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A New Approach to Studying Modern Human Origins: Hypothesis Testing with Coalescence Time Distributions

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A new approach for testing hypotheses about modern human origins using molecular divergence dates is presented. Coalescence times from many unlinked loci are needed to test the alternative models. Hypotheses are evaluated on the basis of their differing predicted distribution patterns of coalescence times from multiple genes. No single coalescence time from one genetic system is sufficient to reject any of the three alternative models. Several nuclear datasets give recent dates for human genetic ancestors, at approximately the mitochondrial coalescence time, while some nuclear datasets support older dates. Given the overall distribution of available mitochondrial and nuclear coalescence times, the rapid replacement hypothesis is the likeliest model for modern human origins. The unusual nature of the human mitochondrial pattern is highlighted by comparative data from nonhuman hominoids. To understand the pattern of modern human genetic variability better, more nuclear data from all hominoid species are needed. © 1996 Academic Press, Inc.

INTRODUCTION

What would we like to know about the evolution of modern humans (*Homo sapiens*)? Basically, when, where, and how they evolved. The earliest hominids incontrovertibly arose in Africa: earliest documented are australopithecines by at least 4.4 my, followed by the genus *Homo* at 2.5 my. The first hominid species to leave Africa was *H. erectus*. New fossil finds from Soviet Georgia (Gabunia and Vekua, 1995), and older fossils from Java which have been newly dated (Swisher *et al.*, 1994), put the time of *H. erectus'* emergence out of Africa at 1.8 my—almost double what it was just a few years ago. This first emergence of hominids out of Africa by *H. erectus* is universally accepted by paleontologists and not under current debate.

The unanswered issue is the fate of those *H. erectus* who left Africa. Was 1.8 my ago, when they migrated out of Africa, the last time all living humans were connected by a common ancestor? One theory says "yes"—our last common ancestor lived in Africa, before the 1.8-my date when *H. erectus* made it out of Africa. An implication of this model is that human populational differences and "races" are very old. It posits significant genetic continuity, for example, between *H. erectus* found in China and the Chinese peoples living there today. One version of this theory holds that the transition from *H. erectus* to *H. sapiens* was made several times in several places around the world with species' cohesiveness maintained by gene flow (Weidenreich, 1947; Van Valen, 1966; Wolpoff *et al.*, 1984). Hence the name "multiregional hypothesis." If gene flow between populations is nonexistent, this becomes the "candelabra hypothesis" (Coon, 1962).

Another theory says "no"—we all had a much more recent ancestor than *H. erectus* at 1.8 my ago. Some time after *H. erectus* spread throughout the Old World, modern humans, *H. sapiens,* emerged in Africa in one speciation event. Subsequently, these modern humans made a second hominid exodus out of Africa, and they eventually replaced the descendants of *H. erectus* as the two different species encountered each other, during *H. sapiens'* migration out of Africa. This is known as the "rapid replacement" or the "out of Africa" or the "Noah's ark" model (Howells, 1976; Stringer and Andrews, 1988; Cann *et al.*, 1987). This theory holds that populational or "racial" differences between living humans are not very old; for example, *H. erectus* fossil individuals from China would not have been direct ancestors of humans living in China today. It is the possible occurrence of a second hominid migration by *H. sapiens* out of Africa giving rise to all modern humans which is debated.

As stated, these three hypotheses represent extreme forms of a whole range of possible hypotheses about modern human evolution. After all, hominids undoubtedly left Africa more than once or twice over the past 2 my; there were probably many range extensions out of Africa over the past 1.8 my. Therefore one could imagine many multiregional hypotheses, each associated with a different time for our last common ancestor. For example, the first band of *H. erectus* to leave Africa may have died off, leaving fossils but no offspring. If

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so, these *H. erectus* individuals could not have contributed to the gene pool of living *H. sapiens,* and we would need instead to test whether a second or third wave of *erectus* emigrants at a somewhat later time contributed their genes to modern humans. Nevertheless, the extreme form of the multiregional hypothesis, emphasizing 1.8 my as the time of our last common ancestor, is important because *H. erectus* individuals were outside of Africa at that time and thus could have been ancestors to regional populations of *H. sapiens.*

It is important to remember that these theories existed before molecular data were available. The term "replacement model" or "Noah's ark" model can mean and has meant different things to different anthropologists, and different dates (and places of origin) have been associated with the "replacement model." Because of the molecular evidence from mitochondrial (mt) DNA (Cann *et al.,* 1987), an approximate date of 250,000 years for the second hominid exodus and Africa as its origin has become associated with the replacement model.

We want to be able to distinguish among these three models for the origins of modern humans. This paper offers a novel approach in showing how molecular data can help support or refute these hypotheses for modern human origins, and it applies this new method to the existing data. Rather than being a comprehensive review of all the molecular data bearing on modern human origins, it focuses on those datasets providing divergence time estimates and in doing so highlights issues which are important for inferring molecular divergence dates generally. It also continues the debate about the usefulness of molecular divergence dates for inferring modern human origins, following up on issues raised by Stoneking (1993, 1994) and Templeton (1993, 1994). Also, the nonhuman hominoid molecular data are used to demonstrate how a comparative context is helpful in thinking about human genetic variation through time. This paper will focus then on what molecular divergence times can tell us about modern human origins, and it does not discuss in detail inferences about the geographic origin of modern humans based on relative extents of genetic diversity or on molecular tree topologies.

USING MOLECULAR DATA TO STUDY MODERN HUMAN ORIGINS

Relating Molecular Divergence Dates to Population History

Molecular data are potentially able to address the questions of when and where modern humans arose, but not how they arose (except in a very limited fashion). For some researchers (Ruvolo *et al.,* 1993; Stoneking, 1993), "when" is more important than "where" for deciding among the theories, since an African origin is compatible with all of them. After all, an African origin at 1.8 my versus an African origin at 250,000 years is essentially the difference between the multiregional (and candelabra) and the rapid replacement models. However, this point has been debated by Templeton (1994): because "coalescence times of 1 million years, 500,000 years, or 100,000 years are completely compatible with a multiregional hypothesis in which human populations experienced low levels of gene flow", Templeton believes in "the complete irrelevancy of the time of origin in testing the alternative hypothesis" (Templeton, 1994). As Templeton (1994) correctly points out, hypothesis compatibility is very different from a hypothesis testing approach. With the hypothesis consistency approach, we ask the question, "do the data fit my preferred hypothesis?" With the hypothesis testing approach, we ask, "do the data fit my preferred hypothesis but none of the other hypotheses?" The goal of this paper is to reconcile these very different viewpoints concerning the usefulness of molecular divergence times by investigating further their use in hypothesis testing.

Molecular data are useful for inferring divergence times between populations. This is possible because, for the most part, genes evolve at a constant rate over time, providing us with a molecular clock (Zuckerkandl and Pauling, 1962). What does a molecular divergence time tell us? As we follow the history of genetic lineages back through time, all of the lineages in a population join up or coalesce into a common ancestral type, known as the coalescent (Kingman, 1982; Hudson, 1990), and the age of this common genetic ancestor is known as the coalescence time. For any gene, all of the different alleles within a population have a coalescent, regardless of the population's history. However, the object for understanding human evolution is to determine whether a tree of genetic haplotypes is at all informative about population history. For this to be true requires that there be some way of relating gene trees to populational trees.

When a population divides, each daughter population takes some sample of alleles from the original population with it, and if the division did not occur too far in the past relative to effective population size, the coalescence time of each population's alleles is usually greater than or equal to the time of population divergence, thus providing an upper bound for when populations split (Nei, 1987; Stoneking, 1993). In some small proportion of cases, however, the coalescence time of observed alleles can occur *after* a population divergence because of allelic loss (Fig. 1). The probability of this happening is greater for smaller populations, in which allelic loss is more frequent, according to population genetic principles. Thus coalescence times are usually greater than population divergence times, but not always.

Coalescence times can also greatly exceed population

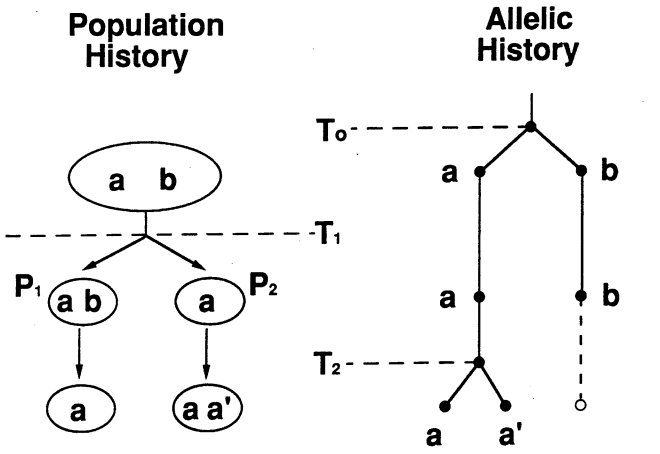

FIG. 1. Scenario showing how a molecular coalescent can occur after a population divergence. (Left) Population history (in which populations are indicated by ovals) shows two alleles (a, b) in an ancestrally polymorphic population. Population divergence occurs at time T_1, and only one population P_1 receives both alleles during population formation. Subsequently, allele b is lost from population P_1; the probability of allelic loss is inversely related to population size. A new allele, a′, arises in population P_2 after population divergence. (Right) Allelic history shows the molecular evolutionary relationships (lines) among alleles (dots). If no alleles had been lost from the populations over time, the coalescence time of all alleles (a, a′, b) would have been T_0, which is greater than the time of population divergence (T_1). Instead, observed alleles (a, a′) have coalescence time T_2, which is less than the time T_1 of population divergence. In this example, what causes the observed molecular coalescence time to be less than the time of population divergence time is the loss of one relatively anciently divergent allele in two descendant populations. Although allelic loss affects all alleles, regardless of their degree of "ancientness" or molecular divergence from other alleles, ancient alleles may nevertheless be lost "preferentially" by chance in some small proportion of cases, causing this effect.

