Mitochondrial DNA polymorphisms in Carib people of Belize

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SUMMARY

Analysis of mitochondrial DNA (mtDNA) control region polymorphisms in 28 Carib people of Belize, former British Honduras, revealed high levels of genetic admixture with West African populations. A previously characterized length mutation consisting of a deletion of nine base pairs in an intergenic mtDNA region was observed in two of the individuals. Phylogenetic analysis of mtDNA control region sequences associated with the mutation suggested that it arose independently in different geographical locations. Whereas in one individual the deletion reflects the Amerindian ancestry of the Caribs, in the second case it seems to be of African origin, as it occurred in conjunction with an mtDNA type found in sub-Saharan Africa. Our results agree with historical accounts on the origins of the Caribs of Belize.

1. INTRODUCTION

People known as Caribs have inhabited three major areas of the Americas. The mainland Caribs originally occupied the area north of the Amazon, spreading to parts of Guyana, Venezuela and Colombia. They are thought to have expanded about 1000 years ago from the South American mainland to the Lesser Antilles and became known as Island Caribs. At the end of the eighteenth century, a third group, the Black Caribs or Garifuna, was established in the mainland of Central America, including Belize, the former British colony of British Honduras (Davidson 1984; Rouse 1986).

The present-day Carib people of Belize have a characteristic ethnic identity and individual language and customs, and derive from the pre-Columbian Island Carib and Arawak inhabitants of the Lesser Antilles, with an important genetic and cultural input from West African slaves. This fusion resulted from raids by Caribs on European colonies as well as admixture with escaped and shipwrecked slaves in the period between 1517 and 1646. The original Island Caribs practically vanished from the Caribbean in the decades following European contact, the British islands of Dominica and St Vincent becoming their last area of refuge, where to this day they still retain some of their original culture (Harvey *et al.* 1967). The Island Carib-African hybrids, or Black Caribs, adopted the language of the Island Caribs and eventually established settlements in St Vincent. By 1646 they numbered about 3000, and the population increased further in the following decades, until after a series of uprisings against the British they were exiled in 1797 to the island of Roatan off the Central American coast (figure 1). The Black Caribs escaped to the mainland and established settlements

along the coasts of what are now Belize, Honduras and Nicaragua (Crawford 1984).

The Black Caribs are a group of significant interest to geneticists, population biologists, and historians because they are the result of unique historical events, migration and marriage patterns, and represent a population which has been relatively isolated for the past 200 years (Crawford 1984). Genetic studies based on blood group systems and other classical genetic markers revealed similarities between some gene frequencies in Black Caribs and West Africans (Weymes & Gershowitz 1984; Custodio *et al.* 1984), but considerable deviation from this pattern was also observed and was explained by founder effects in the settlement history and genetic admixture with other mainland groups like the Maya, Ketchi and Creoles (Matson & Swanson 1964; Devor *et al.* 1984).

The development of molecular techniques has increased the ease and level of resolution of genetic studies on human populations. The analysis of mitochondrial DNA (mtDNA) provides a particularly useful tool for the study of human genetic affinities because the mtDNA genome is well characterized, easy to study due to its small size and simple organization, and inherited uniparentally through the maternal line (see Wallace (1995) for a review). The lack of recombination in mtDNA means that it is passed on from generation to generation unchanged, barring mutation events, and the temporal and spatial relationships between individual maternal lineages can be investigated in the absence of complex gene frequency data. In the present study, we have analysed mtDNA polymorphisms in the hypervariable control region of a sample of Black Caribs which were the subject of an earlier study based on classical genetic markers (Custodio *et al.* 1984), in an attempt to shed light on the ethnohistory of this population group.

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Figure 1. Map showing the migration routes of the Carib people.

2. MATERIALS AND METHODS

(a) *Samples*

Our sample consisted of 30 Carib people from the former colony of British Honduras, now Belize. Blood samples were collected originally for a study of blood group systems (Mourant *et al.* 1976) and stored for more than 20 years as frozen plasma or haemolysates. The individuals sampled and their four grandparents were born in the area designated as British Honduras. No direct family relationship between individuals was known to exist *a priori*. DNA was extracted from 100 µl aliquots of plasma or haemolysate using a commercial DNA/RNA isolation kit (USB Corp., Cleveland, Ohio).

