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Mitochondrial Control-Region Sequence Variation in Aboriginal Australians

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Summary

The mitochondrial D-loop hypervariable segment 1 (mt HVS1) between nucleotides 15997 and 16377 has been examined in aboriginal Australian people from the Darling River region of New South Wales (riverine) and from Yuendumu in central Australia (desert). Forty-seven unique HVS1 types were identified, varying at 49 nucleotide positions. Pairwise analysis by calculation of BEPPI (between population proportion index) reveals statistically significant structure in the populations, although some identical HVS1 types are seen in the two contrasting regions. mt HVS1 types may reflect more-ancient distributions than do linguistic diversity and other culturally distinguishing attributes. Comparison with sequences from five published global studies reveals that these Australians demonstrate greatest divergence from some Africans, least from Papua New Guinea highlanders, and only slightly more from some Pacific groups (Indonesian, Asian, Samoan, and coastal Papua New Guinea), although the HVS1 types vary at different nucleotide sites. Construction of a median network, displaying three main groups, suggests that several hypervariable nucleotide sites within the HVS1 are likely to have undergone mutation independently, making phylogenetic comparison with global samples by conventional methods difficult. Specific nucleotide-site variants are major separators in median networks constructed from Australian HVS1 types alone and for one global selection. The distribution of these, requiring extended study, suggests that they may be signatures of different groups of prehistoric colonizers into Australia, for which the time of colonization remains elusive.

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Introduction

The mitochondrial genome, valuable for studying inter- and intraspecific evolution, because of its predominantly maternal mode of inheritance (Giles et al. 1980; Hauswirth and Laipis 1982), absence of recombination, and rapid evolutionary rate (Brown et al. 1979), has been the molecule of choice for many human studies throughout the world (e.g., Cann and Wilson 1983; Cann et al. 1987; Vigilant et al. 1989, 1991; Schurr et al. 1990; Di Rienzo and Wilson 1991; Ward et al. 1991, 1993; Torroni et al. 1992, 1993*a*, 1993*b*; Lum et al. 1994; Redd et al. 1995; Soodyall et al. 1996). Cann et al.'s (1987) analysis of RFLPs gave rise to the postulation that all modern mitochondrial types have spread from an African source existing ~200,000 years before the present (B.P.). Although this has generated contentious debate among researchers, particularly with regard to estimation of mutation rates and interpretation of phylogenetic analytical methods, subsequent studies have not refuted the model, and mtDNA remains a powerful tool for the examination of recent population history. An added advantage of the study of mtDNA, rather than nuclear DNA, is that, in many places (e.g., Australia and America) where indigenous groups have been dispossessed by colonizers, social history has resulted in many communities where indigenous maternal ancestry has been maintained although paternal lineages are mixed. In these cases, nuclear-DNA studies have limited value, whereas mtDNA studies can provide information about maternal connections that sometimes is lost with the breakup of traditional groups.

Few Australian mtDNA studies have been published (Cann et al. 1987; Griziotis et al. 1987; Hertzberg et al. 1989; Stoneking and Wilson 1989; Lum et al. 1994; Betty et al. 1996), and none of these has described diversity within the continent. Therefore, until now, the use of published Australian data in global comparisons contributes limited information. No mtDNA studies have described diversification within the continent.

Australia has been inhabited for $\geqslant$50,000 years (Roberts et al. 1990) and possibly very much longer. C^{14}-dated archaeological evidence demonstrates the presence of *Homo sapiens* by 30,000 B.P., in several well-documented sites throughout the continent. These sites in-

clude Tasmania, which was isolated from the mainland ~10,000 B.P. and is one of the extreme regions of human expansion (reviewed in Smith et al. 1993; Flood 1995, pp. 15–221). Recent dates from northwestern Australia suggest human activity associated with rock art as early as 115,000 B.P. or even 175,000 B.P. (Fullagar et al. 1996), and, if work in progress provides support for these early dates, models of human evolution and expansion will require revision.

The indigenous inhabitants of Australia exhibit rich and varied life styles in a country of enormous environmental diversity through time and space. It is generally agreed that the first humans entered the landmass known as Sahul (encompassing present-day Australia and New Guinea) from the north, possibly when sea levels were lower than at present (White and O'Connell 1982), although the exact source of founding populations—and whether they arrived in one, two, or more waves–remains contentious.

Within Australia, human groups inhabited diverse regions where conditions also varied greatly as climatic conditions altered. The Darling River and Willandra Lakes region of New South Wales has yielded archaeological material including the oldest known cremation in the world (Bowler et al. 1970), skeletal material showing morphological variation (Thorne 1976; Webb 1989; Pardoe 1994), and evidence of changing resource management as the once rich, wet environment became more arid (Hope 1981, 1993; Balme 1995). Estimates of a population size of ~300,000 at the time of European colonization have usually been regarded as poorly founded (Flood 1995), and, as in other parts of the world, changing conditions during the Pleistocene influenced the rate of population growth, also likely to have varied across the Australian continent (Allen and O'Connell 1995).

A possible common origin for Australian people and Papua New Guinean highlanders as distinct from coastal populations has also been debated on the grounds of archaeological evidence, linguistic and other cultural attributes (White and O'Connell 1982; Allen and O'Connell 1995), and mtDNA analysis (Stoneking and Wilson 1989; Stoneking et al. 1990). More recently, Roberts-Thomson et al. (1996) have suggested common ancestry for Australians and Papua New Guinea highlanders, on the basis of α-globin haplotypes.

Biological data reveal considerable diversity across the continent, in studies that have included metric and nonmetric analyses of skeletal material (e.g., see Brown 1973; Abbie 1975, 1979; Thorne 1976; Webb 1989; Pardoe 1994), morphometric and phenotypic observation of living populations (White and Parsons 1976; Birdsell 1993 [a collation of previous work]), blood-group and enzyme distributions (Kirk 1976; Simmonds 1976), and information about nuclear-gene haplotypes (Yenchitsomanus et al. 1986; Tsintsof et al. 1990; Gao and Serjeantson 1991; Roberts-Thomson et al. 1996). Debate has been concerned with how much diversity can be attributed to different founding populations and/or how much is the result of evolution within Australia, a debate to which information about genetic diversity should be able to contribute.

The aim of the present study is to examine mtDNA sequences in two populations of aboriginal Australians who are descendants of distinct cultural groups from two geographically distant and environmentally different regions. The results are analyzed to discern potential population structure within Australia and to compare them with several published global sequences, in an attempt to see how much can be learned about evolutionary processes and prehistoric migration history.

The targeted sequence for examination is the first hypervariable region of the mitochondrial D-loop (mt HVS1), which has been shown to be informative in other studies (e.g., Vigilant et al. 1989; Di Rienzo and Wilson 1991; Horai et al. 1993). An extension to this strategy has been to screen representative samples of the resulting HVS1 types for the presence or absence of the COII/$tRNA^{Lys}$ 9-bp deletion, which has been shown to be a useful marker in combination with sequence haplotypes in Polynesians (Lum et al. 1994; Redd et al. 1995) and which may have arisen independently in other populations, including some Africans (Soodyall et al. 1996). The deletion previously had been reported as absent in Australians (Hertzberg et al. 1989) but recently has been detected in four northern Australians (Betty et al. 1996); therefore, it was considered useful to examine the samples in this study, for the possible presence of the marker.