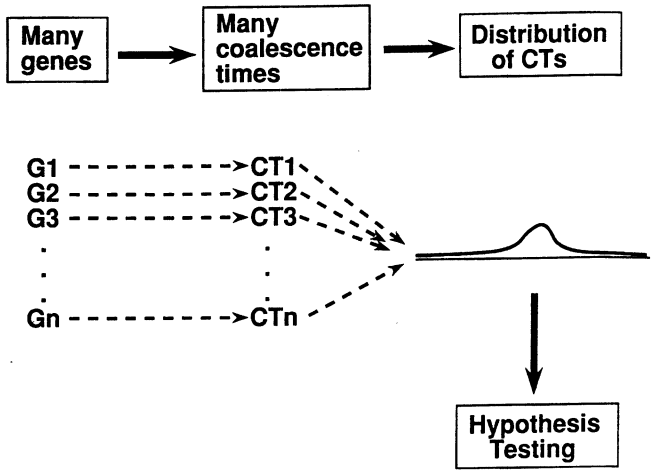

FIG. 2. Outline of the coalescence time distribution method for testing hypotheses of modern human origins. First, datasets from many, preferably unlinked, genes or DNA regions are gathered. Second, coalescence times for all human alleles for each gene or DNA region are estimated. Third, this set of coalescence times is plotted as a frequency distribution. Fourth, the resultant distribution is compared with the three different distributions predicted by the three models of modern human origins (Fig. 3) in order to test them.

divergence times, especially those of anciently polymorphic genes. For example, the HLA gene tree has human alleles which coalesce at 35 my (Ayala *et al.*, 1994). However, the structure of the HLA gene tree excludes it from being a population tree, because, for example, a human allele can be most closely related to a gorilla allele, and not to another human allele (Ruvolo *et al.*, 1994). Therefore the coalescence time of human HLA alleles is not informative for inferring the time of human populational divergence. Although HLA is recognizably an anciently polymorphic system (since many ancient alleles have been preserved, probably because of balancing selection), not every anciently polymorphic gene will similarly preserve a sufficient number of alleles enabling us to identify it as such. Thus we will not necessarily be able to exclude molecular coalescence times from such anciently polymorphic genes as being uninformative for modern human origins. Hence coalescence times from some small proportion of genes are expected to exceed the time of population divergence leading to modern humans by a large factor. Theoretically, whenever modern humans arose, the hominid population giving rise to them is likely to have contained some anciently polymorphic genetic systems, some of which should have coalescence times extending back to *Homo habilis* and further, to the australopithecines.

The Coalescence Time Distribution Method

The above observations underscore the fact that when a population divides, this event creates a distinctive distribution of coalescence times for all the different genes in the genomes of the daughter populations. This leads to a new method of testing among the different models of modern human origins (Fig. 2). The steps are: first, to collect datasets from many genes (preferably unlinked ones); second, to estimate coalescence times for each dataset; third, to plot the frequency distribution of coalescence times; and fourth, to use the observed distribution to test the hypotheses by comparing it with the different distributions predicted by the three models.

What are the predicted distributions for the different models? If there had been a recent divergence of a small population which ultimately gave rise to all living humans, as hypothesized in the rapid replacement model, then this population event should affect *all* haplotype trees for all genes, both mitochondrial and nuclear, and their coalescence times. Therefore as we look at molecular divergence times from many unlinked datasets, if the rapid replacement model is true, there should be a clustering of coalescence times around some relatively recent time (e.g., 250,000 years) corresponding to the population divergence time, with a tapering frequency

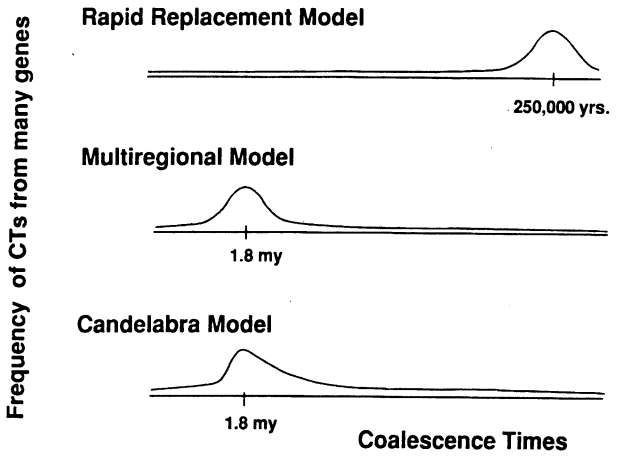

FIG. 3. Qualitatively predicted coalescence time frequency distributions from multiple genes under alternative models for modern human origins. The x-axis shows coalescence times derived from different genetic datasets and the y-axis shows the frequency of coalescence times derived from the datasets. The candelabra model differs from the multiregional model in that more genes indicate lower coalescence times above and below the modal value (due to allelic loss associated with lack of gene flow among populations). All three distributions have long tails at both ends because of the high degree of stochasticity associated with the evolutionary process. The modal coalescence times shown for the different distributions were chosen as reasonable estimates on the basis of fossil (1.8 my) and molecular (mitochondrial, 250,000 years) data in order to illustrate the differences between frequency distributions under the different models. However, the particular values shown are not intrinsic to the models; they can be somewhat greater or smaller and still be compatible with the different alternative models of modern human origins (see text).

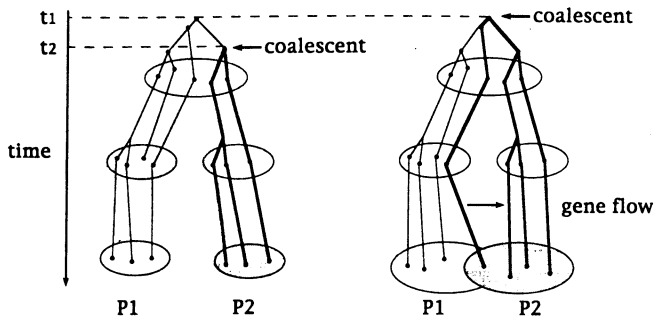

FIG. 4. Gene flow acts to maintain older coalescence times. The history of populations (shown as ovals) through time (y-axis) is shown along with the gene tree of alleles (shown as points connected by lines). Daughter populations P1 and P2 diverge from an ancestral population in both cases. In the absence of gene flow (left), the alleles in population B have a coalescent at time t_2 (with bold lines indicating allelic history). With gene flow occurring between P1 and P2 (right), the alleles of population P2 have an older coalescence time, at t_1.

distribution over the 0- to 250,000-year range because some younger coalescences will occur by chance (as discussed above and shown in Fig. 1). Because different genes give different coalescence times, most of which are expected to be older than the population divergence times, there will also be a tapering distribution of older coalescence times, above 250,000 years. Therefore the rapid replacement model predicts a characteristic distribution of coalescence times inferred from many different human genes, and this distribution is different from those predicted by the other models (Fig. 3). If the multiregional model is correct, there should be a clustering of coalescence times from many different genes predominantly around some ancient date (e.g., 1.8 my), again with a tapering distribution over the 0- to 1.8-my range (Fig. 3), and this will be true for the candelabra model as well. However, the shape of the distribution for the candelabra model differs somewhat from that of the multiregional model (Fig. 3) because of the hypothesized absence of gene flow among populations. For any given gene, the coalescence time for all alleles contained within several populations will be the same whether or not gene flow occurs among populations, provided all alleles have an equal chance of persisting over time. The absence of gene flow reduces the effective population size for each (now separate) population, and this increases the probability of allelic loss. Even though alleles are lost irrespective of their degree of genetic difference, allelic loss on average can only decrease a coalescence time for any given gene. Even without allelic loss, gene flow acts to maintain older coalescence times (Fig. 4). As a consequence, the candelabra model (which hypothesizes no gene flow) has a coalescence time distribution showing more genes with smaller coalescence times compared to the multiregional model at both high and low ends of the distribution. Although many datasets would be needed to distinguish the multiregional model from the candelabra model because their distributions are so similar, both are very different from the rapid replacement model. For the rapid replacement model, there should be a sharper drop-off in the high-end of the distribution (above 250,000 years) than seen in the multiregional distribution due to reduction in population size.

