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Evidence for mitochondrial DNA recombination in a human population of island Melanesia

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Mitochondrial DNA (mtDNA) analysis has proved useful in studies of recent human evolution and the genetic affinities of human groups of different geographical regions. As part of an extensive survey of mtDNA diversity in present-day Pacific populations, we obtained sequence information of the hypervariable mtDNA control region of 452 individuals from various localities in the western Pacific. The mtDNA types fell into three major groups which reflect the settlement history of the area. Interestingly, we detected an extremely rare point mutation at high frequency in the small island of Nguna in the Melanesian archipelago of Vanuatu. Phylogenetic analysis of the mtDNA data indicated that the mutation was present in individuals of separate mtDNA lineages. We propose that the multiple occurrence of a rare mutation event in one isolated locality is highly improbable, and that recombination between different mtDNA types is a more likely explanation for our observation. If correct, this conclusion has important implications for the use of mtDNA in phylogenetic and evolutionary studies.

Keywords: human evolution; Melanesia; mitochondrial DNA; Polynesia; population genetics; recombination

1. INTRODUCTION

Mitochondrial DNA (mtDNA) variation has been used extensively for the study of human evolutionary history because it has a number of useful features, including simple organization, maternal inheritance and a relatively fast rate of evolution (Attardi 1985; Brown *et al.* 1979; Giles *et al.* 1980). The presumed uniparental and clonal nature of mtDNA inheritance means that the variation between individuals is assumed to be the result of mutations accumulated in maternal lineages since divergence from a common ancestor. Studies of mtDNA diversity in present-day human populations have contributed considerably to the debate on the tempo and mode of evolution of anatomically modern humans (Cann *et al.* 1987; Vigilant *et al.* 1991; Templeton 1993). Although the debate is not resolved, mtDNA data indicate that there is relatively little variation in the mtDNA of living humans, suggesting that we descend from a recent (female) common ancestor who probably originated in Africa. Analysis of mtDNA from a bone fragment of the Neanderthal type specimen, aged 30 000 to 100 000 years, showed that the Neanderthal mtDNA fell outside the range of variation for living humans, which supports the general view that archaic humans did not hybridize with anatomically modern humans (Krings *et al.* 1997).

In addition to studies addressing general questions in human evolution, mtDNA is widely used for analysis of the genetic affinities of human populations of different geographic origins. In one of the earliest applications of mtDNA to the study of human migrations, present-day native inhabitants of the New World were shown to belong to four principal mtDNA haplogroups (A, B, C and D) with an ultimate origin in Asia (Wallace & Torroni 1992; Torroni *et al.* 1993*a*,*b*, 1994). The classification of mtDNA types into different haplogroups on the basis of restriction fragment polymorphisms or base substitutions in the mtDNA control region has been extended to populations of other continents, most notably Europe (Torroni *et al.* 1996). This approach has provided a useful nomenclature for mtDNA studies of populations of different geographical regions, and emphasizes the need for accurate phylogenetic identification of the founding sequences of the different mtDNA haplogroups in order to understand migration events (Bandelt *et al.* 1995; Forster *et al.* 1996; Torroni *et al.* 1998).

A number of mtDNA studies have been carried out on Pacific populations to investigate the colonization of Polynesia, the last large area of the world to have been settled by humans (Hertzberg *et al.* 1989; Hagelberg & Clegg 1993; Hagelberg *et al.* 1994; Lum *et al.* 1994; Melton *et al.*