Subjects and Methods

Subjects

Aboriginal people from western New South Wales and Yuendumu in central Australia gave signed and witnessed consent to participation, after consultation within appropriate guidelines and with ethical clearance (National Health and Medical Research Council 1991; van Holst Pellekaan 1992). The participants are from distinct traditional language groups, which also fall into two larger areas, designated "riverine" and "desert" (fig. 1). These terms are labels for 2 of 18 regions broadly classified on the basis of environments encompassing watershed areas, which link several cultural groups sharing some traditions. Although the boundaries of these regions are not sharply delineated, there is general agreement that they are a valid classification for many purposes (Horton 1994, pp. 934–936). By using the regions as sampling separators, we avoid the risk of beginning the study with assumptions regarding the direct rela-

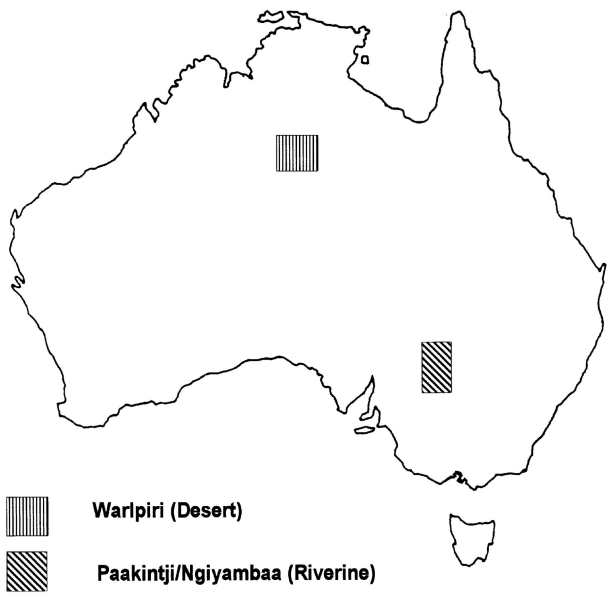

Figure 1 Location of Australian sample areas

tionship between cultural/linguistic development and genetic population history.

The Australian riverine (AR) group comprises 63 blood samples from people in the Darling River area of western New South Wales. Maternal ancestry derives mainly from two language groups, Paakintji and Ngiyambaa, whose land was taken for pastoral purposes, with very little being recorded of the language and history before numbers were drastically reduced by genocide and introduced disease (Hardy 1976; Donaldson 1980, pp. 2–13; Hercus 1982, 1993, pp. 2–18). The Australian desert (AD) group consists of blood samples from 51 individuals now living in Yuendumu, in central Australia, whose language group is Warlpiri. They have also been dispossessed of much of their land but have retained the language and, compared with the AR tribes, more of their traditional customs (Meggitt 1962, pp. 16–136; Bell 1983, pp. 41–109).

DNA Extraction

In the case of the AR samples, genomic DNA was extracted from whole blood, by standard phenol/chloroform methods (Sambrook et al. 1989); in the case of the AD samples, it was extracted from enriched buffy coats by use of Chelex® 100 resin (BioRad), as described by Walsh et al. (1991).

PCR

Amplification was achieved for (*a*) the D-loop segment 1 (HVS1), by use of the primers and conditions described by Di Rienzo and Wilson (1991) to amplify a 600-bp segment, and (*b*) the region of the 9-bp deletion between cytochrome oxidase II and $tRNA^{Lys}$, by use of the same thermal conditions and primers described by Wrischnik et al. (1987).

HVS1 Sequencing

Approximately 50 ng of gel-purified PCR product was sequenced by use of the CircumVent™ Thermal Cycle Dideoxy DNA Sequencing method recommended by the manufacturers (New England Biolabs 1993). The region to be sequenced was targeted by use of either primer L15926 (Di Rienzo and Wilson 1991) or one of the internal primers, L15996 and H16401 (Vigilant et al. 1989), incorporating one of the radioactive labels, [α-^{35}S] dATP or [α-^{33}P] dATP. The reactions were run on 6% denaturing acrylamide:bis (19:1) gels and were fixed, dried, and exposed to autoradiographic film for 24–48 hours before the film was developed. Each sample was sequenced from two separate PCR reactions, in each direction.

Data Analysis

Sequences were entered and edited in MacClade 3.1 (Maddison and Maddison 1992). Unique types were identified, and, in order to compare the Australian sample with other published global types, we selected some from Australia's nearest geographical neighbors and several from Africa, as the postulated home of modern humans. Additional global populations are western Australia (Lum et al. 1994); Pacific (which includes Papua New Guinea coastal), Asian, Indonesian, and Samoan (Redd et al. 1995); Pygmy (Redd et al. 1995; Soodyall et al. 1996); Papua New Guinea Highland (Lum et al. 1994; Vigilant et al. 1991); and !Kung (Vigilant et al. 1991).

Pairwise comparisons were made to generate mean sequence divergence (MSD) and genetic distance (Nei and Tajima 1983), as follows: $D_{NT} = d_{xy} - (d_x + d_y)/2$, where D_{NT} is genetic distance, d_x is MSD within population x, d_y is MSD within population y, and d_{xy} is MSD between populations x and y.

Statistical significance was tested by a modification of the method of Hudson et al. (1992). Those authors tested a range of statistics to differentiate between populations, using a permutation test to assess the significance of the statistic. We have used a different statistic, which may be described as "BEPPI" (between population proportion index).

If there are two populations, with n_1 and n_2 sequences, respectively, then there are, altogether, $n_1 + n_2$ sequences and a total of $((n_1 + n_2)(n_1 + n_2 - 1)/2$ pairs of sequences. Each pair of sequences can be classified as involving two sequences from either the same population (W [within populations]) or different populations (B [be-

tween populations]). Then W_i is the number of pairs, within a population, that differ by i nucleotides, and B_i is the number of pairs, between populations, that differ by i nucleotides. The differences generated in this way are the same as the mismatch/intermatch nucleotide differences described by Rogers and Harpending (1992), but they are used differently in the present study. The maximum number of nucleotide differences between pairs is m. The distribution of pairs may then be summarized as in table 1.

Then

$$\sum_{i=0}^{m} B_i = n_1 n_2 \ ;$$

$$\sum_{i=0}^{m} W_i = \frac{n_1(n_1-1)}{2} + \frac{n_2(n_2-1)}{2} \ ;$$

and

$$\sum_{i=0}^{m} (B_i + W_i) = \frac{(n_1+n_2)(n_1+n_2-1)}{2} \ .$$

If there is a difference between the two populations, pairs that differ by a low number of nucleotides should tend to come from the same population, whereas pairs that differ by a high number of nucleotides should come from different populations. The null hypothesis is that there should be no relationship between d, the number of differences, and $p = B/(B + W)$, the proportion of pairs coming from different populations.

