

# African Populations and the Evolution of Human Mitochondrial DNA

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The proposal that all mitochondrial DNA (mtDNA) types in contemporary humans stem from a common ancestor present in an African population some 200,000 years ago has attracted much attention. To study this proposal further, two hypervariable segments of mtDNA were sequenced from 189 people of diverse geographic origin, including 121 native Africans. Geographic specificity was observed in that identical mtDNA types are shared within but not between populations. A tree relating these mtDNA sequences to one another and to a chimpanzee sequence has many deep branches leading exclusively to African mtDNAs. An African origin for human mtDNA is supported by two statistical tests. With the use of the chimpanzee and human sequences to calibrate the rate of mtDNA evolution, the age of the common human mtDNA ancestor is placed between 166,000 and 249,000 years. These results thus support and extend the African origin hypothesis of human mtDNA evolution.

THE MOLECULAR STUDY OF GENES FROM DIFFERENT ORGANISMS is helping biologists to build trees relating these organisms to one another. These trees contain information about both the order of branching of lineages linking ancestors to modern descendants and the approximate times at which the branching events occurred. The success of the molecular tree approach to the study of evolution results from two major, previous findings: Molecular evolution is dominated by mutations that are inconsequential or nearly so from the standpoint of natural selection (1), and these mutations accumulate at fairly steady rates on surviving lineages (2, 3). Perhaps the best illustration of the second point is the demonstration that the evolutionary rate for silent mutations in protein-coding genes is almost identical among most of the chromosomal genes of plants, animals, and bacteria (3, 4).

In this article, we describe the use of mitochondrial DNA (mtDNA) as a tool for unraveling the genealogical history of our species. There are two reasons why this molecule is attractive for this purpose: It is maternally inherited (5), so that trees relating mtDNA

types are readily interpreted as genealogies reflecting the maternal history of our species, and it evolves quickly (6), so that many differences arise even among mtDNAs from closely related populations.

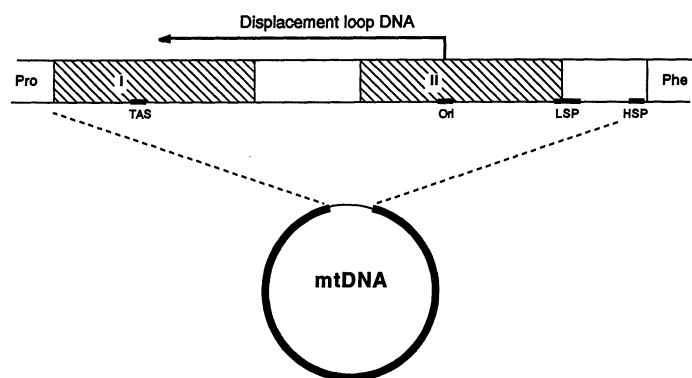
Previously, Cann *et al.* (7) presented the results of a worldwide survey and proposed that all contemporary human mtDNAs trace back through maternal lineages to an ancestral mtDNA present in an African population some 200,000 years ago. At first, this proposal was rejected because of confusion over conceptual issues (8, 9). Now that the conceptual conflicts have largely been resolved, attention has focused on whether the mtDNA data justify the specific hypothesis that the common mtDNA ancestor lived in an African population roughly 200,000 years ago. Among the perceived weaknesses (10–14) of the Cann *et al.* study (7) are that it used an indirect method of comparing mtDNAs, namely restriction analysis; used a small sample made up largely of African Americans to represent native African mtDNAs; used an inferior method (that is, the midpoint method) for placing the common mtDNA ancestor on the tree of human mtDNA types; gave no statistical justification for inferring an African origin of human mtDNA variation; and provided an inadequate calibration of the rate of human mtDNA evolution. Some of these criticisms have been addressed elsewhere (15–19); our purpose here is to present (i) the results of a study of sequences of two hypervariable mtDNA segments from 189 individuals, including 121 native Africans; (ii) a tree relating these sequences to a published chimpanzee sequence, thereby permitting the use of a superior method (that is, the outgroup method) for placing the common human mtDNA ancestor on the tree; (iii) more rigorous statistical tests of the geographic origin of the mtDNA ancestor (including a new test); and (iv) a new estimate, based on a comparison of the chimpanzee and human sequences, of when the human mtDNA ancestor lived.

