# The Root of the Phylogenetic Tree of Human Populations

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Although African populations have been shown to be most divergent from any other human populations, it has been difficult to establish the root of the phylogenetic tree of human populations since the rate of evolutionary change may vary from population to population owing to the fluctuation of population size and other factors. However, the root can be determined by using the chimpanzee as an outgroup and by employing proper statistical methods. Using this strategy, we constructed phylogenetic trees of human populations for five different sets of gene frequency data. The data sets used were two sets of microsatellite loci data (25 and 8 loci, respectively), restriction fragment length polymorphism (RFLP) data (79 loci), protein polymorphism data (15 loci), and *Alu* insertion frequency data (4 loci). All these data sets showed that the root is located in the branch connecting African and non-African populations, and in the four data sets the root was established at a statistically significant level. These results indicate that Africans are the first group of people that split from the rest of the human populations.

## Introduction

Many phylogenetic analyses of human populations have suggested that Africans are most divergent from any other human populations (e.g., Nei and Roychoudhury 1974, 1982, 1993; Cann, Stoneking, and Wilson 1987; Cavalli-Sforza et al. 1988; Nei and Livshits 1989; Bowcock et al. 1991, 1994). However, since the rate of evolutionary change may vary from population to population owing to the fluctuation of population size and other factors, it has been difficult to establish the root of the phylogenetic tree of human populations. Vigilant et al. (1991) attempted to establish the root by using human and chimpanzee mitochondrial DNA (mtDNA) sequences, but they were not successful because the mtDNA tree represents a gene tree rather than a population tree (e.g., Tajima 1983; Neigel and Avise 1986; Pamilo and Nei 1988; Hedges et al. 1991; Templeton 1993). In recent years, a number of authors (e.g., Bowcock et al. 1994; Mountain and Cavalli-Sforza 1994; Deka et al. 1995) published human and chimpanzee gene frequency data for nuclear DNA loci that can be used for this purpose. Unfortunately, these authors either analyzed human and chimpanzee data separately or did not use proper statistical methods. Therefore, the root of the human population tree still remains unresolved.

We have therefore collected all nuclear gene frequency data that can be used for this purpose and constructed phylogenetic trees of human populations with the aim of putting the root to the tree. The results obtained will be presented in this paper.

## Materials and Methods

We collected five different sets of gene frequency data for major human population groups and the chimpanzee. The first data set came from Bowcock et al. (1994), who generated gene frequency data for 30 microsatellite loci for 14 human populations and for 25 loci for the chimpanzee. In this study, we used data for 25 loci that are shared by the human and the chimpanzee. The second set of data was taken from Deka et al. (1995), who published gene frequency data for eight microsatellite loci from eight human populations and the chimpanzee. The human populations and most microsatellite loci studied by these authors were different from those of Bowcock et al. (1994). The third data set was restriction fragment length polymorphism (RFLP) data obtained by Mountain and Cavalli-Sforza (1994) for 79 anonymous DNA marker loci from eight human populations and the chimpanzee. The fourth set was protein polymorphism data shared by three major races of man and the chimpanzee (King and Wilson 1975; Nei and Livshits 1989). There are many polymorphic protein loci that have been examined for human populations and the chimpanzee, but the number of loci shared by the three major races and the chimpanzee we could use was 15. They were ACP1, ADA, AK1, G6PD, GSR, HBB, PGM1, PGD, ORM1, PI, CP, C3, GC, HPA, and TF (see Roychoudhury and Nei 1988 for gene symbols). The fifth set of data was gene frequency data for four polymorphic loci for *Alu* sequence insertion in the human genome (Batzer et al. 1994). This insertion apparently occurred after the human–chimpanzee divergence, so that they are polymorphic only in humans.

