Mitochondrial DNA sequences in ancient Australians: Implications for modern human origins

Gregory J. Adcock*†, Elizabeth S. Dennis‡, Simon Easteal§, Gavin A. Huttley§, Lars S. Jermiin§¶, W. James Peacock‡‖, and Alan Thorne*

*Research School of Pacific and Asian Studies and §John Curtin School of Medical Research, Australian National University, Canberra ACT 0200, Australia; ‡Commonwealth Scientific and International Research Organization Division of Plant Industry, Canberra ACT 2601, Australia; and ¶Australian Genomic Information Centre and School of Biological Sciences, University of Sydney, New South Wales 2006, Australia

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DNA from ancient human remains provides perspectives on the origin of our species and the relationship between molecular and morphological variation. We report analysis of mtDNA from the remains of 10 ancient Australians. These include the morphologically *gracile* Lake Mungo 3 [≈60 thousand years (ka) before present] and three other *gracile* individuals from Holocene deposits at Willandra Lakes (<10 ka), all within the skeletal range of living Australians, and six Pleistocene/early Holocene individuals (15 to <8 ka) from Kow Swamp with *robust* morphologies outside the skeletal range of contemporary indigenous Australians. Lake Mungo 3 is the oldest (Pleistocene) ''anatomically modern'' human from whom DNA has been recovered. His mtDNA belonged to a lineage that only survives as a segment inserted into chromosome 11 of the nuclear genome, which is now widespread among human populations. This lineage probably diverged before the most recent common ancestor of contemporary human mitochondrial genomes. This timing of divergence implies that the deepest known mtDNA lineage from an anatomically modern human occurred in Australia; analysis restricted to living humans places the deepest branches in East Africa. The other ancient Australian individuals we examined have mtDNA sequences descended from the most recent common ancestor of living humans. Our results indicate that anatomically modern humans were present in Australia before the complete fixation of the mtDNA lineage now found in all living people. Sequences from additional ancient humans may further challenge current concepts of modern human origins.

Since its inception more than 25 years ago (1) the debate over recent human origins has focused on two models (1, 2). The regional continuity hypothesis postulates that ever since humans began to migrate out of Africa, more than 1.5 million years ago, there has been a single evolving species, *Homo sapiens*, distributed throughout the Old World, with all regional populations connected, as they are today, by gene flow. Some skeletal features developed and persisted for varying periods in the different regions so that recognizable regional morphologies have developed in Africa, Europe, and Asia.

The other view, the ''recent out of Africa'' model, argues that over the period since humans began to leave Africa there have been several species of *Homo.* In Europe, for example, these species would include *H. erectus* (and/or *H. ergaster*), *H. antecessor*, *H. heidelbergensis*, and *H. neanderthalensis*. Under this model *H. sapiens* emerged in Africa approximately 100 thousand years (ka) before present and spread globally, replacing other species of *Homo* that it encountered during its expansion. This model proposes that all current regional morphologies, especially those outside Africa, developed within the last 100 ka.

These alternative models arose from interpretations of morphological evidence (2). Over the last 15 years, molecular data, particularly nucleotide sequences drawn from populations of living humans, have made an increasing contribution to the debate (3). Analysis has shown that humans have remarkably little mtDNA sequence variation, that many mtDNA lineages are not confined to specific regions, and that the earliest branching lineages are found in East Africa. These findings were interpreted as strongly supporting the recent out of Africa model (4). However, this interpretation failed to recognize that the demographic history of a species cannot be inferred from the pattern of variation of a single nucleotide segment. Patterns of variation in different regions of the genome must be considered and interpreted in the context of paleontological and archeological evidence (3).

Human origins are now being investigated with the use of more general models of population structure and evolutionary genetics (3–6). Different regions of the genome appear to have had different evolutionary histories (7–14), and the idea that the pattern of human evolution can be deduced solely from the pattern of contemporary mitochondrial genome diversity is becoming increasingly untenable (3). There are also indications that the patterns of variation at low-recombining regions of the human genome, including the mitochondrial genome, have been affected by the action of selection and do not solely reflect mutation and genetic drift (15, 16).

