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The Origins of the Polynesians: An Interpretation from Mitochondrial Lineage Analysis

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Summary

Using mitochondrial lineage analysis of 1,178 individuals from Polynesia, the western Pacific, and Taiwan, we show that the major prehistoric settlement of Polynesia was from the west and involved two or possibly three genetically distinct populations. The predominant lineage group, accounting for 94% of Polynesian mtDNA, shares a 9-bp COII/tRNA^{Lys} intergenic deletion and characteristic control region transition variants, compared to the Cambridge reference sequence. In Polynesia, the diversity of this group is extremely restricted, while related lineages in Indonesia, the Philippines, and Taiwan are increasingly diverse. This suggests a relatively recent major eastward expansion into Polynesia, perhaps originating from Taiwan, in agreement with archaeological and linguistic evidence, but which experienced one or more severe population bottlenecks. The second mitochondrial lineage group, accounting for 3.5% of Polynesian mtDNA haplotypes, does not have the 9-bp deletion and is characterized by an A-C transversional variant at nt position 16265. Specific oligonucleotides for this variant were used to select individuals from the population sample who, with other sequences, show that the Polynesian lineages were part of a diverse group in Vanuatu and Papua New Guinea. The very low overall diversity of both lineage groups in Polynesia suggests there was severe population restriction during the colonization of remote Oceania. A third group, represented by only four individuals (0.6%) in Polynesia but also present in the Philippines, shares variants at nt positions 16172 and 16304. Two Polynesians had unrelated haplotypes matching published sequences from native South Americans, which may be the first genetic evidence of prehistoric human contact between Polynesia and South America.

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Introduction

The prehistoric exploration and settlement of the remote Pacific islands between 3,000 and 1,000 years ago ranks as one of man's most remarkable achievements. European explorers from the 16th century onward found that all habitable islands, no matter how remote, were either currently or had once been inhabited. The most wide ranging of these voyagers, the English navigator James Cook, noted the similarity in both appearance and language between islanders from as far apart as New Zealand in the south to Hawaii in the north, and his colleague, Joseph Banks, writing in 1770 (Allen and White 1989), was convinced they all came from the same source, which he believed to have been in the west. Interest in the question of Polynesian origins has not diminished in the intervening 200 years, and, since the 1950s, the debate has been between those who believe that the archaeological record and cultural and linguistic affiliations strongly support a southeast Asian origin (Bellwood 1987), others who prefer a largely Melanesian origin with indigenous development of characteristic traits (Terrell 1986), and a small minority who advance the prevailing westerly direction of both wind and currents, as well as some anthropological evidence, to support a significant settlement from the Americas (Heyerdahl 1950).

Analysis of the distribution of nuclear-encoded polymorphisms, including ABO, MNS, Rh blood groups, red-cell enzymes, serum protein groups, and HLA antigens (Kirk 1989), although in general agreement with the majority view of a colonization from southeast Asia, has not provided a complete solution, partly because of the Asian origin, via Beringia, of native Americans. The presence in Polynesia of α-globin alleles, including a specific $-\alpha^{3.7}$III thalassemia deletion, is evidence for at least some genetic input from Melanesia (Hill et al. 1985; Trent et al. 1988).

Mitochondria are maternally inherited and nonrecombining, and the effectively haploid genome accumulates mutations faster than does nuclear DNA. The most variable region of the mitochondrial genome is the 1,122-bp noncoding control region between bp 16024 and bp 00576 (numbering after Anderson et al. 1981), within which the variation is concentrated in two regions (I and II) (Stoneking et al. 1991). Control region

variation, as well as restriction mapping of the entire mitochondrial genome, has been used extensively to infer population histories (Horai and Hayasaka 1990; di Rienzo and Wilson 1991; Vigilant et al. 1991; Ward et al. 1991; Shields et al. 1993). As a method, it differs from allele frequency–based surveys of nuclear-encoded variants in several respects: (*i*) The variation is extensive and is not scrambled by recombination. (*ii*) Deducing the phylogenetic relationships within and between haplotype clusters is usually straightforward. (*iii*) The effective population size is roughly one quarter that for nuclear variants, which enhances the effect of drift. (*iv*) Since the mitochondrial genome is maternally inherited, only female lineages are relevant.

An initial survey of 13 control region I sequences from Rarotonga, in the southern Cook Islands, revealed three lineages defined by their nucleotide sequence between bp 16000 and bp 16380. The major lineage (9 of 13) differed from the Cambridge reference sequence (CRS) (Anderson et al. 1981) by four transitional substitutions at bps 16189, 16217, 16247, and 16261. Three Cook Islanders possessed a closely related sequence that lacked the transition at bp 16247. Both lineages also contained the 9-bp COII/tRNA^{Lys} intergenic deletion, a variant already known to occur frequently in Polynesia (Hertzberg et al. 1989). However, one Cook Islander possessed a completely different control region sequence, defined by eight transitional substitutions and one transversional substitution, compared to the CRS and by the absence of the 9-bp deletion. Discovery of an almost identical lineage in a native Hawaiian (J. K. Lum and R. L. Cann, personal communication; Lum et al. 1994) suggested that, although it differed greatly from the major lineage group, it may still be an authentic Polynesian haplotype rather than an accidental introduction. This paper reports the results of a search for these and related lineages in 14 locations throughout the Pacific.

Subjects and Methods

DNA Sources

DNA samples for this study were provided by 1,178 individuals from 14 locations in the Pacific (fig. 1), all of whom had agreed to take part in a genetic survey. Taiwanese samples came from Bunum, Atayal, Paiwan, and Ami. Samples from the Philippines came from the island of Luzon. The Sabah (Indonesia) sample came from the vicinity of Kota Kinabalu. The Papua New Guinea (Melanesia) samples were collected at coastal sites. Samples from the Marshall islands (Micronesia) were collected in Majuro. Vanuatuan (Melanesia) samples were from the islands of Maskelyne, Maewo, Tanna, Nguna, and Espirito Santo. The seven Polynesian sampling sites were as follows: Tongan samples were from Tongatapu, and Samoan samples came from Savai'i and Upola. Samples from the Cook Islands were from Rakahanga, Manihiki, Penrhyn, and Puka Puka, in the northern group, and from Atiu, Aitutaki, Mangaia, Mauke, and Rarotonga, in the southern group. Samples from the Australes islands came from Rimatara and Rapa. Marquesan samples were from Hiva Oa. Samples from Aoteoroa (New Zealand) were collected in Auckland.