For each of the five different sets of data we computed pairwise genetic distances using the modified Cavalli-Sforza distance ($D_A$; Nei, Tajima, and Tateno 1983). This distance measure is not proportional to evolutionary time but is efficient for obtaining correct phy-

**Table 1**

**Average Heterozygosities and $D_A$ Distances (×100) for Microsatellite Data Set I**

| Population | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Chimpanzee ...... | 59.8 | | | | | | | | | | | | | | |
| 2. Pygmy (CAR) ..... | 61.0 | 82.5 | | | | | | | | | | | | | |
| 3. Pygmy (Zaire) .... | 64.5 | 27.4 | 75.5 | | | | | | | | | | | | |
| 4. Bantu (Lisongo) ... | 62.1 | 25.3 | 27.0 | 81.1 | | | | | | | | | | | |
| 5. North Italian ...... | 64.4 | 33.9 | 40.1 | 33.7 | 74.4 | | | | | | | | | | |
| 6. North European ... | 61.3 | 36.6 | 39.0 | 33.7 | 14.4 | 73.8 | | | | | | | | | |
| 7. Melanesian ....... | 69.2 | 43.2 | 49.4 | 44.9 | 28.6 | 28.8 | 63.4 | | | | | | | | |
| 8. Australian ........ | 71.4 | 41.1 | 47.5 | 39.0 | 24.5 | 23.7 | 22.9 | 68.2 | | | | | | | |
| 9. New Guinean ..... | 69.4 | 45.5 | 50.8 | 43.7 | 34.2 | 28.4 | 26.1 | 21.8 | 65.3 | | | | | | |
| 10. Amerindian (M) ... | 66.3 | 39.5 | 41.5 | 40.5 | 26.8 | 24.7 | 27.6 | 26.3 | 29.0 | 70.1 | | | | | |
| 11. Amerindian (K) ... | 68.3 | 51.8 | 53.1 | 49.3 | 35.8 | 35.4 | 42.5 | 38.8 | 36.8 | 26.5 | 51.5 | | | | |
| 12. Amerindian (S) .... | 67.5 | 41.7 | 43.9 | 41.6 | 29.9 | 30.7 | 34.3 | 30.5 | 38.2 | 17.9 | 19.4 | 62.7 | | | |
| 13. Japanese ......... | 59.4 | 41.7 | 40.1 | 39.1 | 27.8 | 23.7 | 28.6 | 28.3 | 28.6 | 21.9 | 30.2 | 26.0 | 69.9 | | |
| 14. Chinese .......... | 67.1 | 43.8 | 40.5 | 39.4 | 25.3 | 22.4 | 26.6 | 24.3 | 27.2 | 22.0 | 30.6 | 25.1 | 15.6 | 70.3 | |
| 15. Cambodian ....... | 64.0 | 36.9 | 38.2 | 36.3 | 26.1 | 21.8 | 24.7 | 22.8 | 26.9 | 21.7 | 32.1 | 25.3 | 17.9 | 14.3 | 73.7 |

NOTE.—Diagonal and off-diagonal entries are average heterozygosities and $D_A$ distances, respectively. CAR: Central African Republic. M: Maya. K: Karitiana. S: Surui.

logenetic trees under various evolutionary conditions whether the mutation pattern follows the infinite-allele model or the stepwise-mutation model (see Kimura 1983) or whether population size changes or not (Nei, Tajima, and Tateno 1983; Nei and Takezaki 1994; N. Takezaki and M. Nei, unpublished). Since we are interested primarily in rooting the phylogenetic tree of human populations rather than estimating branch lengths, we used this distance measure. For constructing phylogenetic trees from pairwise distances, we used the neighbor-joining method (Saitou and Nei 1987), because human populations are known to vary considerably over evolutionary time and thus the evolutionary rate would vary from population to population (Livshits and Nei 1990). The reliability of the phylogenetic trees obtained was examined by Felsenstein's (1985) bootstrap test with 500 replications.

## Results
### Microsatellite DNA Data Set I

Table 1 shows the average heterozygosities for the 14 human populations examined and the chimpanzee and the $D_A$ distances for all pairs of populations. Average heterozygosity is higher for African and European populations than for Oceanians (Melanesians, Australians, New Guineans) and Amerindians as in the case of classical loci (Livshits and Nei 1990). This observation is similar to that of Bowcock et al. (1994) for their 30 microsatellite loci.

