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An Asian-specific 9-bp Deletion of Mitochondrial DNA Is Frequently Found in Polynesians

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Summary

One hundred fifty Polynesians from five different island groups (Samoans, Maoris, Niueans, Cook Islanders, and Tongans) were surveyed for the presence of an Asian-specific length mutation of mitochondrial (mt) DNA by using enzymatic amplification with thermostable *Taq* DNA polymerase. Ninety-three percent of Polynesians exhibited this 9-bp deletion, including 100% of Samoans, Maoris, and Niueans. The same deletion was also found in 8% of Tolais from New Britain and in 14% of coastal New Guineans. A deletion frequency of 82% in Fijians confirmed their ethnic affinity to Polynesians. In contrast, the deletion was absent in 30 New Guinea highlanders and 31 Australian aborigines, the only exception being an aborigine who also had the Southeast Asian triplicated ζ-globin gene rearrangement in his nuclear DNA. These data support the theories claiming that an independent group of pre-Polynesian ancestors who colonized into the Pacific were ultimately derived from east Asia.

Introduction

The human mitochondrial (mt) DNA genome is a closed circular molecule consisting of 16,569 nucleotide base pairs (bp) (Anderson et al. 1981). It is characterized by two unique features that make it a valuable tool in the study of evolutionary genetics of modern populations (Wilson et al. 1985). These features include a strictly maternal mode of inheritance (Giles et al. 1980) and a rapid rate of evolution 5–10 times faster than that of nuclear DNA (Brown et al. 1979). As a result, studies of mtDNA sequence variation have provided a magnified view of genetic diversity between individuals within populations as well as between different racial groups (Cann et al. 1987).

Methods for detecting differences between mitochondrial genomes have included restriction-enzyme analysis by either conventional Southern blotting (Johnson et al. 1983) or end-labeling of restriction fragments (Brown 1980; Stoneking et al. 1986; Cann et al. 1987). Both procedures detect restriction-site polymorphisms due to single bp substitutions or more recently described conformational mutations (Singh et al. 1987). Another form of evolutionary change in mtDNA involves length mutations (Cann and Wilson 1983), of which nine have been described in regions designated I–IX. One of these is due to a deletion of one copy of a 9-bp tandem repeat sequence (CCCCCTCTA) within the noncoding region V (Wrischnik et al. 1987). Analysis to date suggests the latter is a valuable anthropological marker for peoples of east Asian origin (Wrischnik et al. 1987). The present study describes the use of the polymerase chain reaction (PCR) with direct visualization of amplified mtDNA by ethidium bromide staining of polyacrylamide gels to identify this deletion in a majority of Polynesians from five distinct island groups. Additionally, the deletion was found in the mtDNAs of coastal New Guineans, in Tolais from the island of New Britain, and in Fijians but not in mtDNAs from Papua–New Guinea (PNG) highlanders or in Australian aborigines. These data are consistent with an east Asian origin for the founding population in Polynesia.

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Material and Methods

Subjects

In toto, 150 Polynesians, consisting of 30 individuals from each of five island groups—Samoans, Maoris, Niueans, Cook Islanders, and Tongans—were studied. Samples were obtained from either random cord bloods derived from Polynesian newborns at the Middlemore Hospital in Auckland or from adult Polynesians living in Auckland. All subjects were of native Polynesian ancestry as determined from the mothers of newborns and adults sampled. Blood for controls was collected from 30 PNG highlanders (Lake Kopiago and Erave—southern highlands), 28 coastal New Guineans (near Madang), 40 Tolais from the island of New Britain, 28 Fijians, and 31 Australian aborigines from Western Australia. Control samples were obtained from individuals living in their respective homelands.