Techniques that recover mtDNA from fossils (17, 18) now permit analysis of sequence variation in ancient populations. The first successful recovery of Pleistocene human remains was from the original Feldhofer Neandertal skeleton (19). The mtDNA sequences of this individual and other Neandertal individuals from Mezmaiskaya Cave in Russia (20) and Vindija Cave in Croatia (21) belong to a lineage that diverged earlier than the most recent common ancestor (MRCA) of sequences found in living humans. The results have been widely argued as evidence that Neandertals did not contribute genes to contemporary Europeans, thus supporting the recent out of Africa model (22–24). This interpretation may not be justified. mtDNA is a small component of the total genome, and the failure of a mitochondrial lineage to survive to the present does not imply a similar failure for the remainder of the genome. There is morphological evidence for the survival of Neandertal genes in Europe after the arrival of Cro Magnon people (25, 26).

The availability of modern human remains from the Pleistocene and early Holocene in Australia, with different morphologies ranging from *gracile* to *robust* (27), provides an additional

Abbreviations: ka, thousand years; KS, Kow Swamp; MRCA, most recent common ancestor; LM, Lake Mungo.

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See commentary on page 390.

†Present address: Laboratoire d'Ecologie, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 7625, Université Pierre et Marie Curie, Bât. A, 7eme Etage, 7, quai St. Bernard, Case 237, F-75252 Paris Cedex 5, France.

‖To whom reprint requests should be addressed. E-mail: jim.peacock@pi.csiro.au.

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context in which to investigate the relationship between mtDNA sequence variation and morphology during recent human evolution. Although the geographic and locally adaptive origins of the different Australian morphologies are controversial (28), most workers have linked them to migration into Australia by morphologically distinct groups (29–33). The morphologies of the more *robust* ancient individuals are outside the range of living indigenous Australians (34), but unlike the situation in Europe, there is a consensus that all prehistoric Australian human remains represent part of the ancestry of living Aboriginal Australians. The dating of Lake Mungo 3 (LM3) to 62 ± 6 ka (35, 36) implies that *gracile* morphology arrived in Australia well before *robust* morphology. The location of LM3 in southeastern Australia (Fig. 1*A*), thousands of kilometers from a likely point of arrival in the north of what was then greater Australia, suggests that people may have first arrived substantially earlier than 60 ka ago. We have recovered mtDNA from LM3 and nine other ancient Australians, including *robust* and *gracile* individuals (from 60 ka to <2 ka ago).

Methods

Samples. Human remains come from two sites in southeastern Australia (Fig. 1*A*). The Pleistocene LM3 skeleton and three other *gracile* individuals from the Holocene (LM4, LM15, and LM55) are from LM and other localities in the Willandra Lakes area of western New South Wales. The remaining six individuals (KS1, KS7, KS8, KS9, KS13, KS16) are from Kow Swamp (KS) in northern Victoria, a burial area dated to the terminal Pleistocene/early Holocene (31). All are *robust*. The bone fragments used had been recovered during excavation, but their anatomical position had not been determined.

DNA Isolation and Sequencing. Preparation of bone samples and the extraction of DNA were carried out in separate laboratories 2 km apart in which human DNA had not been previously isolated or analyzed. Bone samples were handled only by G.J.A. and A.T. G.J.A., the only researcher to move between laboratories, never traveled from the amplification laboratory to the sample preparation laboratory on the same day. All solutions were made up in volumes suitable for single use. Samples were prepared by using sterile materials and solutions in a sterile workplace in a draught-free clean room. Plasticware and solutions were decontaminated by treatment with silica (37) and exposure to UV light. Details of the procedures used to minimize contamination can be found in ref. 38.

Internal bone samples, extracted with a bleach-cleaned dental drill, were ground to a powder. DNA was purified by the protocol of Hagelberg and Clegg (39) with a Qiagen Q-5 column (Qiagen, Dusseldorf, Germany) instead of phenol/chloroform and ethanol precipitation or by the silica method (37). Yields of DNA ranged from 0.1 to 2.3 μg/g of bone sample, estimated by comparison with standard DNA samples on electrophoretic gels. DNA was extracted independently from each bone sample at least twice. Animal (cow and turtle) bones that had been handled by humans and mock preparations made without bone also were processed as controls to detect contamination.