The shapes of the coalescence time frequency distribution curves are very general as described here, and more rigorous formulation of them is needed which could, for example, take into account relative effective population sizes. However, a more rigorous formulation would have to incorporate information about the genome which is currently unknown, such as the frequency distribution of mutation rates for all the genes in the human genome and the proportion of human genes which are anciently polymorphic. Nevertheless, even at this qualitative level, the coalescence time distribution approach is useful because the three alternative models of modern human origins have different and hence distinguishable expected coalescence time distributions associated with them. These predicted coalescence time distributions can therefore be used to test the different hypotheses of modern human origins.

An important implication of the coalescence time dis-

tribution approach is that no *single* coalescence time can be used to reject any of the three models. Philosophically, this is a significantly different approach in which the different models for modern human origins are evaluated according to their relative likelihoods on the basis of all available data, as opposed to the widely used method of outrightly rejecting a model on the basis of data from one gene or genetic system (see, for example, Cann *et al.,* 1987; Vigilant *et al.,* 1991; Ruvolo *et al.,* 1993; Horai *et al.,* 1995). Also, and equally important, coalescence times intermediate between 250,000 years and 1.8 my are expected to occur under all three models and are compatible with all of them. For any gene, the age of a common human genetic ancestor, which gives an upper bound on when populations split, can indeed be useful in testing the different hypotheses for the origin of modern humans. However, we will need to have *many* molecular divergence times inferred from a number of unlinked loci in order to get a *distribution* of coalescence times necessary for testing the relative likelihoods of the different models of modern human origins. Far from being "irrelevant" (Templeton, 1994), molecular divergence times provide an important means of testing alternative models for the origin of modern humans.

Types of Molecular Divergence Dates

Molecular divergence dates are of two types, coalescence times (described above) and times inferred using the method of relative branch lengths, and these are theoretically different. Relative branch length dates have been widely used in the molecular systematic literature over the last 25 years. With the relative branch length method, amounts of genetic distance which have accumulated along branches of the gene tree (i.e., the branch lengths) are used to infer divergence dates, via a mutation rate. A mutation rate is estimated by first choosing a paleontological divergence event with a known date (based on the fossil record) to calibrate the molecular clock. Then for any two living taxa which last shared a common ancestor at this divergence event, the amount of genetic difference between them is divided by twice the amount of time since their common ancestor. (The factor of two means that the mutation rate is expressed as genetic change along a lineage, rather than as genetic divergence between two descendant lineages.) Thus the mutation rate estimates how much genetic difference accumulates along a lineage per unit of time. For any other divergence event, a date can be derived by multiplying the branch length back to the event of interest by the mutation rate.

It is only recently that coalescence times, which have theoretical advantages over relative branch length dates (Templeton, 1993), have started to be estimated from molecular datasets. The advantage of the coalescent approach is that it allows the estimation of error bars associated with the evolutionary process. This source of error in molecular divergence dates, as Templeton (1993) has pointed out, has routinely been ignored by anthropologists and molecular evolutionists working on the problem of modern human origins. Because it is the largest source of error in such calculations, Templeton argues, hypotheses cannot rigorously be tested without considering coalescent error. Whether or not coalescent error is the largest source of error is debatable; Hillis and Moritz (1990) have argued that greatest source of error is associated with calibration times and fossil dates. Nevertheless, coalescent error is significant and should be evaluated and taken into account when inferring divergence times.

Generally, coalescence times are greater than dates derived using relative branch lengths. In the two-allele case, they differ by a factor which is a function of the number of nucleotide differences between the two sequences and the length of the sequence studied (see Appendix for derivation). As the absolute number of nucleotide differences between two human sequences gets larger, this factor approaches one. Therefore, the larger the dataset, the greater the agreement between these two types of dates. In the one case for which both types of dates have been inferred, the difference between them is small (Ruvolo *et al.,* 1993), and this is likely to be true for most DNA sequence datasets investigating human genetic variability. Therefore, for purposes of evaluating the overall pattern of genetic evidence concerning modern human origins, I treat the two types of dates as equivalent and use the general term "molecular divergence date" throughout to describe both coalescence times and relative branch length dates.

GENETIC EVIDENCE PROVIDING MOLECULAR DIVERGENCE TIMES

Mitochondrial Evidence

Restriction Fragment Length Polymorphism (RFLP) Data

In the now classic study of human mitochondrial DNA variation by Cann *et al.* (1987), mtDNA samples of people from around the world were cleaved using restriction enzymes. Sizes of the DNA fragments generated by cutting the circular mtDNA molecule were used to infer the location of restriction enzyme sites. Then the patterns of similarities and differences in cutting sites were used to construct a tree of mtDNA relatedness among individuals. On this tree of mtDNA types, Cann *et al.* (1987) found first that two major clades existed, one consisting exclusively of Africans, and the other containing other Africans plus everyone else. Within this latter clade, African mt types also appeared as most different from others. From this, Cann *et al.* (1987) concluded that Africa was the probable source of all human mtDNA types. Second, only a small degree of genetic difference separated any two humans.

When the greatest genetic distance found between any two human mitochondrial types was converted into time, it corresponded to a molecular divergence date which was very recent, in the range of only 140,000–290,000 years.

The Cann *et al.* (1987) study was criticized on three grounds: (1) it only indirectly surveyed the mitochondrial genome (because restriction enzyme site data are not as exact, and therefore not as good, as DNA sequence data); (2) Africans were not directly surveyed (because African-Americans were used to represent Africans); and (3) their method of tree-rooting did not use an outgroup sequence (chimpanzee) but instead folded the tree in half along its longest branch. Furthermore, more parsimonious trees not having an African origin could be found for these data (Maddison, 1991).

mtDNA Sequence Data

Hypervariable control region sequences. Vigilant *et al.* (1989) sought to address the criticisms of the original Cann study by sequencing DNA. They sequenced the hypervariable subregions of the mitochondrial control region (or D-loop), the fastest evolving mtDNA segment known. They also surveyed Africans and included chimpanzee sequences in order to root their phylogeny. At first, their results (Vigilant *et al.,* 1989) seemed to confirm the original Cann *et al.* (1987) study in indicating both a tree topology supportive of an African origin and a recent divergence time. However, a second publication based on a somewhat larger dataset (Vigilant *et al.*, 1991), although also consistent with an African origin, presented a very different tree from the first study (Vigilant *et al.,* 1989): the first tree showed some !Kung mtDNA types as most different from other human types, while the second tree showed Pygmy types as most different (with the former !Kung types shown as not particularly different). A closer look at the control region data seemed warranted given these differences.

As it turned out, several tree topologies having different implications for modern human origins are consistent with the control region sequence data. The most thorough study to reexamine tree topologies (Maddison *et al.,* 1992) found a minimum of three sets of tree topologies compatible with the control region sequence data. (Trees within a set varied only slightly in the relationships among closely related taxa, but not in their deeper hierarchical structure.) Two of these sets of trees matched the already-published trees with African origins (with either !Kung or Pygmies as most different), but we found a third set of trees not to be consistent with an African origin. This third solution shows several New Guinean types in one clade as sister group to the other human types. However, this third pattern is not necessarily consistent with a New Guinean origin, because within the second clade of all humans New Guineans do not appear as most different. Thus, this third set of trees is not indicative of any one particular geographic origin. Because there are so many possible trees linking the 135 mt types, all of them could not be checked exhaustively to see if there existed additional most parsimonious solutions. Had we let our computers search the space of all possible trees longer, more than three sets of tree consistent with the data might have been found. (Other studies reexamining these data were either less complete or hedged the issue of parsimony. Templeton (1992) examined far fewer trees and found only 100 more parsimonious alternatives compared to the many thousands of equally parsimonious alternative trees found by Maddison *et al.* (1992). Hedges *et al.* (1992) avoided the issue of which most parsimonious trees summarize the data by using a distance analysis, thereby generating only one tree without evaluating alternative topologies.)

The existence of these three different parsimony trees means that an African origin is not unequivocally supported by the human control region DNA sequence data. This is not equivalent to saying that an African origin is disproven or incorrect. Clearly, a contributing factor to this ambiguous situation about tree topology is the quickly evolving nature of hypervariable control region sequences. This same property which makes them so useful for distinguishing among human types is also a disadvantage, because it causes problems in establishing the root of the tree and hence the final topology (Maddison *et al.,* 1992; Kocher and Wilson, 1991). Furthermore, the quickly evolving nature of control region DNA sequences can be problematic for inferring molecular divergence dates. This is because time estimates derived from the control region data are based on *inferred* amounts of genetic difference which involve substantial corrections over the observed DNA sequence differences.

Inferring molecular divergence times from any DNA sequence dataset requires an important step, namely correction of observed sequence differences according to some appropriate model of molecular evolutionary change (see Nei, 1987, for discussion). Why is this necessary? When we compare two DNA sequences between individuals or species, at any particular site the sequences either match or they are different. At a site where they differ, more than one substitution may have occurred along the evolutionary path connecting the two sequences back through their ancestral sequence. The number of observed differences is therefore an underestimate of the true number of substitutions which occurred, and we need to apply a correction method to estimate from the observed DNA sequence differences how many total substitutional events actually occurred. Only this latter value is proportional to time.