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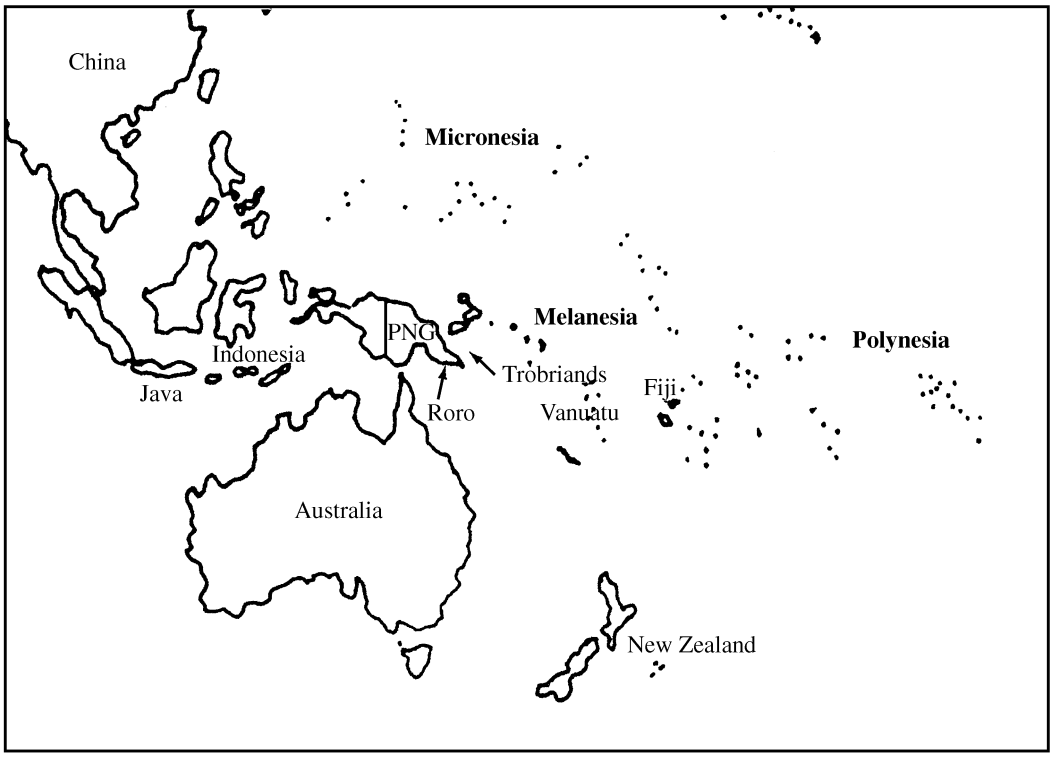

Figure 1. Map of the western Pacific, showing the places of origin of the human DNA samples used in this study. The populations included are PNG highlanders (37); Roro people of the south coast of PNG (22); Trobriand Islanders (56); Indonesians from Java (10); and Vanuatu Islanders (327). Numbers in parentheses refer to the number of individuals from each location.

1995; Redd *et al*. 1995; Sykes *et al*. 1995). The peoples of Polynesia are genetically homogeneous, suggesting that they expanded recently from a small founding population. Polynesians have an extremely high frequency (100% in some islands) of a well-known marker, a deletion of nine bases (CCCCCTCTA) from a tandem repeat in a small non-coding mtDNA region between the genes for cytochrome oxidase II and lysyl transfer RNA (Wrischnick *et al*. 1987; Hertzberg *et al*. 1989). This mutation is also an identifying marker for Amerind haplogroup B, and reflects the common Asian origin of the Polynesian and Amerind people. Polynesians exhibit several specific base substitutions in the non-coding mtDNA control region, at positions 16189, 16217, 16247 and 16261 of the human mtDNA reference sequence (Anderson *et al*. 1981). This Polynesian mtDNA motif, together with a variant lacking the substitution at 16247, accounts for approximately 95% of the mtDNA types in Polynesia. The low mtDNA variability in Polynesia supports the view that the area was settled very recently and rapidly by a small number of settlers from island south-east Asia (Diamond 1988).

The people of the western Pacific, including Papua New Guinea (PNG) and island Melanesia, exhibit higher levels of genetic diversity because this part of the Pacific has a much older settlement history than Polynesia (for a review, see Bellwood 1989). It is thought that modern humans might have reached PNG about 60 000 years ago, and archaic humans possibly much earlier, as there is evidence that *Homo erectus* might have crossed the open sea to reach as far as the island of Timor in eastern Indonesia (Morwood *et al*. 1998). There are still relatively few mtDNA data on populations of the western Pacific (Stoneking *et al*. 1990; Melton *et al*. 1995; Redd *et al*. 1995), and these focus mainly on individuals with the nine base pair (bp) deletion to reconstruct the path of the proto-Polynesian migrations.