Methods

DNA amplification.—Total genomic DNA was prepared by conventional phenol/chloroform extraction from 10 ml of frozen whole blood (Old and Higgs 1983). Amplification of mtDNA by PCR was carried out with the thermostable *Taq* DNA polymerase (Perkin Elmer-Cetus) (Saiki et al. 1988). Two 20-base oligonucleotide primers were synthesized by using the Applied Biosystems 381A DNA Synthesizer and had the following sequences as described by Wrischnik et al. (1987): primer A = 8196-5′-ACAGTTTCATGCCCATCGTC-3′8215; primer B = 8316-5′-ATGCTAAGTTAGCTTT-ACAG-3′-8297. This set of primers amplifies a 121-bp segment of mtDNA as determined from the one published sequence (Anderson et al. 1981). Amplification conditions were as follows: 100-μl reaction mixtures containing 500 ng total DNA, 1 μg of each primer, 25 mM Tris pH 8.8, 5 mM $MgCl_2$, 50 mM KCl, 5 mM ammonium sulphate, 5% (w/v) PEG 8000, 400 μM each of dATP, dCTP, dGTP, and dTTP, and 1.5 units of *Taq* polymerase. The reaction mixture was overlayed with paraffin to prevent evaporation. DNA was denatured at 94°C for 10 min, then for 1 min in subsequent cycles. Annealing of primers was carried out at 56°C for 30 s, followed by DNA synthesis for 1 min at 65°C. Cycles of denaturation, primer hybridization, and DNA extension were repeated 34 more times by using fixed-temperature waterbaths. Five microliters (5%) of the final reaction mixture were subject to high-resolution electrophoresis through 12% vertical polyacrylamide gels (Biorad Mini-PROTEAN™ dual slab cell apparatus) for 2 h at 200 V in Tris-borate-EDTA buffer (Maniatis et al. 1982). A standard size marker, *Hae*III-digested φX174, was run in parallel, and the amplified fragments were stained with ethidium bromide and were visualized by means of fluorescence under UV light.

DNA sequencing.—Sequencing of one of the mtDNA amplification products was obtained by blunt-end ligation into a *Sma*I-digested M13mp18 vector. Amplified DNA was eluted from a 12% polyacrylamide gel, phenol extracted twice, and ethanol precipitated prior to ligation into the M13 vector and subsequent transformation into *E. coli*. Single-stranded M13 DNA template was prepared from two positive clones. Each clone was sequenced using the dideoxy chain termination method (Sanger et al. 1977). Attempts at using Sequenase were unsuccessful, and this was subsequently replaced with *Taq* DNA polymerase. Conditions for sequencing included 800 ng ssDNA template, 5 ng universal M13 primer (Pharmacia), 50 mM KCl, 10 mM Tris pH 8.3, 1.5 mM $MgCl_2$, and 0.01% gelatin (Cetus buffer). The reaction mixture was heated to 95°C for 3 min and was transferred to a 55°C waterbath to allow annealing to occur. *Taq* DNA polymerase (2 U) was added together with ^{32}PdCTP, and the mixture incubated for 30 s. Aliquots (3.5 μl) of the annealing reaction were then added to each of the G, A, T, and C tubes containing the following deoxy/dideoxy nucleotide mixes: ddG = 0.01 mM dGTP, 0.05 mM dTTP, 0.01 mM dCTP, 0.05 mM dATP, and 0.25 mM ddGTP; ddA = 0.05 mM dGTP, 0.05 mM dTTP, 0.01 mM dCTP, 0.005 mM dATP, and 0.25 mM ddATP; ddT = 0.05 mM dGTP, 0.005 mM dTTP, 0.01 mM dCTP, 0.05 mM dTTP, and 0.25 mM ddTTP; ddC = 0.05 mM dGTP, 0.05 mM dTTP, 0.01 mM dCTP, 0.05 mM dATP, and 0.25 mM ddCTP. Following extension at 56°C for 10 min, formamide stop dye mix was added. Electrophoresis was carried out through a 4% polyacrylamide gel by using the LKB Macrophor™, system and sequences were interpreted after overnight autoradiography using Kodak X-Omat™ AR 5 film.

