	C	·	·	·	T	·	·	·	·	**C**	C	C	**T**	·	·
187	C	·	·	·	T	·	·	·	·	**C**	C	C	**T**	·	·
154	C	·	·	·	T	·	·	·	·	**C**	C	C	**T**	·	·
182	C	·	·	·	T	·	·	·	·	**C**	·	C	**T**	·	·
71	C	·	·	·	T	·	·	·	·	**C**	·	C	**T**	·	·
48	C	·	·	·	T	·	·	·	·	**C**	·	C	**T**	·	·
191	C	·	·	·	T	·	·	·	·	**C**	·	C	**T**	·	·
162	C	·	·	C	T	·	·	·	·	**C**	·	C	**T**	C	·
53	C	·	·	C	T	·	·	·	·	**C**	·	C	T	C	·
170	C	·	·	·	T	·	·	·	·	**C**	·	·	**T**	C	·
54	C	·	·	·	T	·	·	C	·	**C**	·	·	**T**	·	·
51	C	·	·	·	T	·	·	·	T	**C**	·	C	**T**	·	·
58	C	·	·	·	·	·	G	·	·	**C**	·	C	**T**	C	·
163	C	C	·	·	·	·	·	·	·	**C**	·	C	**T**	·	·

Base positions are compared to the Cambridge reference sequence (Anderson *et al.* 1981).

product; for instance, it was possible to amplify the A haplogroup region in almost all the samples (25 out of 27); in contrast, only 16 samples yield PCR products for the D haplogroup. In some of the C and D haplogroup amplifications, the bands were so faint that the final result remained unclear. It should be noted that amplification is independent of the results of the enzymatic digestion and, therefore, the amplification efficiency does not bias the haplogroup attribution.

The amplification efficiency was higher for the control region, maybe due to a better primer design (Table 2). In the best preserved specimens (ten samples), it was possible to obtain the 354 bp region with only two overlapping fragments (L16055-H16218 and L16209-H16410). The widely described degradation of ancient DNA into fragments, usually smaller than 200 bp (Pääbo, 1989; Lalueza-Fox, 1996*a*), made necessary the amplification of nine samples (71, 182, 53, 154, 187, 196, 58, 51, 170) in four fragments. A partial sequence, with a G in np 16212 (L16131-H16218 fragment), was obtained for the no. 197 sample; however, since it was not possible to extend the sequence or reproduce it subsequently, this has not been included in Table 2. In addition, seven samples failed to yield amplifiable DNA. The low amplification efficiency and low reproducibility of some samples most likely reflects severe DNA degradation and a small number of template molecules.

The sequence markers obtained, corresponding to the mtDNA lineages (C in np 16325 and C in np 16362 for the haplogroup D, and C in np 16298, C in np 16325 and T in np 16327 for the haplogroup C), confirmed the haplogroup attribution (Forster *et al.* 1996). Taking into account the consensus haplogroup assignation, inferred from both the haplogroup markers and the sequences, it can be summarized that 75% of the Tainos studied belonged to the C haplogroup (N = 18) and 25% (N = 6) to the D haplogroup. No presence of the two other major Amerindian haplogroups (A and B) was detected. The highest limit for a 95% confidence interval for no observation in a sample size (N) of 24 individuals can be estimated through the Poisson distribution $1-e^{-FN} = 0.95$, were F is the frequency of an unobserved haplogroup; therefore, other haplogroups could be present in the Taino

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520137.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 3. *MtDNA lineage frequencies in Amerindian populations*