To investigate the genetic affinities of the peoples of the western Pacific, we generated sequence information from the mtDNA hypervariable region of 452 individuals from various localities, namely the highlands of PNG, south coast of PNG (Roro people), Trobriand Islands, the archipelago of Vanuatu, and Java in Indonesia (figure 1). Our results show three distinct mtDNA lineages in the Pacific, present at different frequencies in different locations. However, our data contradict the view that mtDNA is inherited in a strictly maternal fashion, without recombination. We observed a rare mtDNA mutation in people of one island, who belong to the three major Pacific groups. This observation suggests some type of horizontal transmission of the mtDNA region containing this mutation might have occurred in the ancestors of the present inhabitants. If our results are correct, hybridization of human mtDNA types is a relatively frequent event. This has important implications for the reconstruction of mtDNA lineages in human evolutionary studies.

2. MATERIALS AND METHODS

(a) *DNA samples*

We analysed DNA extracted from blood samples taken, with full informed consent, from 452 people from the following locations: PNG highlands (37); Roro people of the south coast of PNG (22); Trobriand Islands, New Guinea (56); Java, Indonesia (10); and the Vanuatu archipelago (327), including the islands of Futuna, Tanna, Aniwa, Malakula, Maewo, Maskelyne, Nguna,

Lamen and Paama. Numbers in parentheses refer to the number of individuals from each location.

(b) *Mitochondrial DNA analysis*

The intergenic mtDNA 9-bp deletion was detected by PCR analysis as described previously (Wrischnick *et al.* 1987; Hagelberg & Clegg 1991). For mtDNA sequence analysis, DNA samples were PCR amplified in 20 µl reaction volumes, using primers HVR1 (5′-CTAACCTGAATCGGAGGACAAC-3′) and HVR4 (5′-GCATACCGCCAAAAGATAAAA-3′) specific for a 1239 bp fragment of human mtDNA, under the conditions described previously (Hagelberg & Clegg 1991). The PCR fragments were subjected to agarose gel electrophoresis and visualized under UV light. A 1 µl aliquot of each PCR product was re-amplified with internal primers and subjected to automated DNA sequencing on a LI-COR 4200 sequencing system (MWG-Biotech, Milton Keynes, UK). We obtained an approximately 360 bp sequence between nucleotide positions 16 041 and 16 400 of the mtDNA reference sequence for most individuals. Samples exhibiting a T to C transition at position 16 189 had a homopolymeric tract of 12 C residues that prevented sequencing past this point. In these cases, two partial single sequences that joined at the C tract were obtained. The DNA sequences were aligned manually.

(c) *Phylogenetic analysis of the DNA sequences*

Pairwise distances between the 175 mtDNA types were calculated using the substitution model of Tamura & Nei (1993), which makes allowances for unequal nucleotide frequencies and transition–transversion bias. Rate heterogeneity among sites was modelled with a gamma distribution with shape parameter $\alpha = 0.17$ (Yang & Kumar 1996). This substitution model is implemented in the MEGA program (Kumar *et al.* 1993). Table 1 was derived from intra- and inter-lineage summary statistics of these distances.

Phylogenetic analysis was performed using the neighbour-joining method (Saitou & Nei 1987), as implemented in the NEIGHBOR program of the PHYLIP package (Felsenstein 1993), applied to the pairwise distances described above. The resulting phylogenetic tree is shown in figure 2. Computational constraints meant that bootstrap analysis could not include all 175 haplotypes. Therefore, an initial bootstrap analysis of a subsample of individuals from each haplogroup (20 in total) was performed using the MEGA program to assess the robustness of the division into groups I to III. A second, more rigorous, bootstrap analysis was carried out after calculating pairwise distances between haplotypes using a model based on a Markov process of nucleotide substitution which allows for transition–transversion bias and unequal nucleotide frequencies, as implemented in version 3.57 of the PHYLIP package (Felsenstein 1995). Three data subsets, the first containing all haplotypes of groups I and II, the second all haplotypes of I and III, and the third all haplotypes of II and III, were used in this bootstrap analysis to test the separation of the haplotypes into three independent mtDNA groups.