Results

mtDNA in Polynesians

Region V is a small noncoding mtDNA segment located between the cytochrome oxidase II and the lysyl tRNA genes (Cann and Wilson 1983). It includes two tandemly arranged copies of a 9-bp sequence, CCCCC-TCTA (Anderson et al. 1981; Wrischnik et al. 1987). Deletion of one of these copies has been shown to oc-

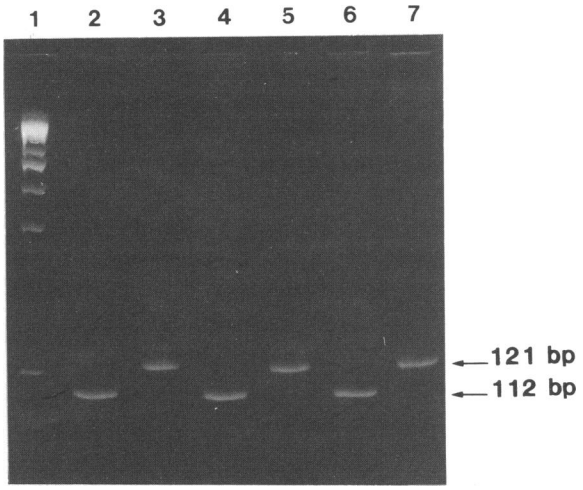

Figure 1 Examples of amplified mtDNA region V electrophoresed on a 12% polyacrylamide gel and stained with ethidium bromide. Lane 1 contains the size marker ϕX 174 digested with *Hae*III. Lanes 2, 4, and 6 contain samples of 112-bp fragments, carrying the 9-bp deletion, derived from Polynesian mtDNAs. Lanes 3, 5, and 7 have mtDNA fragments of 121 bp, consisting of two copies of the 9-bp repeat sequence. These are derived from a PNG highlander and two Australian aborigines, respectively.

cur in individuals of east Asian origin (Wrischnik et al. 1987). Figure 1 shows a comparison of the 112-bp and 121-bp fragments of amplified regions of mtDNA that correspond, respectively, to the presence of one and two tandem copies of the 9-bp sequence. In all cases, amplified DNA segments were easily differentiated and each sample exhibited one of the two expected fragments. Overall, 93% of Polynesians had the 9-bp deletion in region V of their mtDNA (table 1). Samoans, Maoris, and Niueans were each characterized by a mutation frequency of 100%. Indeed, in only 7 of 30 Tongans and 4 of 30 Cook Islanders were the normal two-tandem-copy arrangements detected. Frequencies of the deletion were 77% (23/30) and 87% (26/30), respectively, in these two groups. One of the amplified 112-bp fragments derived from a Niuean individual was sequenced following purification and cloning into M13 (fig. 2). This confirmed the presence of the 9-bp deletion, which includes nucleotides between either 8272 and 8280 or 8281 and 8289 as numbered by Anderson et al. (1981). It was subsequently assumed for other samples that DNA bands of the exact same size (112 bp) visualized on stained 12% polyacrylamide gels represented the 9-bp deletion.

mtDNA in Controls

Amplification of mtDNA was also performed using samples derived from five control populations described above (table 1). No example of deletion V was detected in a group of 30 PNG highlanders. Similarly, only 1 individual from a sample population of 31 Australian aborigines exhibited the 9-bp deletion. Of note was the fact that this individual also carried in his nuclear DNA the Southeast Asian (SEA) type of triplicated zeta-globin gene rearrangement ($\zeta\zeta\zeta/$). Eight percent of Tolais (New Britain) and 14% of coastal New Guineans exhibited deletion V. On the other hand, Fijians had a very high frequency (82%) of the mtDNA deletion.

Table 1

Asian-specific 9-bp Deletions of mtDNA in Polynesians and Control Groups

Population	Sample Size	mtDNA Deletion	
		No. Detected	% Positive
Samoans	30	30	100
Maoris	30	30	100
Niueans	30	30	100
Cook Islanders	30	26	87
Tongans	30	23	77
Polynesians overall	150	139	93
Fijians	28	23	82
Tolais	40	3	8
Coastal PNGs (Madang)	28	4	14
PNG highlanders	30	0	0
Australian aborigines	31	1[a]	3

[a] Individual also has SEA-type $\zeta\zeta\zeta/$ configuration.