In mitochondrial DNA, the extent of intraspecific genetic diversity is known to be higher in the chimpanzee than in the human (Ferris et al. 1981; Ruvolo et al. 1994). Our results, however, show that average heterozygosity is somewhat lower in the former than in the

latter. Similar results were also obtained by Deka et al. (1995) for eight microsatellite loci. This slightly lower heterozygosity might reflect the possible sampling bias against polymorphic loci in the chimpanzee, because the loci used were originally chosen for human populations. However, since microsatellite loci are subject to a high rate of mutation and in this type of study they are chosen primarily on the basis of the number of repeats of nucleotides (usually more than 10 repeats; D. Goldstein, personal communication), it is not clear whether the bias really exists or not. The fact that Amerindians tend to show as low heterozygosity as the chimpanzee in both data sets by Bowcock et al. (1994) and Deka et al. (1995) indicates that the relatively low heterozygosity in the chimpanzee could also be due to a small effective size of the chimpanzee population. At any rate, there is no evidence that nuclear genes are more polymorphic in the chimpanzee than in the human.

The phylogenetic tree obtained from $D_A$ distances is given in fig. 1. This figure shows that the root of the human phylogenetic tree is located in the branch between Africans and non-Africans, and a bootstrap test indicates that both interior branches connecting the chimpanzee and Africans (97%) and the chimpanzee and non-Africans (99%) are statistically significant. Bowcock et al. (1994) gave the root to the same place by using the midpoint method, but this method gives no statistical assurance. They did not use the chimpanzee as an outgroup population.

However, if we consider the branching orders of human populations only, our results are identical with Bowcock et al.'s except for three African populations and three Asian populations, of which the branching order is not statistically significant. They are also similar
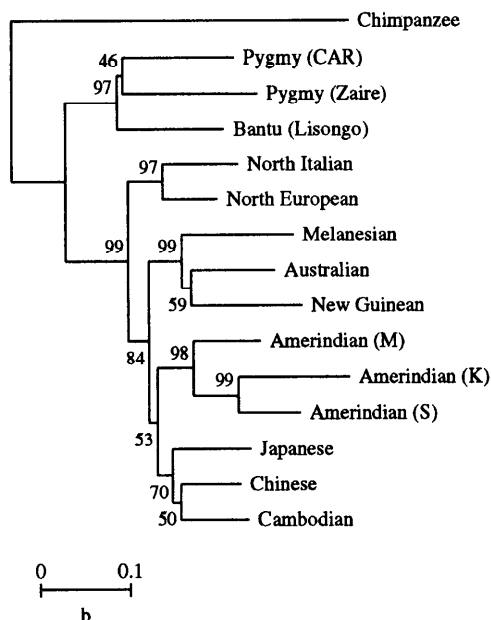
Fig. 1.—Phylogenetic tree microsatellite data set I. The tree was constructed by using $D_A$ distances in table 1. The number for each interior branch is the bootstrap value. M: Maya. K: Karitiana. S: Surui. CAR: Central African Republic. b: Scale for branch length ($D_A/2$). Note that the branch lengths are not proportional to evolutionary time.



Fig. 2.—Phylogenetic tree for microsatellite data set II. The tree was constructed by using $D_A$ distances. The number for each interior branch is the bootstrap value. The number in parentheses for the interior branch leading to the non-African population cluster is the probability that the branch is different from zero (confidence probability).

to those of Nei and Roychoudhury (1993) for classical genetic markers, though in their tree Oceanians were slightly closer to East Asians than Amerindians are. In none of these studies, however, has the branching order of the three groups of populations been statistically established.

## Microsatellite DNA Data Set II

Deka et al. (1995) already constructed the neighbor-joining tree for this set of data with $D_A$ distances. However, for the sake of comparison we reproduced the tree (fig. 2). This tree is identical with Deka et al.'s, although it is presented in a different form and the bootstrap values are slightly different. In the present tree, the
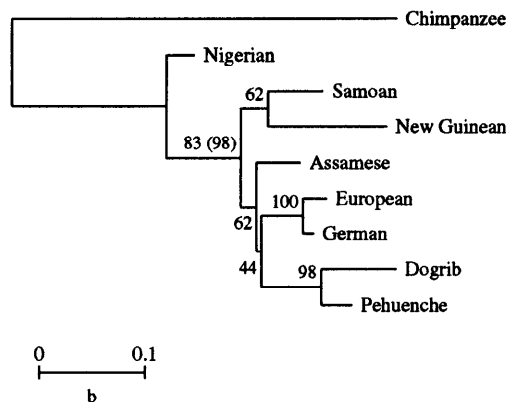
bootstrap value (83%) for the branch separating Africans from non-Africans is lower than 95%. This is probably due to the fact that the number of loci used is small. However, Felsenstein's bootstrap test is known to be conservative (e.g., Zharkikh and Li 1992a, 1992b; Hillis and Bull 1993; Sitnikova, Rzhetsky, and Nei 1995). By contrast, Dopazo's (1994) interior branch test by means of bootstrapping generally gives more accurate test results (T. Sitnikova, unpublished). We therefore applied this method to the branch between Africans and non-Africans and obtained a confidence probability of 98%. This suggests that the root of the human population tree is again located between the African and non-African populations, though some caution is required in this case because the number of loci used is small. Little can be said about the branching order of Oceanians, Europeans, Asians, and Amerindians with this data set.