Three overlapping segments (HA, HB, HC) were amplified by nested touchdown PCR. Initial reactions using primer pairs 1 and 3 for each region (HA1, HA3, etc., below) were followed by reactions with external primer 3 with an internal primer 2 (HA2–HA3, etc.). The amplifications used 200 nM of each dNTP, PCR buffer (40), and oligonucleotides at 5 ng/reaction. The primer sequences (5′–3′) were HA1 (L16004) CCATTAGCACCCAAAGCTAAGATTC, HA2 (L16040) TGTTCTTTCATGGGGAAGC, HA3 (H16193) GTACTTGCTTGTAAGCATG, HB1 (L16127) CTGCCAGCCACCATGAATATTGYAC, HB2 (L16153) ATAAATACTTGACCACCTG, HB3 (H16306) AATGGCTTTATGTRCTATG, HC1 (L16239) CCTTCAACTRTCACACATC, HC2 (L16257) AACTGCARCTCCAAAGCC, and HC3 (H16401) TGATTTCACGGAGGATGGTG.

Amplification conditions (annealing temperature/time; ex-

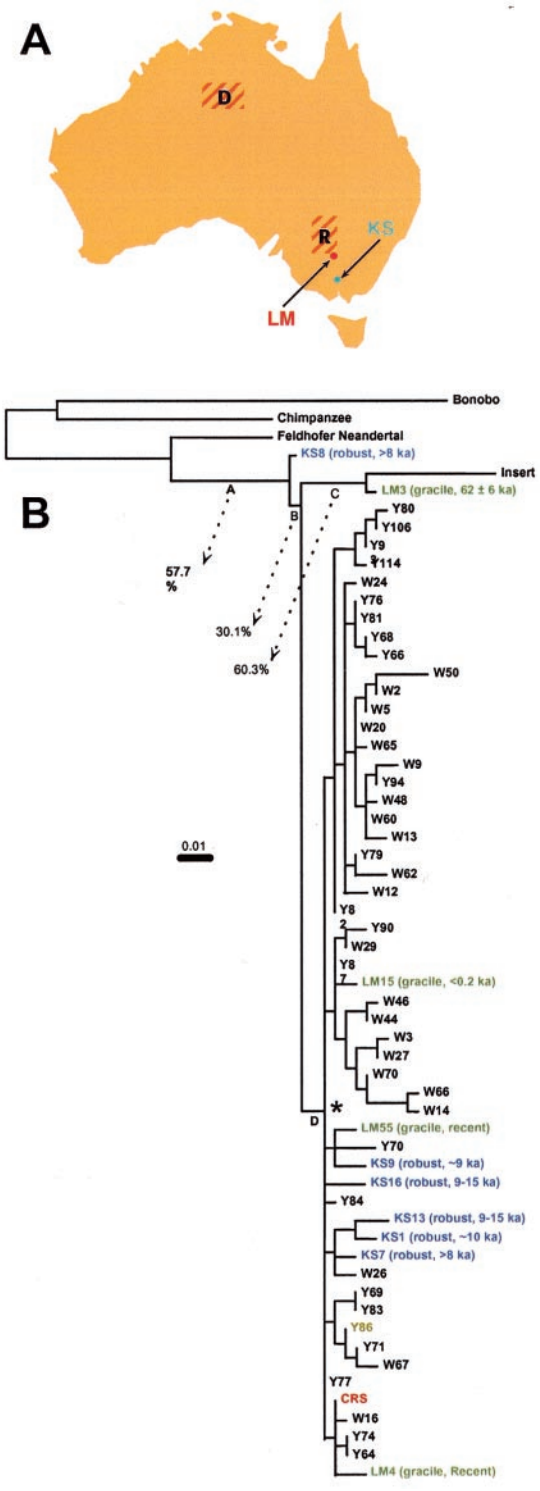

Fig. 1. (*A*) Location of KS and LM and areas from which desert (D) and riverine (R) populations were sampled (41). (*B*) Phylogenetic tree of mtDNA sequences, with their ages, from ancient (blue, *robust*; green, *gracile*) and living Aboriginal Australians (black), the Feldhofer Neandertal, the mitochondrial genome-derived nuclear insert (Insert), and the Cambridge Reference Sequence, with chimpanzee and bonobo sequences used as outgroups. Relative likelihood support (47) is shown for branches A, B, and C.