(b) *PCR amplification and mtDNA sequencing*

DNA amplifications were done in a Perkin-Elmer DNA thermal cycler, in 25 µl reactions containing 0.5 units of SuperTaq DNA polymerase (HT Biotechnology, Cambridge, UK) in the buffer supplied by the manufacturer, with the addition of 200 µM of each dNTP, 20 pmol of each primer and 1 µl of DNA template.

The DNA samples were screened for the presence of the markers that define the four major haplogroups in Amerindians by PCR (polymerase chain reaction) amplification and subsequent restriction enzyme analysis, as described previously (Torroni *et al.* 1992). A DNA fragment encompassing the 9 bp deletion in the intergenic region between cytochrome oxidase II and lysil transfer RNA (COII/tRNA^{Lys}) (Wrischnick *et al.* 1987) was amplified with primers RMV1 (5' AGGGCCCGTATT-TACCCTATAG 3') and RMV2 (5' ATTTAGTTGGGG-CATTTCATG 3'), which specify a product of 133 or 124 bp for non-deleted or deleted samples, respectively. Amplifications consisted of 35 cycles of 94 °C (1 min),

57 °C (1 min) and 72 °C (1 min); the denaturation step of the first cycle was lengthened to 5 min to ensure complete denaturation of the template, and the final extension step was 10 min. PCR products were electrophoresed on 3% Nu-Sieve/1% agarose gels (FMC BioProducts) and the bands visualized under ultraviolet light after staining with ethidium bromide.

A fragment of 277 bp in the hypervariable control region of mtDNA was amplified using primers L16067 (5' CTCACCCATCAACAACCGCTAT 3') and H16344 (5' GGACGAGAAGGGATTGACTG 3'). PCR conditions were as above, but with the annealing step at 64 °C. Following amplification, the PCR products were electrophoresed on 1% agarose gels to separate non-specific amplification products and excess primers. Bands of the desired size were excised from the agarose gel and the products purified using the QIA-quick Gel Extraction Kit (Qiagen Ltd., Dorking, Surrey). PCR products were sequenced in both directions using the amplification primers as sequencing primers. Sequencing reactions were carried out in a thermal cycler with 30 ng of DNA template and 3.2 pmol primer, using the ABI PRISM Dye Terminator Cycle Sequencing Kit and AmpliTaq DNA polymerase, FS (Perkin-Elmer, Warrington, Cheshire). Excess dideoxy chain terminators were removed from the sequencing reactions by ethanol precipitation. Fluorescence-based DNA sequencing was done in an Applied Biosystems ABI 373A DNA Sequencer, on a 6% polyacrylamide gel. The sequences were aligned with the program SeqEd (Applied Biosystems, Warrington, Cheshire, UK).

(c) *Data analysis*

The control region sequences of the Carib individuals, encompassing the 277 nucleotides between mtDNA positions 16 067 and 16 344, were compared with the previ-

et al. 1993), Africans and Asians (Horai & Hayasaka 1990), Europeans (Sajantila *et al.* 1995; Richards *et al.* 1996), Pacific islanders (Lum *et al.* 1994) and sub-Saharan Africans (Soodyall *et al.* 1996). The Carib sequences were compared with those in a published worldwide concordance of control region substitutions. The transversions at positions 16 144 (T → G), 16 278 (C → A) and 16 342 (T → A) and the transition at position 16 190 (C → T) have not been previously reported (Miller *et al.* 1996). The Carib sequences were arranged by motifs sharing similar patterns of substitutions, some not previously observed in human populations, but others identical to published sequences of Africans, including Hazda and Yoruba (Vigilant (1990) quoted by Miller *et al.* (1996)), Mandenka (Graven *et al.* 1995) and sub-Saharan Africans (Soodyall *et al.* 1996), as well as Chinese (Vigilant 1990) (table 2).

The genetic affinities between the Carib people and other Amerindian and African groups were investigated by phylogenetic mismatch and intermatch analysis. A neighbour-joining tree based on 17 Carib, 78 Amerindians and 47 African mtDNA types was constructed (figure 2). Branches with bootstrap values below 60 were collapsed. The deepest nodes of the tree gave rise to solely African lineages, the most ancient belonging to !Kung, East Pygmy, West Pygmy and Carib. The second node gave rise to !Kung mtDNA types exclusively. These observations agree with previously published phylogenetic analyses which placed the !Kung in the deepest branches of a human mtDNA maximum parsimony tree (Vigilant *et al.* 1989). Most of the Amerindian sequences clustered in the shallowest nodes of the tree, although a small number was found in the oldest branches. The Carib sequences were scattered throughout the tree, many clustering with African sequences, which suggests that these people have undergone extensive genetic admixture.