## Sequences and Geographic Specificity

The control region (Fig. 1) is an 1122-base pair segment of noncoding DNA that is the most rapidly evolving and polymorphic region of the human mtDNA genome (16, 17, 20, 21). Sequence analysis of this region therefore affords the maximum resolution for distinguishing among even very closely related mtDNAs. The 189 individuals studied included 121 native Africans from the following sub-Saharan populations (and places): 25 !Kung (Botswana and Namibia), 27 Herero (Botswana), 1 Naron (Botswana), 17 Hadza (Tanzania), 14 Yorubans (Nigeria), 20 Eastern Pygmies (Zaire), and 17 Western Pygmies (Central African Republic). The locations of the African populations are indicated in Fig. 2. The 68 additional individuals include 20 Papua New Guineans, 1 native Australian, 15 Europeans, 24 Asians, and 8 African Americans. The New Guineans

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**Fig. 1.** The human mtDNA control region. The circle represents the circular human mtDNA genome, with the thin line showing the relative size of the control region. Indicated are the flanking proline (*Pro*) and phenylalanine (*Phe*) transfer RNA genes, the two hypervariable segments that were amplified and sequenced in this study (shaded portions), and regulatory elements: sequences associated with premature termination of replication (TAS); the promoters for initiating transcription of the light strand (LSP) and heavy strand (HSP); and an origin of replication (Ori) that initiates the displacement loop (D-loop).

are from various parts of coastal and highland Papua New Guinea; the Australian is from Perth. The Europeans, Asians, and African Americans are heterogeneous with respect to geographic origin (22).

DNA purification, enzymatic amplification of the two hypervariable segments of the mtDNA control region, and direct sequencing of the amplification product were as described by Vigilant *et al.* (16). Comparison of approximately 610 nucleotides from each individual revealed substitutions at 179 sites and length changes at 22 sites. Each unique sequence is termed an mtDNA type; these 201 polymorphic sites defined 135 types among the 189 individuals (23).

Sixteen mtDNA types were found to occur more than once in the sample of 189 individuals (Table 1). People with identical mtDNA types were not found among the geographically heterogeneous Europeans and Asians, but only within individual populations of Africans or within the Papua New Guinea population. There was no sharing of mtDNA types among people from different populations, with one apparent exception. This exception involves a Yoruban and an African American with identical mtDNA types and thus is consistent with the view that African Americans stem mainly from West Africa. Even groups as closely related as the Eastern and Western Pygmies (24) did not share any mtDNA types (16). We conclude that mtDNA types defined by control region sequences display strong geographic specificity, in agreement with other studies of human mtDNA variation that used techniques capable of distinguishing among closely related mtDNAs (7, 16, 20, 25).

An additional implication is that most women have moved their home bases extremely slowly, especially in nonagricultural populations (16, 26). This encourages the expectation that it should be possible, in principle, to determine where the last common ancestor of extant mtDNAs lived. To achieve this, it was first necessary to build a tree relating mtDNAs.

## Tree Analysis

A genealogical tree (Fig. 3) relating the 135 mtDNA types was built using the parsimony method (27, 28), in which a branching network is constructed in an effort to minimize the number of mutations required to relate the types. To convert the resulting network into a tree, the ancestor or root must be placed, which

requires additional information or assumptions.