Table 1. Nucleotide sequence variation at sites that vary among ancient Australian, the Feldhofer, and the Insert sequences

	Nucleotide site
	1111111111111112222222222222222222222222222233333333333333
	7900112234566888900122334444455556667788889990111234555668 8
	8378126998439349919834041347936892344846780391178071567281 7
CRS	ATCCCCTGACTACACTTCTCCTACATGATACACCTCGCACCTCAACTAACCTCTTTTTA
Bonobo	......CAT...T..CCTA.TCGA.CACCAA...C.......AG..CCCT..A.CCC..
Chimpanzee	....T..ATT.....AA.C.TCGA.CA...A......TG....CG..CT.T.T.C.C..
Feldhofer	GCTTTT.ATTC.T-.CC.C.T.GT..A...AG.T...T......G.C..T.....C...
Insert	-......A.......AA..T..G.....C...A.CTATG..CTC.TC.....TC..C..
LM3	...................T.G..........CT.T....T..T......TC....G
LM4	.................T..........G.............C............
LM15	....................T.......................T.......C....G
LM55	..........G.......................T.....................
KS1	.C............T.....T.........................CG..T........
KS7	............T.....T..................T..........C.......
KS8	...................T.G..............TG.......C............
KS9	.C..................T..............T............C.........G
KS13	.C............T.....T....C.G.................TC............
KS16	....................T...................T.............C..C.
Aboriginal	TG CT TCC A CA TCG C C A T CC CT T
Polymorphism	CA TC CTT T TC CTA T T G C TT TC C
GJA	......................C.........................C............
AT	......C...
Contaminant	......................C.........................C............
K13 Cntmnt	-------.............T.........................----------

Nucleotide sites are numbered as in the CRS less 16,000 (50), so that, for example, our site 78 corresponds to site 16,078 in the CRS. Variation in living Aboriginal Australians is shown for individual sites rather than as continuous linear sequences. The following additional sites are variable in samples from living Aboriginal Australians (41): 51, 72, 75, 86, 137, 158, 172, 176, 179, 188, 192, 193, 213, 221, 239, 245, 260, 261, 266, 270, 271, 291, 294, 295, 303, 304, 319. Only differences from the CRS are shown. Information about the bone samples is in refs. 48 and 52, and references therein. GJA, Greg J. Adcock; AT, Alan Thorne.

tension temperature/time; no. cycles) were HA1/HA3 (54–52/25; 73/45; 2, 2, 24 cycles); HA2/HA3 (54–52/25; 73/45; 4, 34 cycles); HB1/HB3 (52–50/25; 73/45; 2, 2, 26 cycles); HB2/HB3 (52–51/25; 73/45; 4, 36 cycles); HC1/HC3 (57–55/25; 73/45; 6, 6, 15 cycles); HC2/HC3 (56–55/25; 73/45; 2, 35 cycles).

Only one set of PCRs was performed per day, and particular PCR primers were never used more than 5 days in succession. Each segment was amplified independently at least three times. Amplification products were sequenced by direct cycle sequencing with the Applied Biosystems *Taq* dideoxy terminator kit and the primers used in the secondary PCRs. This procedure minimizes the chance of detecting sequences of minor contaminants or of amplification errors. Of 157 positive amplification reactions, 10 were identical to the sequence of the researcher performing the amplification experiments (G.J.A.), and the 11th appeared to be an artifact of the PCR technology (K13 contaminant in Table 1). These 11 sequences were excluded from the analysis.

Sequence Analysis. The ancient Australian sequences were compared first with 45 sequences from living Aboriginal Australians (ref. 41 and Table 1). Other Aboriginal Australian sequences have been described (42), but they were not used in the analysis, as their provenance is not well established. The ancient sequences were compared with sequences from populations in different parts of the world (43). Maximum-likelihood analyses of three sequence groups were performed.