3. RESULTS

In most cases we obtained a 360 bp double-stranded mtDNA sequence between nucleotide positions 16 041 and 16 400 of the reference sequence (Anderson *et al.* 1981), with a total of 115 variable sites and 245 invariable sites in our data set. The 452 individual DNA sequences fell into 175 different mtDNA types. The 175 mtDNA types could be divided into three major haplogroups or lineages on the basis of a number of characteristic substitutions in each lineage. The first lineage, which we have called lineage or haplogroup I, had the previously described Polynesian motif (Hagelberg & Clegg 1993; Lum *et al.* 1994), characterized by transitions at positions 16 189 (T to C), 16 217 (T to C), 16 247 (A to G) and 16 261 (C to T), and variants thereof. In addition, sequences with this motif have two A to C transversions at positions 16 182 and 16 183, effectively lengthening the homopolymeric C tract in this mtDNA segment. Twenty-five per cent of our total sample of 452 individuals belonged to this mtDNA group. The exact Polynesian motif with no additional private polymorphisms was the single most frequent mtDNA type found in the entire sample, shared by 64 individuals. It was abundant in the Trobriand Islands near the east coast of PNG, and in the Roro, a population group on the south coast of PNG. It was also present at varying frequencies in different islands of the Vanuatu archipelago.

Table 1. *Genetic distances within and between the three principal Pacific mtDNA lineages*

(Values given are mean inter- and intra-group distances (expected number of substitutions per site). The bracketed numbers are the standard deviations of the inter- and intra-group distances.)

		mtDNA lineage		
mtDNA lineage	*n*	I	II	III
I	114	0.003 (0.004)	0.040 (0.008)	0.039 (0.007)
II	217	—	0.018 (0.012)	0.038 (0.010)
III	70	—	—	0.011 (0.006)

The second major mtDNA lineage, lineage II, was the largest and most diverse (table 1), accounting for 48% of our sample. The identifying base substitutions for this group were 16 129, 16 144, 16 148, 16 223, 16 241, 16 265 (A to C transversion), 16 311, 16 343 and 16 362, although each individual haplotype only had a subset of these substitutions. The G to A transition at position 16 129 was observed in 214 out of the 217 people belonging to this group (98.6%). There was significant haplotype sharing between the PNG highlands and Vanuatu, although the highest haplotype diversity was observed in PNG (above 0.99). A variant of lineage II, identified by transitions at positions 16 129, 16 148, 16 223 and 16 362, was the second most common single mtDNA haplotype in our sample, observed in 43 individuals. It was found only in Vanuatu and probably derived from an ancestral type in PNG.

The third major mtDNA lineage, lineage III, was the smallest, present in 16% of our sample. It is characterized by transitions at position 16 176, 16 266 and 16 357 with respect to the reference sequence (Anderson *et al.* 1981). Lineage III is found in the highlands of PNG and in Vanuatu and, like the second lineage, its geographical

Table 2. *Mitochondrial DNA control region substitutions in 41 inhabitants of the island of Nguna in Vanuatu, island Melanesia*

(Nucleotide positions are as in the reference sequence (Anderson *et al*. 1981), minus 16 000. The positions reported are between 16 041 and 16 400. The blanks denote the positions identical to the reference sequence and the bold typeface indicates the individuals carrying the rare substitution in position 16 076. The number of individuals of each haplotype is shown in the right-hand column.)

	mtDNA position																													
	41	76	83	129	140	148	176	182	183	189	217	223	241	245	247	261	263	265	266	270	294	311	318	342	343	355	357	362	
	reference sequence																												
	A	C	T	G	T	C	C	A	A	T	T	C	A	C	A	C	T	A	C	C	C	T	A	T	A	C	T	T	*n*
haplogroup I		**T**						C	C	C	C				G	T													3
haplogroup II				A		T						T																C	17
	G			A		T						T																C	2
				A		T						T													T			C	4
		T		A	C	T						T																C	2
		T				T						T									T		T					C	1
		T	C	A		T							G				C	C				C		C	G				1
haplogroup III		**T**					T												T	T							C		8
		T					T												T								C		1
		T					T							T					T								C		1
other		**T**							C	C		T									T								1

distribution probably reflects ancient links between PNG and island Melanesia. The remaining 11% of individuals either did not fall clearly into any of the three major groups or had substitutions common to more than one group. The islands of Vanuatu varied considerably in the frequency of the major mtDNA lineages, ranging from islands with a strong Polynesian presence, like Futuna, to almost exclusively Melanesian islands such as Tanna.