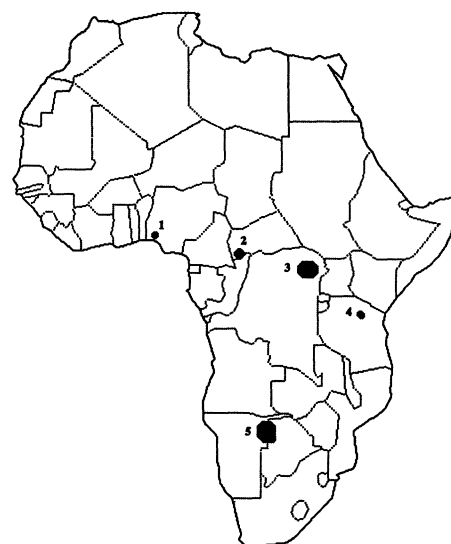
Cann *et al.* (7) used the midpoint method of rooting their tree, assuming that the rate of evolution has been the same in all lineages. If, however, mtDNA evolution were faster in Africans, then the deep African lineages revealed by midpoint rooting of their tree would not indicate an African origin. Instead, these ostensibly deep African lineages would actually be shallow lineages along which more mutations had accumulated. Hence, the tree might not yield any information regarding the geographic origin of the mtDNA ancestor (12).

The outgroup method (28) is a preferable method of rooting a tree because it does not rely on the assumption that the rate of evolution is the same in all lineages. This method uses a sequence from another species (the "outgroup"), such as an African ape, to place the human mtDNA ancestor on the network. The outgroup attaches to the network relating the human mtDNA types at the position that minimizes the total number of mutations in the tree. The point of attachment is the position of the human mtDNA ancestor on the tree. Although Cann *et al.* (7) could not use the outgroup method because high-resolution restriction maps of African ape mtDNA were not available, a control region sequence from a common chimpanzee has now been published (29), and this chimpanzee sequence was used to root the tree in Fig. 3 (30).

**African Americans.** A perceived shortcoming of the restriction map study (7) concerned the use of 18 African Americans as sources of authentic African mtDNAs (11, 13). Evidence supporting the African classification of these types has already been discussed (7, 18). This study greatly extends the previous sampling of sub-Saharan Africans (16, 20) and reveals that the control region sequences of a subset of the 18 African Americans link them closely to native Africans (31). Indeed, as already mentioned, an identical mtDNA type is shared between an African American and a Nigerian Yoruban (type 63 in Fig. 3). Thus, the majority of African Americans may be considered sources of African mtDNA.

## Geographic Origin

One feature of particular interest is the inferred place of origin of the last common ancestor of human mtDNA. The outgroup rooting divides the tree into two primary branches, one consisting of six African mtDNA types, and the other consisting of all of the remaining mtDNA types, including many African types. This is exactly the pattern found before, based on restriction analysis, which



**Fig. 2.** Locations of the native African populations included in this study. Numbered locations correspond to the populations as follows: 1, Yorubans; 2, Western Pygmies; 3, Eastern Pygmies; 4, Hadza; and 5, !Kung, Herero, and Naron.

led to the original hypothesis of an African origin of human mtDNA (7, 19). Furthermore, the next 13 branches (consisting of 16 types) lead exclusively to African types. These 14 deep African branches provide the strongest support yet for the placement of the common human mtDNA ancestor in Africa (32); the statistical significance of the branching pattern can be assessed by two different methods.

**Winning sites method.** First, we can ask how many additional mutations would be required to produce a tree whose branching order implies that the common mtDNA ancestor lived in some geographic location other than Africa. In particular, we ask whether this number is significantly more than the number of mutations in the minimum length, African origin tree (Fig. 3). The winning sites method of phylogeny testing (16, 27, 33) does this by comparing, at each variable nucleotide position, the number of mutations required by two alternative trees. A tree is said to win at a nucleotide position if fewer changes are required at that position by that tree. The binomial distribution is then used to determine whether there is a significant difference in the number of winning sites favoring one tree over the alternative.

To apply this test to the African origin hypothesis, we note that the shortest tree with a non-African origin is obtained by moving the deepest non-African lineage in Fig. 3 from the second primary branch (made up of types 7 to 135) to a position that is deeper than the first primary branch (types 1 to 6). In this case, the deepest non-African lineage leads to type 23, which is from an Asian. Moving type 23 to the common ancestral stem (directly below the arrow in Fig. 3) produces a tree that requires 11 more mutations than the minimum length, African origin tree (34). The winning sites test determines whether these 11 mutations are significantly more than would be expected if both trees are consistent with the data. By applying the test, the African origin tree wins at 13 sites whereas the alternative tree wins at only two sites. The probability of observing a 13 to 2 ratio by chance, if both trees were equally consistent with the data, is about 0.004. Thus, the African origin tree does require significantly fewer mutations than does the shortest alternative tree with a non-African origin.