All sequence datasets need correction to infer divergence times, but quickly evolving DNA regions require more correction than slowly evolving ones, because they are more likely to undergo multiple substitutions at any given site. This would not be problematic, except

that several correction methods exist based on different underlying models of evolutionary change. For two DNA sequences which are only slightly different, the different models only make slight corrections, so the corrected values tend to agree. But as the two DNA sequences become more different, inferences from the different models begin to disagree, often increasingly so, in terms of how many substitutions they estimate to have actually occurred over evolutionary time. For studying the origin of modern humans, this means that dates inferred from mitochondrial DNA sequences (because of their 10-fold higher average rate of change) require more correction and are more model-dependent than those based on nuclear sequences (Ruvolo *et al.*, 1993). Corrected genetic distances between human nuclear DNA sequences are approximately equal to uncorrected values, because humans show few differences; however, correction is still necessary for most nuclear datasets because of the larger interspecific sequence differences frequently used for calibrating the molecular clock.

Several different dates for the mitochondrial common ancestor have been derived from the hypervariable control region sequence data. Although all are relatively recent, there is considerable variation among them. Vigilant *et al.* (1991) inferred a date of 166–249,000 my using the relative branch length method and assuming humans and chimpanzees diverged at 4–6 my. However, the correction method used by Vigilant *et al.* (1991) is highly parameter-sensitive, and it produces a much wider range of dates consistent with the data than that given above (Ruvolo *et al.*, 1993; Table 2). Using the maximum likelihood method, Hasegawa and Horai (1991) inferred a date of 280,000 years for a 4-my human–chimpanzee divergence; recalibrating this date with a 6-my interspecies divergence time gives 420,000 years. A coalescence time of 256,000 years (with confidence range 153–389,000 years) has been calculated by Templeton (1993), assuming a 6-my human–chimpanzee divergence time.

With the control region data, the *degree* of correction is great, and therefore worrisome in terms of the accuracy of inferred molecular divergence dates. However, mitochondrial DNA regions vary in molecular evolutionary rate, and study of more slowly evolving protein-coding mitochondrial genes provides an advantage. Although fewer differences are expected among such human sequences, fewer differences means less correction is needed. Therefore estimated divergence times from protein-coding mitochondrial genes are potentially more accurate than those provided by the hypervariable control region.

Mitochondrial protein-coding sequences. The mitochondrial cytochrome oxidase subunit II (COII) gene, which I and my colleagues have been using to study primate phylogeny generally, is an example of a slowly evolving mitochondrial gene useful for studying modern human origins. Because COII shows so few differences among humans (a maximum of 0.9%), we combined the COII data with that from another slowly evolving mitochondrial region (the ND4-5 region; Kocher and Wilson, 1991) in order to derive time estimates for the human mitochondrial ancestor (Ruvolo *et al.*, 1993).

In combining datasets, the different selective profiles on these two slowly evolving mitochondrial regions have to be taken into account. Basically, substitutional changes at first and second codon positions accumulate in different ways for the two regions. However, third codon position substitutions accumulate in the same way for both regions (see Fig. 4 in Ruvolo *et al.*, 1993). This is not surprising given the nature of the mitochondrial genetic code; first and second codon position substitutions tend to cause amino acid replacements, while third position substitutions generally do not. This suggests that third position substitutions are relatively free of selective constraints. This observation led us to use just this subset of the data, the third positional substitutions, for deriving molecular divergence dates for humans. (For older divergence events, this approach is not accurate, because third position sites become saturated with multiple substitutions events; instead, all substitutions need to be considered, despite their selective constraints.)

For correcting the COII/ND4-5 data, we applied a maximum likelihood correction model because it is based on a more general model of DNA change than the others, and it also allows the frequencies of A's, C's, G's, and T's to be unequal. With this correction method, and based on relative branch lengths, the age of the common mitochondrial human ancestor is 1/27th that of the human–chimpanzee divergence time. Assuming a human–chimpanzee divergence at 6 my (Hill and Ward, 1988), this corresponds to a molecular divergence time among modern humans of 222,000 years (Ruvolo *et al.*, 1993).

We also used the combined mitochondrial sequence data from COII and ND4-5 to estimate coalescence times for human alleles. We used a variety of mutation rates, which is equivalent to calibrating with different divergence times between humans and chimpanzees. Using 6 my for the human–chimpanzee divergence, the mean coalescence time for human alleles is 298,000 years (somewhat older than the date derived above using a relative branch length approach) with 95% coalescent error bars of 129,000 to 536,000 years. Even if we use 10 my for the human–chimpanzee divergence, which is probably much too ancient, the mean coalescence time is 506,000 years with 95% coalescent confidence limits of 220,000 to 910,000 years. Although the 95% error bars on coalescence times for the mitochondrial data are wide, these values do not include 1.8 my (Ruvolo *et al.*, 1993). Thus, even when the stochastic

nature of the coalescent process is considered, an ancient coalescence time is not compatible with the protein-coding mitochondrial data.

Complete mitochondrial genomic sequences. Recently, entire mitochondrial genomes from two humans (an African and a Japanese) were reported along with those for the other hominoids (Horai *et al.,* 1995) and compared with the existing human reference sequence (Anderson *et al.,* 1981) as a representative European. Using only substitutions which are not under selective constraints (as in Ruvolo *et al.,* 1993), a recent date for modern humans was also inferred: 143,000 ± 18,000 years. In this case, the confidence limits are not associated with the coalescent process, but are error bars associated with length of the DNA region studied and with the mutation rate. It is still necessary to compute coalescent error bars for these data in order to test that the confidence limits do not include 1.8 my (although it is likely they do not). In relative terms, since the date of 143,000 years assumes a human–chimpanzee divergence time of 4.9 my, the molecular divergence date of modern humans is approximately 1/34th of the human–chimpanzee divergence. Assuming instead 6 my for the human–chimpanzee divergence gives 176,000 years for the modern human divergence.

One aspect of these data should be viewed as tentative. As Horai *et al.* (1995) observe, their estimate of 70,000 years for the inferred divergence date between European and Japanese mtDNA is older than that calculated from gene frequency data (55,000 years). Because of suspected inaccuracies with the standard human reference sequence, Horai *et al.* (1995) used an edited version of it, changing bases at seven sites, based on data from other humans and hominoids. However, the original representative "European" mtDNA sequence comes from more than one individual; most is presumably of European origin but some of the sequence (unspecified amounts at unspecified positions) was derived from HeLa cells (Anderson *et al.,* 1981). Since this cell line was established from Henrietta Lacks, an African-American woman (Karp, 1976), some of the "European" reference sequence (even the slightly edited version) is likely to be closer to the African sequence in certain mitochondrial regions. If on average Africans are mitochondrially more distant from other populations, this could potentially make the measured "European"–Japanese genetic difference greater and give an artificially older date for their divergence. This is indeed what the data show: the "European"–African difference is slightly less than the Japanese–African difference: 93 versus 106 bp different, respectively (from Tables 1, 2; Horai *et al.,* 1995). Also, the "European" reference sequence is not exclusively human: five bp at known sites could not be determined unambiguously and were therefore taken to match that of the cow (Anderson *et al.,* 1981). Use of this mosaic "European human" reference sequence potentially affects inferences about divergences between Europeans and other human populations. Its use should have only a negligible effect on the inferred divergence date for modern humans, given what is known about the degree of genetic difference of true European mitochondrial sequences from those of other populations (e.g., Vigilant *et al.,* 1991). Nevertheless, it highlights the need to sequence at least one complete mitochondrial genome, from a single European, to use in place of the standard reference sequence. This same "European" reference sequence has been used in several other mtDNA studies (Cann *et al.,* 1987; Vigilant *et al.,* 1989, 1991; Kocher and Wilson, 1991; Ruvolo *et al.,* 1993).

The approach of using total mtDNA sequences to address modern human origins is powerful, because only in this way can the maximum number of slowly evolving mitochondrial sites be studied. Since these require little data correction, dates derived from them are potentially more accurate. While the problem of the extreme genetic closeness of all living humans cannot be side-stepped, sequencing complete mt genomes reveals the maximal possible existing differences. This is a fruitful avenue of research, which should be pursued not only for what it can tell us about modern human origins, but because comparisons of closely related human DNA sequences can help us elucidate molecular evolutionary processes.

To summarize the mitochondrial data, the different datasets and analyses give a range of molecular divergence times. Assuming a 6-my divergence between humans and chimpanzees, these are: 290,000 years for RFLP data (Cann *et al.,* 1987); 176,000 years for entire mtDNA sequences (Horai *et al.,* 1995); 298,000 years for the combined COII and ND4-5 sequence data (Ruvolo *et al.,* 1993); and for the hypervariable control regions sequences, 249,000 years (Vigilant *et al.,* 1991), 420,000 years (Hasegawa and Horai, 1991), and 256,000 years (Templeton, 1993). Although we need an entire distribution of coalescence times from many genetic systems in order to test the different alternative hypotheses, the mitochondrial divergence date, as a one-point estimate at roughly 250,000 years, supports the rapid replacement model as the likeliest hypothesis. This single estimate, however, cannot strictly rule out the other two alternative models.

General Issues Concerning Molecular Divergence Date Estimation Illustrated by the Mitochondrial Studies

The mitochondrial DNA data are basically in good agreement in indicating a recent genetic common ancestor. However, arriving at this conclusion has not been quite as straightforward as it might first appear from casual inspection of the literature. There are some general (and instructive) observations about using DNA sequence data to derive molecular divergence

dates which have arisen during the analysis of the human mitochondrial data.