The island of Nguna, with a population of approximately 1000, has a language with Polynesian influences. Out of 41 Nguna individuals studied, 11 different mtDNA haplotypes were obtained, one belonging to lineage I, six to lineage II and three to lineage III. One Nguna individual had a sequence that did not fall into any of the three lineages. Eighteen Nguna people, or close to half the sample, exhibited a C to T transition at position 16 076, described earlier in a northern European individual (Sajantila *et al*. 1995), but not observed elsewhere in a major database of human mtDNA control region sequences (Kogelnik *et al*. 1996) or in our own study. The 16 076 substitution was present in eight different Nguna haplotypes representing each of the three major lineages (table 2).

In order to obtain a measure of confidence in the separation of the haplotypes into three major lineages, we carried out phylogenetic analysis of the complete sequences (variable and fixed sites). The Nguna haplotypes with the 16 076 substitution are shown by the large arrows on the phylogenetic tree (figure 2). Bootstrap analysis on the Nguna haplotypes and a subset of typical haplotypes of the whole data set provided excellent support for the division into three major groups. Computational constraints meant this analysis could not be performed using all 175 mtDNA types; instead, a subset was used of ten of the most abundant types (two from lineage I, five from lineage II and three from lineage III) and the ten Nguna types falling within these lineages. This gave confidence levels of 98, 85 and 91% for the branches defining lineages I, II and III, respectively. An alternative method of bootstrap analysis using all of the haplotypes of the three different groups, but focusing in turn on just two of the three major haplogroups (the first set containing groups I and II, the second groups I and III, and the third groups II and III), also gave high confidence levels for the separation of each pair of groups, namely 79% for the separation of groups I and II, 93% for the separation of groups I and III, and 65% for the separation of groups I and III. This suggests that haplogroups II and III are closer to each other than each is to group I, and agrees with the distance values given in table 1.

4. DISCUSSION

The results of mtDNA analysis of 452 individuals of the western Pacific indicate that human populations in this area derive from three principal mtDNA lineages. Lineage I is characterized by the 9-bp deletion and exhibits relatively low nucleotide diversity. It contains all the individuals with the Polynesian motif and related mtDNA types, and has been observed in south-east Asia, the coast of New Guinea, and island Melanesia (Melton *et al*. 1995; Redd *et al*. 1995; Sykes *et al*. 1995; Hagelberg *et al*. 1998). Studies of the distribution of haplogroup I in several Asian and Pacific populations suggest that the spread of this haplogroup was associated with the demographic expansion which resulted from the development of agriculture in east and south-east Asia (Hagelberg *et al*. 1999). The