DNA Amplification

DNA samples were amplified in a rapid-cycling water bath (Autogene II; Dale Scientific) in volumes of either 10 μl (for deletion detection) or 50 μl (for sequencing and hybridization), with final concentrations of 200 μM of each dNTP, 2 ng/μl of each primer, ~50 ng of genomic DNA, 0.3 μl of DNA polymerase (Biotaq; Bioline), and the KCl buffer supplied with the enzyme. The presence or absence of the 9-bp deletion was detected by amplification by using primers and conditions suggested by Wrishnik et al. (1987), and products were resolved by electrophoresis in 4% agarose. Mitochondrial control region I was amplified by using primer con H1 (5′ CCT GAA GTA GGA ACC AGA TG) and a 5′-biotinylated primer con L2B (5′ CAC CAT TAG CAC CCA AAG CT). Amplification conditions were 94°C × 4 min, 55°C × 1 min, and 72°C × 1 min (cycle 1) then 94°C × 1 min, 55°C × 1 min, and 72°C × 1 min, for a further 34 cycles with a final extension at 72°C of 8 min. Biotinylated amplification products were bound to streptavidin-coated magnetic beads (Dynal) then alkali-denatured and were washed to prepare bound single-stranded sequencing templates. Sequencing reactions were carried out by using Sequenase 2.0 kits (Amersham) after priming with conH2 (5′ TTG ATT TCA CGG AGG ATG GT). Sequencing gels were run with adjacent tracking of samples with the same terminator, to facilitate the detection of variants after autoradiography.

Oligonucleotide Screening

To screen for the presence of the A-C transversion at nt position 16265, two oligonucleotides were synthesized: 265A (5′ ACC CCT CAC CCA CTA) to detect the CRS and 265C ((5′ ACC CCT CCC CCA CTA) to detect the transversion. Control region–amplified DNA samples were alkali-denatured and spotted onto duplicate nylon membranes (Hybond N+; Amersham) by using a dot-blot apparatus. End-labeling and hybridization were performed according to the method of Hewett et al. (1994) by using hybridization temperatures of 46°C (265A) and 48°C (265C).

Statistical and Phylogenetic Analysis

Lineage diversity within populations was estimated in two ways. The simple haplotype diversity (*h*), which is

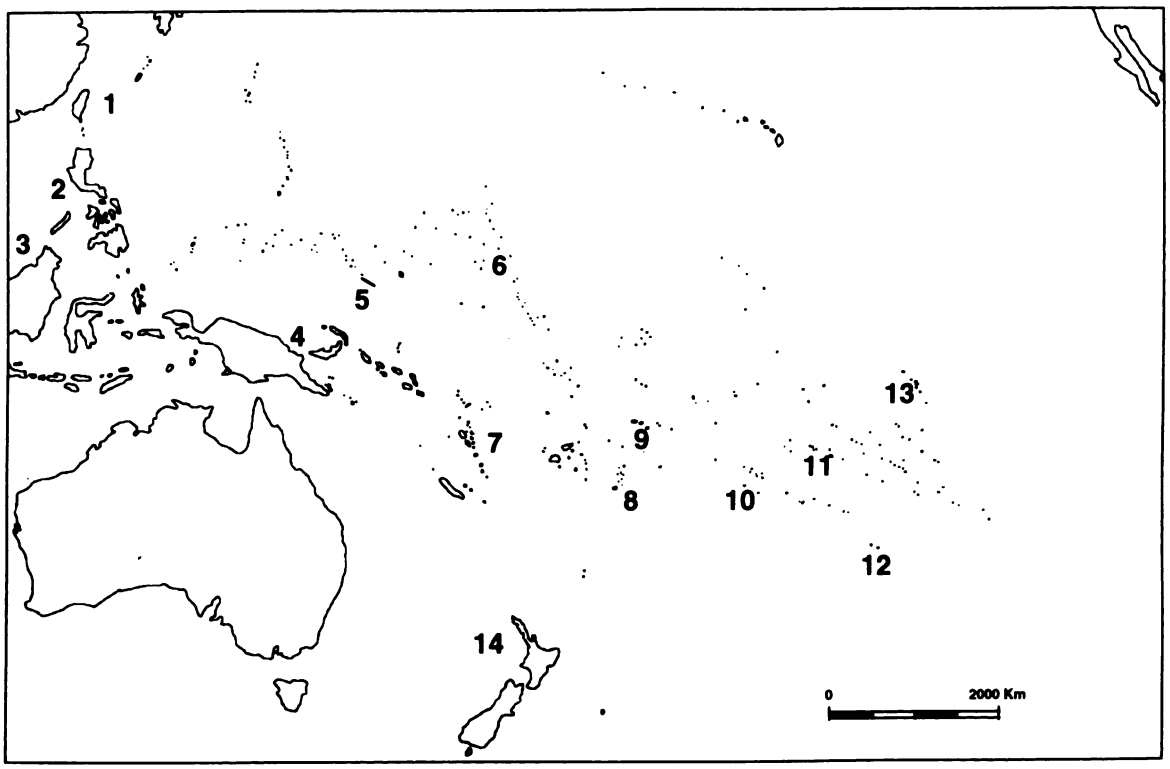

Figure 1 Location of sampling sites. (1) Taiwan, (2) Philippines, (3) Sabah (Borneo), (4) Papua New Guinea, (5) Kapingamarangi, (6) Marshall Islands, (7) Vanuatu, (8) Tonga, (9) Samoa, (10) Cook Islands, (11) Tahiti, (12) Australes, (13) Marquesas, and (14) Aoteoroa (New Zealand).

an estimate of the probability that two individuals picked at random from a population have different haplotypes but disregards their phylogenetic relationships, was estimated by using the methods of Tajima (1989) and Nei (1987). The mean and variance of the pairwise differences were computed using the program MacPairwise (Micklem 1994). Minimum divergence times were estimated from these differences by using transversional divergence between the human and chimpanzee consensus sequences (Morin et al. 1994), assuming a transition-to-transversion ratio of 30:1 (Ward et al. 1991), and calculated separately for different regions sequenced. Phylogenetic analyses were performed by the method of median networks (Bandelt et al., in press), which illustrate all most parsimonious phylogenetic relationships (MPs) supported by the data in a single diagram. The theoretical prediction that each median network includes all the MPs was confirmed for groups I–III by using the phylogenetic package PAUP, version 3.1.1 (Swofford 1993). The resulting MP trees were used to attribute consensus values to reticulation links in the network. Statistical support for lineage groups was investigated by bootstrap analysis (500 replications) of neighbor-joining trees constructed with haplotypes occurring more than once in the data by using MEGA (Kumar et al. 1993).