Linguistic classification and Population	*n*	mtDNA lineages frequencies (%) A	B	C	D	Others	References
ESKIMO-ALEUT							
Old Harbor, Eskimos	115	61.7	3.5	0.0	34.8	0.0	Merriwether *et al.* 1995*b*
Ouzinbie, Eskimos	41	73.2	0.0	4.9	14.6	7.3	Merriwether *et al.* 1995*b*
Gambell, Eskimos	50	58.0	0.0	14.0	26.0	2.0	Merriwether *et al.* 1995*b*
Savoonga, Eskimos	49	93.9	0.0	0.0	2.0	4.1	Merriwether *et al.* 1995*b*
St. Paul, Aleuts	72	25.0	0.0	1.4	66.7	6.9	Merriwether *et al.* 1995 *b*
CONTINENTAL NA-DENE							
Dogrib	30	100.0	0.0	0.0	0.0	0.0	Torroni *et al.*, 1993*a*
Dogrib	124	88.7	0.0	2.4	0.0	8.9	Merriwether *et al.* 1995*b*
Navajo	48	58.3	37.5	0.0	0.0	4.2	Torroni *et al.* 1993*a*
Apache	25	64.0	16.0	12.0	8.0	0.0	Torroni *et al.* 1993*a*
HAIDA NA-DENE							
Haida	38	92.1	0.0	7.9	0.0	0.0	Ward *et al.* 1993
Haida	25	96.0	0.0	0.0	4.0	0.0	Torroni *et al.* 1993*a*
NORTHERN AMERIND							
Bella Coola	25	60.0	8.0	8.0	20.0	4.0	Torroni *et al.* 1993*a*
Bella Coola	32	78.1	6.25	9.4	6.25	1.6	Ward *et al.* 1993
Nuu-Chah-Nulth	63	44.5	3.1	19.1	26.7	13.3	Ward *et al.* 1991
Nuu-Chah-Nulth	15	40.0	6.7	13.3	26.7	13.3	Torroni *et al.*, 1993*a*
Ojibwa	28	64.3	3.6	7.1	0.0	25.0	Torroni *et al.* 1993*a*
Mohawk	18	46.4	10.5	13.8	0.6	28.7	Merriwether *et al.* 1995*b*
Maya	27	51.9	22.2	14.8	7.4	3.7	Torroni *et al.* 1993*a*
Mixe	16	62.5	31.3	6.2	0.0	0.0	Torroni *et al.* 1994
Muskoke	71	36.6	15.5	9.9	38.0	0.0	Merriwether *et al.* 1995*b*
CENTRAL AMERIND							
Mixtec Alta	15	73.4	13.3	13.3	0.0	0.0	Torroni *et al.* 1994
Mixtec Baja	14	92.9	7.1	0.0	0.0	0.0	Torroni *et al.* 1994
Zapotec	15	33.3	33.3	33.3	0.0	0.0	Torroni *et al.* 1994
Pima	30	6.7	50.0	43.3	0.0	0.0	Torroni *et al.* 1993*a*
CHIBCHA-PAEZAN							
Teribe	20	80.0	20.0	0.0	0.0	0.0	Torroni *et al.* 1994
Guatuso	20	85.0	15.0	0.0	0.0	0.0	Torroni *et al.* 1994
Boruca	14	21.4	71.5	0.0	7.1	0.0	Torroni *et al.* 1993*a*
Kuna	16	100.0	0.0	0.0	0.0	0.0	Torroni *et al.* 1993*a*
Kuna	63	71.4	28.6	0.0	0.0	0.0	Batista *et al.* 1995
Guaymi	16	68.8	31.2	0.0	0.0	0.0	Torroni *et al.* 1993*a*
Bribi/Cabecar	24	54.2	45.8	0.0	0.0	0.0	Torroni *et al.* 1993*a*
Huetar	27	70.4	3.7	0.0	25.9	0.0	Santos *et al.* 1994
Ngöbé	46	67.4	32.6	0.0	0.0	0.0	Kolman *et al.* 1995
Cayapa	120	29.1	40.0	9.2	0.0	21.7	Rickards *et al.* 1999
Atacama	13	23.1	69.2	7.7	0.0	0.0	Bailliet *et al.* 1994
Atacameño	50	12.0	72.0	10.0	6.0	0.0	Merriwether *et al.* 1995*b*
Yanomama	24	0.0	16.7	54.2	29.2	0.0	Torroni *et al.* 1993*a*
ANDEAN							
Quechua	19	26.3	36.8	5.3	31.6	0.0	Merriwether *et al.* 1995*b*
Aymara	172	6.4	67.4	12.2	14.0	0.0	Merriwether *et al.* 1995*b*
Mapuche	39	15.4	38.5	20.5	25.6	0.0	Ginther *et al.* 1993
Mapuche	58	5.3	32.7	20.6	31.1	10.3	Bailliet *et al.* 1994
Huilliche	38	5.3	28.9	18.4	47.4	0.0	Bailliet *et al.* 1994
Huilliche	80	3.75	28.75	18.75	48.75	0.0	Merriwether *et al.* 1995*b*
Pehuenche	100	2.0	9.0	37.0	52.0	0.0	Merriwether *et al.* 1995*b*
Aonikenk	15	0.0	0.0	26.7	73.3	0.0	Lalueza-Fox 1996
Kawéskar	19	0.0	0.0	15.8	84.2	0.0	Lalueza-Fox 1996
Yámana	11	0.0	0.0	90	10	0.0	Lalueza-Fox 1996
Selk'nam	13	0.0	0.0	46.2	46.2	7.7	Lalueza-Fox 1996
EQUATORIAN TUCANOAN							
Piaroa	10	50.0	0.0	10.0	40.0	0.0	Torroni *et al.* 1993*a*
Wapishana	12	0.0	25.0	8.3	66.7	0.0	Torroni *et al.* 1993*a*
Ticuna	28	17.9	0.0	32.1	50.0	0.0	Torroni *et al.* 1993*a*
Zoro	30	20.0	6.7	13.3	60.0	0.0	Ward *et al.* 1996