A visual test can be made by plotting a graph of $B/(B + W)$ values. Such a test needs to take into account the fact that some classes contain large numbers of values and that some classes contain small numbers of values, so that a test that weights all classes equally will be imprecise. A weighting factor is therefore needed, which we have taken to be $B_i + W_i$ for each class$_i$. The width of bars in a histogram can be taken as proportional to $B_i + W_i$, in a visual test (see Results).

We have included only one copy of each sequence within a population (i.e., HVS1 types) and have ignored the zero class, as indicated in the above table. Sampling of closely related individuals within a population—for example, mother-offspring or sib pairs—might otherwise exert a disproportionate influence on the test.

A single statistic that tests the relationship between d and p is the weighted regression, which we have used as the BEPPI statistic. The significance of the weighted regression can be evaluated by a conventional t-test. However the values of $p = B/(B + W)$ in different classes

Table 1

Distribution of Number of Pairs of Sequences, Between and Within Populations, Classified in Terms of Number of Nucleotide Differences

	Distribution of No. of Pairs of Sequences When No. of Nucleotides =				
	[0]	1	2..	*i*..	*m*
Between-population pairs	$[B_0]$	B_1	B_2	B_i	B_m
Within-population pairs	$[W_0]$	W_1	W_2	W_i	W_m
p	$[\frac{B_0}{B_0+W_0}]$	$\frac{B_1}{B_1+W_1}$	$\frac{B_2}{B_2+W_2}$	$\frac{B_i}{B_i+W_i}$	$\frac{B_m}{B_m+W_m}$

are not independent of each other, so the validity of the test needs to be established. Following Hudson et al. (1992), we randomly permuted the $n_1 + n_2$ sequences, which gives a uniform probability that any pair of sequences will be a between-population pair, and recalculated the weighted regression. This procedure was repeated 10^6 times, giving a population of values against which to test the observed value for the BEPPI statistic.

We note that there is a close relationship between the weighted regression statistic and a statistic measuring the between- and within-population distances. Algebraic simplification of the weighted regression formula shows that it contains a term that is equal to $(\bar{B} - \bar{W})$, where $\bar{B}$ is the mean between-pair difference,

$$\sum_{i=0}^{m} iB_i/n_1 n_2 \ ,$$

and where $\bar{W}$ is the mean within-pair difference,

$$\sum_{i=0}^{m} iW_i/[n_1(n_1-1)/2 + n_2(n_2-1)/2] \ .$$

Thus, any case in which the mean difference between sequence pairs from different populations exceeds the mean difference between sequence pairs within populations gives rise to a positive weighted regression coefficient. (A computer program [for Macintosh computers] is available that handles all aspects of the BEPPI test, including the plotting of the histogram and the performing of the permutation test.)

Sequence trees were constructed in two ways—namely, the neighbor-joining method and the median-network method. Neighbor-joining consensus trees were derived from 1,000 bootstrap replicates of the sequence data, by use of the PHYLIP programs SEQBOOT, DNADIST (using the maximum-likelihood

method with transition:transversion ratio of 30:1, suggested as appropriate by Lundstrom et al. [1992]), Neighbor Joining (NJ) and CONSENSE (Felsenstein 1995). In generating the NJ trees, input order was randomized both for the 1,000 trees and in the resulting CONSENSE tree. Phylograms were drawn by the TREEVIEW program (Page 1995).

Median-network portraits (Bandelt et al. 1995) were constructed by use of the frequency of nucleotide variants from the Cambridge reference sequence (CRS) (Anderson et al. 1981), as the criterion for separation of HVS1 types. Since no computer software is yet available for this technique, the networks were constructed by hand and were drawn by Computereasy Draw (Jacobs 1991). An advantage of the median-network technique, also demonstrated in other studies (Sykes et al. 1995; Richards et al. 1996), is that possible parallel mutation events are identifiable, which thus avoids the artificial resolution of polytomies that can occur in distance calculations used by other tree-building methods. Nucleotide variants that have been treated as independent events in the median-network portraits are referred to in the Results section and are indicated in the legends of the appropriate figures.

Results

mt HVS1 Diversity

One hundred fourteen individual HVS1 sequences for 380 bp between nucleotides 15997 and 16377 were obtained and aligned by use of CRS (Anderson et al. 1981) as a reference. The frequency with which pairs of individuals differ at 1,...,*i* nucleotide sites was calculated to give a mean of 6.77 (see fig. 2*a*). Forty-seven unique sequences were identified as HVS1 types (GenBank [http://www.ncbi.nlm.nih.gov] accession numbers for HVS1–HVS47 are AF039317–AF039363, respectively), 46 of which vary from the CRS, at a total of 49 sites (table 2). Of these sequences, 24 HVS1 types are from the AR region, 26 HVS1 types are from the AD region , and 3 HVS1 types are seen from both regions. No 9-bp deletions between cytochrome oxidase II and $tRNA^{Lys}$ were detected in any of the HVS1 types. Pairwise comparison of HVS1 types indicated that variation within the two separate regions was not very different from the variation between the regions, although some genetic distance is calculable (table 3). In the manner of Rogers and Harpending (1992), the distribution of the frequency with which pairs differ within (mismatch) and between (intermatch) AR and AD are shown in figure 2*b*. It is notable that the AR mismatch distribution suggests bimodality, which may reflect that the sample from this region is drawn from two language groups although,

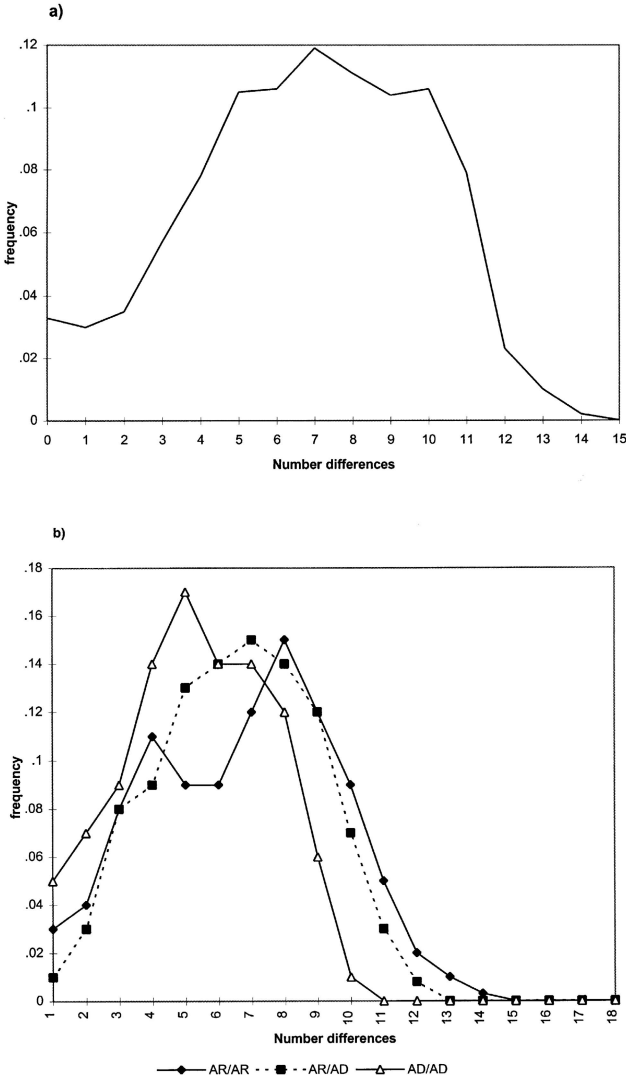

Figure 2 Frequency with which (*a*) pairs of Australian individuals ($n = 114$) differ at 1,...,*i* nucleotide sites (mean = 6.7; variance = 9.5) and (*b*) pairs of HVS1 types (26 AR and 24 AD) differ, within (mismatch) AR and AD and between (intermatch) AR and AD (according to the method of Rogers and Harpending [1992]).