Screening and Sequencing Strategy

We screened 655 individuals from sites in Polynesia (table 1) for the deletion and control region sequences obtained from the 43 individuals who lacked the deletion. To trace related lineages into the western Pacific, 523 samples from Melanesia, Micronesia, Indonesia, the

Table 1

Distribution of Deleted and Nondeleted Lineages

Location	Sample	Deleted (%)	Nondeleted
Taiwan	88	32 (36)	56
Phillipines	74	20 (26)	54
Sabah	74	30 (41)	44
Papua New Guinea	114	50 (44)	64
Kapingamarangi	62	62 (100)	0
Marshall Islands	55	53 (97)	2
Vanuatu	56	22 (40)	34
Tonga	88	82 (93)	6
Samoa	83	81 (98)	2
Cook Islands	224	204 (91)	20
Tahiti	114	110 (95)	4
Australes	68	65 (96)	3
Marquesas	47	41 (87)	6
Aoteoroa	31	29 (94)	2
Total	1,178	881	297

Table 2

Haplotypes and Their Distributions

Group and Haplotype	Variant[a]	Geographical Distribution[b]													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
I:															
1	189 217 274 335	2	1	...	...	...	...	...	...	...	...	...	...	...	...
2	189 217 292	...	...	1	...	...	...	...	...	...	...	...	...	...	...
3	189 217 365	3	...	...	...	...	...	...	...	...	...	...	...	...	...
4	189 217	1	...	2	...	8	...	...	...	...	...	...	...	...	...
5	189 217 261 311	3	...	...	...	...	...	...	...	...	...	...	...	...	...
6	189 217 261	3	2	...	...	5	...	5	...	3	10	...	...	1	2
7	189 217 261 324	3	...	...	...	...	...	...	...	...	...	...	...	...	...
8	189 217 261 272 324	2	...	...	...	...	...	...	...	...	...	...	...	...	...
9	189 217 261 272 288 324	1	...	...	...	...	...	...	...	...	...	...	...	...	...
10	189 217 261 312	...	...	...	...	...	...	...	...	...	1	...	...	...	...
11	189 217 247 261	...	...	...	4	3	...	3	2	14	46	...	...	12	20
12	189 217 247 261 287	...	...	...	...	...	1	...	...	...	...	...	...	...	...
13	189 217 247 261 291	...	...	...	...	...	...	...	...	...	1	...	...	...	...
14	189 217 247 261 354C–A	...	...	...	...	...	...	...	...	1	...	...	...	...	...
15	189 217 247 261 274G–C	...	...	...	...	...	...	...	...	1	...	...	...	...	...
16	189 217 247 261 342	...	...	...	...	...	...	...	...	1	...	...	...	...	...
17	189 217 247 261 293A–C	...	...	...	1	...	...	...	...	...	...	...	...	...	...
18	189 217 247 261 362	...	...	...	...	...	...	...	...	2	...	...	...	...	...
19	189 217 247	...	...	...	...	...	...	...	...	...	1	...	...	...	...
II:															
20	129 144 148 223 241 265A–C 293 311 343	...	...	...	...	...	...	...	...	1	11	2	3	...	1
21	129 144 148 223 241 265A–C 311 343	...	...	...	1	...	...	...	1	1	...	...	...	...	...
22	129 144 148 223 241 265A–C 274 311 343	...	...	...	...	...	...	...	...	...	3	...	...	...	...
23	129 144 148 223 241 265A–C 311	...	...	...	...	...	...	...	2	...	...	...	...	...	...
24	129 148 223 241 265A–C 311 343	...	...	...	3	...	...	...	...	...	...	...	...	...	...
25	129 144 148 223 265A–C 311 343	...	...	...	...	...	...	1	...	...	...	...	...	...	...
26	129 144 148 172 265A–C 311 343	...	...	...	...	...	...	2	...	...	...	...	...	...	...
27	129 148 223 311 362	...	...	...	...	...	...	2	...	...	...	...	...	...	...
28	129 148 223 362	...	...	...	...	...	...	7	...	...	...	...	...	...	...
29	129 148 223 355 362	...	...	...	...	...	...	1	...	...	...	...	...	...	...
30	129 148 223 355 362 365	...	...	...	...	...	...	1	...	...	...	...	...	...	...
31	148 223 362	...	...	...	...	...	...	1	...	...	...	...	...	...	...
32	148 223 295 362	...	...	...	...	...	2	...	...	...	...	...	...	...	...
33	148 223 261 362	...	1	...	...	...	...	...	...	...	...	...	...	...	...
III:															
34	172 304	...	...	...	...	...	...	...	...	...	...	...	...	2	...
35	172 189 304	...	...	...	...	...	...	...	...	...	...	1	...	...	...
36	172 304 311	...	1	...	...	...	...	...	1	...	...	...	...	...	...
37	129 172 304	...	1	1	...	...	...	...	...	...	...	...	...	...	...
38	172 180 304 311	...	1	...	...	...	...	...	...	...	...	...	...	...	...
39	164 172 189 294 304	...	1	...	...	...	...	...	...	...	...	...	...	...	...

40	263	...	...	...	...	...	...	...	...	...	1	...	...	...	...
41	312	...	...	...	...	...	...	...	...	...	1	...	...	...	...
42	356	...	...	...	...	...	...	...	...	...	1	...	...	...	...
43	221 264	...	...	...	...	...	...	...	...	...	1	...	...	...	...
44	210 256 269	...	...	...	...	...	...	...	...	...	1	...	...	...	...
45	093 223 298 327	...	...	...	...	...	...	...	...	...	1	...	...	...	...
46	192 218 270 291	...	...	...	...	...	...	...	...	...	...	...	...	...	1
47	223 290 319 362	...	...	...	...	...	...	...	...	...	...	1	...	...	...
48	235 291 293	...	...	...	...	...	...	...	1	...	...	...	...	...	...
49	189 223 291	...	...	...	...	...	...	...	1	...	...	...	...	...	...
50	172 223	...	...	...	...	...	...	...	...	...	...	...	...	1	...
51	298 311	...	...	...	...	...	...	...	...	...	...	...	...	1	...
52	126 153 294	...	...	...	...	...	...	...	...	...	...	...	...	1	...
53	223 224 270 274 311	...	...	...	...	...	...	...	...	...	...	...	...	1	...
54	0	...	...	...	...	...	...	2	...	...	...	...	...	...	...
55	176	...	...	...	...	...	...	1	...	...	...	...	...	...	...
56	192	...	...	...	...	...	...	1	...	...	...	...	...	...	...
57	223	...	...	1	...	...	...	...	...	...	...	...	...	...	...
58	320	...	...	...	...	...	...	1	...	...	...	...	...	...	...
59	126 311	...	...	1	...	...	...	...	...	...	...	...	...	...	...
60	129 223	...	...	1	...	...	...	...	...	...	...	...	...	...	...
61	185 223	1	...	...	...	...	...	...	...	...	...	...	...	...	...
62	188 250	...	...	...	...	...	...	2	...	...	...	...	...	...	...
63	189 223	1	...	...	...	...	...	...	...	...	...	...	...	...	...
64	189 243	...	3	...	...	...	...	...	...	...	...	...	...	...	...
65	189 266C-G	4	...	2	...	...	...	...	...	...	...	...	...	...	...
66	189 278	...	...	1	...	...	...	...	...	...	...	...	...	...	...
67	189 278 362	...	...	1	...	...	...	...	...	...	...	...	...	...	...
68	189 362	...	...	1	...	...	...	...	...	...	...	...	...	...	...
69	209 266	...	...	...	...	...	...	1	...	...	...	...	...	...	...
70	223 257C-A	1	...	...	...	...	...	...	...	...	...	...	...	...	...
71	223 291	...	...	2	...	...	...	...	...	...	...	...	...	...	...
72	223 293	...	1	...	...	...	...	...	...	...	...	...	...	...	...
73	223 295	1	...	4	...	...	1	...	...	...	...	...	...	...	...
74	256 261	...	...	...	...	...	...	1	...	...	...	...	...	...	...
75	256 304	...	...	1	...	...	...	...	...	...	...	...	...	...	...
76	266 357	...	...	...	...	...	...	1	...	...	...	...	...	...	...
77	278 362	...	...	1	...	...	...	...	...	...	...	...	...	...	...
78	291 362	...	1	...	...	...	...	...	...	...	...	...	...	...	...
79	304 362	...	...	1	...	...	...	...	...	...	...	...	...	...	...
80	066 241 223	...	...	...	...	...	...	1	...	...	...	...	...	...	...
81	086 297 324	...	1	...	...	...	...	...	...	...	...	...	...	...	...
82	126 223 291	...	...	1	...	...	...	...	...	...	...	...	...	...	...
83	126 231 311	...	1	...	...	...	...	...	...	...	...	...	...	...	...
84	129 223 261	1	...	...	...	...	...	...	...	...	...	...	...	...	...
85	130 185 223	...	1	...	...	...	...	...	...	...	...	...	...	...	...
86	153 223 362	1	...	...	...	...	...	...	...	...	...	...	...	...	...
87	157 256 304	...	...	1	...	...	...	...	...	...	...	...	...	...	...
88	169 176 209	...	...	...	...	...	...	1	...	...	...	...	...	...	...