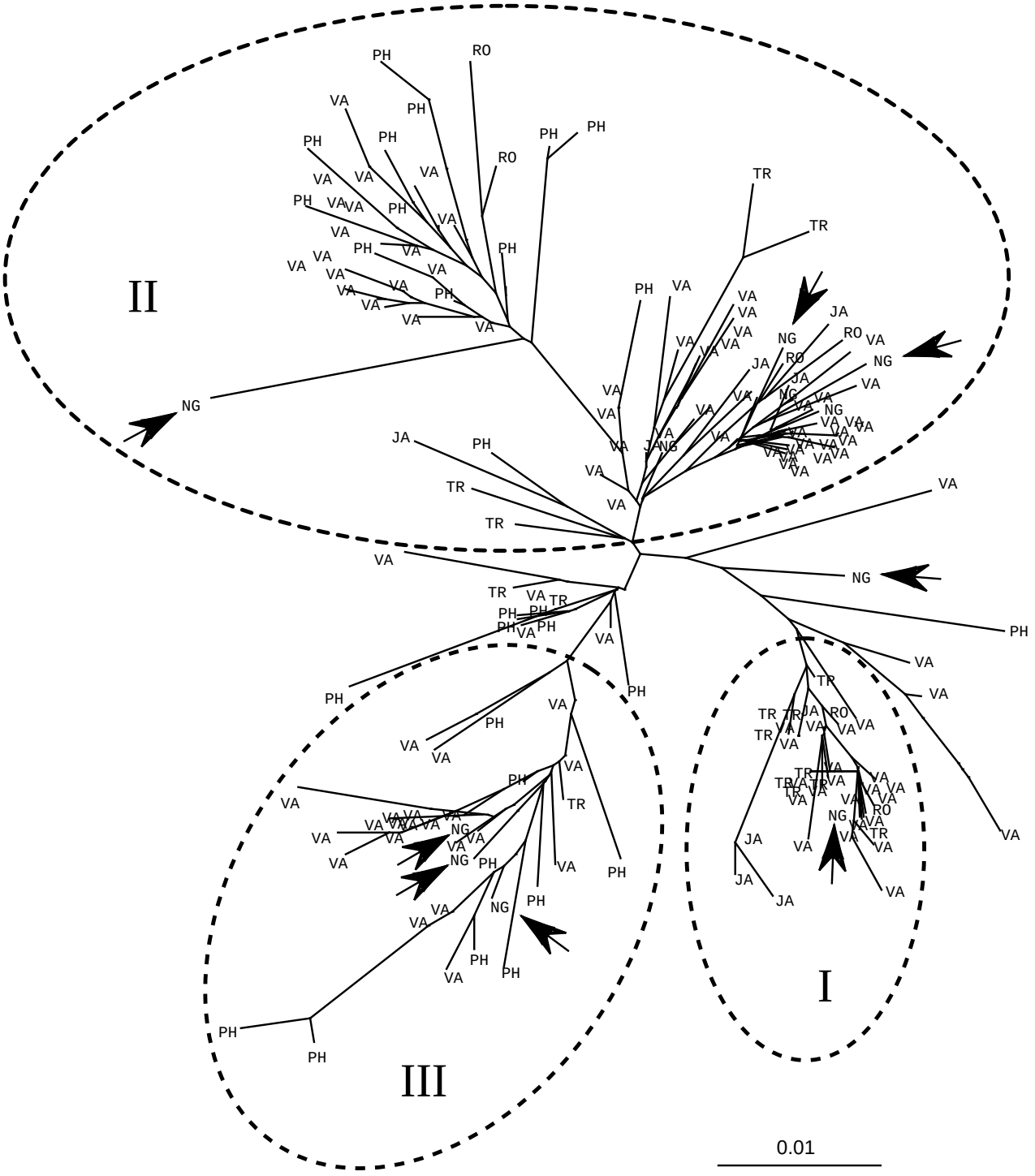

Figure 2. Neighbour-joining tree of the 175 mtDNA haplotypes, indicating the three major mtDNA lineages. Lineage II is composed of two subgroups which might have diverged in PNG. The Nguna haplotypes with the 16 076 substitution are indicated by arrows. Abbreviations are PH (PNG highlands), RO (Roro), TR (Trobriands), JA (Java), VA (Vanuatu excluding Nguna), and NG (Nguna).

relatively low amount of diversity in this haplogroup suggests that it expanded relatively recently in human history (in our data, the intergroup genetic distance in group I is only 0.3%, compared to 1.8% in group II and 1.1% in group III). Lineages II and III seem to be typical of New Guinea highlanders, and it is likely that the people belonging to this group descend from the original Pleistocene settlers of south-east Asia and New Guinea, who probably expanded east into island Melanesia well before the arrival of the proto-Polynesian migrants. Lineage II has further diversified and spread to parts of island Melanesia such as Vanuatu, where the predominant mtDNA type is characterized by the motif 16 129; 16 148; 16 223; 16 362. We might be justified in calling this set of substitutions an 'island Melanesian motif', to distinguish it from the formerly described 'Polynesian motif'.

There is strong statistical support for the existence of three separate human mtDNA lineages in our data.