First, the same dataset handled in different ways can yield very different dates. For example, the hypervariable control region data (Vigilant *et al.,* 1991) gives relative dates varying by a factor of two or more for the same transition/transversion parameter, depending on whether one uses the transversion method or the Brown *et al.* (1982) correction method. Using the same correction method consistently, but varying the transition/transversion ratio, can cause over a threefold difference in molecular divergence date (Ruvolo *et al.,* 1993; Table 2). In this case, all of the inferred dates are fairly recent. Nevertheless, it emphasizes that more attention should be paid to data correction methods in inferring molecular divergence dates.

Second, datasets which would give very different dates if corrected by the same method and using the same parameters can appear to agree "coincidentally" when different methods and parameters are used. For example, using the same method (Brown *et al.,* 1982) and the same parameter (a transition/transversion ratio of 15) and assuming a human–chimpanzee divergence at 6 my, the hypervariable control region dataset (Vigilant *et al.,* 1991) gives a date for the modern human divergence of 462,000 years, while the ND4-5 (Kocher and Wilson, 1991) dataset gives 200,000 years (Ruvolo *et al.,* 1993). This is more than a twofold difference. The published date for the control region data, based on a different correction method (the transversion method), is 250,000 years (Vigilant *et al.,* 1991), which is in much better apparent agreement with the ND4-5 date. Although both dates are recent, they agree less than first appearances would suggest.

Third, comparing "absolute" molecular divergence dates between datasets can be misleading, unless the same calibration point and time are used. An example was given earlier, comparing dates from hypervariable control regions (cf. Vigilant *et al.,* 1991; Hasegawa and Horai, 1991). Generally, it is preferable to express molecular divergence dates in relative or noncalibrated terms, independent of any particular fossil date. Relative molecular proportions from any given dataset remain the same, even if new fossils are found or old ones redated.

Fourth, basing molecular divergence dates on nucleotide sites which are free of selective constraints, rather than on all available sites, is preferable. Dates based on different subsets of the data (selectively constrained vs unconstrained) can be very different. For example, relative dates for the human mitochondrial ancestor from the COII dataset are 1/19 and 1/27 using, respectively, all available sites and only unconstrained sites (with the maximum likelihood method and a 30:1 transition/transversion ratio). Comparable dates from the ND4-5 region are 1/29 and 1/27 (Ruvolo *et al.,* 1993). A large difference can also be seen with complete mitochondrial genomes (Horai *et al.,* 1995); nonsynonymous and RNA genes give a relative date of 1/11, whereas the unconstrained, synonymous sites give 1/34. (Note, however, that these latter proportions from Horai *et al.* (1995) are based on a different correction method and calibration point from that in Ruvolo *et al.* (1993).) The sites which are selectively constrained are evolving with different selective profiles and at different rates in different mitochondrial regions (Ruvolo *et al.,* 1993), and this is another reason to eliminate them from molecular divergence date calculations whenever possible.

Fifth, there are several different sources of error in inferring molecular divergence dates. The two chief kinds are error associated with the coalescent process and error associated with calibration points and times. While the first can be estimated mathematically in a uniform manner, the latter relies on paleontological dating and interpretation of fossils, and it is therefore less predictable in magnitude. Nevertheless, both need to be taken into account. Not including all types of error bars means, in the case of modern human divergence times, inadequately testing whether 1.8 my is compatible with the data. To date, the analyses of mitochondrial DNA sequence datasets which have taken both error types into consideration (Hasegawa and Horai, 1991; Ruvolo *et al.,* 1993; Templeton, 1993) are consistent with a recent mitochondrial (genetic) ancestor.

In order to judge whether two molecular datasets genuinely agree or not in their estimated divergence dates, it is necessary to compare correction methods, parameters, and calibration points and times, not just inferred molecular divergence dates.

Mitochondrial Mismatch Data and Environmental Catastrophes

A different and complementary approach to the mitochondrial data has been taken by Rogers and Harpending (1992). Instead of estimating the time of the common mitochondrial ancestor, they use the data to figure out when population expansions or bottlenecks have occurred. The idea is that mitochondrial lineages have a greater chance of being preserved when a population expansion occurs. Many pairs of people will show roughly the same amount of sequence difference, and this can be used to date the time of the expansion or bottleneck.

In this approach, one plots on the x-axis the amount of pairwise sequence difference between each pair of people in a population, and the y-axis gives the frequency of those differences for all pairs. The crest of the wave tells us something about when the expansion (or what is mathematically equivalent, a bottleneck) occurred. Mismatch analyses of mitochondrial control region sequence data and their population waves have also been used to rule out the multiregional hypothesis

(Rogers and Jorde, 1995). The population wave data show that the human population had a small effective population size at a time when the multiregional hypothesis posits that hominids were spread over large expanses of the Old World. Basically, there were too few humans in existence to be compatible with the multiregional hypothesis (Rogers and Jorde, 1995). This interesting approach has also been used to forward a so-called "weak Garden of Eden" model of modern human evolution, in which population divergence occurs before population expansions.

A genetic bottleneck (i.e., a substantial reduction in genetic diversity due to population size reduction) of recent occurrence is the usual explanation for why humans are different with their short (young) mitochondrial lineages. Some believe it to have been culturally driven, but lacking evidence, this remains speculative. Rogers and Jorde (1995) suggest that a recent environmental catastrophe might have affected human population size and been the cause of low genetic diversity within humans. This follows from their analysis of mitochondrial hypervariable control region sequences from 37 eastern common chimpanzees (*Pan troglodytes schweinfurthii*) (Morin *et al.,* 1994), which they interpret as showing a population wave peak occurring at the same time as in comparable human data. They reason that it is unlikely for both peaks in the two species to occur at the same time by chance; therefore an outside (environmental) factor, such as the Toba volcanic eruption at 73,500 years BP, may have been responsible for the genetic pattern. However, this argument is debatable on two points. First, the chimpanzee data they used do not fit nicely into a wave form as do the human data (see Fig. 6 in Rogers and Jorde, 1995). Preliminary analysis of another, much larger dataset, of approximately 260 eastern common chimpanzee hypervariable control regions, shows bimodal distributions in some chimpanzee populations, unlike the unimodal human plots, with some chimpanzee peaks corresponding to older times than those found in humans (T. Goldberg and M. Ruvolo, unpublished data). Second, if we now step back and look at a bigger portion of the primate evolutionary tree, the human mitochondrial results can be appreciated in a comparative context (Fig. 5; Ruvolo *et al.,* 1994). The mitochondrial COII data clearly show that most other hominoid species have longer (older) mitochondrial lineages than those existing in humans. This means that whatever caused relatively short mitochondrial lineages in humans did not cause them in other hominoids. On the COII gene tree, common chimpanzees have much longer mitochondrial lineages than do humans, extending back 600,000 and 900,000 years. While some of these dates differentiate hominoid subspecies (e.g., the orangutan date is for Bornean versus Sumatran subspecies), not all of them do. Within the western lowland gorilla subspecies, for example, a comparatively ancient mitochondrial divergence time of 705,000 years exists. If there *had* been some environmental catastrophe, such as the Toba volcanic eruption at 73,500 years BP, which affected human mitochondrial diversity, it certainly did not shorten mitochondrial lineages within other hominoid species or subspecies. Any significant environmental or climatological event producing dramatic genetic effects within humans should have affected chimpanzees and gorillas, as well as a diverse array of other species. Evidently this is not the case, and it is therefore unlikely that a global environmental catastrophe produced low genetic diversity within humans. There must be some alternative explanation for the unusual (from a hominoid perspective) mitochondrial pattern found within humans.

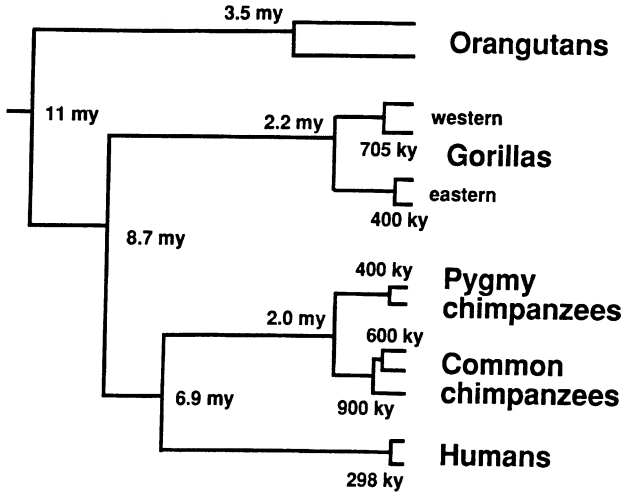

FIG. 5. Hominoid mitochondrial tree. Dates for hominoid divergences are based on mitochondrial cytochrome oxidase subunit II gene (COII) sequences from Ruvolo *et al.* (1994). Total substitutions were corrected by the maximum likelihood method with a transition/transversion ratio of 15 and using the phylogenetic analysis software program PHYLIP (Felsenstein, 1993). The tree was constructed using the Fitch–Margoliash (1967) algorithm with contemporaneous tips, also within PHYLIP. Dates were derived by equating the human divergence to 298,000 years, the coalescence time found in Ruvolo *et al.* (1993), using COII and ND4-5 substitutions. For branch lengths shown here, all substitutions were used, not just third codon position substitutions, because the latter begin to reach saturation for the older divergence dates shown here.