We examined 44,927,025 of the possible trees relating the 10 ancient Australian sequences and the 45 sequences from living Aboriginal Australians with the use of TREXML (44). A transition–transversion ratio of 18.8 was assumed following Tamura and Nei (45). Based on model-averaging theory, two consensus trees were obtained with the use of a Kishino–Hasegawa test (46) and a standardized exponential weighting scheme (47). A consensus tree was generated from the 13,000 most likely trees. Because these all had the same basal topology, this part of the phylogeny was further examined by exhaustive comparison of all 105 possible trees involving sequences from chimpanzee (*Pan troglodytes*) (48), bonobo (*Pan paniscus*) (16), Feldhofer Neandertal, KS8, a mtDNA nuclear-genome insert on chromosome 11 (Insert) (49), the Cambridge Reference Sequence (50), LM3, and a grouping of the remaining sequences, arranged as in the topology shown in Fig. 1*B*. Forty trees resulting from this analysis were not significantly less likely ($\alpha = 0.05$) than the maximum-likelihood tree. These were combined with the maximum-likelihood tree to produce a weighted consensus tree (47).

Internal branch support was evaluated for the ancient sequences in combination with 3,453 globally distributed sequences by likelihood mapping (51) with the use of the PUZZLE program (52). The analysis was performed on subsets of up to 250 sequences (the limit of the program). The relative likelihoods of the three possible trees relating each of 140,000 sequence quartets were estimated. Sufficient comparisons were made to ensure that the probability that any one of the 3,453 human sequences was not included in the analysis was $\gg 0.01$.

We performed an analysis similar to the first analysis in which the original set of sequences was supplemented by the Mezmaiskaya Neandertal and 21 sequences from the mtDNA database (database numbers: 14, 30, 31, 32, 35, 36, 38, 44, 46, 51, 79, 84, 102, 116, 125, 181, 187, 209, 860, 945) representing all major lineages known from Africa (53, 54). Analyses were performed with the use of various substitution models and character weighting schemes, and the likelihoods of more than 10^{12} trees were explored.

Median-joining networks connecting the sequences were constructed with the use of the program NETWORK 2.0b (www.fluxus-

EVOLUTION

engineering.com) and the median-joining algorithm (55). Distances between sequences were estimated with the use of the Kimura two-parameter model and the Tamura and Nei model (45), and these were used to construct neighbor-joining (56) trees. Neighbor-joining trees also were obtained for 2,000 bootstrap resamplings of the data.

Results and Discussion

The Ancient mtDNA Sequences Are Authentic. We attempted to isolate mtDNA from six individuals from Willandra Lakes (LM in Fig. 1*A*) and six individuals at KS. We were not able to isolate DNA from two of the samples from the Willandra Lakes region. These samples were from cremation burials. We were successful with the 10 other samples from these two Pleistocene/Holocene deposits (Fig. 1*A*).

In isolating DNA from ancient bone samples, a major concern is the authenticity of any recovered sequence. Because PCR technologies are used, extreme care has to be taken to avoid the amplification of contaminating sequences that may, for example, be contributed by the investigators associated with the samples.

We are confident that the sequences we have obtained from the ancient bone samples are authentic. In each case we made two independent DNA isolates and carried out at least three independent PCR amplifications from each individual, with at least one from each of the two independent isolates, and obtained the same sequences. We used PCR primers to produce overlapping segments in the amplification reactions. In each case the sequences of the overlapping regions were identical for any given individual, indicating that the amplifications were derived from the same DNA source (Table 1). Each individual produced a different sequence in the region of mtDNA that we analyzed. All of the sequences differed from the sequence of the mtDNA segments of the two individuals handling the bone samples, and each of the sequences differed from an invariant mitochondrial insert sequence that occurs in the nuclear genome of many living humans (49).