Interestingly, we detected a base substitution at mtDNA position 16 076 in people belonging to eight separate mtDNA types falling into the three separate Pacific lineages in the small island of Nguna. The presence of a rare mutation in people of different lineages is puzzling. There can be several explanations for this observation: The 16 076 mutation occurred several times by chance in the island of Nguna if position 16 076 was hypervariable. Alternatively, the 16 076 substitution might have occurred just once before the split of the three lineages, although this would mean that several subsequent reverse mutations at other sites must have happened to account for the data observed. A third explanation is that the 16 076 mutation occurred once in an ancestor of the present Nguna population and was transferred to other lineages by paternal leakage of mtDNA and subsequent recombination.

Hypervariability in mtDNA is a well-recognized phenomenon (Hasegawa *et al.* 1985; Wakeley 1993). In current human mtDNA studies (for example, Forster *et al.* 1996; Richards *et al.* 1996; Watson *et al.* 1996), the multiple occurrence of base substitutions in separate branches of a phylogenetic tree is generally attributed to independent mutations at hypervariable sites of the mtDNA control region. According to this view, the 16 076 mutation would have had to have occurred independently several times in Nguna, but virtually nowhere else in world populations studied to date, and in none of the other locations of our study. This seems unlikely. It is also unlikely that the 16 076 mutation happened before the three lineages diverged, as this would mean that several back-mutations would have had to occur at this site to explain the existence of the other mtDNA types without this substitution in Nguna and throughout the western Pacific.

Although at odds with current dogma on mtDNA inheritance, paternal contribution and genetic recombination are possible explanations for the phenomenon observed in Nguna. Views on the strict maternal inheritance of mtDNA have been challenged. Paternal inheritance of mtDNA was observed in mice progeny at a frequency of one in 10 000 (Gyllensten *et al.* 1991). In contrast to popular belief, the sperm tail with its mitochondria is not excluded from the embryo during human fertilization (Ankel-Simons & Cummins 1996), although experimental evidence for an active exclusion mechanism has been presented (Shitara *et al.* 1998). If such a mechanism exists, it has the potential to fail on occasions, which would enhance the rates of paternal mtDNA leakage.

It seems likely that paternal mtDNA molecules might make a small, albeit significant, contribution to mtDNA lineages, particularly if measured over prolonged time periods of evolutionary history. Heteroplasmy arising from paternal mtDNA leakage would provide the opportunity for recombination between different mtDNA types. MtDNA recombination has long been known to contribute to genetic variability in unicellular eukaryotes, fungi and plants (Gray 1989), and has recently been demonstrated experimentally in nematodes (Lunt & Hyman 1997) and a natural fungi population (Saville *et al.* 1998). Evidence for a rare recombination event in a large matrilineal human pedigree has also been tentatively presented (Howell *et al.* 1996). However, there are many unanswered questions about the mechanism and rate of recombination. The exact position of the recombination breakpoints in our human study would need to be identified and corroborated in other populations. It is likely that a recombination hotspot exists between position 16 041 and 16 129 and it would be desirable to extend the amount of sequence information of the recombinant mtDNAs to detect the second breakpoint.

If our conclusion is correct, genetic recombination probably occurred at least twice (and possibly as many as eight times) in Nguna and is therefore a relatively frequent event in human evolution. This has important implications for evolutionary and phylogenetic studies of human mtDNA. First, recombination would perturb estimates of the time of divergence of mtDNA types, raising questions about the suggested time and mode of recent human evolution (Cann *et al.* 1987; Vigilant *et al.* 1991). Second, the occurrence of recombination would cast doubts on the labelling of some mtDNA control region nucleotide positions as mutation hotspots (Wakeley 1993). It is just as likely that the frequent occurrence of some substitutions in unrelated human lineages might be the result of recombination, notably in populations like those of Europe which have undergone high levels of genetic admixture in recent millennia (Richards *et al.* 1996).