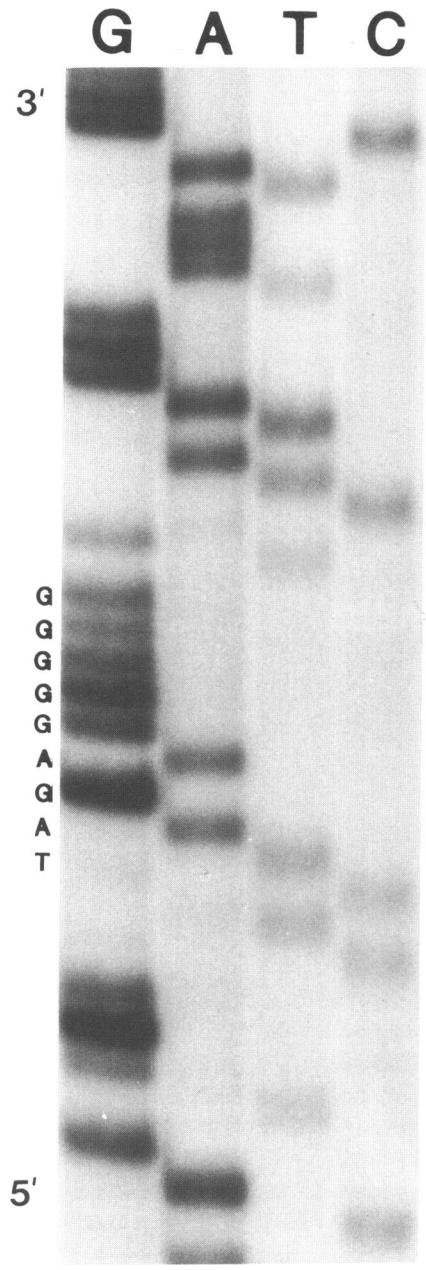

Figure 2 Sequence of an mtDNA region V. Autoradiograph of a 4% polyacrylamide gel containing the sequence of region V which carries the 9-bp deletion. This illustrates the sequence 3'-GGGG-GAGAT-5' from the antisense strand corresponding to the deletion 5'-CCCCCTCTA-3' in the sense strand. In this example only one copy of the 9-bp segment is present, in contrast to the published sequence, which contains a tandem repeat of two copies.

Discussion

Evidence derived from archeological, anthropological, and linguistic studies has enabled models for the colonization of the Pacific to be constructed. It has been proposed that an australoid population was the first to penetrate into PNG and Australia more than 50,000 years ago (B.P.) (reviewed in Bellwood 1987). In New Guinea this population was subsequently replaced by three successive waves of Papuan-speakers who spread across PNG and also reached adjacent Melanesian islands, New Britain, New Ireland, and perhaps the Solomons between 15,000 and 6,000 years B.P. (fig. 3) (Wurm 1967). Settlement of the remainder of the Pacific region was achieved by Austronesian speakers (Spriggs 1984). By 5,000 years B.P. early Austronesians had reached the northern coast of PNG and settled in the New Britain/New Ireland area. It was in this region that they came into contact with Papuan-speakers (Tryon 1984), prior to undertaking further migrations southeast to the Bismarck and Solomon Islands. The first Austronesian colonists who penetrated into Polynesia were associated with the Lapita cultural complex, which was also present throughout much of island Melanesia (Green 1979). Spreading eastward, these colonists settled Fiji by about 3,500 years B.P. and subsequently settled Tonga and Samoa in western Polynesia. The colonization of central and eastern Polynesia occurred from either or both Tonga and Samoa (Kirch 1984). The Marquesas, Society, and Southern Cook Island groups were settled by about 1,700 years B.P. The marginal extremes of Hawaii and Easter Island were colonized by 1,500 years B.P., followed by New Zealand (Maoris) a further half millennium later (Kirch 1984).

In respect of the theories proposed above, a number of questions remain unresolved. One concerns the ultimate origins of the Lapita settlers and whether they derived from Austronesians already present in northwestern Melanesia (Spriggs 1984) or represented an instantaneous introduced phenomenon (Green 1985). Genetic data described elsewhere lend support to the latter contention. HLA gene analysis shows that while Polynesians and island Melanesians share some HLA markers in common (DRw6 and DRw8), additional distinctive HLA-A,B linkage arrangements HLA-A9,Bw22 and HLA-A2,B40 are present in Polynesians (Serjeantson et al. 1987). Polynesians also differ from Melanesians by the presence, at significant frequencies, of α-globin gene haplotypes Ia and IIa (Hertzberg et al. 1988) and by a high incidence of the SEA ζζζ/ rearrangement (26% of individuals) (Trent et al. 1986). Nuclear gene analyses, including that from HLA and α-globin loci, have also supported an Asian origin of the pre-Polynesian ancestors, although these analyses have not been conclusive (Serjeantson et al. 1987; Hertz-