it has been analyzed as a single population. This separation was not supported statistically when the sample was split, so the AR classification was retained. The separation, however, does emerge in the median network (discussed below).

MSD and genetic distance were calculated (see table 3). It is again notable that MSD is greater within the AR group than it is either within the AD or between the two, although positive genetic distance is calculable.

All of the Australians are least distant from the Papua New Guinea highlanders and then from the Pacific populations, whereas farther away are the !Kung and far-

Table 2

Forty-Nine Variable Sites within 380-bp HVS1 Sequence, for 47 HVS1 Types

HVS1 Type	Differences from CRS[a]	No. of Individuals		
	0000111111111111122222222222222222222222333333333 5778223577788899011223445566666777899999001123556 1256697826948923938139156801346014701459341907562 ACTTTGAATCCCCTCCTGCCCCACCACCTCCCTGCCCCCAGTTGCCCTT	AR	AD	Total
T1	..C.....C..........T.....................C.....	5	2	7
T2	..C.....C..........T.....................CC.....	1	3	4
T3	..C.....C..........T.....T.............C.....		5	5
T4	..C.....C..........T.....T.....		1	1
T5	....C.........T....TT........T....T...........C.	4		4
T6	..............T....TT........T....T...........C.	4		4
T7	..........TTTC......TT...........T...........C.	15		15
T8	..........TTTC......T............T.....	1		1
T9	..................T.........................C.		2	2
T10	....C.	1	1	2
T11	....................................T........T...	1		1
T12	....................................T........T..C	6		6
T13	.T........TT.....	3		3
T14	........................C..C....A..T........T..C	1		1
T15	.....A...................C..C.........T.........C	1		1
T16	........................C.......A..T..........C		1	1
T17	..............................A..T.......TT..C	3		3
T18	..............................A..T..........C	2		2
T19	............C...................A..T..........C	2		2
T20	............C.....................T..........C	1		1
T21	....................................T..........C	2		2
T22	....................................T..........C	1		1
T23	..........T....C.............................C	2		2
T24	.....A.....	3		3
T25	......G............T............T..T.........C.	1		1
T26	......G............T.........A...T..T.........C.	1		1
T27	............T.........T......T...TT.....	1		1
T28	............C.....TT............T...........C.	1		1
T29	..............C.T.........................C.		3	3
T30	....T..		4	4
T31	G...T..		4	4
T32	.........T...........T....T.................C		4	4
T33	...C.....T..............T.................C		1	1
T34	...C.....................T.................C		1	1
T35	........................T.................C		3	3
T36	....................................T.........C		1	1
T37	..............A..T.................AC......C		2	2
T38	.......G.........A..T.................AC......C		1	1
T39	.......G.........A..T.................A.......C		1	1
T40	..................T.....................C		1	1
T41	..................T.....		1	1
T42	..............A..T.....		2	2
T43	..............A..T.................A..A....C		1	1
T44	........C...........T..T.......A.....		1	1
T45	............T....T.......CT..A......G.....		1	1
T46	G.......C...........T..T.......A.....		1	1
T47	.....		3	3

[a] Variable sites (from which the prefix "16" has been deleted) are identified by means of the three sets of numerical rows, read from top to bottom; e.g., 051 represents nucleotide site 16051. The CRS is given immediately below the three sets of numerical rows.

Table 3

D_{NT} (above the Diagonal) and MSD, Within (Underlined) and Between 112 HVS1 Types (below the Diagonal, Italicized), for Seven Groups Comprising the AR and AD Populations and Five Previously Published Populations

	Genetic Distance and MSD for Population Pair						
Population[a]	AR	AD	AWA	P	PY	PH	!K
AR	6.77	*.45*	*.67*	*2.56*	*8.06*	*1.19*	*5.49*
AD	6.50	5.34	*.26*	*2.67*	*7.82*	*.98*	*5.7*
AWA	6.65	5.52	5.18	*2.94*	*7.53*	*.75*	*4.9*
P	7.96	7.36	7.55	4.03	*8.96*	*3.69*	*6.18*
PY	12.78	11.83	11.46	12.31	2.67	*6.91*	*4.58*
PH	7.54	6.62	6.31	8.09	11.21	5.93	*4.31*
!K	11.63	11.12	10.24	10.95	8.67	10.03	5.50

[a] AR = 24 AR samples; AD = 26 AD samples; AWA = 7 samples from western Australia (Lum et al. 1994); P = 35 Pacific (Asian, Indonesian, Samoan, and coastal Papua New Guinea samples) (Redd et al. 1995); PY = 3 Pygmy samples (Redd et al. 1995) (these samples equate with types in the work of Soodyall et al. [1996]); PH = 8 Papua New Guinea highland samples (Vigilant et al. 1991; Lum et al. 1994); and !K = 9 !Kung samples (Vigilant et al. 1991).

thest away is the Pygmy group. It is also notable that the Papua New Guinea highland group is more distant from the Pacific sample (which includes Papua New Guinea coastal individuals) than from any of the Australians.

Population Structure

The distribution of the frequency with which pairs of HVS1 types differ by 1,...,*i* nucleotide sites within (mismatch) and between (intermatch) the population groups, shown in table 2, reflects genetic distance, demonstrating that the Australians are genetically more distant from the Africans than from either Papua New Guinea highlanders or Pacific (fig. 3). In order to investigate population structure more rigorously, we calculated the BEPPI statistic as described above and have provided a histogram to display the results for AR and AD (fig. 4). The weighted regression coefficient for the comparison of AR and AD sample is .0200. The significance as judged by a *t*-test is 2.93 with 12 df, which is significant at the 1% level, for a one-tail test. A permutation test with 10^6 replicates gave 11,330 values equal that were $\geqslant .0200$, in rough agreement with the *t*-test. When the two Australian samples were compared with the global selection, the test revealed statistically significant values (table 4) for all except the seven western Australians in the study by Lum et al. (1994), who would be better included as AD in future analyses.