(*continued*)

Table 2 (continued)

Group and Haplotype	Variant[a]	Geographical Distribution[b]													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
89	172 223 264	...	...	...	...	...	...	1	...	...	...	...	...	...	...
90	185 189 324	...	1	...	...	...	...	...	...	...	...	...	...	...	...
91	185 223 291	...	...	2	...	...	...	...	...	...	...	...	...	...	...
92	189 223 294	...	...	...	...	...	...	1	...	...	...	...	...	...	...
93	189 243 311	...	1	...	...	...	...	...	...	...	...	...	...	...	...
94	189 265 266C–A	...	1	...	...	...	...	...	...	...	...	...	...	...	...
95	189 266C–G 362	1	...	...	...	...	...	...	...	...	...	...	...	...	...
96	189 290C–A 374A–C	...	...	...	...	...	...	1	...	...	...	...	...	...	...
97	192 278 325	...	1	...	...	...	...	...	...	...	...	...	...	...	...
98	209 223 324	...	1	...	...	...	...	...	...	...	...	...	...	...	...
99	223 241 319	...	...	...	...	...	...	1	...	...	...	...	...	...	...
100	223 278 295	...	...	2	...	...	...	...	...	...	...	...	...	...	...
101	223 291 362	...	1	2	...	...	...	...	...	...	...	...	...	...	...
102	223 295 362	...	6	1	...	...	...	...	...	...	...	...	...	...	...
103	223 301 362	...	2	...	...	...	...	...	...	...	...	...	...	...	...
104	290 198 362	...	...	...	...	...	...	1	...	...	...	...	...	...	...
105	129 223 257C–A 261	1	...	...	...	...	...	...	...	...	...	...	...	...	...
106	129 223 295 362	1	...	...	...	...	...	...	...	...	...	...	...	...	...
107	157 256 304 335	...	1	...	...	...	...	...	...	...	...	...	...	...	...
108	172 223 248 291	1	...	...	...	...	...	...	...	...	...	...	...	...	...
109	176 209 266 357	...	...	...	...	...	...	2	...	...	...	...	...	...	...
110	176 266 270 357	...	...	...	...	...	...	3	...	...	...	...	...	...	...
111	189 223 291 362	...	1	...	...	...	...	...	...	...	...	...	...	...	...
112	214 223 295 362	...	...	...	...	...	1	...	...	...	...	...	...	...	...
113	223 224 291 294	1	...	...	...	...	...	...	...	...	...	...	...	...	...
114	223 291 356 362	...	1	...	...	...	...	...	...	...	...	...	...	...	...
115	258A–C 265 298 362	...	...	1	...	...	...	...	...	...	...	...	...	...	...
116	266 291 311 357	...	...	...	...	...	...	1	...	...	...	...	...	...	...
117	066 129 168 172 223	...	...	...	...	...	...	1	...	...	...	...	...	...	...
118	157 256 304 335 362	...	...	3	...	...	...	...	...	...	...	...	...	...	...
119	168 220A–C 265 298 362	...	1	...	...	...	...	...	...	...	...	...	...	...	...
120	172 223 264 311 320	...	...	...	...	...	...	1	...	...	...	...	...	...	...
121	176 209 266 274 357	...	...	...	...	...	...	1	...	...	...	...	...	...	...
122	189 223 263 286 311	...	...	1	...	...	...	...	...	...	...	...	...	...	...
123	209 223 233 274 304	...	1	...	...	...	...	...	...	...	...	...	...	...	...
124	223 224 291 294 362	1	...	...	...	...	...	...	...	...	...	...	...	...	...
125	176 250 266 291 311 257	...	...	...	...	...	...	1	...	...	...	...	...	...	...
126	189 261 266C–A 290 347 370	...	...	2	...	...	...	...	...	...	...	...	...	...	...
127	223 234 241 269 292 297 311 362	...	...	...	...	...	...	1	...	...	...	...	...	...	...

[a] Variant positions are as CRS—16000. Transversions are defined. Other positions indicate transitions. Group I sequences are reported between 16189 and 16375. Others, with the exception of sequences with a transition at 16189, are reported between 16065 and 16375.

[b] Numbers correspond to locations in fig. 1.

Philippines, and Taiwan were screened for the presence of the 16265 A-C transversion by allele-specific hybridization. This picked out seven transversion-positive individuals (from Papua New Guinea and Vanuatu) whose control region sequences showed they carried one lineage that is identical and three that are related to those found in Polynesia. To trace related lineages that may not have the transversion variant, the remaining 516 samples were screened for the 9-bp deletion, and control region sequences were determined for 109 of the 254 nondeleted lineages detected. To estimate the extent of variation and the relationships within deleted lineages, control region sequences were obtained for 118 of 612 and 70 of 269 deleted lineages from Polynesia and the western Pacific, respectively.