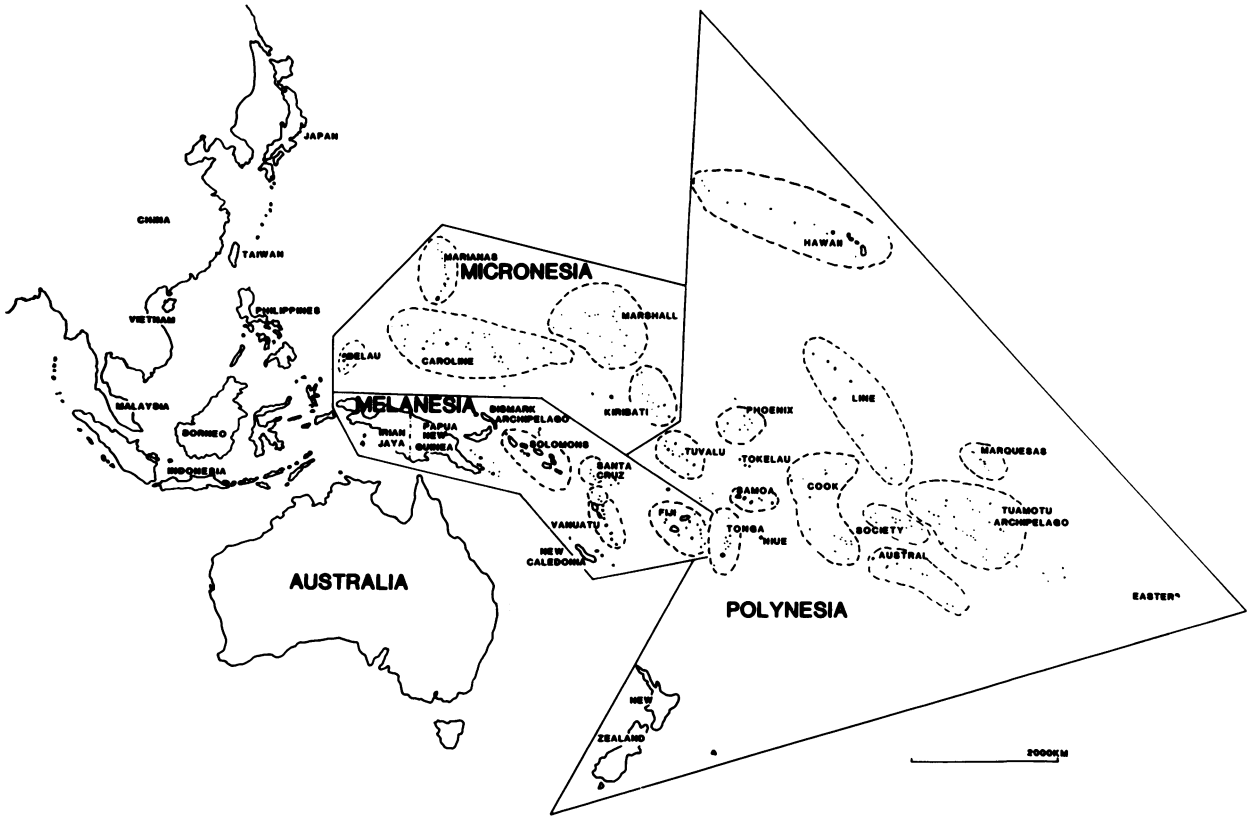

Figure 3 Map of the Pacific region, illustrating Polynesia, Melanesia, Micronesia, and SEA. New Britain and New Ireland (not shown) lie immediately to the northeast of PNG.