## RFLP Data

The average heterozygosities and $D_A$ distances obtained for 79 RFLP loci are presented in table 2. The

**Table 2**
**Average Heterozygosities and $D_A$ Distances ($\times 100$) for RFLP Data**

| Population | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1. Chimpanzee ...... | 4.9 | | | | | | | | |
| 2. Pygmy (CAR) .... | 41.2 | 31.2 | | | | | | | |
| 3. Pygmy (Zaire) .... | 39.5 | 2.3 | 29.7 | | | | | | |
| 4. European ......... | 43.9 | 6.8 | 6.0 | 38.5 | | | | | |
| 5. Melanesian ....... | 45.8 | 9.4 | 8.9 | 6.4 | 27.6 | | | | |
| 6. Australian ........ | 46.3 | 8.7 | 8.4 | 4.6 | 4.5 | 30.9 | | | |
| 7. New Guinean ..... | 46.1 | 9.2 | 8.7 | 5.2 | 3.5 | 1.4 | 26.3 | | |
| 8. Chinese .......... | 46.1 | 9.7 | 8.5 | 3.9 | 5.9 | 6.1 | 5.6 | 34.6 | |
| 9. Japanese ......... | 46.1 | 9.4 | 8.0 | 4.2 | 6.2 | 5.9 | 5.3 | 1.4 | 32.3 |

NOTE.—Diagonal and off-diagonal entries are average heterozygosities and $D_A$ distances, respectively. CAR: Central African Republic.
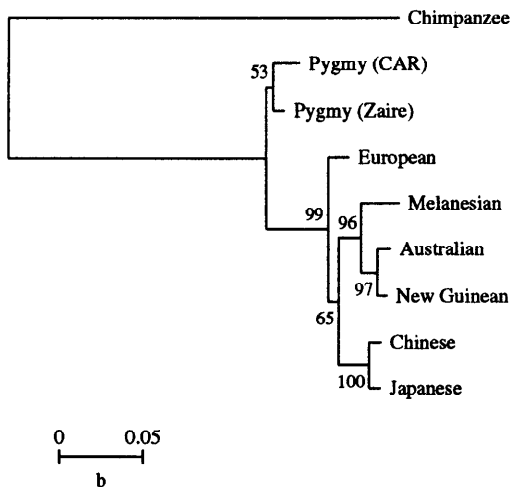
FIG. 3.—Phylogenetic tree for RFLP data. The tree was constructed by using $D_A$ distances in table 2. The number for each interior branch is the bootstrap value. CAR: Central African Republic.

RFLP loci used here were initially identified in Europeans and then examined in other human populations and the chimpanzee. Probably for this reason, the European population has the highest heterozygosity and the chimpanzee population has the lowest. RFLP loci do not seem to stay polymorphic for a long evolutionary time.

Figure 3 shows the phylogenetic tree obtained from this data set. The topology of this tree is the same as that of the tree in figure 1, though the number of populations examined is smaller here. The root of the tree is again placed in the branch connecting Africans and non-Africans, and the interior branch connecting the chimpanzee and non-African human populations is supported at a bootstrap probability of 99%, indicating that Africans were the first to split from other human populations.

### Protein Polymorphism Data

Table 3 shows the average heterozygosities and $D_A$ distances for the data for 15 polymorphic protein loci. Average heterozygosity is considerably lower in chimpanzees than in humans, which is consistent with the previous observation (Nei and Graur 1984). The phylogenetic tree given in figure 4 again shows that the root exists between Africans and non-Africans and that the location of the root is statistically supported.