The isolated DNA segments were short, as expected from degraded mtDNA. None of the samples from the ancient bones was capable of generating the entire 354-bp segment defined by the two terminal primers. In contrast, amplification of this same segment was achieved readily with the mtDNA isolated from the two individuals carrying out the DNA amplifications. To guard against amplification artifact, we sequenced the PCR products directly. Rare contamination was recognized readily and excluded from the analysis (see *Methods*).

We conclude that the mtDNA sequences we report from each of the 10 ancient bone samples are derived from the excavated remains.

The Ancient mtDNA Sequences Do Not Differentiate *Gracile* and *Robust* Morphologies. Direct inspection of the alignment of the ancient Australian sequences with mtDNA sequences from living Aboriginal Australians (Fig. 1*B*) shows that the ancient sequences differ from the Cambridge Reference Sequence at between two (LM55) and 10 (LM3) nucleotide sites (Table 1). Two sequence sites (230, 278) separate the KS8, LM3, Feldhofer, and Insert sequences from all others, suggesting that two of the ancient Australian sequences (KS8 and LM3) diverged before the MRCA of sequences in living Aboriginal Australians (Table 1). Among the ancient sequences, three sites (263, 290, 355) indicate a separation of the LM3 and Insert sequences from all others (including KS8), suggesting that LM3 and the Insert sequences belong to a distinct early lineage.

Maximum-likelihood analysis shows that branches A, B, C, and D in Fig. 1*B* are present in all of the 13,000 most likely trees and in the weighted consensus tree derived from these. They are also present in the weighted consensus of all 105 possible trees involving bonobo, chimpanzee, Feldhofer, KS8, Insert, LM3, and a grouping of the remaining sequences. Branches C and A have approximately twice as much relative-likelihood support as branch B. If the lengths of branches A, C, or D are set to zero, then the resultant trees are significantly less likely ($P < 0.0001$ for each tree) than the maximum-likelihood tree, whereas the tree with the length of B set to zero was not significantly less likely ($0.2 > P > 0.1$). The A, B, C, and D branches were found in 96%, 26%, 76%, and 37%, respectively, of the neighbor-joining trees obtained by bootstrap resampling.

These results show that, with the possible exception of KS8 and LM3, the ancient Aboriginal sequences, including those from individuals with both *robust* and *gracile* morphologies, are within a clade that includes the sequences of living Aboriginal Australians, and that they therefore diverged after the MRCA of contemporary Aboriginal sequences. mtDNA lineages fail to differentiate individuals with clearly distinct morphologies.

Branch C indicates that the LM3 and Insert sequences belong to separate lineages. The existence of branch D indicates that these lineages diverged before the node that marks the MRCA of the living Aboriginal sequences (asterisk in Fig. 1*B*). There is relatively little support for branch B and therefore for a separate lineage leading to the KS8 sequence. We confirmed the grouping of LM3 and the Insert sequences relative to a larger, global sample of contemporary human sequences by likelihood mapping. The tree grouping the LM3 and the Insert sequences was the most likely in 100% of 140,000 quartets that included the LM3 and Insert sequences combined with two sequences sampled at random from the 3,453 human sequences. In all 140,000 comparisons there was absolute support for this grouping.

The LM3 Sequence Belongs to an Early Diverging mtDNA Lineage. The divergence of the LM3 sequence before the MRCA of contemporary human sequences is indicated by its grouping with the Insert sequence (Fig. 1*B*), which other reports have suggested diverged before the MRCA of sequences in living humans (49, 53). Expansion of the maximum-likelihood analysis to include the Mezmaiskaya Neandertal sequence and sequences representing the major African lineages (53, 54) further confirmed the grouping of LM3 with the Insert sequence. The grouping was found in all trees with high likelihood values for all parameter configurations. The grouping of these two sequences would occur if the LM3 sequence were an allelic variant of the Insert sequence. This possibility can be ruled out because two of the primers (HA1 and HA2) used to amplify the LM3 DNA are complementary to regions of the mitochondrial genome that are not present in the Insert, and because there are 14 nucleotide differences between the regions sequenced for LM3 and the Insert. These differences are more than would be expected between two alleles at a locus in the human nuclear genome. Previous sequence analysis revealed no allelic variation of the Insert individuals from different parts of the world (49).