Results

Sequence haplotypes and their geographical distributions are shown in table 2, and a phylogenetic analysis of the data is presented in figures 2 and 3. Blurring of the bands distal to the segment 16184-16193 was experienced when this was an uninterrupted homopolymeric tract, as in all 9-bp-deleted lineages, making the sequences 16000–16183 impossible to read accurately. This is due to heteroplasmy for tract length (Bendall and Sykes 1995). Several deleted lineage samples from Polynesia sequenced from the opposite direction showed this region to be as the CRS in all cases and thus uninformative, an observation made by others (Lum et al. 1994). As a consequence, the sequence in these individuals is reported only between positions 16189 and 16365. Others are reported between 16065 and 16365. Simple haplotype diversity, mean pairwise differences, and some minimum divergence times are in table 3. Divergence times are not given where there is an expectation of diversity in the founding population.

A reduced median network was constructed by using haplotypes represented at a frequency of ≥ 2 in the entire Pacific data set to indicate the overall topology of Pacific sequences (fig. 2). The frequency criterion ensures that the topology is more reliable than if the entire data set is used by overcoming, to some extent, the effect of the high level of homoplasy, which acts to disrupt the ancestral structure through recent parallelisms and also by excluding "accidentals" from the phylogenetic analysis. It is clear from inspection that certain branches of this skeleton network appear quite specific to either island southeast Asia or Melanesia. In addition, while there is a great deal of variation in Island Southeast Asia and Melanesia, Polynesian haplotypes can be seen at the tips of three of the major branches, falling into just three lineage groups.

Group I

This lineage group is defined by the sharing of variants at positions 16189 and 16217 and by the presence of the 9-bp deletion (fig. 3*a*). It was supported by 43% of bootstrap replications on sequence alone, indicating only a tentative identification as a distinct lineage group, but bootstrap support increased to 75% when the 9-bp deletion was treated as an additional character. It is by far the most common lineage group within Polynesia, and, since all of the sequenced deleted lineages are contained within it, group I accounts for 94% of mtDNA haplotypes within the region. Its most striking feature within Polynesia, particularly east of Samoa, is the extremely restricted diversity. The percent mean pairwise sequence difference in Samoa is 0.39, and this drops to 0.18 for all islands further east (table 3). Of 94 individuals from islands east of Samoa (but including Aoteoroa), 91 belong to one of two haplotypes (6 and 11 in fig. 3*a*) which themselves only differ by a single transition at position 16247.

Their relative frequencies are roughly maintained at all locations within Polynesia, haplotype 11 being 4–10 times more common. Group I is rather more diverse in Samoa, with four haplotypes in addition to 6 and 11 in a sample of 22 individuals. Figure 3*a* shows that haplotypes 12–19 are almost certainly single-step derivatives of haplotype 11, and the only other group I haplotype in Polynesia, haplotype 10, is likely to be a single-step derivative of haplotype 6. Further to the west, haplotype 6 is traceable to the Philippines and Taiwan, while haplotype 11 is not found west of Papua New Guinea. It is interesting that the sample from the Polynesian "outlier" Kapingamarangi is entirely group I with three haplotypes present in the sample (4,6,11), of which the most common, haplotype 4, is not found in Polynesia. Group I is at its most diverse in Taiwan (% mpd = 1.33) with eight haplotypes in 18 individuals. Haplotype 11 has also been reported to be the predominant haplotype in Hawaii (Lum et al. 1994), and the same study reports finding two derivative haplotypes. One, our haplotype 12 (found, interestingly, in our Marshall Island sample but nowhere else), is also found in one Hawaiian, while two Samoans carry haplotype 13, which we find in the Cook Islands.

The data for group I are entirely consistent with its introduction into Polynesia from southeast Asia rather than from the Americas. Although the 9-bp deletion and the transitions at positions 16189 and 16217 are comparatively common in native Americans (Ward et al. 1991; Ginther et al. 1993; Torroni et al. 1993) the predominant Polynesian haplotype (11) has not been described.

Group II

Twenty-five of the 43 Polynesians with nondeleted mtDNA were contained within four closely related haplotypes (20–23, in table 2). This group is defined, in Polynesia, by an A-C transversion at position 16265 and

by transitions at positions 16129, 16148, and 16311. It was supported in 70% of bootstrap replications. This group (fig. 3*b*) accounts for 3.8% of Polynesian lineages and, with the exception of the Marquesas islands, was found in all locations. The unusual haplotype found during preliminary work in the Cook Islands (22) is almost identical to the native Hawaiian lineage reported by Lum et al. (1994). They also report a Samoan lineage that matches haplotype 20 (in the region 16193-16368, which they report) except that it, too, lacks the transition at position 16343.

Allele-specific screening of the western Pacific sample picked out a further seven individuals who shared the characteristic A-C transversion at position 16265, three from Vanuatu and four from Papua New Guinea. Although one Papuan haplotype (21) exactly matched a Polynesian lineage, the other (24) and the two Vanuatuan haplotypes (25 and 26) were not found within Polynesia but were clearly closely related (fig. 3*b*). Like group I, the diversity is very restricted in Polynesia but considerably higher in Melanesia. In addition, other haplotypes possibly related to group II (haplotypes 27–33) are relatively frequent in Vanuatu. However, none of the haplotypes in Vanuatu exactly matched any in Polynesia.

Group II is highly distinctive, separated by three transitions and a transversion from its nearest neighboring Melanesian haplotype (27), and its ultimate origin is obscure. However, there is an interesting possible phylogenetic connection between group II and a group of