**Geographic states method.** The second method of testing the statistical significance of an African origin is not concerned with the number of

mutations, but with the distribution of geographic states for a particular tree. This method centers attention on the fact that the first 14 branches in the tree in Fig. 3 lead exclusively to African mtDNA types. The geographic-states test estimates the probability that this pattern of the 14 deepest branches in the tree all being African would have arisen by chance alone, while taking into consideration the fact that the majority of mtDNAs examined were African.

The 135 mtDNAs in the tree fall into 31 clusters that are exclusively African and 24 clusters that are exclusively non-African. The probability that the first  $n$  branches of the tree are of geographic state  $x$ , when the total number of geographically assigned clusters is  $x + y$ , is given by the following formula, based on the hypergeometric distribution (35)

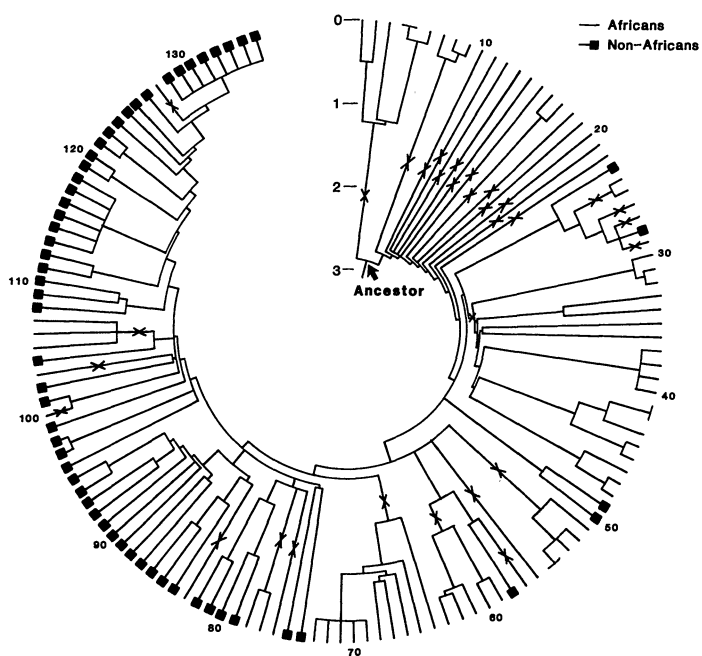
$$P = [x/(x + y)] [(x - 1)/(x + y - 1)] \dots [(\alpha - n + 1)/(\alpha + y - n + 1)]$$

For the minimum length tree,  $n = 14$ ,  $x = 31$ ,  $y = 24$  and therefore  $P = 0.00006$ . Thus, there is a 0.006% probability that the observed distribution of geographic states, in which the first 14 branches lead exclusively to Africans, could occur by chance.

The results of two different statistical tests are therefore consistent with the hypothesis of an African origin of human mtDNA, as evidenced by deep African branches in the phylogenetic tree (36). On the basis of these tests, it is not likely that this pattern of deep African branches arose merely because the majority of the individuals studied (121 of 189) were African. This same result was found in the initial study in which less than 14% of the individuals were African (7). Also, an attempt was made to maximize the possibility of finding a deep non-African branch by including in this study individuals who were previously found on the deepest non-African branches in trees based on restriction map analysis (7, 25). In addition, an independent study of sequence variation in the mtDNA control region of 91 non-Africans and only 10 Africans also supports an African origin (20).