Mismatch distributions, or pairwise nucleotide differences within a population, were calculated for the Caribs, Amerindians and five African groups (East Pygmy, West Pygmy, Herero, !Kung and Yoruba) (table 3). The intermatch distributions, or pairwise comparisons between populations, were calculated for all the above population groups (Nei & Li 1979). The mismatch pattern of the Carib sequences indicates that genetic admixture occurred in these people. The corrected intermatch distance indicates a close relationship between the Caribs and the Yorubas (graphs not shown).

4. DISCUSSION

We analysed mtDNA polymorphisms in a sample of 28 Carib people from Belize, thought to be the descendants of the original Carib islanders with a genetic contribution from African slaves. Previous studies suggest that Amerindians belong to four major mtDNA haplotypes that can be traced back to Asia (Wallace *et al.* 1985; Schurr *et al.* 1990; Torroni

Table 2. *Mitochondrial DNA haplotypes in Caribs arranged by motifs*
Nucleotide positions shown in bold are the substitutions shared by individuals belonging to the motifs denoted as I, II, III, IV and V, respectively.

motif	mitochondrial type																
I	1	16148[T]	16172[C]	16187[T]	16188[G]	16189[C]	16230[G]	16270[T]	16278[T]	16293[G]	16311[C]	16320[T]	CAR[3]	SUB[18]			
	2			16187[T]		16189[C]		16270[T]	16278[T]	16293[G]	16311[C]		CAR[4]				
	3	16141[G]		16166[G]		16189[C]	16230[G]	16270[T]	16278[T]	16293[G]	16311[C]	16330[C]	CAR[1]	16342[A]			
	4	16138[G]		16187[T]		16189[C]	16230[G]	16270[T]	16278[T]	16293[G]	16311[C]		CAR[2]				
	5	16126[C]		16187[T]		16189[C]	16230[G]	16270[T]	16278[T]	16293[G]	16311[C]		CAR[1]	YOR[2]	CHIN[1]	MAN[14]	
II	6						16223[T]	16278[T]	16278[T]	16294[T]	16309[G]		CAR[3]	HDZ[2]			
	7					16189[C]	16192[T]	16278[A]	16278[T]	16294[T]	16309[G]		CAR[1]				
	8						16223[T]	16278[T]	16278[T]	16294[T]	16309[G]		CAR[1]				
	9					16189[C]	16192[T]	16278[T]	16278[T]	16294[T]	16309[G]		CAR[1]				
III	10	16138[G]	16141[G]	16166[G]	16185[T]		16223[T]				16311[C]	16327[T]	CAR[1]				
	11			16166[G]	16185[T]		16223[T]				16311[C]	16327[T]	CAR[1]				
	12			16166[G]	16187[T]		16223[T]				16311[C]	16327[T]	CAR[1]				
IV	13					16189[C]	16190[T]	16264[G]	16292[T]	16294[T]	16311[C]		CAR[1]				
	14						16209[C]		16292[T]	16294[T]	16311[C]		CAR[2]				
V	15			16166[G]			16223[T]				16309[G]		CAR[2]				
	16						16223[T]				16309[G]		CAR[2]				
	17		16172[C]			16189[C]	16217[C]	16223[T]	16260[T]				CAR[1]				

Abbreviations: CAR, Carib (this study); SUB, sub-Saharan Africa (Soodyall *et al.* 1996); MAN, Mandenka (Graven *et al.* 1995); CHIN, Chinese, HDZ, Hazda, YOR, Yoruba (Vigilant (1990) quoted by Miller *et al.* (1996)). The number of individuals in each population is shown in brackets.

Table 3. Genetic distances between mtDNA control region sequences of Caribs, Amerindians and Africans (East Pygmy, West Pygmy, Herero, !Kung, Yoruba)

(Bold type shows the mean pairwise difference within populations. Values above the diagonal are mean pairwise differences between populations and below the diagonal are the corrected intermatch distances (distance between two populations minus the mean of the mismatch distances within each population (Nei & Li 1979)).)