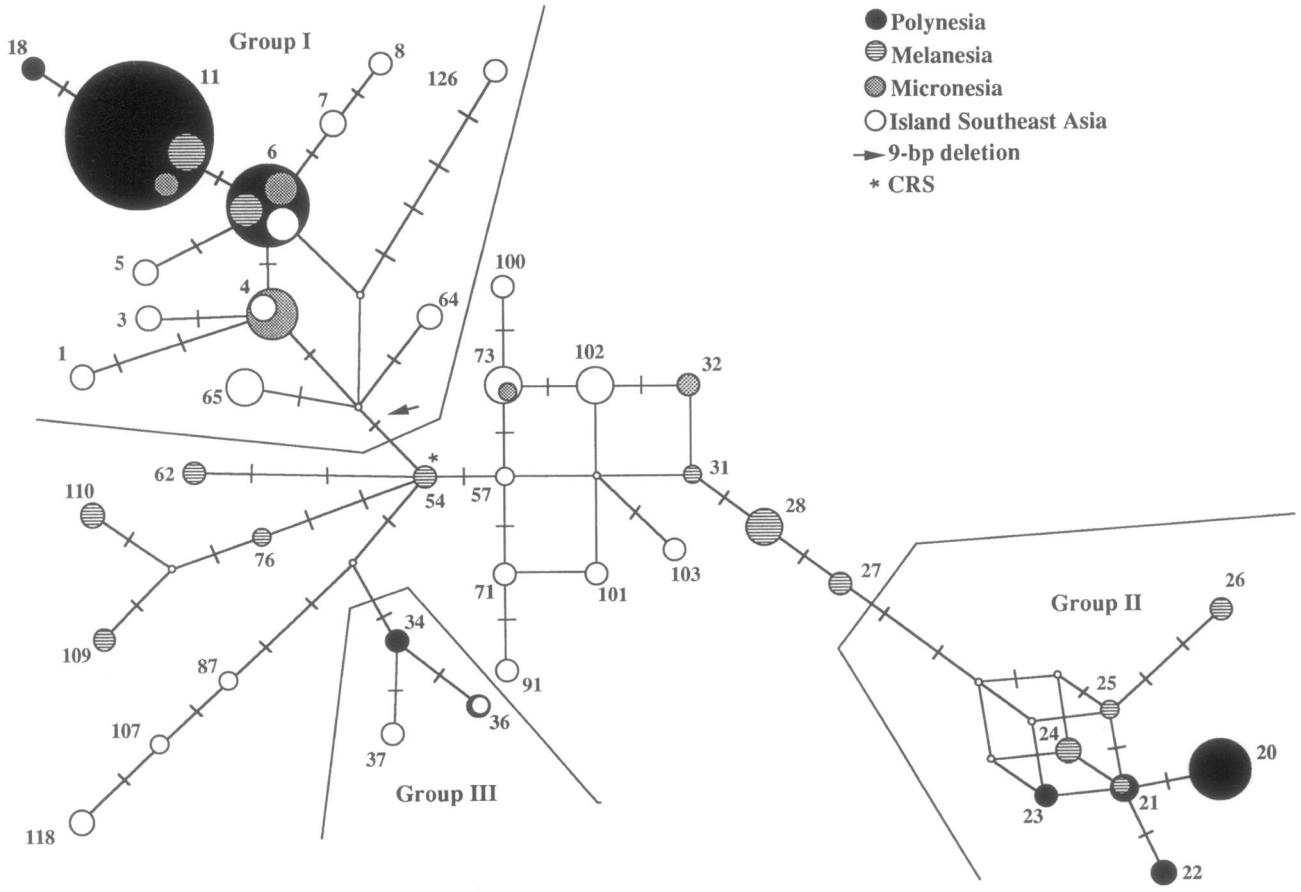

Figure 2 Reduced median network constructed from all 36 haplotypes present at a frequency of ≥2 in the entire Pacific data set. Six intermediates present only once in the data set were then added to empty internal nodes. Numbered circles are haplotypes, as described in table 2. The areas of the circles are proportional to the numbers of individuals sharing that haplotype. Small unfilled circles are theoretical intermediates. The branches represent evolutionary events that separate the haplotypes; single base substitutions are each indicated by a slash on the branch. Parallel mutational events distinguished by median network reduction are suffixed with a lower-case letter. Reticulations in the network are an indication of parallel events that cannot be resolved. Notation of the base is only given once in any reticulation but is the same in parallel connections within it. Haplotypes are shaded according to geographic region (Island Southeast Asia = 1–3; Micronesia = 5–6; Melanesia = 4–7; Polynesia = 8–14). The likely position of the 9-bp deletion event is indicated by an arrow (→). CRS = Cambridge reference sequence, indicated by asterisk (*) (Anderson et al. 1981). Solid lines demarcate lineage groups. Note that deleted lineages were sequenced from 16189–16375, other lineages from 16065–16375.