**MtDNA sequence differences.** An African origin is also suggested by the finding that mtDNA sequence differences are bigger among Africans than among Asians or Europeans (Table 2). The average amount of sequence difference that is shown in Table 2 directly

**Fig. 3.** Phylogenetic tree relating the 135 mtDNA types found among the 189 individuals in this study. Markings on the branches indicate the 31 African clusters of mtDNA types; the remaining 24 non-African clusters are not labeled. This tree was constructed by first eliminating uninformative nucleotide positions (nonvariable positions and those variable positions that would have the same number of mutations regardless of the branching order of the tree). The 119 informative sites were then used to determine the branching order by the computer program PAUP (50). The program found 100 trees with a minimal length of 528 steps; there are many more (perhaps thousands) of trees of this length, and there could be shorter trees. The 100 trees we examined differed only in the arrangement of some of the terminal twigs, thereby making it possible to draw conclusions about features common to all, such as the presence of deep African branches on both sides of the root. One of the 100 trees of length 528 was chosen at random and is reproduced here; the consistency index for this tree was 0.34 for the 119 informative sites. The tree was rooted by using a chimpanzee mtDNA control region sequence (29) as an outgroup and the ancestral nodes (branch points) have been approximately placed with respect to the scale of accumulated percent sequence difference, as defined in Table 2. Three pairs of mtDNA types (4 and 5, 41 and 42, and 54 and 55) have 0.0% sequence difference because they do not differ at variable nucleotide positions, but they do differ by length mutations and hence are considered to represent distinct types. The population affinities of the mtDNA types are as follows: Western Pygmies (1, 2, 37-48); Eastern Pygmies (4-6, 30-32, 65-73); !Kung (7-22); African Americans (3, 27, 33, 35, 36, 59, 63, 100); Yorubans (24-26, 29, 51, 57, 60, 63, 77, 78, 103, 106, 107); Australian (49); Herero (34, 52-56, 105, 127); Asians (23, 28, 58, 74, 75, 84-88, 90-93, 95, 98, 112, 113, 121-124, 126, 128); Papua New Guineans (50, 79-82, 97, 108-110, 125, 129-135); Hadza (61, 62, 64, 83); Naron (76); Europeans (89, 94, 96, 99, 101, 102, 104, 111, 114-120).



**Table 1.** Mitochondrial DNA types found more than once in human populations. Types are numbered according to the tree in Fig. 3. Type 63 was in one Yoruban and one African American; all other types occurred only within a population.

Population	<i>n</i>	Type number
Eastern Pygmies	20	4 (2)*, 5 (3), 68 (2), 73 (2)
Western Pygmies	17	2 (3), 48 (2)
!Kung	25	7 (6), 14 (4), 20 (2)
Herero	27	55 (19), 105 (2)
Hadza	17	64 (10), 83 (5)
Yoruba	14	26 (2)
Papua New Guinea	20	135 (4)

\*Numbers in parentheses represent the number of individuals with that type.

reflects the average number of mutations separating mtDNAs from the same population. Because the occurrence and accumulation of these mutations is primarily a function of time, we infer that the greater mtDNA sequence differences in sub-Saharan Africa indicate that this population is older (37).

## Rate of Evolution

An estimate of the rate of sequence divergence of the hypervariable segments of the mtDNA control region was obtained by comparing the average amount of sequence difference between humans and chimpanzee. The average apparent sequence difference between the hypervariable segments of the control region from humans and chimpanzee is 15.1%. Because some nucleotide positions may have mutated more than once, this is an underestimate of the amount of evolution that has occurred in the past several million years since the divergence of the chimpanzee and human lineages. In particular, transitions (mutation of a pyrimidine base to another pyrimidine or a purine base to another purine) are known to occur much more frequently than transversions (mutation of a purine base to a pyrimidine or vice versa) in primate mtDNA (16, 17, 20, 21, 38). Transitions are therefore likely to be underrepresented, and a multiple hit correction is needed to account for the loss of the record of mutations over time.

The apparent mtDNA sequence difference between chimpanzee and humans of 15.1% is adjusted in the following manner in order to account approximately for multiple substitutions at the same nucleotide position. From the distribution of inferred mutations on the minimum length tree, transitions have outnumbered transversions by a ratio of 15 to 1. That is, each transversion can be considered the equivalent of 15 transitions. Because there are an average of 26.4 transversions between the chimpanzee and human hypervariable control region segments, the equivalent number of transitions is 396, and the adjusted estimate of the amount of sequence divergence becomes about 69.2%.