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520137.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 3. (*cont.*)

Linguistic classification and Population	*n*	mtDNA lineages frequencies (%) A	B	C	D	Others	References
Gavião	27	14.8	14.8	0.0	70.4	0.0	Ward *et al.* 1996
Tainos	24	0.0	0.0	75.0	25.0	0.0	Present study
GE-PANO-CARIB							
Makiritare	10	20.0	0.0	70.0	10.0	0.0	Torroni *et al.* 1993*a*
Macushi	10	10.0	20.0	30.0	40.0	0.0	Torroni *et al.* 1993*a*
Kraho	14	28.6	57.1	14.3	0.0	0.0	Torroni *et al.* 1993*a*
Marubo	10	10.0	0.0	60.0	30.0	0.0	Torroni *et al.* 1993*a*
Mataco	28	10.7	35.7	0.0	53.6	0.0	Torroni *et al.* 1993*a*
Xavante	25	16.0	84.0	0.0	0.0	0.0	Ward *et al.* 1996
LINGUISTIC CLASSIFICATION NO-SPECIFIED							
Colombians	20	50.0	20.0	25.0	5.0	0.0	Horai *et al.* 1993
Chileans	45	4.5	22.2	40.0	33.3	0.0	Horai *et al.* 1993
ANCIENT GROUPS							
Norris Farm-Oneota	108	31.5	12.0	42.6	8.3	5.6	Stone and Stoneking 1998
Great Salt Lake Fremont	32	0.0	73.0	13.0	7.0	7.0	Parr *et al.* 1996

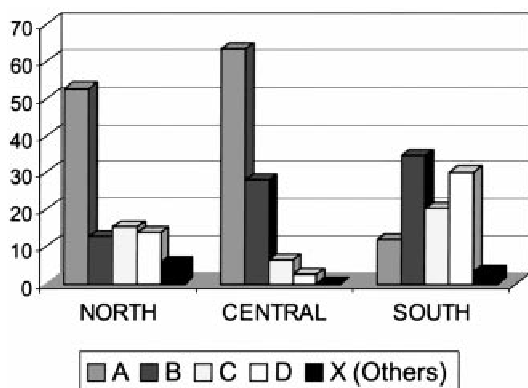

Fig. 2. MtDNA lineage frequencies in Amerindian populations grouped by broad geographic regions (North, Central and South America). X haplogroup has only been found in North America, the black bar in South America correspond to a lineage described in the Capayas.

population with frequencies up to 12.5% and would not have been detected with the present sample size of 24 individuals.

Data on the haplogroup frequencies for other Amerindian populations have been compiled from previously published papers, and have been grouped according to linguistic and geographic criteria (Table 3). The most obvious pattern of variation in these frequencies is still geographical, as some authors have suggested (Merriwether *et al.* 1995; Lalueza-Fox, 1996*b*). When grouped in the three main geographic entities of the continent (North American, Central American and South American Amerinds) marked differences in the distribution of the mtDNA lineages can be observed (Fig. 2).

Cloning results

The sequence of the clones obtained for one sample (no. 163) consistently shows the substitutions found in the direct sequencing of the sample (16298 [C] 16325 [C] and 16327 [T]), as well as some singletons (Fig. 3). Since none of the singletons are shared in two or more clones, these most probably correspond to cloning artifacts and not to template damage and *Taq* misincorporations; the latter would yield multiple clones sharing the substitution (Krings *et al.* 1997). Thus, the DNA from the no. 163 sample is remarkably well preserved (only 5 singleton substitutions in 2864 nucleotides sequenced, error rate/1000 bp of 1.75). Since there did not seem to be important taphonomic differences among samples from the La Caleta site related to preservation, the cloning results suggest a low ratio of putative misincorporations due to template damage and *Taq* errors in our sample.

Diversity parameters

Nucleotide diversity of the Tainos was estimated at 0.0084 for the 354 bp fragment; this value is lower than that found in most of the

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520137.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Amerind populations studied; only the Kuna (0.0092), the Huetar (0.0097) and the Xavante (0.0083) show a similar level of reduced diversity. The sequence diversity obtained for the Tainos was 0.918; in this case, this value is higher than most of the other Amerind populations, similar to the Amazonas (0.933), Embera (0.942), Mapuche (0.912) or Wounan (0.920). In addition, the Tainos present a bell-shaped pairwise difference distribution, with a mean value of 2.96, the smallest of the Amerind populations used for comparison, but close to the values found in the Xavante (3.00), the Kuna (3.30) and the Huetar (3.50).