	Carib <i>n</i> = 28	Amerindian <i>n</i> = 101	E. Pygmy <i>n</i> = 15	Herero <i>n</i> = 8	!Kung <i>n</i> = 30	W. Pygmy <i>n</i> = 14	Yoruban <i>n</i> = 41
Carib	10.91	9.53	10.01	7.79	10.60	11.86	8.35
Amerindian	1.81	4.54	9.15	5.75	9.40	11.41	6.21
E. Pygmy	1.76	5.08	5.58	6.01	8.08	6.63	7.71
Herero	0.26	1.41	1.42	4.14	7.10	7.67	4.91
!Kung	3.13	5.12	3.28	3.02	4.02	8.96	8.34
W. Pygmy	3.09	5.82	2.52	2.23	3.63	6.63	9.48
Yoruban	0.11	1.16	2.14	0.60	3.55	3.38	5.56

et al. 1992; Horai *et al.* 1993), and additional haplotypes have also been reported (Ward *et al.* 1991; Bailliet *et al.* 1994; Bianchi *et al.* 1995). One of the four major haplogroups, haplogroup B, is defined by the presence of the 9 bp deletion in the COII/tRNA^{Lys} intergenic region (Cann & Wilson 1983) observed frequently in people of Asian origin. Two of the Caribs carried the deletion, but phylogenetic analysis of their mtDNA control region sequences revealed that whereas in one individual the mutation was of Asian origin, in the second individual it was probably the result of an independent mutation in an African mtDNA lineage. The deletion has been observed in African populations (Vigilant *et al.* 1990; Merriwether *et al.* 1994; Chen *et al.* 1995; Soodyall *et al.* 1996) and it is likely that it arose independently in sub-Saharan Africa and Asia, and probably more than once in Africa (Redd *et al.* 1995; Soodyall *et al.* 1996).

In our sample, 13 out of 28 individuals from whom sequence information could be obtained had a nucleotide transition at position 16278. This substitution is abundant in some African populations, such as the Herero (89%) of Southern Africa, Biaka Pygmies (71%), Hazda (71%) and Mbuti Pygmy (70%) of Central Africa, and the Yoruba (64%) of West Africa (Vigilant (1990) quoted by Miller *et al.* (1996)). In addition, it is found at a frequency of 85% in the Mandenkalu of Senegal, also West Africa (Graven *et al.* 1995).

The 16278 substitution has also been observed at lower frequencies in Amerindian groups such as the Nu-Chah-Nulth (16%) (Ward *et al.* 1991), and was used to define a fifth major Amerindian haplotype, haplotype V (Bailliet *et al.* 1994). However, in the case of our Carib sample, it is likely that the sequences with the substitution reflect African admixture, as several of the control region motifs associated with the substitution (see table 2) were identical to motifs observed previously in the West African Yoruba and Hazda (Vigilant (1990) quoted by Miller *et al.* (1996)) and Mandenka (Graven *et al.* 1995). Although the 16278 substitution is found in many different populations, it is particularly abundant in

Africa and it is not inconceivable that its presence in Amerindians is the consequence of African admixture and that Amerindian haplotype V does not exist.

Our analysis of mean nucleotide differences within and between populations showed that the sequences of the Carib people were extremely diverse, with a mean of 10.91 nucleotide differences between individual sequences. This was about double the value observed for the same segment of the first hypervariable mtDNA region in Amerindian or African populations. Previous studies indicate that Africans exhibit the highest degree of mtDNA diversity (Johnson *et al.* 1983; Cann *et al.* 1987; Scozzari *et al.* 1988; Chen *et al.* 1995), and our result suggests that the high variability in our sample is due to the presence of both African and Amerindian mtDNA types in the Black Caribs of Belize. The measurement of genetic distance, or corrected intermatch distances, showed that the Caribs were more closely related to the Yoruba (distance 0.11) than to the Amerindians (distance 1.81) and other African groups examined.

Our results agree with the historical reports on the derivation of these people from Island Caribs and African slaves in the island colonies of the Lesser Antilles. However, the specific provenance of the African progenitors of the Black Carib population is a matter of conjecture. Analysis of intrapopulation sequence divergence in this study indicates a closer relationship of the Caribs with West Africans than with the Southern African !Kung or East African Pygmies. The slaves brought to the Americas from West Africa before emancipation in the 19th century were mostly from the area between Senegal and Angola. The Yoruba were the African group with the strongest impact on the culture of the Caribbean and Central and South America, in particular Cuba and Brazil (Bascom 1969). However, the origin of the Yoruba people is unclear. Archaeological evidence suggests similarities with the material culture of Northern Nigeria (Bascom 1969), while linguistic evidence indicates that Yoruban belongs to the Niger-Congo branch of the great Congo-Cardofanian language family which includes most West African languages (Greenberg 1963).