Sequence Trees

A consensus neighbor-joining phylogenetic tree of 47 Australian HVS1 types (see fig. 5) suggests very few ancestral lineages but gives very poor (<50%) bootstrap support for any groups other than those of very similar types, so this interpretation must be regarded as tenuous. More information is represented in a median-network portrait of the AR and AD HVS1 types (fig. 6). Several sites were identified as possibly independently occurring mutations and were treated separately in the network when inconsistencies made connections difficult; the sites treated thus are 16051, 16129, 16184, 16189, 16192, 16263, 16291, 16295, 16337, and 16362. It is notable that, in other studies, the region between 16179 and 16192 is frequently identified as exhibiting variation in the form of substitutions—and, possibly, length mutations—in a group of cytosines (Horai and Hayasaka 1990; Bendall and Sykes 1995; Wakeley 1993). Several substitutions are noted in this region in the Australian samples, but no length variations are noted. Difficulty with regard to these variants was experienced in the construction of the network, and we decided to treat them as parallel mutations. The connections displayed in figure 6 suggest three main groups of HVS1 types: those from AD, those from northern AR, and those from southern AR. The nucleotide sites that control the main separations are 16223, 16287, 16291, 16356, and 16362, which are discussed below.

A global network was constructed, although, because the manual procedure is difficult with large numbers, some selection was made. We chose several distinctive Australian HVS1 types—1, 3, 5, 7, 26, 12, 17, 19, 29, 32, 37, and 47 (see fig. 6)—and several from the population groups examined by pairwise analysis. Also included are four HVS1 types from the native South American haplotypes identified by Horai et al. (1993). With regard to the other region representing one of the extremes of human expansion, haplotypes have been examined by several researchers interested in human movement out of northern Asia into the Americas (e.g., see Schurr et al. 1990; Ward et al. 1991, 1993; Torroni et al. 1992, 1993*a,* 1993*b;* Bonatto et al. 1996). Although that question is not addressed here, the South American haplotypes are included in order to facilitate examination of particular substitutions that may be important in the understanding of evolutionary processes and human migrations. The resulting median network (fig. 7) shows a distinct African group, several distinctive Australian HVS1 types forming a discrete group, a Pacific group, and others clustering less discretely. A consensus NJ tree of the same selection again shows a similar general pattern, but, again, there is very poor bootstrap support for main branches (fig. 8). The nucleotide sites that control the main network separations are 16223, 16311, 16362, 16356, and 16287. As a means of utilizing information from large sets of published data reported separately, we have calculated the empirical frequency with which these nucleotides vary in published mtDNA sequences, suggesting that this is a useful strat-

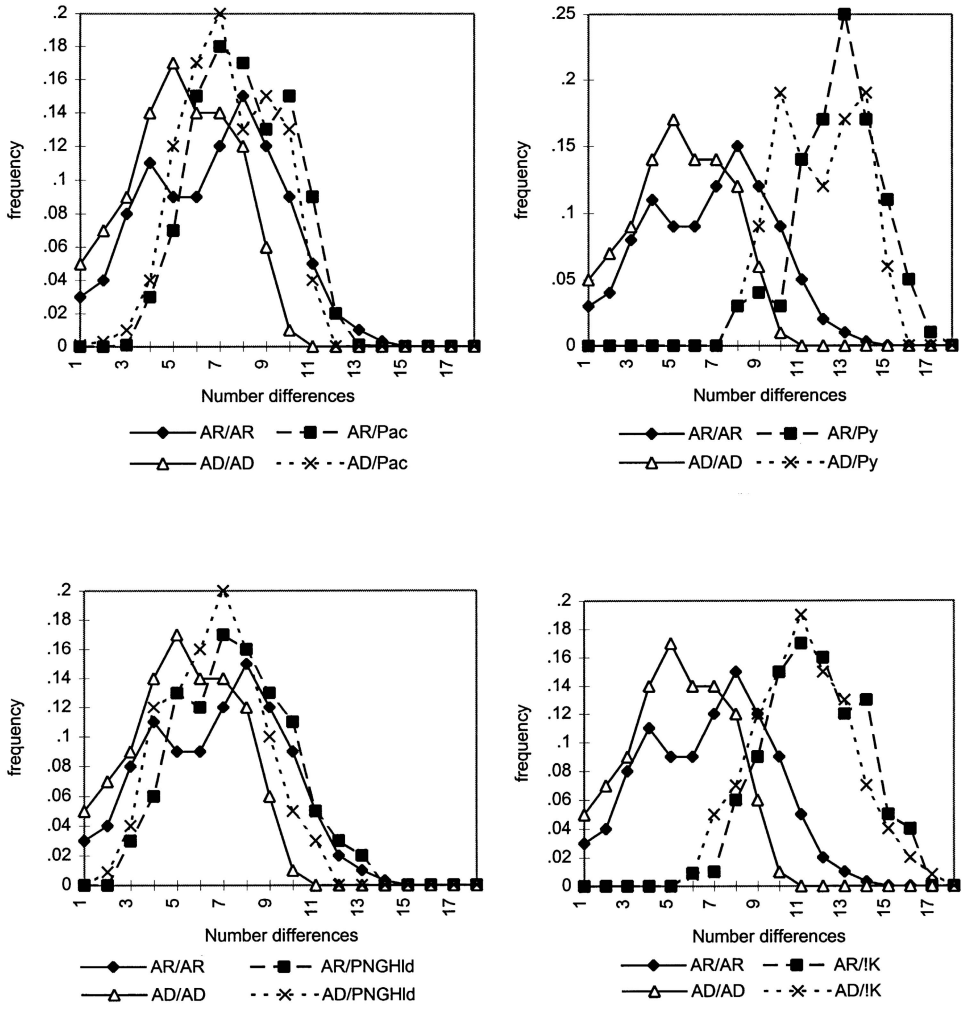

Figure 3 Frequency with which pairs differ at 1,...,*i* nucleotide sites, within AR and AD HVS1 types and between both Australian groups and other populations.

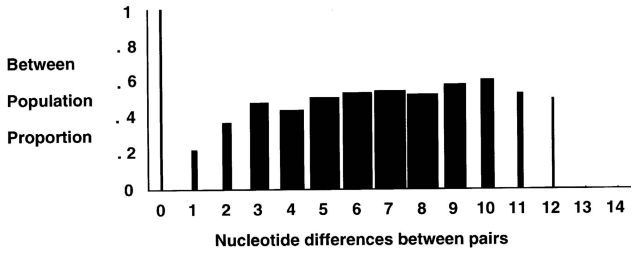

Figure 4 Relationship between proportion of pairs between populations (*y*-axis), for different values of the number of nucleotide differences between pairs (*x*-axis). The width of the histogram bars indicates the number of pairs on which each value is based.

egy for the management of large data sets and for investigation of possible signatures of evolutionary process (van Holst Pellekaan et al. 1997).