Sequence sharing

Some of the sequences found have been already described, especially those close to the root of the D and C haplogroups: (1) 16223 [T] 16325 [C] 16362 [C], (2) 16223 [T] 16298 [C] 16311 [C] 16325 [C] 16327 [T], and (3) 16223 [T] 16298 [C] 16325 [C] 16327 [T]. Interestingly, two previously undescribed sequences, 16223 [T] 16242 [T] 16311 [C] 16325 [C] 16362 [C] 16400 [T] and 16189 [C] 16223 [T] 16298 [C] 16325 [C] 16327 [T] 16362 [C] are very close to two Mapuche sequences already described by Ginther *et al.* (1993). In addition, 16263 [C] has been described in a Mongolian individual in association with some of the substitutions (16223 [T] 16298 [C] 16327 [T]) also found in our sample, while 16254 [G] and 16129 [A] substitutions have been found in Asian individuals. 16265 [T] is an unusual substitution although it has been found in some Panamanian individuals (Kolman & Bermingham, 1997), while 16126 [C] has been widely described in other populations.

Taino genetic affinities

The results of the principal coordinate analysis on the mismatch-intermatch genetic distance matrix are represented in Fig. 4; the first two principal coordinates account for 68.5% of the total variance of the genetic distance matrix (the first coordinate accounts for 42.2% and the second for 26.3%). The neighbor-joining tree (not shown) displays a topology that reflects a similar structure. It can be observed that most groups from Central America (the Ngöbé, Kuna, Huetar and Quiche) are separated from the other groups, but related quite closely to one another, while the Tainos and Yanomami are opposite to them; in between them are all the other South American populations as well as the Chocó speakers from Panamá/Colombia, the Embera and the Wounan. The Xavante, a group from the south of Brazil, seem to be the most differentiated population within South America.

Phylogenetic analysis of sequences

In the median network of the South American sequences, the Taino individuals tend to be distributed around the central nodes of the C and D haplogroups, clustering with or close to the inferred ancestral sequences, suggesting a relative antiquity of these sequences. The C haplogroup median network is clearly star-like (Fig. 5); a visual fact supported by the low values of kurtosis (0.258 ± 0.778) and skewness (1.026 ± 0.398) of the network's distribution branch length (Mateu *et al.* 1997). This kind of star-shaped phylogeny suggests a population expansion (Forster *et al.* 1996). It can be observed that most of the Taino sequences are related to the founding sequence by just one, or very few, substitutions; the main inconsistencies are due to reversions in position 16362, which has already been described as a highly unstable position (Forster *et al.* 1996) and has a substitution rate five times higher than the control region average (Meyer *et al.* 1999). Interestingly, the longest branches in the network correspond to sequences found in the Amazonas region, either in some Yanomami or in the tribes included within the 'Amazonas' group.

The position of most of the Taino sequences close to the root and their lack of dispersion along the network suggests that a population expansion occurred before the formation of long branches in South American lineages, and/or before a very narrow bottleneck in the peopling

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520157.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

```
           16210
ANDERSON   ACAGCAATCAACCCTCAACTATCACACATCAACTGCAACTCCAAAGCCACCCCTCACCCACTAGGATACCAACAAACCTACCCACCCTTAACAGTACATAGTACATAAAGCCATTTACCGTACATAGCACA
CLONE 1A   .............T..........................................................................C..........................C.T.............
CLONE 2A   ........................................................................................C..........................C.T.............
CLONE 3A   ....................................................................T...................C..........................C.T.............
CLONE 4A   ........................................................................................C..........................C.T.............
CLONE 5A   ...................G....................................................................C..........................C.T.............
CLONE 6A   ........................................................................................C..........................C.T.............
CLONE 1B   ........................................................................................C..........................C.T.............
CLONE 2B   ........................................G...............................................C..........................C.T.............
CLONE 3B   ........................................................................................C..........................C.T.............
CLONE 4B   ........................................................................................C..........................C.T.............
CLONE 5B   ........................................................................................C..........................C.T.............
                                                                     16409
ANDERSON   TTACAGTCAAATCCCTTCTCGCCCCCATGGATGACCCCCCTCAGATAGGGGTCCCTTGACCACCATCCT
CLONE 1A   .....................................................................
CLONE 2A   .........................................T...........................
CLONE 3A   .....................................................................
CLONE 4A   .....................................................................
CLONE 5A   .....................................................................
CLONE 6A   .....................................................................
CLONE 1B   ...............16355
CLONE 2B   ...............
CLONE 3B   ...............
CLONE 4B   ...............
CLONE 5B   ...............
```

Fig. 3. Sequences from clones generated from two different amplifications (L16,209-H16,410 and L16,209-H16,356) of the no. 163 Taino sample. Anderson is the Cambridge reference sequence (Anderson *et al.* 1981); substitutions shared in all the clones obtained are C in 16,298, C in 16,325 and T in 16,327.