In order to estimate the rate of mtDNA evolution, we need to know not only the amount of sequence divergence between chimpanzee and human mtDNA control regions, but also when human and chimpanzee mtDNAs diverged. The best estimate for the human-chimpanzee mtDNA divergence is about 4 to 6 million years ago (39). The rate of divergence of the hypervariable segments is thus roughly 11.5% to 17.3% per million years.

## Age of the Common Ancestor

This rate of divergence can be used to infer the time of existence of the most recent common ancestor of human mtDNA. The

ancestor corresponds to the deepest node of the tree in Fig. 3 and is placed at 2.87% on the scale of accumulated sequence differences (40). Accordingly, the ancestor existed about 166,000 to 249,000 years ago; even if the time of the chimpanzee-human divergence were as much as 9 million years ago (13), the date for the common mtDNA ancestor would still be only 373,000 years ago. The range of 166,000 to 249,000 years ago is consistent with the range of 140,000 to 290,000 years ago that was derived from restriction maps (7). It is also consistent with an estimate of 172,000 years ago from mtDNA sequences of a protein-coding region (17), and with an estimate of 280,000 years ago from a maximum likelihood analysis of sequences of the control region (41).

However, the above estimates of the age of the common human mtDNA ancestor should be regarded as preliminary, because differences in the pattern of nucleotide substitutions in the control region between humans and chimpanzees may render the correction for multiple substitutions inaccurate (17, 41). There is a need for an intraspecific calibration of the rate of sequence evolution in the human control region, similar to the intraspecific calibration based on restriction maps (42), that does not rely on comparing human and chimpanzee sequences. This will require sequencing many mtDNAs from populations that were founded at known times.

## Modern Human Origins

The present study strongly supports the contention that all the mtDNAs found in contemporary human populations stem from a single ancestral mtDNA that was present in an African population approximately 200,000 years ago (43). The mtDNA evidence is thus consistent with an origin of anatomically modern humans in Africa within the last 200,000 years, with subsequent migrations out of Africa that established human populations in Eurasia (7, 18, 19, 26, 44). Evidence from chromosomal genes and their products is also consistent with the African origin hypothesis (24, 45).

The absence of human mtDNAs that diverged from one another more than about 280,000 years ago leads to the inference that the migrating human populations from Africa probably replaced the resident Eurasian populations that descended from earlier migrations of *Homo erectus* from Africa 800,000 to 1,000,000 years ago (46, 47). This replacement hypothesis runs counter to one view of the fossil evidence; proponents claim that there has been genetic continuity between modern and archaic Eurasian populations (10, 48). However, there are other interpretations of the fossil record that do support the African origin hypothesis (44, 49). A more

**Table 2.** Sequence differences (*d*) among African, Asian, and Caucasian mtDNAs as measured in three studies: 1, this study; 2, Horai and Hayasaka (20); and 3, Cann *et al.* (7). Sequence difference (percent) is the average number of nucleotide differences per 100 bp for comparing pairs of individuals from a population. The variation among values for a given population reflects mainly the differences in the regions of the molecule studied.

Population	1, Control region (610-bp sequences)		2, Control region (482-bp sequences)		3, Restriction maps (entire molecule)	
	<i>n</i>	<i>d</i>	<i>n</i>	<i>d</i>	<i>n</i>	<i>d</i>
African	21*	2.08	10	2.38	20	0.47
Asian	24	1.75	71	1.37	34	0.35
Caucasian	15	1.08	20	0.94	47	0.23

\*Includes three individuals chosen randomly from each of the six native African populations and from the African Americans.

quantitative morphological approach, employing statistical testing, is essential before the African origin hypothesis is either rejected or accepted on the basis of the fossil evidence. It may be that the number of phylogenetically informative traits available from the fossil record is too small to achieve significance in any statistical test.

In conclusion, our study provides the strongest support yet for the placement of our common mtDNA ancestor in Africa some 200,000 years ago. Still, mtDNA is but a single genetic locus; similar high-resolution studies of appropriate segments of Y chromosome and other nuclear DNA markers are needed to arrive at a comprehensive understanding of the evolutionary history of our species.