Figure 2. Neighbour-joining tree of mtDNA control region sequences, based on the PHYLIP computer package (Felsenstein 1993) and using the chimpanzee sequence as an outgroup. The tree consists of 17 Carib, 78 Amerindian and 47 African (East Pygmy, Herero, !Kung, West Pygmy and Yoruban) mtDNA types. Caribs marked with a star carry the 9 bp deletion.

Human DNA samples derived from blood and tissue archives are a valuable resource for the study of

genetic variation. A mass of genetic data based on classical genetic markers and more recently generated molecular data constitute a wealth of genetic information on human populations which can help shed light on past events. Our analysis of mtDNA control region sequences in an archival sample of Black Caribs, or Garifuna, of Belize, confirms historical reports that suggest high levels of admixture of the original Island Caribs with West African people, and show specific genetic affinities with the Yoruba people. The presence of an identical mtDNA substitution at position 16 278 in Africans and haplotype V Amerindians suggests that this haplotype could be the result of African admixture, as in the case of our Carib sample, and indicates the need for caution in interpreting DNA data in the absence of independent historical information about a population group.

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REFERENCES

- Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, H., Smith, A. J. H., Staden, R. & Young, I. G. 1981 Sequence and organisation of the human mitochondrial genome. *Nature* **290**, 457–465.
- 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.
- Bascom, W. 1969 *The Yoruba of Southwestern Nigeria*. New York: Holt, Rinehart and Winston.
- Bianchi, N. O., Bailliet, G. & Bravi, C. 1995 Peopling of the Americas as inferred through the analysis of mitochondrial DNA. *Brazil. J. Genet.* **18**, 661–668.
- Brown, G. G. & Simpson, M. V. 1982 Novel features of animal mtDNA evolution as shown by sequences of two rat cytochrome oxidase subunit II genes. *Proc. Natn. Acad. Sci. USA* **79**, 3246–3250.
- Cann, R. L. & Wilson, A. C. 1983 Length mutations in human mitochondrial DNA. *Genetics* **104**, 699–711.
- Cann, R. L., Stoneking, M. & Wilson, A. C. 1987 Mitochondrial DNA and human evolution. *Nature* **325**, 31–36.
- Chen, Y. S., Torroni, A., Excoffier, L., Santachiara-Benerecetti, A. S. & Wallace, D. C. 1995 Analysis of mtDNA variation in African populations reveals the most ancient of all human continent-specific haplogroups. *Am. J. Hum. Genet.* **57**, 133–149.
- Crawford, M. 1984 Problems and hypotheses: an introduction. In *Current development in anthropological genetics*. Vol. 3. *Black Caribs: a case study in biocultural adaptation* (ed. M. H. Crawford), pp. 1–9. New York: Plenum.
- Custodio, R., Huntsman, R. M., Newton, D., Tills, D., Weymes, H., Warlow, A., Kopec, A. C. & Lord, J. M. 1984 Blood groups, haemoglobin, and plasma protein polymorphisms in Black Carib populations. In *Current development in anthropological genetics*. Vol. 3.