Fig. 4. Principal Coordinate (PC) Analysis of the distance matrix obtained from Central and South American populations. The score values have been multiplied by 100. Abbreviations are: AMA, Amazonas, CAY, Cayapa, EMB, Embera, GAV, Gavião, HUE, Huetar, KUN, Kuna, NGO, Ngöbé, QUI, Quiche, TAI, Tainos, WOU, Wounan, XAV, Xavante, YAN, Yanomami, ZOR, Zoro.

of the Antilles. To test whether both putative population growths correspond to the same demographic event, we have estimated the pairwise distribution of the C sequences of the Taino and one South American population (the Yanomami), whose distribution may clearly reflect a past population expansion (Harpending *et al.* 1993). Since both mean values are quite different (2.11 for the Tainos and 1.25 for the Yanomami), this suggests we are observing the consequences of two population expansions, one associated to a founding event in the Yanomami population and another, more ancient, to the peopling of the Caribbean.

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520157.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

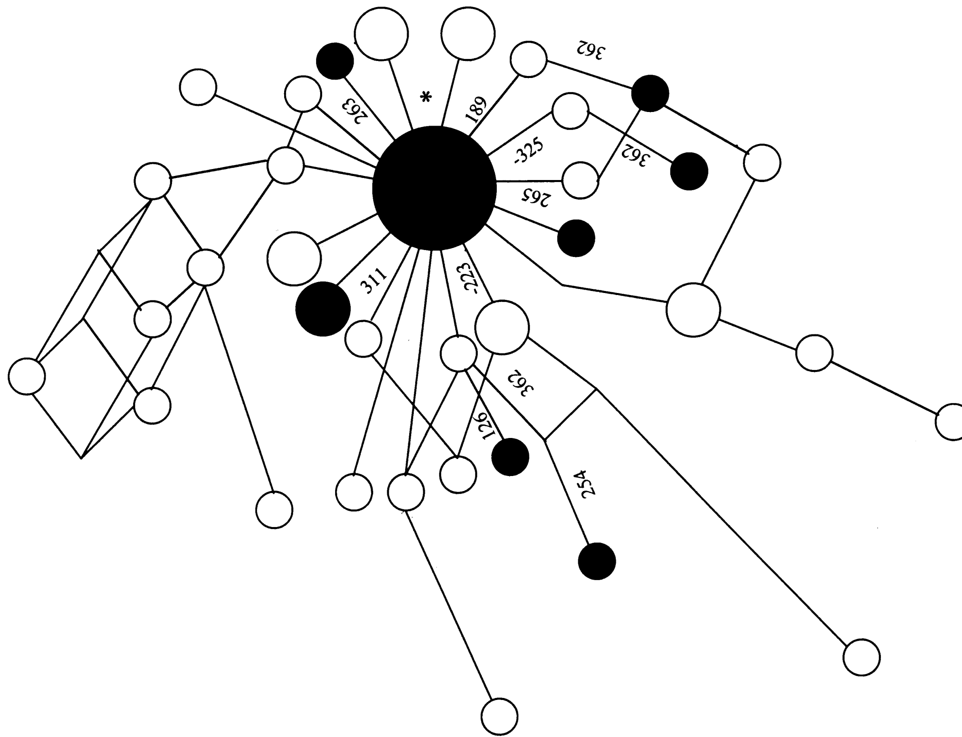

Fig. 5. Reduced median network of the C haplogroup mtDNA sequences from South America. Taino sequences are in black; the circles are proportional to the frequency of the sequences they represent and the substitutions involved in the Taino sequences are listed in the branches. Central node (*) includes sequences with T in 16,223, C in 16,298, C in 16,325 and T in 16,327.

DISCUSSION

The closest phylogenetic affinities of the Tainos are with South American populations, since high frequencies of C and D lineages are more common in South American than in Central or North American populations, in accordance with the observed clinal pattern in the geographic distribution of these lineages along the continent (Merriwether *et al.* 1995; Lalueza-Fox, 1996*b*). There is little genetic structure among the Central and South American populations; the most notable feature seems to be the clustering of most Central American populations in a distinct group from the rest. In fact, AMOVA (Analysis of Molecular Variance) revealed that, when populations (excluding the Tainos) were divided into Central and Southern American, the difference among sub-continents accounted for 10.8% of the genetic variance (significantly different from zero; $p = 0.00196$), while 13.05% of the genetic variance could be explained by differences among the populations in each sub-continent. Geography, considered in a latitudinal sense, is probably the main differentiating factor in the genetic history of the Amerind populations, the main exception being the Tainos. Considering the position of the Tainos in the genetic analysis, it is clear that, despite being geographically close to the Central American groups, their affinities are with South American groups. In particular, the closest group to the Tainos are the Yanomami, the only South American sample available near the Orinoco Valley, a suggested area for the Taino ancestors (Rouse, 1986). The Taino sequences cluster close to the ancestral founding sequences in the median network analyses; this suggests a considerable antiquity for the origin of the mtDNA variation found in the pre-Columbian Caribbean populations and a narrow bottleneck in the founding population.