In our data on Pacific populations, we detect little evidence for the existence of hypervariable sites that could not be explained by the occurrence of recombination events. For example, position 16 129 is considered to be one of the most hypervariable in human mtDNA, with a rate approximately eight times higher than the background mutation rate in the first hypervariable segment of the human mtDNA control region (Handt *et al.* 1998; H.-J. Bandelt, personal communication; A. von Haeseler, personal communication). However, this site is apparently fixed in our haplogroup II (the G to A transition at 16 129 is present in almost 99% of our sample of individuals of haplogroup II), although haplogroup II appears to be the oldest and exhibits the highest nucleotide diversity of the three Pacific haplogroups. In fact, the substitution at 16 129 is the principal identifier for group II. If position 16 129 was hypervariable, it is extraordinary that it has not changed in the western Pacific in the long time since the expansion of people into New Guinea and island Melanesia, a period of 30 000 to 60 000 years. How likely is it for position 16 129 to be fixed in the Pacific but hypervariable in the rest of the world? It has been argued that certain mtDNA positions can become fixed in some populations, or mutation rates might be affected by the 'context' of the DNA sequences. For example, Forster *et al.* (1996) observed that a transition at position 16 519, said to be extremely variable in Amerind and African populations (Torroni *et al.* 1993*a*,*b*; Chen *et al.* 1995), is frozen in Amerind haplogroup B, for 'some unknown reason'. The mechanism underlying these observations merits consideration.

We would like to suggest that some of the so-called hypervariable sites, for example 16 129, 16 223, 16 311 and 16 362, are in fact extremely ancient substitutions in human mtDNA, which accounts for their widespread distribution in human populations. The occurrence of these substitutions in different mtDNA lineages, used as

evidence of their hypervariability, could simply be the result of their extreme antiquity, coupled with the effects of occasional recombination events. It is interesting that the distribution of these substitutions is particularly high in African populations (Watson *et al.* 1996), as well as in New Guinea highlanders, who are thought to descend from some of the earliest anatomically modern humans to migrate out of Africa.

It is fortunate that we have been able to analyse mtDNA diversity in comparatively isolated populations like those of island Melanesia, where mtDNA lineages are still fairly distinct and where extensive admixture was limited until relatively recently by geographical barriers. Recombination events might go unnoticed in highly admixed populations because they would appear to be the result of parallel mutations in different human lineages. Likewise, recombination events might proceed undetected in homogeneous populations such as the Polynesians, the majority of whom belong to the same mtDNA haplogroup. Interestingly, few authors sequence the portion left of position 16 189 in Polynesians because it is thought to lack informative sites (Sykes *et al.* 1995). However, if a recombination hotspot existed in the region around 16 076, this is the segment which would exhibit evidence of a different mtDNA type, donated by European males. The most likely place to detect possible recombination events would be in locations whose populations had been relatively isolated but in recent times had suffered an influx of people of a very different mtDNA type. Our observations fit well with the recent suggestion that low levels of mitochondrial transmission between parents, which provide the opportunity for mtDNA heteroplasmy and recombination, might increase when closely related species or long-separated subpopulations come into direct contact (Saville *et al.* 1998). This is exactly the situation in Nguna and other Vanuatu islands, which were probably settled originally by Melanesians during the Lapita expansion (Bellwood 1989) but were invaded by Polynesians in the last few hundred years (Spriggs 1997). The comparative isolation of Nguna would mean that the hybrid mtDNA types caused by recombination were not lost from the island.

One of the worldwide effects of recombination would be to blur the differences between human mtDNA lineages, as recombination would create hybrid haplotypes and eradicate ancient haplotypes. This would make it harder to reconstruct the ancestral relationships of human populations. If paternal mtDNA leakage and subsequent recombination or some analogous gene conversion event are significant in human evolution, it will be necessary to revise the conclusions of many current studies based on mtDNA, including views on the relationship between Neanderthals and modern humans. The occurrence of a mtDNA type which differs at 27 positions from an average modern human mtDNA sequence would not be sufficient to conclude that a single Neanderthal individual was of a species that did not interbreed with anatomically modern humans (Krings *et al.* 1997), as a lineage of such antiquity could easily be lost in the intervening 30 000 or more years since the individual lived. Despite these caveats, mtDNA has been shown to be an extremely useful genetic marker for population studies, and it will undoubtedly continue to offer valuable insights into human evolutionary history, provided all the information contained in the data can be taken into account.

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