Nuclear Evidence

Protein Polymorphism Evidence

Trees constructed from protein polymorphism data are different in kind than mitochondrial trees because the taxa appearing on protein trees correspond to populations, not to genetic types of individual people. Topologically, protein trees are consistent with an African origin, in that African populations sort together in one clade as sister group to other human groups (Cavalli-Sforza *et al.,* 1988; 1994; Nei and Roychoudhury, 1993). However, this is an observation about consistency, not

hypothesis testing; this particular tree topology does not necessarily rule out other geographic origins. Here controversy still remains over the lower-level relationships among human groups (e.g., Nei and Roychoudhury, 1993). At least one reconstruction for human populations shows remarkable consistency with the tree of human linguistic groupings (Cavalli-Sforza *et al.,* 1988), which fits in well with the observation that linguistic barriers are often barriers to gene flow (Barbujani and Sokal, 1990). Dates derived from protein polymorphism evidence have supported a recent nuclear ancestor for all humans: 116,000 years with a confidence interval of 75,000–287,000 (Nei and Roychoudhury, 1982).

Protein polymorphism data have also been important in elucidating how genetic variation within humans is apportioned among human groups. An overwhelming proportion, 85% of our species' total genetic diversity, is contained with human populations, while higher level groupings of humans into ethnic groups and classically defined races contain a much smaller percentage of overall genetic variation (Lewontin, 1972). This has implications for the discovery of further human variation. It suggests that surveying living peoples more completely is not likely to result in the discovery of human groups which are genetically very different from those already characterized. This does not mean that rare and previously uncharacterized alleles will not be discovered, only that considerable overlap is expected among human groups. Because of the nature of hominoid social structure, in which females usually transfer between groups, this expectation also holds for hominoid mitochondrial variation (Ruvolo *et al.,* 1994). To some degree, protein polymorphism studies have been biased in their measurement of within-group diversity, particularly in their underestimate of African genetic variability. The discovery of this bias is now being revealed through more direct surveys of human genomes using restriction fragment length polymorphism data (Mountain and Cavalli-Sforza, 1994) and microsatellite data (Bowcock *et al.,* 1994).

Nuclear DNA Sequence Evidence

In a study of human Y-chromosomal diversity, Dorit *et al.* (1995) surveyed DNA sequence variation in a 729-basepair intron among human ZFY genes and found no variation within humans, while five differences were found between humans and chimpanzees. The Y-chromosome coalescence time estimate for humans based on the ZFY data is 270,000 years with a 95% confidence interval of 0 to 800,000 years (Dorit *et al.,* 1995). These data illustrate the difficulty of using nuclear gene or intron sequences to study the origin of modern humans. Because of the relatively slow nuclear mutation rate, the absolute number of differences observed between individual humans and between human and chimpanzee species for a 1-kb DNA region is small, therefore error bars on coalescence times can be large. Nevertheless, these nuclear data from the sex-specific portion of the Y-chromosome support a recent date for the common genetic ancestor of modern humans, roughly equivalent to that observed with mitochondrial data.

Some nuclear datasets suggest older dates for the common human genetic ancestor, but there are some complicating aspects. For example, DNA sequence data from the unconverted $\gamma^1 - \gamma^2$ globin region (Bailey *et al.,* 1992) show the difference between two human sequences equal to roughly one-fifth the distance between human and chimpanzee alleles. Assuming that humans and chimpanzees diverged at 6 my, these sequences would have diverged at 1.2 my. However, in this comparison, one of the human sequences is not actually a single allele, but rather a composite sequence from two individuals (Bailey *et al.,* 1992). Furthermore, selection may well be acting on γ-globin genes; if so, alleles with heterozygote advantage could be maintained longer than under neutrality. The ψη-globin pseudogene is different because it is noncoding and presumably selectively neutral, and human ψη-globin sequences diverged approximately 1.3 my. However, each of the two available human sequences are composites of two alleles (Bailey *et al.,* 1992), as in the γ-globin case. These data are suggestive of a relatively old molecular divergence date, but complete sequences of single human alleles are needed to explore this further, as well as error bars on the inferred molecular divergence date.

DNA sequence data from the apolipoprotein C-II gene have been variously interpreted. Two apolipoprotein C-II deficiency alleles arose more than 500,000 years ago, and this has been viewed as support for the multiregional hypothesis (Xiong *et al.,* 1991). Takahata (1993) disagrees with this interpretation, arguing that, because of the fourfold difference between mitochondrial and nuclear autosomal effective population sizes, the coalescence time for a nuclear gene is expected to be four times greater than for mtDNA, under neutrality. Therefore, an 800,000-year nuclear coalescence time is consistent with a 200,000-year mitochondrial coalescence time (Takahata, 1993). The equivalence or compatibility of nuclear and mitochondrial coalescence times raises a larger number of significant issues than can be addressed here (such as whether the assumptions involved are realistic). However, for the new method of coalescence time distributions proposed here, there is no need to judge whether a single date is compatible with another date or a particular hypothesis, because it is the entire distribution of coalescence dates which constitutes the basis for testing alternative models.

One genetic dataset provides an even more ancient molecular divergence date: a coalescence time of 3 my between two human sequences from a region upstream of the δ-globin gene (Maeda *et al.,* 1983). This date assumes a human–chimpanzee divergence at 4.5 my. Since this is a noncoding region, selection presumably

has not acted on these alleles. Again, although compatibility of molecular divergence dates is not an issue for hypothesis testing as proposed here, Takahata (1993) has noted that 3 my is not compatible with a 200,000-year mitochondrial divergence time because the probability of these two δ-globin alleles persisting over 3 my is very low, assuming that first, the polymorphism is neutral; second, the effective population size is on the order of 10^4; and third, no gene conversion or unequal crossing-over occurred. Of all the currently available data, the δ-globin gene flanking sequences provide the oldest human molecular divergence date; but it is only one point of many which will be used to evaluate the alternative models.

Anciently Polymorphic Genetic Systems: Two Special Cases

As discussed earlier, anciently polymorphic systems produce gene trees which are not interpretable in terms of population history. Nevertheless, some aspects of these genetic systems have been used to interpret the different models of modern human origins.

Opsin genes. One genetic dataset which has been used to argue (albeit weakly) against the African replacement model is based on patterns of amino acid polymorphisms in hominoids, rather than on molecular divergence dates. Ayala *et al.* (1994) observe that the green opsin gene polymorphism for threonine-isoleucine at amino acid position 65 is found in orangutans and in humans of European descent, but not in Africans or Asians (Deeb *et al.,* 1994). They argue that one possible interpretation is loss of this ancient polymorphism during the long time period since *H. erectus* emerged from Africa by Africans and by Asians, while Europeans retained the polymorphism. However, this argument ignores data from the other hominoids. Gorillas have the polymorphism, but common chimpanzees do not (and the one pygmy chimpanzee examined has the identical amino acid to common chimpanzees). Since Ayala *et al.*'s (1994) hypothesis assumes the human ancestor had the polymorphism, it requires three changes overall: two changes within humans (loss in Africans, Asians) and one change (loss) along the lineage leading to chimpanzees. However, there is an alternative hypothesis: the polymorphism was lost first along the lineage leading to humans and chimpanzees after the gorilla divergence, and then regained recently in Europeans. Overall, this is a more parsimonious explanation, as it requires only two changes among hominoids, while the hypothesis forwarded by Ayala *et al.* (1994) requires three changes. Placing the human data in the context of hominoid phylogeny shows that the opsin data do not support the multiregional hypothesis, even in a weak sense.

HLA (major histocompatibility system) genes. Another genetic dataset which has been used to evaluate alternative models for modern human origins is from the HLA gene system. Although these data have been interpreted as consistent with both the rapid replacement and the multiregional models, the HLA data rule out a particular version of the rapid replacement model, the "Noah's ark" model, which hypothesizes a very small bottleneck for modern humans (Ayala *et al.,* 1994); a recent modern human population reduction to less than several thousand individuals conflicts with the HLA data. The HLA data further imply that, over the 30 my prior to 200,000 years, the populations ancestral to humans should have had a mean effective population size of 10^5, rather than 10^4, assuming a model of balancing selection and constant population size (Takahata, 1991). However, the value of 10^5 could be an overestimate if there was not a single, random mating population or if intragenic recombination occurred (Takahata, 1993). This large effective population size is inferred because of the large number of existing human alleles which are anciently arisen and which must have persisted over a very long time period, roughly 35 my (Ayala *et al.,* 1994).