Discussion

The analysis tells us that samples from living populations retain mitochondrial types that distinguish the AD and AR populations from each other and from other global populations, although three distinctive HVS1 types (types 1, 2, and 10) are seen in both AR and AD samples, suggesting gene flow in the past, perhaps a long time ago (rather than recently, as it is not supported by

Table 4

Significance, by *t*-Test, of BEPPI Statistic

Population 1/Population 2	*t*-Test	*p*[a]
AR/AWA	.27	NS
AR/P	9.14	<.001
AR/PY	5.84	<.01
AR/PH	2.92	<.02
AR/!K	12.10	<.001
AD/AWA	.10	NS
AD/P	21.66	<.001
AD/PY	5.48	<.001
AD/PH	6.23	<.001
AD/!K	9.49	<.001
AWA/P	8.66	<.001
AWA/PY	6.93	<.001
AWA/PH	2.60	<.05
AWA/!K	9.53	<.001
P/PY	6.58	<.001
P/PH	8.66	<.001
P/!K	7.71	<.001
PY/PH	6.46	<.001
PY/!K	5.67	<.001
PH/!K	11.36	<.001

NOTE.—Populations are as described in table 3.

[a] NS = not significant.

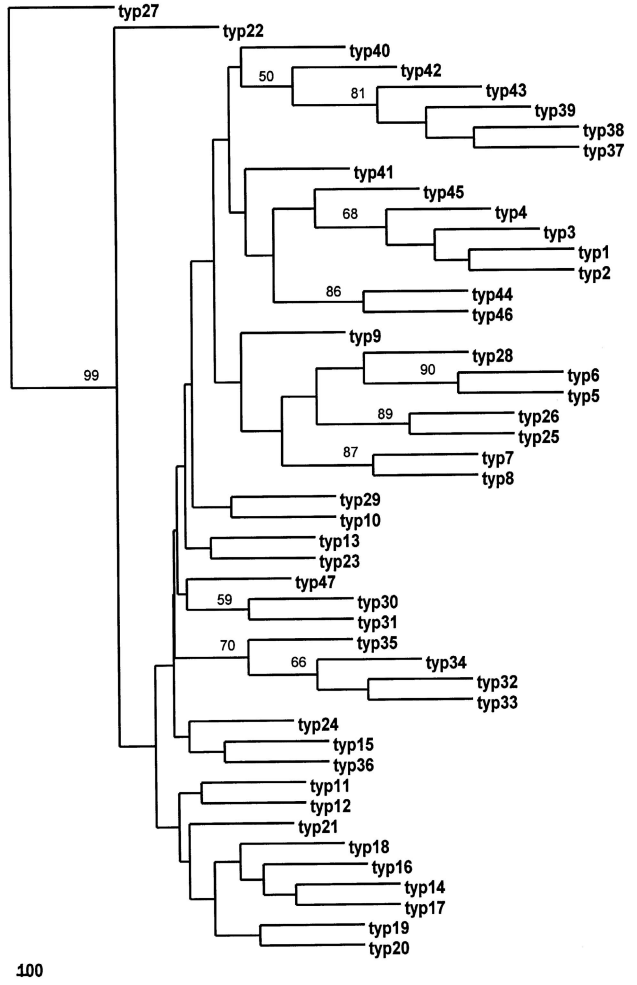

Figure 5 Consensus NJ tree based on 1,000 replicates of the 47 HVS1 types in table 2, according to the maximum-likelihood distance method with transition:transversion ratio of 30:1. Numbers indicate >50% bootstrap support for the group to the right of the fork (all other groups are less supported). The regional source for the types is indicated in table 2. Type 27 is likely to be of nonaboriginal maternal ancestry.

recollection). Social structure of recent traditional groups clearly defined the tribal sections with which marriage was acceptable, so that migration between the Warlpiri and AR language groups would be extremely unlikely, although not impossible. The Warlpiri have been described as strongly ethnocentric, although trade routes over long distances certainly existed, and creation stories of the Warlpiri and groups to the southwest and southeast contain common elements (Meggitt 1962, pp. 16–136) that may reflect shared ancient ancestry. During the colonization period, dispersion of families may also have led to gene flow in non-traditional directions.

Some HVS1 types vary little from the CRS, and one type (type 47), which occurs in three individuals from Yuendumu, is identical to the CRS. Since, in living memory, there is nothing in the family history to suggest nonaboriginal ancestry, the two remaining possibilities are that admixture occurred in the more distant past or that the CRS also evolved in Australian populations. This would be in contrast to the very distinctive Australian HVS1 types that are seen in specific regions (e.g., southern AR, types 18 and 19; northern AR, types 5 and 7; and AD, types 37, 38, and 45).

With regard to the founding populations of Australia, several points emerge. The genetic-distance calculations place Papua New Guinea highland sequences nearest to both Australian groups, lending support to the model of more-recent shared ancestry. However, the specific nucleotide variants in the sequences are different, so acceptance of common ancestry also implies that the ancestral mitochondrial gene pool from which both populations arose was diverse at the time of entry into Sahul and/or that considerable diversification has occurred since that time.

Evolution within Australia may account for most diversity within and both between the AD and AR groups and between the northern and southern AR sequences. It is also possible that the most diverse HVS1 types represent immigrant mitochondrial lineages that are different and possibly earlier than those that vary little or not at all from the CRS, which may be descendants of later immigrants. The tribal groups as described at the time of European colonization may represent comparatively recent culturally defined populations, with the mito-