- Black Caribs: a case study in biocultural adaptation* (ed. M. H. Crawford), pp. 289–301. New York: Plenum.
- Davidson, W. V. 1984 The Garifuna in Central America. In *Current development in anthropological genetics*. Vol. 3. *Black Caribs: a case study in biocultural adaptation* (ed. M. H. Crawford), pp. 13–35. New York: Plenum.
- Devor, E., Crawford, M. H. & Bach-Enciso, V. 1984 Genetic population structure of the Black Caribs and Creoles. In *Current development in anthropological genetics*. Vol. 3. *Black Caribs: a case study in biocultural adaptation* (ed. M. H. Crawford), pp. 365–379. New York: Plenum.
- Felsenstein, J. 1993 *PHYLIP* (Phylogeny inference package), v. 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.
- Ginther, C., Corach, D., Penacino, G. A., Rey, J. A., Carnese, F. R., 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 genes. In *DNA fingerprinting: state of the science* (ed. S. D. J. Pena, R. Chakraborty, J. T. Epplen & A. J. Jeffreys), pp. 211–219. Basel: Birkhäuser.
- Graven, L., Passarino, G., Semino, O., Boursot, P., Santachiara-Benerecetti, S., Langaney, A. & Excoffier, L. 1995 Evolutionary correlation between control region sequence and restriction polymorphisms in the mitochondrial genome of a large Senegalese Mandenka sample. *Molec. Biol. Evol.* **12**, 334–345.
- Greenberg, J. H. 1963 The languages of Africa. *Int. J. Am. Linguistics* **XXIX**, 1.
- Harvey, R. G., Godber, M. J., Kopec, A. C., Mourant, A. E. & Tills, D. 1967 Frequency of genetic traits in the Caribs of Dominica. *Hum. Biol.* **41**, 342–364.
- Horai, S. & Hayasaka, K. 1990 Intraspecific nucleotide sequence differences in the major noncoding region of human mitochondrial DNA. *Am. J. Hum. Genet.* **46**, 828–842.
- Horai, S., Hayasaka, K., Kondo, R., Tsugane, K. T. N. 1995 Recent African origin of modern humans revealed by completed sequences of hominid mitochondrial DNAs. *Proc. Natn. Acad. Sci. USA* **92**, 532–536.
- Horai, S., Kondo, R., Nakagana-Hattori, Y., Hayashi, S., Sonoda, S. & Tajima, K. 1993 Peopling of the Americas, founded by four major lineages of mitochondrial DNA. *Molec. Biol. Evol.* **10**, 23–47.
- Johnson, M. J., Wallace, D. C., Ferris, S. D., Ratazzi, M. C. & Cavalli-Sforza, L. L. 1983 Radiation of human mitochondrial DNA types analysed by restriction endonuclease cleavage patterns. *J. Molec. Evol.* **19**, 255–271.
- Kolman, C. J., Bermingham, E., Cooke, R., Ward, R. H., Arias, T. D. Guionneau-Sinclair, F. 1995 Reduced mtDNA diversity in the Ngobe Amerindians of Panama. *Genetics* **140**, 275–283.
- Lum, J. K., Rickards, O., Ching, C. & Cann, R. L. 1994 Polynesian mitochondrial DNAs reveal three deep maternal lineage clusters. *Hum. Biol.* **66**, 567–590.
- Matson, G. A & Swanson, J. 1964 Distribution of hereditary blood antigens among Indians in Middle America. *Am. J. Phys. Anthropol.* **22**, 271–284.
- Merriwether, D. A., Huston, S. L., Bunker, C. A. & Ferrell, R. E. 1994 Origins and dispersal of the mitochondrial DNA region V 9 bp deletion and the insertion in Nigeria and the Ivory Coast. *Am. J. Hum. Genet.* **55**, A128.
- Miller, K. W. P., Dawson, J. & Hagelberg, E. 1996 A concordance of site mutations for the first and second hypervariable segments of the human mtDNA control region. *Int. J. Legal Med.* **106**, 107–113.
- Monsalve, M. V., Cardenas, F., Guhl, F., Delaney, A. D. & Devine, D. V. 1996 Phylogenetic analysis of mtDNA in South American mummies. *Ann. Hum. Genet.* **60**, 293–303.
- Mourant, A. E., Kopec, A. C. & Domaniewska-Sobczak, K. 1976 *The distribution of the human blood groups and other polymorphisms*, pp. 143–871. Oxford University Press.
- Nei, M. & Li, W. H. 1979 Mathematical models for studying genetic variation in terms of restriction endonucleases. *Proc. Natn. Acad. Sci. USA* **76**, 5269–5273.
- Redd, A. J., Takezaki, N., Sherry, S. T., Sofro, A. S. M., Stoneking, M. & McGarvey, S. T. 1995 Evolutionary History of the COII/tRNA^{Lys} intergenic 9 base pair deletion in human mitochondrial DNAs from the Pacific. *Molec. Biol. Evol.* **12**, 604–615.
- Richards, M., Corte-Real, H., Forster, P., Macaulay, V., Wilkinson-Herbots, H., Demaine, A., Papiha, S., Hedges, R., Bandelt, H.-J. & Sykes, B. 1996 Paleolithic and Neolithic lineages in the European mitochondrial gene pool. *Am. J. Hum. Genet.* **59**, 185–203.
- Rogers, A. & Harpending, H. 1992 Population growth makes waves in the distribution of pairwise differences. *Molec. Biol. Evol.* **9**, 552–569.
- Rouse, I. 1986 *Migrations in prehistory. Inferring population movement from cultural remains*. Yale University Press.
- Saitou, N. & Nei, M. 1987 The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molec. Biol. Evol.* **4**, 406–425.
- Sajantila, A., Lahermo, P., Anttinen, T., Lukka, M., Sistonen, P., Savontaus, M.-L., Aula, P., Beckman, L., Tranebjaerg, L., Gedde-Dahl, T., Issel-Tarver, L., DiRienzo, A. & Paabo, S. 1995 Genes and languages in Europe: an analysis of mitochondrial lineages. *Genome Res.* **5**, 42–52.
- Schurr, T. G., Ballinger, S. W., Gan, Y., Hodge, J. A., Merriwether, D. A., Lawrence, D. N., Knowler, W. C., Weiss, K. M. & Wallace, D. C. 1990 Amerindian mitochondrial DNAs have rare Asian mutations at high frequencies, suggesting they derived from four primary maternal lineages. *Am. J. Hum. Genet.* **46**, 613–623.
- Scozzari, R., Torroni, A., Semino, O., Cruciani, F., Spedini, G. & Santachiara-Benerecetti, A. S. 1988 Genetic studies on the Senegal population. I. Mitochondrial DNA polymorphisms. *Am. J. Hum. Genet.* **43**, 534–544.
- Sherry, S. T. 1994 *IWAVE: an interpopulation sequence comparison module*, version 1.0. The Pennsylvania State University, University Park, PA 16802, USA.
- Shields, G. F., Schmiechen, A. M., Frazier, B. L., Redd, A., Voevoda, M. I., Redd, J. & Ward, R. H. 1993 mtDNA sequences suggest a recent evolutionary divergence for Beringian and Northern North American populations. *Am. J. Hum. Genet.* **53**, 549–562.
- Soodyall, H., Vigilant, L., Hill, A. D., Stoneking, M. & Jenkins, T. 1996 mtDNA control region sequence variation suggests multiple independent origins of an 'Asian-specific' 9 bp deletion in sub-Saharan Africans. *Am. J. Hum. Genet.* **58**, 609–616.
- Stone, A. C. & Stoneking, M. 1993 Ancient DNA from a Pre-Columbian Amerindian population. *Am. J. Phys. Anthropol.* **92**, 463–471.
- Torroni, A., Schurr, T. G., Yang, C., Szathmary, E. J. E., Williams, R. C., Schandfield, M. S., Troup, G. A., Knowler, W. C., Lawrence, D. N., Weiss, K. M. & Wal-