The HLA system differs in its evolutionary history from many of the genes which have commonly been used to infer phylogenies and molecular divergence times in that alleles do not cluster according to species on the gene tree. Therefore the HLA gene tree cannot possibly be a population tree. However, a method has been developed to derive genetic distances between species from such anciently polymorphic gene trees, called the minimum-minimum method (Satta *et al.,* 1993). The method works first by observing that orangutans, for example, are an outgroup to and equally distant from humans, chimpanzees, and gorillas (on the basis of other evolutionary data). Second, we find the orangutan and human alleles which are least different, then find the least different orangutan and chimpanzee alleles, and then the least distant orangutan and gorilla alleles. Finally, we take the minimum of those three minimum genetic distances as representative of the genetic distance between the orangutan species and the other three species. This "minimum-minimum" genetic distance is then equated to some fossil calibration time in order to calculate a mutation rate for HLA genes.

While the use of ancestrally polymorphic gene trees for understanding bottlenecks in human evolution is laudable, this method of extracting mutation rates from anciently polymorphic data is problematic because the minimum-minimum method will tend to overestimate the true genetic distance between species. Unless each species, including all of the nonhuman hominoids, is well surveyed, it is easy to miss obtaining the minimum value, if the alleles showing the least distance are not sampled. If genetic distance is underestimated then, so is the mutation rate, the inferred molecular divergence date, and the effective population size, which would then become even greater than 10^5.

Using the mutation rates derived from currently available alleles, the HLA data hypothesizes what

seems to be too large an estimate for long-term effective population size in early hominids and hominoid ancestors, roughly 100,000, over a 30-my time period. If effective population size fluctuates over time, the average long-term effective population size is given by the harmonic mean of the different effective population sizes. Because harmonic means are close to the smallest values which contribute to them, 10^5 would then be a *minimum* value for the effective population sizes of hominids and hominoids over the last 30 my. Effective population sizes for humans, chimpanzees, and gorillas have been estimated at 10^4 from population surveys and protein heterozygosities (Nei and Graur, 1984). This is consistent with values obtained from most human DNA sequence data. From the mitochondrial COII data, the total effective population size is 14,900 individuals, with a range of 6,450–26,800 (Ayala *et al.*, 1994). The Y-chromosomal data (Dorit *et al.*, 1995) support a mean effective population size of 13,500; even using the maximum coalescence time for the ZFY dataset (800,000 years), the effective population size would be only 40,000. The $\gamma^1 - \gamma^2$ globin data (Bailey *et al.*, 1992), for which human alleles coalesce at 1.2 my, give an effective population size estimate of 30,000 individuals. Thus the inferred HLA effective population size value of 10^5 is at variance with the mitochondrial results and at least some nuclear datasets.

It is also unclear that nonhuman African hominoids, which are primarily restricted to forest environments, could have maintained such large effective population sizes over time. Many climatic fluctuations occurred over the last 30 my, some of which had profound environmental effects on African forests (Roberts, 1992). Examining only one of them illustrates the types of environmental changes over time which need to be considered in discussions of effective population size. At the last glacial maximum 20,000 years ago, African forests were much smaller than they are today, approximately 6–25% of their current size, and closer probably to the lower estimate, based on reconstructions of past African refugia (Colyn, 1991; Maley, 1987). Effective population sizes for chimpanzees and gorillas could potentially have been reduced as African forests shrunk in size. Whether or not hominoid population sizes were reduced proportionately with forest size is unknown, but significant reduction of past populations is likely, perhaps by a factor of 10 from what they are today. Since many forest reductions with profound ecological effects occurred over the last 30 my, this argues against the maintenance of relatively large long-term effective population sizes in hominoids and their ancestors.

It is not obvious how to reconcile the HLA findings of a relatively large long-term effective population size with smaller estimates from mtDNA, Y-chromosomal data, and other nuclear data. The assumptions involved in inferring relatively large hominid and hominoid effective population sizes certainly merit further investigation, such as relative constancy of hominoid population sizes over time and the appropriateness of the balancing selection model for HLA. Although trees of anciently polymorphic genes cannot be used to infer molecular divergence dates, they contain other valuable information. More discussion of how to use data from ancestrally polymorphic systems like HLA and of the inherent assumptions and the methods for analyzing them would be productive for future research on human evolution.

Microsatellite Variation

Another type of genetic data used to study modern human origins is based on microsatellite variation. A microsatellite locus consists of tandemly duplicated DNA segments, each from two to five nucleotides in length. Loci are highly variable within species, in the number of repeats per allele. These are nuclear loci, and many different ones exist in the human genome. Usually, for a given microsatellite locus, each allele is typed by size to determine the number of repeats it contains. Their high rate of molecular evolutionary change makes them particularly useful for studying genetic differentiation and phylogenetic relationships among closely related taxa, such as human populations, although less useful for interspecific reconstructions (Bowcock *et al.*, 1994).

The fast rate of microsatellite evolutionary change produces a tree for humans which is qualitatively different than the mitochondrial haplotype tree: it shows strong geographic clustering of individuals according to their original populations (Bowcock *et al.*, 1994), in contrast to the geographically "scrambled" mitochondrial haplotype trees (Cann *et al.*, 1987; Vigilant *et al.*, 1989, 1991). This clustering is not uniformly strong for all populations, however; East Asian and some African populations appear more diffusely on the tree. Essentially, the microsatellite data give a more magnified view of recent evolutionary events than do the mitochondrial data, a difference which is due to their relative molecular evolutionary rates of change. The use of microsatellite data is an important development for the study of human populations, not only because it provides a different "time window" into the past, but because it is also a "nuclear window," and thus complementary to the mitochondrial view.

Several different genetic distance measures based on microsatellite data have been developed (Bowcock *et al.*, 1994; Goldstein *et al.*, 1995a,b; Slatkin, 1995), and these incorporate different models of molecular evolutionary change for microsatellite variation. One of the more exciting new aspects of microsatellite data is that an "absolute" dating method is now possible (Goldstein *et al.*, 1995a). Dating is absolute because the mutation rate for a sample of microsatellite loci has been measured directly in the laboratory from untransformed cells of parents and offspring. This mutation rate esti-

mation method circumvents the usual reliance on a molecular clock calibrated by equating an amount of genetic difference to some evolutionary divergence event and time. Applying this absolute dating method to the microsatellite data of Bowcock *et al.* (1994), Goldstein *et al.* (1995a) found a date of 156,000 years linking all modern humans, with a 95% confidence interval of 75,000 to 287,000 years (where error due to coalescence is included but error associated with mutation rate is not). This date is based on 30 microsatellite markers on chromosomes 13 and 15, many of which are in close linkage.

Our understanding of microsatellite evolution is currently still developing. For the most part, microsatellite alleles change over time by gaining or losing one repeat. However, from empirical data, the mutational process is not exclusively "one-step," suggesting that sometimes more than one repeat can be gained or lost (Di Rienzo *et al.*, 1994; Garza *et al.*, 1995; references in Nei, 1995). An additional complication is that alleles may be under size constraints (Bowcock *et al.*, 1994), so that once they reach an upper size limit, they can only evolve by losing repeats, and when they reach a lower size boundary, they can only get bigger. Empirical data on interspecific microsatellite variation support the existence of size constraints (Bowcock *et al.*, 1994; Garza *et al.*, 1995). This aspect of microsatellite evolution is potentially troublesome for inferring molecular divergence dates. If we imagine two populations which just diverged from each other, each having an allele with average size length, this allele may grow in size in one population over time, while decreasing in average size in the other. Eventually, these alleles in the different populations will reach their maximal and minimal sizes, so that the large allele will start to become smaller, and the small allele will become larger. Thus their average size difference will not necessarily reflect time of divergence between them, nor does the difference between anciently related alleles stay fixed at some asymptotic limit. Because the underlying molecular evolutionary processes of microsatellite variation are still being discovered along with estimates for their basic parameters (e.g., mutation rates), current conclusions about the timing of modern human origins based on microsatellite data should be viewed tentatively.

Unique and "Irreversible" Molecular Markers

A property intrinsic to almost all characters changing under the evolutionary process is their ability to undergo homoplasic change. Any character which does not undergo homoplasic change would be invaluable for evolutionary reconstructions (Ruvolo, 1995). Insertion of *Alu* repetitive elements is a stable evolutionary event over millions of years, and no molecular process has been characterized which specifically removes them from the genome (see Hammer, 1994, for references and discussion). They have been observed to be removed "nonspecifically" from the genome, in which case, pieces of the original *Alu* insertion element are left behind, or some flanking sequence is removed along with the *Alu* element. Although further study of their molecular evolutionary dynamics is needed to investigate the possibility of homoplasious change, *Alu* insertions appear to qualify as irreversible changes, useful for evolutionary reconstructions (Perna *et al.*, 1992; Batzer *et al.*, 1994).

"One-shot" molecular events with this degree of uniqueness and apparent irreversibility are unable to provide divergence times by themselves. However, if combined with other genetic information in the region around an *Alu* insertion site, they can provide important data about times of human population divergence and populational movements. Recently, a large-scale global survey of a human *Alu* deletion polymorphism and of a short tandem repeat polymorphism, both of which are located within a 9-kb DNA segment at the CD4 locus on chromosome 12, has been carried out. The pattern and the degree of linkage disequilibrium between the two loci suggest that a single population, existing 90–140,000 years ago was ancestral to all non-Africans and that all humans had a recent African origin (Tishkoff *et al.*, 1994).