In the phylogenetic analysis, trees in which the LM3/Insert lineage branched before the MRCA of contemporary human sequences were not significantly more likely than trees in which this lineage diverged after the MCRA of contemporary human sequences. The branching order, and the basal topology of the tree generally, are highly sensitive to parameter configurations, and they could not be established with confidence. Median-joining network analysis of the ancient Australian and the Neandertal sequences with the representative contemporary African sequences also resulted in uncertainty about the basal topology of the tree. In the minimum-spanning tree the numbers of changes at sites 16,278, 16,311, 16,129, 16,230, 16,093, 16,189, 16,223, and 16,262 were 10, 9, 7, 7, 5, 5, 5, and 5, respectively.

Although this analysis did not reliably establish an early divergence of the LM3/Insert lineage, it demonstrated that the lineage is unusually long. We confirmed the latter conclusion by comparing the distribution of pairwise differences between the LM3 and 3,453

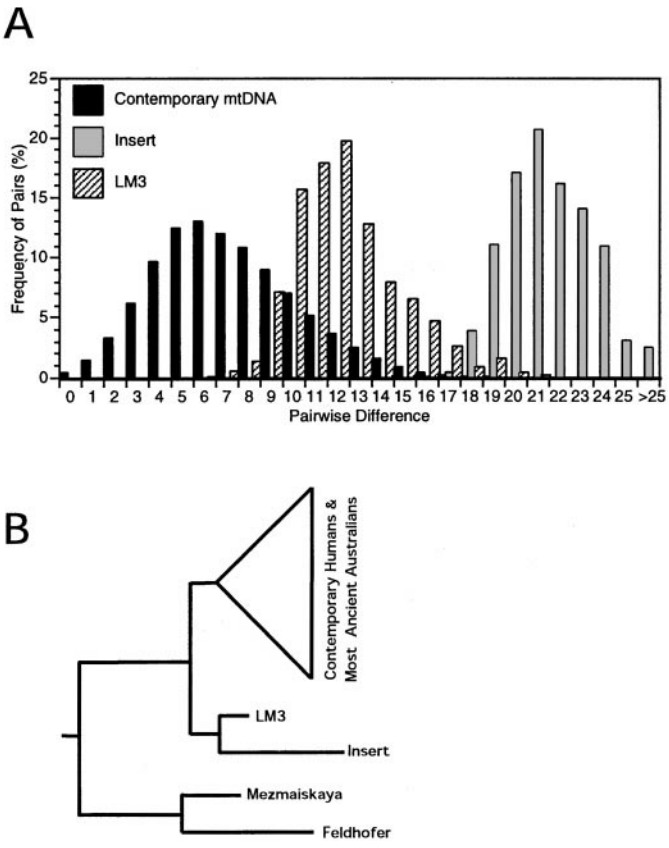

Fig. 2. (*A*) Distribution of pairwise differences ($\approx 6 \times 10^6$ comparisons) of mtDNA sites 12089–16387 among 3,453 contemporary human sequences from the mtDNA database (43), between these sequences and the Insert and LM3 sequences. The distributions are normalized to sum to 100. (*B*) Likely phylogeny of mtDNA sequences in ancient and contemporary humans.

contemporary human mtDNA sequences, the distribution between the Insert sequence and the same sample of contemporary sequences, and the distribution of differences among the 3,453 contemporary sequences (Fig. 2*A*). The range of differences between the LM3 sequence and the contemporary sequences (6–21, mode = 12) is at the upper end of the range of differences among contemporary sequences (0–23, mode = 6). The range of differences between the Insert sequence and the contemporary sequences (16–28, mode = 21) extends well beyond the range of differences among contemporary sequences, indicating either that the Insert has evolved faster than sequences in the mitochondrial genome or that the LM3/Insert lineage diverged earlier. The first possibility is unlikely because in mammals the nuclear genome evolves much more slowly that the mitochondrial genome, and because a high rate of substitution at the Insert locus would be associated with a high level of sequence diversity within human populations, which is not observed (49). There is also no indication of an accelerated rate of evolution in other nuclear genome inserts from the mitochondrial genome (57). The more likely explanation is that the lineage leading to the Insert and LM3 sequences diverged before the MRCA of living human mtDNA sequences.