In the genetic analyses, the Tainos do not seem to be particularly close to the other Equatorian-

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520137.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Tucanoan speakers, the Zoro and the Gavião; instead, they cluster close to the Yanomami, who have a Chibcha-Paezan language. However, the grouping of the three Chibchan-speaker groups (Kuna, Huetar and Ngöbé) could either be attributed to geographic or linguistic affinities. Therefore, although the three-migration hypothesis of Greenberg *et al.* (1986) for the Americas is not supported by genetic data, the correlation between language and mtDNA variation in particular areas, like Central America, may be noticeable.

It has been suggested that some archaic archaeological horizons in the Antilles, such as the Casimiroid flints, originated in Central America, which would indicate a population movement from Yucatan into Cuba and Hispaniola, maybe around 5000 BC. (Rouse, 1986). In contrast, the subsequent Ceramic traditions in the Caribbean can be traced back to the Orinoco Valley in South America, and most probably correspond to a movement of people still ancestral to the Tainos migration into the Caribbean. It is unknown whether these migrating people replaced or mixed with pre-existing populations; however, the absence in the Tainos of the A and B haplogroups, that have high frequencies in Central American populations, points to an extensive replacement of the ancestral Caribbean populations. Also, some contacts between the Caribbean groups and Central American populations have been suggested in more recent times (maybe from 1000 B.C. to Columbian times), especially to explain the diffusion of ball-court structures very similar to those found in Maya cultures (Rouse, 1986). Despite the possible existence of some contacts with Central America, the genetic impact of these should have been quite small, again given the absence of the A and B lineages.

The Tainos seem to be one of the Amerind groups studied so far with lowest genetic diversity. Reduced mtDNA diversity has also been described in some groups from Panama and Costa Rica, like the Huetar, Ngöbé and Kuna (Santos *et al.* 1994; Kolman *et al.* 1995; Batista *et al.* 1995), and has been attributed to either post-contact demographic decline or a small founding population (Kolman *et al.* 1995). In the case of the Tainos, as they predate the Spanish contact, the reduced genetic diversity has to be attributed to the ethnogenesis of this people. Taking into consideration that they were almost at the end of the Caribbean migration arch, most probably this is a reflection of one or more demographic bottlenecks which occurred during the peopling of the Caribbean.

This research was supported by Dirección General de Investigación Científica y Técnica in Spain (project PB-98–1064). We are grateful to J. Mishari Al-Adwani for correcting the English manuscript and to two anonymous referees for their helpful comments.

REFERENCES

Anderson, S., Bankier, A. T., Barrell, B. G., de Brujin, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, R. & Young, I. G. (1981). Sequence and organization of the human mitochondrial genome. *Nature* **290**, 457–465.

Avise, J. C. (1986). Mitochondrial DNA and the evolutionary genetics of higher animals. *Phil. Trans. R. Soc. Lond. B* **312**. 325–342.

Bailliet, G., Rothhammer, F., Carnese, F. R., Bravi, C. M. & Bianchi, N. O. (1994). Founder Mitochondrial Haplotypes in Amerindian Populations. *Am. J. Hum. Genet.* **54**, 27–33.

Bandelt, H. J., Forster, P., Sykes, B. C. & Richards, M. B. (1995). Mitochondrial portraits of human populations using median networks. *Genetics* **141**, 743–753.

Batista, O., Kolman, C. J. & Bermingham, E. (1995). Mitochondrial DNA diversity in the Kuna Amerinds of Panamá. *Hum. Mol. Genet.* **4** (5), 921–929.

Boles, T. C., Snow, C. C. & Stover, E. (1995). Forensic DNA Testing on Skeletal Remains from Mass Graves: A Pilot Study in Guatemala. *J. For. Sci.* **40** (3), 349–355.

Crawford, M. H. (1992). *Antropología biológica de los Indios Americanos*. Madrid, Spain: Col. MAPFRE.

Excoffier, L., Smouse, P. E. & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131** (2), 474–491.

Forster, P., Harding, R., Torroni, A. & Bandelt, H. J. (1996). Origin and Evolution of Native American mtDNA Variation: A Reappraisal. *Am. J. Hum. Genet.* **59**, 935–945.