APPLICATION OF THE COALESCENCE TIME DISTRIBUTION METHOD TO EXISTING DATA

The above review of the genetic evidence shows that several datasets give recent dates for human genetic ancestors, at approximately the same time, roughly 200,000 years. These are from protein polymorphisms, mitochondrial DNA, Y-chromosomal sequences, microsatellite variation on chromosomes 13 and 15, and microsatellite variation plus *Alu* insertion/deletion polymorphism on chromosome 12. The C-II apolipoprotein gene (on chromosome 19) gives a somewhat older molecular divergence time of 500,000 years. Even older dates come from the globin loci on chromosome 11; the γ and ψη globins give a date of approximately 1.3 my, and the 5′ δ-globin region, an ancient coalescence time of 3 my. Using all the molecular divergence dates available from existing data as discussed above, the coalescence time distribution clearly shows a recent peak around 200,000 years BP (Fig. 6). Given this overall coalescence time distribution of mitochondrial and nuclear coalescence times, the rapid replacement hypothesis is the likeliest model for modern human origins.

There is another factor to be considered in evaluating coalescence time distributions. Ideally, coalescence times from many loci, which are unlinked and have independent evolutionary histories, are needed to test the different models for modern human origins using

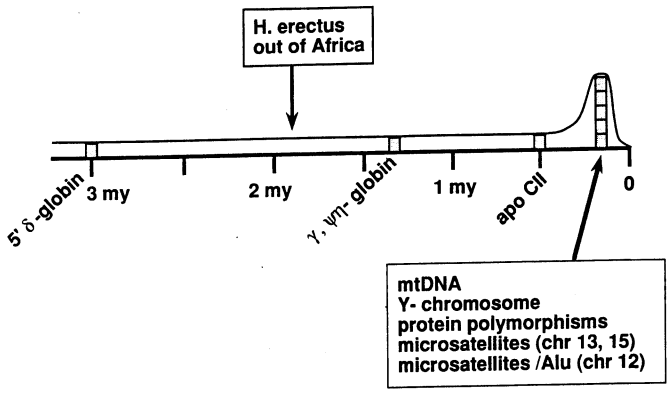

FIG. 6. Distribution of coalescence times from the currently available genetic evidence. Coalescence times (or more strictly, molecular divergence times) from the different datasets are discussed in the text. These are from the 5′ flanking region of δ-globin (Maeda *et al.*, 1983), the $\gamma^1 - \gamma^2$ and ψη globins (Bailey *et al.*, 1992), apolipoprotein CII (Xiong *et al.*, 1991), mtDNA (see text for references), Y-chromosomal data (Dorit *et al.*, 1995), protein polymorphisms (Nei and Roychoudhury, 1982); microsatellites on chromosomes 13 and 15 (Bowcock *et al.*, 1994; Goldstein *et al.*, 1995a); and microsatellite plus *Alu* variation on chromosome 12 (Tischkoff *et al.*, 1994). The clustering of values around a relatively recent time supports the rapid replacement model as the likeliest hypothesis for modern human origins.

the new coalescence time distribution method proposed here. Datasets can be considered to be independent if they are based on genes or DNA regions coded for on different, nonhomologous chromosomes or, if on the same chromosome, from widely separated loci with a high probability of recombination. For the existing data, creating independent datasets would mean separately reanalyzing coalescence times after partitioning the protein polymorphism dataset (Nei and Roychoudhury, 1982) into several datasets according to chromosomal localization of the different loci, dividing the microsatellite dataset from chromosomes 13 and 15 (Bowcock *et al.*, 1994) according to chromosomal location, and combining the data from δ, γ and ψη globins (Bailey *et al.*, 1992; Maeda *et al.*, 1983) into one dataset because these loci are tightly linked on chromosome 11. This is likely to increase the number of datasets with recent coalescence times, while decreasing the number of datasets with older coalescence times (from three to two). The overall effect of repartitioning the data into independent datasets would probably be to make the rapid replacement model even more likely.

How likely are the other alternative hypotheses for modern human origins? An analytical answer would require knowing more about the exact coalescence time distribution curves for the different hypotheses, which in return would require greater knowledge of genomic composition and evolution. Qualitatively, it is interesting and perhaps significant that of the three oldest coalescence times, only one falls near to the time of *H. erectus*' appearance outside of Africa (around 1.3 my for the γ and ψη globin loci). Although the three older coalescence time points are a limited sample, they show no tendency to cluster around any one date nor, in particular, around any date considered to be important in the classically formulated multiregional and/or candelabra models.

CONCLUSIONS AND PROSPECTS FOR THE FUTURE

Molecular divergence dates are useful for testing alternative hypotheses about modern human origins, but only collectively. Hypotheses are tested on the basis of the coalescence time distribution pattern from all available data. The method of using coalescence time distributions to test hypotheses about modern human origins is a new approach to data evaluation, representing a strong philosophical departure from the currently used method of interpreting the evidence. In particular, this approach differs in that no single coalescence time based on one genetic system is viewed as being sufficient for definitively rejecting any of the three alternative models.

The currently available genetic evidence produces a coalescence time distribution which supports the rapid replacement model as the likeliest hypothesis for modern human origins. More nuclear data are needed to evaluate further these hypotheses about modern human origins. In general, the absolute number of differences observed between individual humans and between humans and chimpanzees is small for the nuclear DNA sequence datasets. These small absolute differences produce a high degree of error associated with (some) nuclear molecular divergence dates, and only more nuclear data from longer stretches of DNA, showing a greater number of absolute differences among humans, will help solve this problem. Also, new data should preferably come from many different, unlinked nuclear loci and from autosomes not previously surveyed. For example, the recent large microsatellite survey of Bowcock *et al.* (1994) used many loci, but these were from only two autosomes and some are closely linked. Studies of this type need to be extended to other chromosomes for additional independent estimates of molecular divergence times. Fortunately, there are several types of genomic data to be collected and interpreted, each with its own time window into the past.

Since the issue of modern human origins is being intensely studied using molecular data, the fields of molecular evolution and molecular systematics are benefiting by such close scrutiny, through the development of more sophisticated methods of divergence time estimates, of more realistic models of molecular evolutionary change, and of more sophisticated tree-building algorithms. For example, continuing discussion about

how best to estimate the number of transitions and transversions from human mitochondrial DNA sequence data and on the assumed models for molecular evolutionary change is ongoing (e.g., Tamura and Nei, 1993; Ruvolo *et al.,* 1993; Wakeley, 1994; Perna and Kocher, 1995). Newly generated genetic data on human variation need to be analyzed with an understanding of the dynamics of molecular evolutionary change, our knowledge of which is changing as the data are being collected. This illustrates how studying the problem of modern human origins has been advantageous to the fields of molecular systematics and molecular evolution, and generally, the symbiotic nature of both fields. We can look forward to further improvements in how to extract information about the past from molecular data. Those who study humans will lead the way in this pursuit, because having a wealth of data forces one to think more about how to interpret the data and to develop new analytical methods.

Last, more molecular data from the nonhuman hominoids are needed, particularly from different subsets of the nuclear genome. Humans have been genetically well-characterized, but our close sister groups less so. Putting human data into a hominoid context is powerful, because it illustrates how we are different from other species. It also shows us what our evolutionary history might have been like, but was not. The ways in which we are genetically different from other hominoids will help us understand, all the better, what forces have molded human genomes, including perhaps cultural ones.

APPENDIX

This section derives the relationship between the two different measures of inferred molecular divergence times discussed in the text (coalescence time and time inferred by relative branch lengths) for the two-allele case.

To calculate expected coalescence time, we first need the expected nucleotide heterozygosity θ. Ewens' (1983) formulation of θ is given by

$$\theta = k^*/[1 + 1/2 + 1/3 + \ldots + 1/(n - 1)], \quad (1)$$

where k^* is the number of sites at which two or more different nucleotides occur and n is the number of individuals sampled (see also Ruvolo *et al.,* 1993). In the two-allele case, this becomes simply

$$\theta = k^*. \quad (2)$$

Time to coalescence is given by

$$T = \theta(1 + k)/[2\mu(1 + n\theta)], \quad (3)$$

where k is the pairwise divergence among haplotypes (in number of nucleotide differences), n is the number of sampled nucleotides, μ is the mutation rate (in substitutions per site per year), and θ is the expected nucleotide heterozygosity (using Templeton's (1993) formulation of Tajima's (1983) equation 20, as in Ruvolo *et al.,* 1993).

In the two-allele case, $k^* = k$, so that

$$T = k(1 + k)/[2\mu(1 + nk)], \quad (4)$$

or equivalently,

$$T = (k/2n\mu)[(1 + k)/\{(1/n) + k\}]. \quad (5)$$

Molecular divergence time inferred using the relative branch length method, T_{rb}, is

$$T_{rb} = (k/2n\mu); \quad (6)$$

therefore

$$T = T_{rb}[(1 + k)/\{(1/n) + k\}]. \quad (7)$$

Thus the two types of molecular divergence dates differ by a factor f, where

$$f = (1 + k)/\{(1/n) + k\}. \quad (8)$$

If n, the number of nucleotides surveyed, is large, this is approximately

$$f = (1 + k)/k. \quad (9)$$

This factor is greater than one, so that the coalescence time T is larger than the relative branch length time T_{br}. However, as k becomes large, f approaches one, and the two time measures approach equality.

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