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8. For example, some biologists were uncomfortable with the idea that all of the maternal lineages of a species must trace back to one female member of some ancestral population. However, the variation present within a population at any mtDNA or nuclear DNA site must ultimately trace back to a single common ancestral nucleotide at that site (1). This ancestral nucleotide was necessarily present in a single individual in that ancestral population (9). Although this single individual would of course have had ancestors that were identical at that DNA site, our focus is on the most recent of these ancestors, referred to as the last common ancestor or more simply the common ancestor. For DNAs that recombine (such as nuclear DNA), the genealogical histories for different sites do not have to be identical, so that the variation at different DNA sites most likely traces back to different ancestral individuals.
9. It has been argued that the last common mtDNA ancestor identified by genealogical analysis (as described above) might represent an early population in which there were not just one female but rather many females bearing the ancestral mtDNA type. Although this appears to be correct, the time required for the ancestral mtDNA type to rise in frequency from one individual to many is likely to have been brief (a few hundred to a few thousand years) compared to the time elapsed since the common ancestral mtDNA type arose (about 200,000 years). Hence the time of common ancestry estimated by genealogical analysis closely approaches the true time.
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23. Not all of these sites were included in the analyses. Length mutations were omitted because they presumably arise by mechanisms different from point mutations; most occurred in only one or two individuals and hence would not alter the tree topology obtained by phylogenetic analysis. Eight of the 179 sites of nucleotide substitution were omitted because they were characterized in only a few individuals. Information for nucleotide sites that were missing from an mtDNA type were ignored when placing that type on the tree. The sequences have been deposited in GenBank under accession numbers (M76235–M76368) and are also available from the authors upon request.
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30. A similar placement of the root was obtained when mtDNA control region sequences from other common chimpanzees (17) and from a pygmy chimpanzee (29) were used as outgroups. The gorilla control region sequence (29) is not a suitable outgroup because of the multiple deletions it has undergone.
31. The eight African American mtDNA types are numbers 3, 27, 33, 35, 36, 59, 63, and 100 in the tree (Fig. 3). With one exception (type 100), the types linked most closely to the African Americans include one or more native Africans.
32. An African origin will minimize the number of intercontinental migration events needed to explain the present geographic distribution of mtDNA types, although the actual number of inferred events is strongly influenced by sample size (A. C. Wilson, M. Stoneking, R. L. Cann, *Sys. Zool.*, in press). Placing the ancestor elsewhere requires postulating on an ad hoc basis a significant amount of back migration from Africa to the non-African source and a considerable loss of mtDNA lineages from the presumed non-African source.
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36. While a bootstrap analysis to derive confidence estimates for various branch points would be desirable as a further statistical test (27), the data set is too large to permit such an analysis.
37. Genetic drift could be an alternative explanation. However, while random loss of mtDNA lineages by genetic drift can alter the frequency of mtDNA types in a population, the number of mutations carried by those lineages that are lost is expected to be the same as the number of mutations carried by those lineages that persist. Hence, drift alone is unlikely to account for the greater differences among African mtDNAs.
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40. The amount of sequence divergence corresponding to the common human mtDNA ancestor that is reported here, 2.87%, is larger than the value of 2% reported previously (16). Similarly, the uncorrected estimate of the average amount of sequence divergence between human mtDNAs and the chimpanzee mtDNA reported here, 15.1%, is larger than the value of 13.6% reported previously (16). This is the result of including more individuals with extremely divergent mtDNA types in the present study and does not substantially alter the resulting estimates of the rate of control region sequence evolution and the time of the common ancestor.
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47. Much of the controversy over the implications of the mtDNA evidence for the origin of modern humans stems from the data of approximately 200,000 years for the time of the common mtDNA ancestor. If, instead, that ancestor were much older (on the order of 1,000,000 years ago), the migrations out of Africa evident from the mtDNA phylogeny would correspond to the well-known movement of *Homo erectus* from Africa into Eurasia. There would then be no replacement controversy. However, it is unlikely that the date for the common ancestor is that old, because in addition to our results there are four other independent calibrations of the rate of human mtDNA evolution (6, 17, 41, 42). All lead to dates for the common human mtDNA ancestor that fall within the range of 140,000 to 280,000 years ago, well after the first hominid colonization of Eurasia.
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