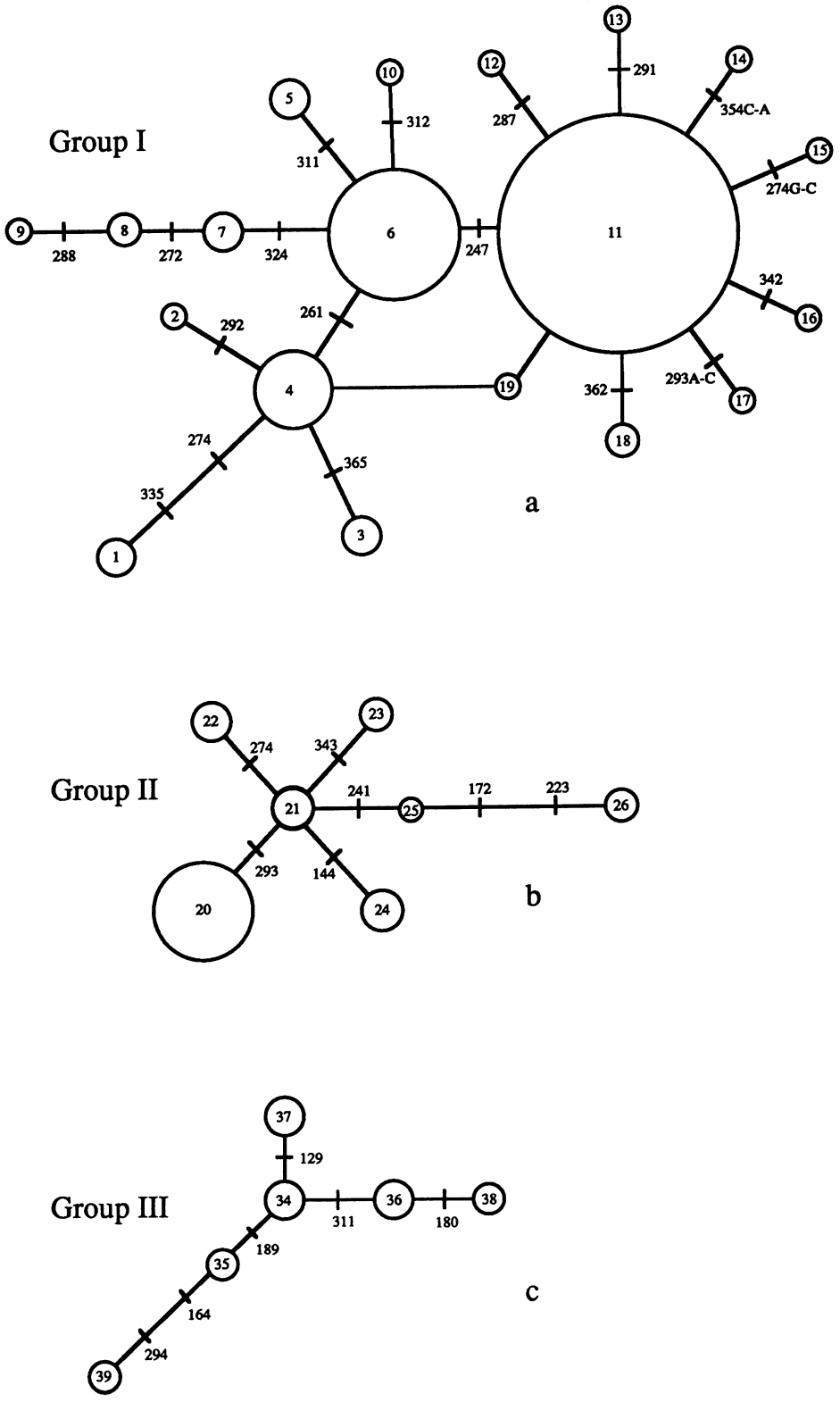

Figure 3 Phylogenetic networks for group I, group II, and group III. Base substitutions are denoted by a three-figure number, which is the last three digits of the Cambridge reference sequence so that, for example, position 16261 is abbreviated to 261. Transversions are further specified; other events are transitions. Each network contains all most parsimonious relationships supported by the data. Maximum parsimony (MP) trees were checked using each subset of data using the branch and bound program of PAUP 3.1.1 (Swofford 1993). In group I, each side of the single reticulation is supported by three of the four (75%) distinct MP trees generated. Links without consensus values are supported in all PAUP-derived trees. Groups II and III both represent the single most parsimonious tree for each group, which are also delivered by PAUP.

Table 3

Diversity, Mean Pairwise Differences, and Minimum Divergence Times in the Pacific

Lineage Group	Location	Diversity (h) ± SE	Mean Pairwise Difference ± SE[a]	Mean Pairwise Difference ± SE[b] (%)	Minimum Divergence Time[c] (years before present)
I	Taiwan	.908 ± .013	2.353 ± .112	1.329 ± .063	31,300
I	Vanuatu and Papua New Guinea	.603 ± .050	.667 ± .067	.376 ± .038	Not given
I	All Polynesia	.349 ± .037	.384 ± .007	.217 ± .004	Not given
I	Samoa	.589 ± .079	.693 ± .045	.392 ± .025	Not given
I	Islands east of Samoa[d]	.295 ± .039	.320 ± .008	.181 ± .005	Not given
I Hap 11-19	Samoa	.462 ± .095	.515 ± .045	.291 ± .025	6,800
I Hap 11-19	Islands east of Samoa	.050 ± .024	.050 ± .001	.028 ± .001	660
II	Vanuatu and Papua New Guinea	.810 ± .064	2.095 ± .322	.674 ± .104	22,000
II	All Polynesia	.473 ± .079	.793 ± .051	.255 ± .016	Not given

[a] Diversity and mean pairwise difference values based on 177 bp for group I and 311 bp for group II haplotypes.
[b] Expressed as mean pairwise difference per 100 bp sequenced.
[c] Estimated using divergence rate of 1 base per 13,300 years for group 1 (bp 16189–16365) and 1 base per 10,500 years for group II (bp 16065–16375). Not given where founding populations are known to be diverse.
[d] Includes Aoteoroa (New Zealand), which, although west of Samoa, was settled much later.

African pygmy sequences from Zaire and the Central African Republic (Vigilant et al. 1991). Only four transitions separate an intermediate node between haplotype 27 and group II and an internal node in a network constructed from the African data (fig. 2, Bandelt et al. 1995).

Group III

Four individuals from Polynesia, two from the Marquesas islands, and one each from Tahiti and Tonga (table 2), as well as five individuals from the western Pacific, share related nondeleted haplotypes with transitions at positions 16172 and 16304 (fig. 3*c*). It is very rare in Polynesia, occurring at a frequency of only 0.6%, and not common elsewhere in the Pacific sample. Lum et al. (1994) describe a single individual from Samoa with transitions at positions 16294, 16304, and 16362. Horai and Hayasaka (1990) report four individuals from Korea, China, and Japan who also share the 16172 and 16304 transitions with two being identical to our haplotype 34. It is only weakly supported as a distinct lineage group, with a bootstrap value of 54%.

Other Lineages

Fourteen lineages (40–53 in table 2) from Polynesia did not fit into any of the recognized lineage groups. None are closely related, and each is carried only by a single individual in the sample. When compared with other available sequences, two haplotypes, one from Tahiti (47) and one from the Cook Islands (45), matched published native American sequences. One, defined by transitions at position 16223, 16290, 16319, and 16362, is identical to a sequence found in Mapuche Indians from Patagonia (Ginther et al. 1993) and one in Beringians (Shields et al. 1993), and the other (positions 16093, 16223, 16298, and 16327) was identical to a Chilean lineage except that the latter sequence did not cover position 16093 (Horai et al. 1993). Other matches were with haplotypes 40 (United Kingdom Caucasian [Piercy et al. 1993]), 42 (Iceland, Turkey, Cornwall, Portugal, and Bavaria), 48 (United Kingdom Caucasian [Piercy et al. 1993] and Cornwall), 51 (Bavaria), and a lineage closely related to haplotype 52, with an additional transition at position 16296, among the Basques (Bertranpetit et al. 1994). Unattributed sequences are the unpublished observation of M. Richards, H. Côrte-Real, and B. Sykes.

Discussion

Our data from the analysis of 127 mtDNA haplotypes from 655 Polynesians and 523 individuals from the western Pacific strongly support the view that, despite the considerable navigational problems, Polynesia was entirely settled from the west. We recognize three mitochondrial lineage groups among Polynesians that together account for 98% of the sample. Two of them (groups I and II) are reasonably well supported by bootstrap analysis and clearly correspond to the clusters reported from a sample of 45 individuals mainly from Hawaii (Lum et al. 1994). The third, which has weaker statistical support, resembles the lineage group defined by a single Samoan individual in the same study.

The large majority of Polynesian lineages (94%) be-

long to group I and are clearly derived from a diverse group present in the western Pacific. DNA recovered from 12 archaeological bone samples from Rapanui (Easter Island) show that group I lineages were carried right to the eastern margin of Polynesia (Hagelberg and Clegg 1993). Group I is at its most varied in Taiwan, with a minimum divergence estimate of 31,000 years (table 3). Linguistic and archaeological reconstructions suggest that Austronesian-speaking populations began to expand from the general region of Taiwan into the Philippines and Indonesia ~5,000 years ago, eventually reaching eastern Polynesia ~3,000 years before present (BP) (Bellwood 1987). Our data lend some, but not complete, support to that reconstruction in that Taiwan contains a diverse set of group I lineages including haplotype 6, which is found widely throughout Polynesia. However, the predominant Polynesian haplotype 11 was not found in Taiwan (nor in the Philippines or Sabah, though the sampling there was not extensive). On the basis of the assumption that haplotype 11 is a derivative of haplotype 6, it may be that the transforming mutation at position 16247 did not occur in Taiwan but further east, perhaps between Sabah and Papua New Guinea, its most westerly occurrence in our sample. Regardless of whether haplotype 11 originated in Taiwan, it does not seem to have been common.