Implications for Human Origins. LM3, whose sequence is reported here, is the oldest individual dated accurately, and possibly the oldest human, from which DNA has been successfully recovered and analyzed. We conclude that his mtDNA and the Insert sequences form a monophyletic group relative to all of the other human sequences that probably diverged before the MRCA of living human mtDNA sequences (Fig. 2*B*). This conclusion implies that the most divergent known mtDNA lineage from an "anatomically modern" human is from an Australian individual. This finding does not imply that all living people originated in Australia, any more than previously described deep lineages in Africa demand a recent origin of humans on that continent. Deep lineages in Africa and our finding of an even deeper lineage in Australia are consistent with a number of possible models of the demographic and evolutionary history of our species.

Sequences from the lineage that includes LM3's mtDNA no longer occur in human populations, except as the nuclear Insert on chromosome 11. The fact that LM3's morphology is within the range of living indigenous Australians indicates that the lineages of the alleles contributing to this *gracile* phenotype have survived. In contrast, the mtDNAs of the *robust* KS individuals belong to the contemporary human lineage. Their distinct *robust* morphology has not survived intact, implying that the allelic lineages of many of the genes that contribute to this phenotype have been lost.

Lack of association between the survival of nuclear and mtDNA lineages is expected because they have different transmission patterns between generations. This point is emphasized by the high frequency of the Insert on chromosome 11 in many human populations (49). Despite having the Insert, none of these populations have the LM3/Insert mtDNA lineage from which the Insert must originally have been transferred. There must have been genetic exchange between people with mtDNA genomes from the LM3/Insert lineage and those with the contemporary lineage. Similar exchanges between people with other Pleistocene mtDNA lineages, like that of the Feldhofer Neandertal individual, may have occurred.

The genealogies of mtDNA sequences in most human populations, including Aboriginal Australians (41), characteristically have very little hierarchical branching structure. This pattern of sequence variation is consistent with a population expansion following a population bottleneck (58) and is generally taken as supporting the recent out of Africa model. Under this model, all contemporary sequences spread globally with an expanding population that replaced all other people and all other lineages. Africa has been postulated as the source of the expansion because some populations in Africa have more sequence diversity than populations anywhere else.

Our data present a serious challenge to interpretation of contemporary human mtDNA variation as supporting the recent out of Africa model. A separate mtDNA lineage in an individual whose morphology is within the contemporary range and who lived in Australia would imply both that anatomically modern humans were among those that were replaced and that part of the replacement occurred in Australia.

An alternative explanation is that the LM3/Insert mtDNA lineage was replaced by a spread of the "contemporary" mtDNA lineage through late Pleistocene human populations under directional selection pressure. A selective sweep of the contemporary mtDNA lineage through the early Australians after initial colonization is consistent with the pattern of mtDNA variation in Aboriginal populations (41). It explains the divergent LM3/Insert lineage in skeletally *gracile* people without implying the replacement of these people by a later wave of immigrants from Africa. If the mtDNA lineage was replaced as a result of selection for a new mtDNA sequence it would not follow that the lineages of nuclear genes would have changed. The lineages would have been retained and may well be represented in contemporary indigenous Australians. LM3 and his contemporaries, as well as the more recent *robust* KS individuals, all could have been ancestors of living indigenous Australians.

Our success in isolating and analyzing mtDNA from several late Pleistocene and early Holocene individuals should encourage fur-

ther attempts to recover mtDNA from ancient human remains. Additional ancient mtDNA sequences may reveal other distinct mtDNA lineages in Pleistocene human populations. Our analysis has shown that anatomical features and the mtDNA of particular individuals may have different evolutionary paths, and some nuclear gene lineages have genealogical and/or geographical patterns that are different from those of mtDNA (8–10, 14). This difference limits the use of ancient DNA in tracing human evolutionary history because, in most cases, only mtDNA can be isolated and analyzed from ancient material. A fuller understanding of the genetic basis of recent human evolution will require more extensive investigation of nuclear genome variation.

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