Ginther, C., Corach, D., Penacino, G. A., Rey, J. A., Carnese, F. H., Hutz, M. H., Anderson, A., Just, J., Salzano, F. M. & King, M.-C. (1993). Genetic variation among the Mapuche Indians from the Patagonian region of Argentina: Mitochondrial DNA sequence variation and allele frequencies of several nuclear

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520157.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

genes. In DNA fingerprinting: state of the science (eds. S. D. J. Pena, R. Chakraborty, J. T. Epplen & A. J. Jeffreys), pp. 211–219. Switzerland: Birkhauser Verlag Basel.

Greenberg, J., Turner II, C. G. & Zegura, S. L. (1986). The settlement of the Americas: a comparison of the linguistic, dental and genetic evidence. *Curr. Anthrop.* **4**, 477–497.

Greenberg, J. (1987). *Language in the Americas.* Stanford, CA: Stanford University Press.

Handt, O., Krings, M., Ward, R. H., & Pääbo, S. (1996). The retrieval of ancient human DNA sequences. *Am. J. Hum. Genet.* **59**, 368–376.

Harpending, H. C., Sherry, S. T., Rogers, A. R. & Stoneking, M. (1993). The genetic structure of ancient human populations. *Curr. Anthrop.* **34**, 483–496.

Horai, S., Kondo, R., Nakagawa-Hattori, Y., Hayashi, S., Sonoda, S., & Tajima, K. (1993). Peopling of the Americas, founded by four major lineages of mitochondrial DNA. *Mol. Biol. Evol.* **10**, 23–47.

Höss, M. & Pääbo, S. (1993). DNA extraction from Pleistocene bones and analysis by a silica-based purification method. *Nucleic Acid Res.* **21**, 3913–3914.

Kiple, K. F. (1984). The Caribbean Slave: A Biological History. Cambridge: Cambridge University Press.

Kolman, C. J., Berminghman, E., Cooke, R., Ward, R. H. &rias, T. D. & Guionneau-Sinclair, F. (1995). Reduced mtDNA diversity in the Ngöbé Amerinds from Panama. *Genetics* **140**, 275–283.

Kolman, C. J. & Bermingham, E. (1997). Mitochondrial and nuclear DNA diversity in the Choco and Chibcha Amerinds of Panama. *Genetics* **147** (3), 1289–1302.

Krings, M., Stone, A., Schmitz, R. W., Krainitzki, H., Stoneking, M. & Pääbo, S. (1997). Neandertal DNA sequences and the origin of modern humans. *Cell* **90**, 19–30.

Lalueza-Fox, C. (1996*a*). Ancient mtDNA analysis in extinct aborigines from Tierra del Fuego/Patagonia. *Ancient Biomolecules* **1**, 43–54.

Lalueza-Fox, C. (1996*b*). Mitochondrial DNA haplogroups in four tribes from Tierra del Fuego-Patagonia: inferences about the peopling of the Americas. *Hum. Biol.* **68**, 855–871.

Mateu, E., Comas, D., Calafell, F., Perez-Lezaun, A., Abade, A. & Bertranpetit, J. (1997). A tale of two islands: population history and mitochondrial DNA sequence variation of Bioko and São Tomé, Gulf of Guinea. *Ann. Hum. Genet.* **61**, 507–518.

Merriwether, D. A., Rothhammer, F. & Ferell, R. E. (1995). Distribution of the four-founding lineage haplotypes in native Americans suggests a single wave of migration for the New World. *Am. J. Phys. Anthropol.* **98**, 411–430.

Merriwether, D. A. & Ferell, R. E. (1996). The four founding lineage hypothesis for the New World: a critical reevaluation. *Mol. Phyl. Evol.* **5** (1), 241–246.

Meyer, S. Weiss, G. & Haeseler, A.v. (1999). Pattern of nucleotide substitution and rate heterogeneity in the hypervariable regions I and II of human mtDNA. *Genetics* **152**, 1103–1110.

Monsalve, M. V. & Hagelberg, E. (1997). Mitochondrial DNA polymorphisms in Carib people of Belize. *Proc. R. Soc. Lond. B.*, **264**, 1217–1224.

Nei, M. (1987). *Molecular evolutionary genetics.* New York, NY: Columbia University Press.

Pääbo, S. (1989). Ancient DNA: Extraction, characterization, molecular cloning, and enzymatic amplification. *Proc. Natl. Acad. Sci. USA* **86**, 1939–1943.

Parr, R. L., Carlyle, S. W., & O'Rourke, D. H. (1996). Ancient DNA analysis of Fremont Amerindians of the Great Salt Lake Wetlands. Am. J. Phys. Anthropol. **99**, 507–518.

Rickards, O., Martinez-Labarga, C., Lum, J. K., De Stefano, G. F. & Cann, R. L. (1999). Mitochondrial DNA history of the Cayapa Amerinds of Ecuador: detection of additional founding lineages for the native American populations. *Am. J. Hum. Genet.* **65**, 519–530.