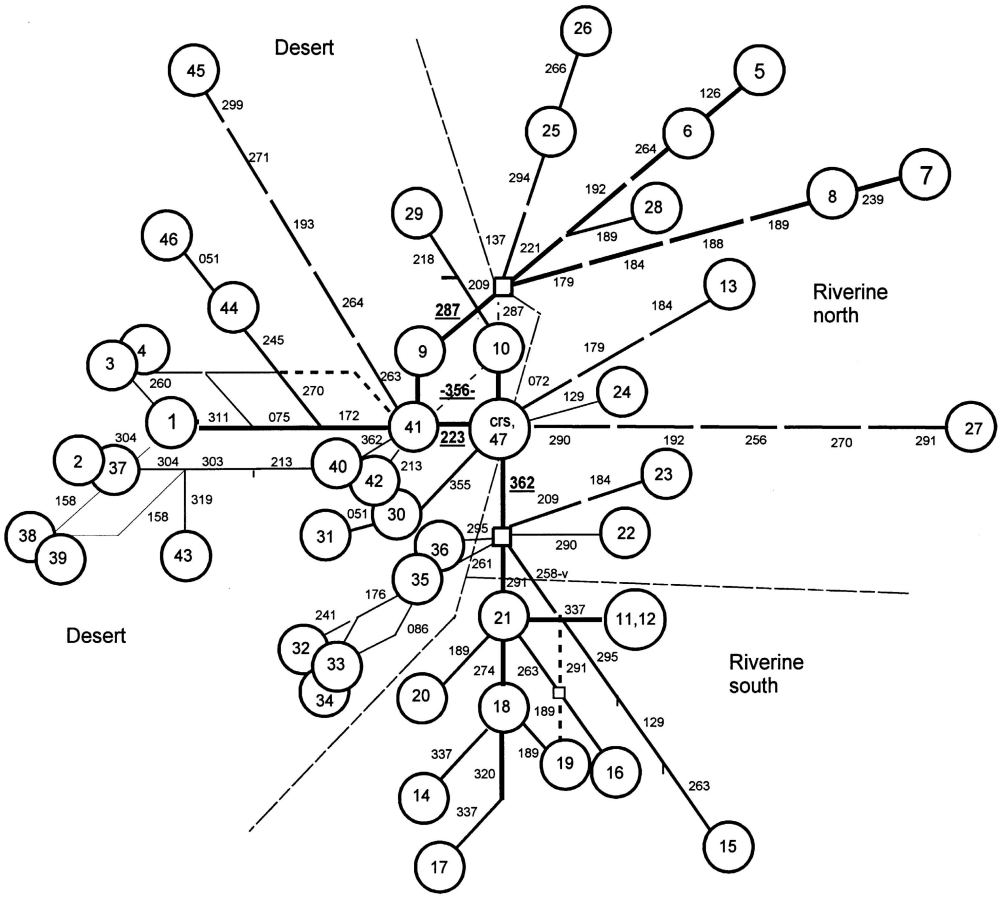

Figure 6 Median network of Australian HVS1 types (numbers in circles correspond to types in table 2). Nucleotide sites (from which the prefix "16" has been deleted) are marked along the lines. A transversion in three types (types 14–16) is considered before a more frequently occurring substitution (i.e., 291). This creates difficulty in the placement of type 11, which has two common variants with type 12 but does not vary at 362. Types 11 and 12 have nevertheless been placed together in the network. Nucleotide sites 051, 129, 263, 291, 295, 337, and 179-192 appear more than once and are suggested to have occurred independently. Main separators are 223, 287, 291, 356 and 362 (also treated as independent but leading to two main groups).

chondrial genotypes defining a broader population structure with identifiable nucleotide-substitution sites that act as "signatures" of earlier population links. These are the nucleotide-substitution sites determining the main separations and connections in the median networks, as already noted—namely, nucleotides 16223, 16287, 16291, 16356, and 16362. Site 16223 is a "T" in most HVS1 types from either the AD region or the northern AR region but is a "C" in exclusively southern AR types, which vary at nucleotide(s) 16291 and/or 16362. Distinctive types 5, 6, 7, 8, 25, and 26 from the northern AR area are distinguished by substitutions at sites 16287 and 16356, as well as at site 16223.

Examination of the global median network (fig. 7) also reveals 16223 as a major separator, as is 16311, which leads to a group encompassing two Australian types, two Papua New Guinea highlander types, three Pygmy types, and three !Kung types. Nucleotide sites 16287 and 16356 lead only to Australians, whereas 16362 and 16291 are major separators in a group that includes southern AR Australians, one AD individual, and South American lineages II–IV. Interestingly, the South American lineage I, Papua New Guinea coastal, and Pacific types are in an exclusive group having no substitution sites in common with the other types in the network—except for sites 16184 and 16189, which, as has been contended above, are sites that have mutated independently in many populations and that therefore are treated last in the network.

Resolution of the NJ trees proceeds a little differently, since the method cannot make decisions about likely parallel events and, for example (fig. 5), has taken account of frequent variation in the region between 16179 and 16192, which we have treated last in the median

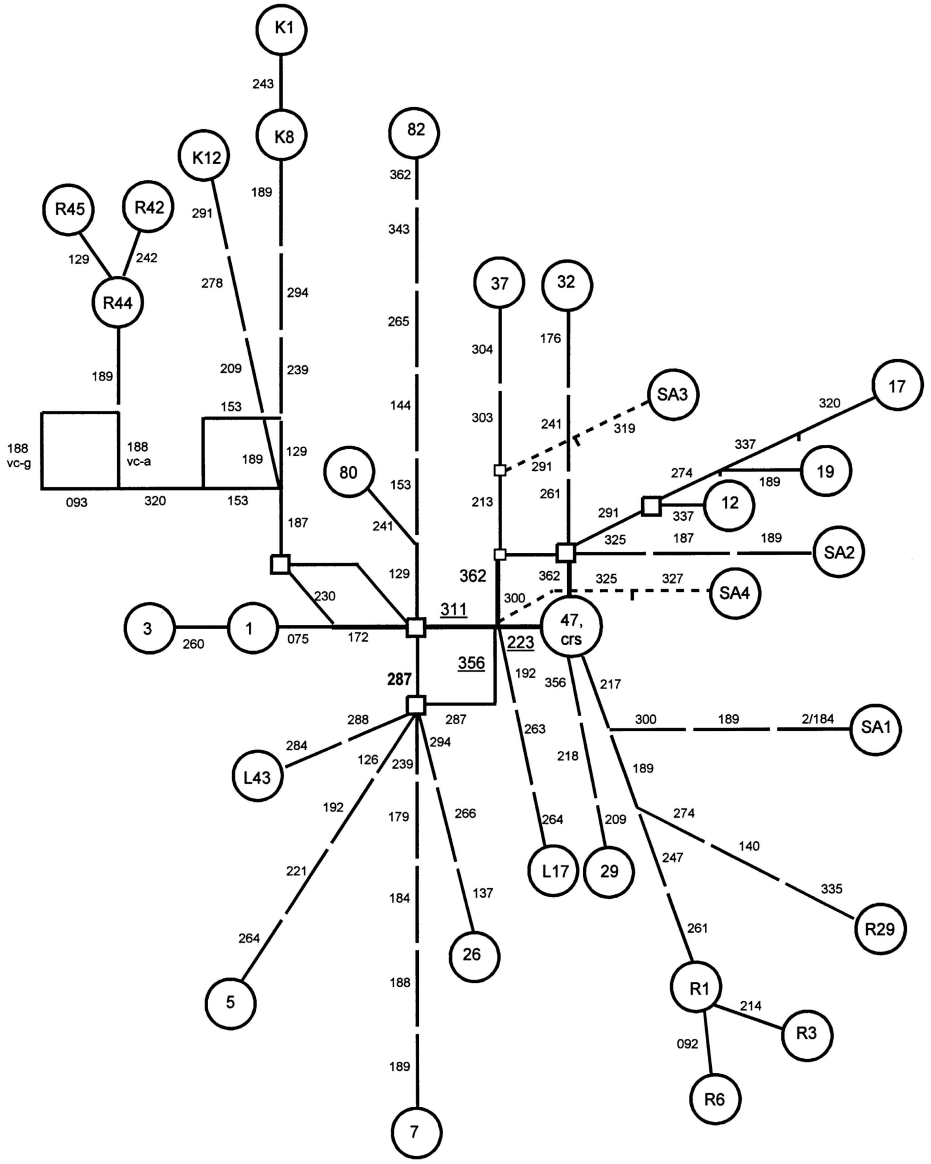

Figure 7 Median network for Australian HVS1 types and several global types: 1 and 3 = AR and AD; 5, 7, and 26 = northern AR; 12, 17, and 19 = southern AR; 29, 32, 37, and 47 = AD; L17 and L43 = western Australia (Lum et al. 1994); R1 and R29 = Pacific; R3 and R6 = Papua New Guinea coastal; R42, R44, and R45 = Pygmy (Redd et al. 1995); 80 and 82 = Papua New Guinea highland (Vigilant et al. 1991); K1, 2, 3, 4, K8, and K12 = !Kung (Vigilant et al. 1991); and SA–S4 = South American lineages I–IV, respectively (Horai et al. 1993). Nucleotide sites (from which the prefix "16" has been deleted) treated as independent events are 291 and 179-192 (treated last). The sites that control the main separations are 217, 223, 311, 356, 287, and 362.