- lace, D. C. 1992 Native American mitochondrial DNA analysis indicates that the Amerind and the Na-Dene 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 Asian affinities and continental radiation of the four founding Native American mtDNA. *Am. J. Hum. Genet.* **53**, 563–590.
- Vigilant, L. 1990 Control region sequences from African populations and the evolution of human mitochondrial DNA. Ph.D. thesis, University of California at Berkeley.
- Vigilant, L., Pennington, R., Harpending, H. & Kocher, T. D. 1989 Mitochondrial DNA sequences in a single hair from a southern African population. *Proc. Natn. Acad. Sci. USA* **86**, 9350–9354.
- Wallace, D. C. 1995 Mitochondrial DNA variation in human evolution, degenerative disease, and aging. *Am. J. Human. Genet.* **57**, 201–233.
- Wallace, D. C., Garrison K. & Knowler, W. C. 1985 Dramatic founder effects in Amerindian mitochondrial DNAs. *Am. J. Phys. Anthropol.* **68**, 149–155.
- Ward, R. H., Frazier, B. L., Dew-Jager, K. & Paabo, S. 1991 Extensive mitochondrial diversity within a single Amerindian tribe. *Proc. Natn. Acad. Sci. USA* **88**, 8720–8724.
- Weymes, H. & Gershowitz, H. 1984 Genetic structure of the Garifuna population in Belize. In *Current development in anthropological genetics*. Vol. 3. *Black Caribs: a case study in biocultural adaptation* (ed. M. H. Crawford), pp. 365–379. New York: Plenum.
- Wrischnick, L. A., Higuchi, R. G., Stoneking, M., Erlich, H. A., Arheim, N. & Wilson, A. C. 1987 Length mutations in human mitochondrial DNA: direct sequencing of enzymatically amplified DNA. *Nucleic Acids Res.* **15**, 529–542.

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