Rogers, A. R. & Harpending, H. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol. Evol.* **9**, 552–569.

Rouse, I. (1986). *Migrations in Prehistory: Inferring Population Movement from Cultural Remains.* New Haven: Yale University Press.

Rouse, I. (1993). *The Tainos: Rise and Decline of the People who greeted Columbus.* New Haven: Yale University Press.

Ruhlen, M. (1991). *A guide to the world's languages.* Stanford, CA: Stanford University Press.

Santos, M., Ward, R. H. & Barrantes, R. (1994). MtDNA variation in the Chibcha Amerindian Huetar from Costa Rica. *Hum. Biol.* **66**, 963–977.

Santos, S. E., Ribeiro-Dos-Santos, A. K., Meyer, D. & Zago, M. A. (1996). Multiple founder haplotypes of mitochondrial DNA in Amerindians revealed by RFLP and sequencing. *Ann. Hum. Genet.* **60** (4), 305–319.

Schneider, S., Roessh, D. & Excoffier, L. (2000). Arlequin ver. 2000. A software for population genetics data analysis. Laboratorie d'Anthropologie, Université de Gênéve. Geneva, Switzerland.

Schurr, T. G., Ballinger, S. W., Gan, Y. Y., Hodge, J. A., Merriwether, D. A., Lawrence, D. N., Knowler, W. C., Weiss, K. M. & Wallace, D. C. (1990). Amerindian mitocondrial DNAs have rare Asian mutations at high frequencies suggesting they derived from four primary maternal lineages. *Am. J. Hum. Genet.* **46**, 613–623.

Stone, A. C. & Stoneking, M. (1998). MtDNA analysis of a prehistoric Oneota population: implications for the peopling of the New World. *Am. J. Hum. Genet.* **62**, 1153–1170.

Stoneking, M. (1993). DNA and recent human evolution. *Evol. Anthropol.* **2** (2). 60–73.

Torroni, A., Schurr, T. G., Yang, C.-C., Szathmary, E. J. E., Williams, R. C., Schanfield, M. S., Troup, G. A., Knowler, W. C., Lawrence, D. N., Weiss, K. M. & Wallace, D. C. (1992). Native American mitochondrial DNA analysis indicates that the Amerind and NaDene populations were founded by two independent migrations. *Genetics* **130**, 153–162.

Torroni, A., Schurr, T. G., Cabell, M. F., Brown, M. D., Neel, J. V., Larsen, M., Smith, D. G., Vullo, C. M. & Wallace, D. C. (1993*a*). Asian Affinities and Continental Radiation of the Four Founding Native American mtDNAs. *Am. J. Hum. Genet.* **53**, 563–590.

Torroni, A., Sukernik, R. I., Schurr, T. G., Starikovskaya, Y. B., Cabell, M. F., Crawford, M. H., Comuzzie, A. G. & Wallace, D. C. (1993*b*). mtDNA Variation of Aboriginal Siberians Reveals Distinct Genetic Affinities with Native Americans. *Am. J. Hum. Genet.* **53**, 591–608.

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520157.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Torroni, A., Neel, J. V., Barrantes, R., Schurr, T. G. & Wallace, D. C. (1994). Mitochondrial DNA ''clock'' for the Amerinds and its implications for timing their entry into North America. *Proc. Natl. Acad. Sci. USA* **91**, 1158–1162.

Ubelaker, D. H. (1992). Prehistoric New World population size: Historical review and current appraisal of North American estimates. *Am. J. Phys. Anthropol.* **45**, 661–666.

Ward, R. H., Frazier, B. L., Dew-Jager, K. & Pääbo, S. (1991). Extensive mitochondrial diversity within a single Amerindian tribe. *Proc. Natl. Acad. Sci. USA* **88**, 1–5.

Ward, R. H., Redd, A., Valencia, D., Frazier, B. L. & Pääbo, S. (1993). Genetic and linguistic differentiation in the Americas. *Proc. Natl. Acad. Sci. USA* **90,** 10663–10667.

Ward, R. H., Salzano, F. M., Bonatto, S. L., Hultz, M. H., Coimbra, Jr C. E. A. & Santos, R. V. (1996). Mitochondrial DNA polymorphisms in three Brazilian Indian Tribes. *Am. J. Hum. Biol.* **8**, 317–323.

Wrischnik, L. A., Higuchi, R. G., Stoneking, M., Erlich, H. A., Arnheim, N. & Wilson, A. C. (1987). Length mutations in human mitochondrial DNA: direct sequencing of enzymatically amplified DNA. *Nucleic Acids Res.* **8**, 529–542.

14691809, 2001, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1469-1809.2001.6520157.x by Readcube (Labtiva Inc.), Wiley Online Library on [29/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License