network. Comparison of the global median network with the consensus neighbor-joining tree (fig. 8) identifies artificial resolutions, the most noteworthy of which is the placement of L43 (Lum et al. 1994) on a separate branch parallel to the "pseudogene" (Zischler et al. 1995) used as an outgroup (which is not used in the network, since there are 19 sites that vary from the reference sequence, which thereby places it too far away). This resolution is on the basis of two common substitution sites (16284 and 16288) that do not occur in any other types included in the median network. However, L43 is easily placed in the median network with other Australians (HVS1 types 5, 7, and 26).

Both the variable rate of substitution and the likelihood of multiple hits at some nucleotide sites that we and others have observed highlights the difficulty in the estimation of evolutionary rates for the mitochondrial D-loop, and, in agreement with Ruvolo (1996), we are

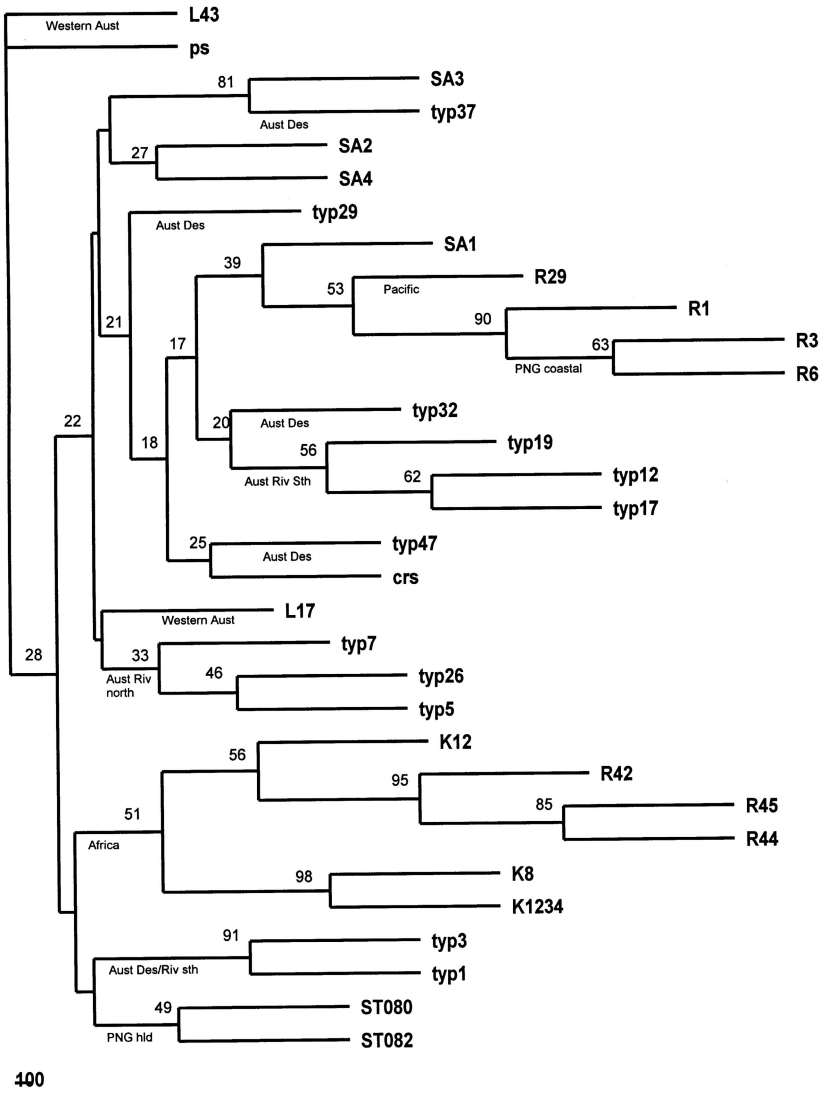

Figure 8 Consensus NJ tree based on 1,000 bootstrap replicates, according to the maximum-likelihood distance method with transition: transversion ratio of 30:1. The data set is as in figure 3. ps = pseudogene (Zischler et al. 1995). Numbers at the fork indicate bootstrap support for the group to the right of the fork.

reluctant to use hypervariable sequences alone to make new estimates based on this data set. We anticipate that more usable information will follow when HVS2 sequences and RFLP data are obtained for the HVS1 types. However, if one uses the rate of 33%/million years, as estimated by Ward et al. (199l), and the MSD estimates (table 3), the implied common ancestry for AR/AD is 51,500 B.P., that for AR/New Guinea highlanders is 60,000 years B.P., and that for AD/New Guinea highlanders is 52,700 B.P. If the estimate of 16.6%/million years, made by Soodyall et al. (1996), is used, then the implied common ancestry for AR/AD is 102,400 B.P., that for AR/New Guinea highlanders is 119,300 B.P., and that for AD/New Guinea highlanders is 104,800 B.P. All of these estimates are feasible in the light of archaeological evidence for the occupation of Australia.

In conclusion, definite population structure is identifiable in mt HVS1 types from living aboriginal participants from Australia, although some gene flow is suggested between AD (Warlpiri) and AR. Genetic distance and mismatch/intermatch frequencies support the model of longer time separation between Australians and Africans than between Australians and either New Guineans or other Pacific groups. The model for separate migrations into New Guinea, leading to the consolidation of earlier immigrants in the highlands, is supported by the observation of highland people being closer, in genetic distance, to the Australians than to New Guinean

coastal and Pacific people. This model is also supported by the confirmed absence of the 9-bp deletion in Australian samples, in the light of Redd et al.'s (1995) confirmation of its presence in Pacific populations, including coastal New Guineans. However, the Australian and New Guinea highland mitochondrial sequences are different, so further work is required in order to address suggestions of descent from the same biological group.

Considerable diversity was also generated within populations more recently, and it has increased as later immigrants have moved into the continent. This fact warrants further investigation, especially in areas such as Australia and the Americas, which, evidence suggests, are the extreme regions into which human populations expanded in prehistoric times. Pleistocene population expansion has frequently been argued as the trigger for the movement of human groups into previously unoccupied areas such as Sahul. Population dynamics in prehistoric Australia are recognized as complex and compounded by postcolonization reduction by genocide and introduced disease, and estimation of population-expansion parameters used in other studies (Rogers and Harpending 1992) is unlikely to be informative. Further study is warranted, to elucidate evolutionary history, especially in areas such as the AR regions, which were well populated in the Pleistocene (Allen and O'Connell 1995; Pardoe 1995).

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