berg et al. 1988). Data from the present study provide convincing genetic evidence of an ultimate east Asian derivation for the Polynesians and their immediate Lapita forebears. Phylogenetic analysis by Wrischnik et al. (1987) has revealed that a length mutation consisting of a 9-bp deletion in region V of the mitochondrial genome may have arisen once only throughout human evolution. This deletion has proved a very valuable marker in examining populations with Asian affinities, since, with rare exceptions, all identified individuals carrying this deletion are of east Asian or Japanese origin (Cann and Wilson 1983; Horai and Matsunaga 1986). Recently, Beck et al. (submitted) have observed this mtDNA deletion in six Hawaiians and one Tongan. In the present study the great majority of Polynesians from five different island groups exhibit the Asian-specific deletion of mtDNA. An overall frequency for the deletion of 93% (139/150 individuals) is the highest reported to date for any population including East Asians (18%) (Cann and Wilson 1983; Wrischnik et al. 1987) and Japanese (16%) (Horai and Matsunaga 1986). The finding of a single mtDNA lineage, as demonstrated by the deletion, in the great majority of individuals from five Polynesian groups would be consistent with an extreme bottleneck in the number of colonizing females. This could even represent the contribution of a single female. Subsequently, an additional (nondeletional) mitochondrial marker might then have been introduced. These data support the view of the Polynesians as a relatively small and independent colonizing group in the Pacific who derived from an east Asian precursor population.

Analyses presented in the present study are consistent with linguistic, globin, and HLA gene evidence that Austronesian colonists underwent a period of intermingling with resident populations on the coast of PNG as well as with resident populations on parts of island Melanesia (Wurm 1967; Serjeantson et al. 1982; Hill et al. 1985). Either early Austronesian speakers arriving in Melanesia 5,000–3,500 years B.P. or subsequent

Austronesian Lapita settlers may have contributed some of their nuclear and mitochondrial genomes to resident Papuan populations. This would account for the presence of the 9-bp deletion of mtDNA in coastal New Guineans and in Tolais at frequencies of 14% and 8%, respectively. These frequencies are somewhat lower than that reported by Stoneking and Wilson (in press), who found the deletion in 42% of 55 coastal New Guineans. The reason for the discrepancy may reflect sampling of different coastal populations in whom variable degrees of admixture between Papuans and Austronesians may have occurred. It is noteworthy that the deletion is absent from PNG highlanders, including 30 individuals examined in the present study and 64 analyzed by Stoneking and Wilson (in press). This is consistent with the theories outlined above—i.e., that colonization of PNG occurred some 50,000 years ago, well before any expansion of Papuan or Austronesian speakers into the southwest Pacific. Similarly, Australian aborigines are characterized by absence of the deletion in 31 individuals (considered in the present study) and in another 21 reported elsewhere (Stoneking and Wilson, in press). Since Australia was settled soon after PNG, by a similarly derived population, it is not surprising that the deletion is absent in this group. The one exception was an aborigine who also carried the SEA ζζζ/ rearrangement. This probably reflects genetic admixture, in a manner similar to that reported in an American black (Wrischnik et al. 1987). In the latter case the mtDNA appears to have derived from an Amerindian, whose ancestors are purported to have an east Asian origin (Beck et al., submitted).

Admixture estimates based on nuclear DNA may provide some insights into the sequence of migrations into a given area when the migrant contributions are small. However, when the admixture coefficients are approximately 50%, as in the estimate for the Polynesian and Melanesian contributions to the Fijian gene pool (Serjeantson et al. 1982), it is not possible to discriminate the colonizers from later arrivals. Wurm (1967) has argued on linguistic grounds that Fiji was colonized by Polynesians, with Melanesian elements introduced by later Melanesian expansion; and, although the early archaeological record did not support this viewpoint (Bellwood 1978), more recent findings suggest that Wurm was probably correct (Bellwood, in press). If women are underrepresented in migrant groups, as may well have been the case in long sea voyages, then the mtDNA contribution by the later arrivals may be less than the nuclear DNA contribution. In Fiji, nuclear DNA shows a Polynesian component of 50%, whereas the Polynesian component of mtDNA is 82% (Serjeantson et al. 1982; Trent et al. 1988). This observation strongly supports Wurm's (1967) contention that Polynesians were the earlier arrivals in Fiji. The fact that some Tongans have the normal length region V may similarly represent Melanesian admixture or may reflect the position of Tonga as the original destination of the early Lapita settlers in western Polynesia.

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