The most notable aspect of the Polynesian group I data is the extreme restriction on haplotype diversity. Diversity in Polynesia is confined to rare haplotypes that are almost certainly one-step derivatives of haplotype 11 (and one from haplotype 6 [fig. 3*a*]) not found further west. There are no two-step derivatives anywhere in the data, and, together with the absence of haplotype 11 west of New Guinea, this suggests that haplotype 11 is itself a relatively recent derivative of haplotype 6, which is found in the Philippines and Taiwan. Regardless of whether haplotype 11 is young, there must have been one or more severe population bottlenecks restricting the eastward gene flow of group I lineages.

There are two possible explanations. Either these Polynesian derivatives of haplotype 11 first occurred in Melanesia (or further west) and were carried into Polynesia by a relatively large number of colonists, which ensured their survival, or, alternatively, they developed in situ within Polynesia. In the latter case, the number of colonizing females could be much smaller, the absolute minimum requirement being for two females to carry haplotypes 6 and 11 beyond Vanuatu and into the remote Pacific. However, a relatively homogeneous group I source population in Melanesia would have allowed larger numbers of colonizing females to cross into Polynesia without a significant increase in introduced diversity. A larger survey of Melanesian group I lineages will give more information on the intrinsic diversity and reveal any matches with haplotype 11 derivatives in Polynesia.

Unlike group I, group II lineages are fairly diverse in Melanesia (table 3). The estimated minimum divergence time for group II in Vanuatu and Papua New Guinea from the data is 22,000 years BP, which, in light of the small sample size, may be consistent with the end phase of Pleistocene occupation, which carried modern humans across the relatively short sea crossings, eastward through New Guinea, New Britain, and the Solomon Islands and perhaps to Vanuatu, without the need for complex navigation. (Irwin 1992). Further exploration had to wait for improvements in nautical design and navigation, perhaps brought by new immigrants from the west, to cross the 850-km distance to Fiji then out into the remote Pacific. Archaeological evidence dates the first occupation of Fiji at 3,200 years BP and of Tonga and Samoa at 3,000 years BP (Kirch and Hunt 1988). Beyond Samoa, the diversities of both groups fall steeply, suggesting further bottlenecks as the colonists moved out further into the remote Pacific. There is an even more dramatic decrease in diversity within lineages 11–19 east of Samoa. If, as we propose, this diversity was largely generated in situ, the reduction suggests there was a long period of settlement in Samoa prior the next phase of eastward colonization.

It is not possible to tell from the data alone whether the two lineage groups were carried into the Pacific by a single or separate colonization events. The similarities in the overall diversities of group I (% mpd = 0.22) and of group II (% mpd = 0.26%) in Polynesia suggest that if there were separate colonizations then they would have occurred at much the same time. However, it is interesting to speculate how, if it were mixed, the colonizing voyages carried sufficient group I females to establish the 24:1 I/II ratio throughout Polynesia without introducing any (surviving) variants from further west. If the relative proportions found in Polynesia today resemble those in a single, large-scale, admixed colonization, then the group I component must already have passed through a very severe bottleneck before setting off into the remote Pacific. Also curious is the absence in Polynesia of any other haplotypes that are common in Vanuatu. The only Melanesian lineages to cross to Polynesia are closely related members of group II. Although the ravages of genetic drift could be responsible, another possible explanation for the genetic data is that the admixture that occurred between group I and group II colonists happened not in the easterly limits of Melanesia, but further west, perhaps in New Guinea.

The relevance of group III is hard to assess. Although Lum et al. (1994) are correct when they say that group III lineages, or any other for that matter, might once have been more frequent, it is difficult to argue, because they are so rare and yet diverse, that they arrived in Polynesia with the first voyagers. It is possible that they arrived later, yet still before European contact. The same

is true of the 14 unrelated lineages represented only once in Polynesia. Of these, the two possibly Amerindian lineages are the most intriguing, providing the first evidence of a direct genetic link with Polynesia. While one cannot conclusively disprove that they are not the sole survivors of an earlier colonization of Polynesia from the Americas, and while it is possible that they represent ancestral Amerindian sequences extant in Asia, it is also possible that they came into the Pacific as a result of secondary contact of the kind that also introduced the Andean sweet potato (Yen 1961; Irwin 1989). Another intriguing finding is a closely related lineage among the Basques. Although the theory that many Polynesians have a Basque ancestry is not new (Langdon 1975), drawing conclusions from single unrelated lineages out of place in a population is dangerous. In the absence of paternal transmission of mtDNA, the more prosaic explanation is a modern, yet unremembered, introduction.

This study provides very strong support for the archaeological and linguistic evidence of a colonization of Polynesia from the West involving at least two distinct populations and puts a figure on their relative contributions. Of the current models, the mtDNA analysis lends greatest support to that of Bellwood (1987) who proposed a relatively rapid expansion from southeast Asia rather than the indigenous development model put forward by Terrell (1986), although there is no doubt that aboriginal Melanesian lineages are also found in Polynesia, and we cannot rule out their earlier arrival in Polynesia. There is no support in our data for Heyerdahl's hypothesis (1950) for a substantial colonization from the Americas.

In many respects, we agree with the conclusions of Lum et al. (1994) based on their largely Hawaiian sample. Where we differ with their findings and interpretations concerns the extent of diversity within Polynesia. In our, larger, sample we find the diversity among both group I and group II to be extremely low, especially east of Samoa. This could, in our view, be substantially accounted for by transformations of haplotypes 6 and 11 within Polynesia rather than being indicative of a large number of "discrete maternal ancestors who crossed the geographical filter into Polynesia" (Lum et al. 1994, p. 584). In both studies, Samoa, with Tonga, the most westerly of the Polynesian islands sampled, is shown to have the highest diversity. This could be explained either by its longer occupation than islands further east, allowing for in situ development of new haplotypes or by the later introduction of new variants from the west. To answer this question, more extensive sampling in west Polynesia and Melanesia is needed in order to look for matches, especially in haplotype 11 derivatives, but the lack of other Melanesian lineages in Samoa suggests that the former explanation is the more likely to be correct.

It has to be remembered that these conclusions are drawn from only a single locus and one that is particularly vulnerable to allele loss through drift in small populations (Avise 1991). That said, the intrinsic advantages of mtDNA in being able to group haplotypes and recognize connections between individuals from geographically remote locations are well illustrated in this study, to say nothing of the advantage of restricting observations to maternal lineages. This simplifies matters in an area like Polynesia, where potentially complicating contributions to the gene pool in recent times have been almost entirely provided by males.

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