### *Alu* Insertion Polymorphism Data

The final data set is *Alu* insertion polymorphism data. Batzer et al. (1994) examined the allele frequencies of 16 human populations, including African Americans, two populations from Indonesia, two Amerindian populations in South America, and two pygmy populations. In this study we eliminated African Americans because this population has been admixtured with Caucasians

**Table 3**
**Average Heterozygosities and $D_A$ Distances ($\times 100$) for Protein Polymorphism Data**

| Population | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1. Chimpanzee . . . | 5.9 | | | |
| 2. African . . . . . . . | 56.8 | 23.1 | | |
| 3. European . . . . . . | 58.6 | 6.2 | 24.4 | |
| 4. Asian . . . . . . . . . | 58.4 | 5.6 | 2.4 | 21.5 |

NOTE.—Diagonal and off-diagonal entries are average heterozygosities and $D_A$ distances, respectively.

(Reed 1969). We also combined each of the two Indonesian populations, two Amerindian populations, and two pygmy populations into one group, because the number of individuals examined was small. The average heterozygosities and $D_A$ distances are given in table 4. Average heterozygosity is lower in Australians and South Amerindians than in other human populations, but other populations seem to have similar heterozygosities, though they are based on only four loci. The average heterozygosity for the chimpanzee is 0, because the *Alu* insertions used here do not exist in the chimpanzee.

The phylogenetic tree based on $D_A$ distances is given in figure 5. Since only four loci were used, the reliability of the branching pattern is low. However, it is interesting to note that the root of the tree is still located in the branch between Nigerians and other populations.

### Discussion

Currently a great controversy is going on over the origin of *Homo sapiens*. The two rival hypotheses are the multiregional theory proposed by Wolpoff, Zhi, and Thorne (1984) and the out-of-Africa theory by Cann, Stoneking, and Wilson (1987) and Stringer and Andrews (1988). In the former theory our ancestral species, *H. erectus*, whose brain size was considerably smaller than that of *H. sapiens*, is believed to have moved out of Africa and spread to various parts of Eurasia more than
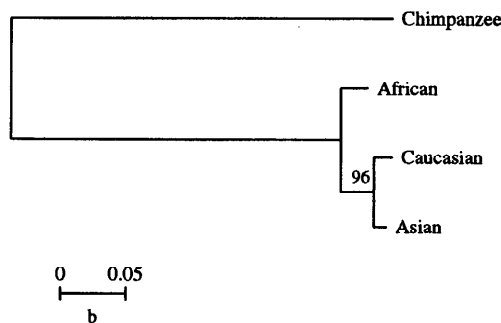


FIG. 4.—Phylogenetic tree for protein polymorphism data. The tree was constructed by using $D_A$ distances in table 3. The number for each interior branch is the bootstrap value.

**Table 4**
**Average Heterozygosities and $D_A$ Distances ($\times 100$) for *Alu* Insertion Frequency Data**

| Population | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Chimpanzee .............. | 0.0 | | | | | | | | | | | | |
| 2. Nigerian ................. | 18.0 | 40.5 | | | | | | | | | | | |
| 3. Pygmy ................. | 23.7 | 2.8 | 36.6 | | | | | | | | | | |
| 4. N. European ............. | 36.7 | 4.4 | 4.2 | 34.2 | | | | | | | | | |
| 5. Greek (Cyprus) ........... | 36.1 | 4.8 | 2.5 | 0.5 | 36.5 | | | | | | | | |
| 6. Turkish (Cyprus) .......... | 39.8 | 6.9 | 3.4 | 1.3 | 0.3 | 35.7 | | | | | | | |
| 7. New Guinean (Coastal) ..... | 28.0 | 4.7 | 3.0 | 6.0 | 5.1 | 6.9 | 41.1 | | | | | | |
| 8. New Guinean (Highland) .... | 28.4 | 4.8 | 4.0 | 5.7 | 5.4 | 7.6 | 0.3 | 36.6 | | | | | |
| 9. Australian ................ | 37.0 | 9.7 | 7.9 | 7.2 | 7.2 | 9.5 | 2.8 | 1.5 | 21.9 | | | | |
| 10. Alaskan ................. | 47.9 | 12.3 | 5.9 | 5.5 | 3.4 | 3.0 | 5.2 | 6.2 | 6.9 | 35.3 | | | |
| 11. S. Amerindian ............. | 61.9 | 21.9 | 13.3 | 12.6 | 9.9 | 8.7 | 10.9 | 12.6 | 12.7 | 2.0 | 24.5 | | |
| 12. Indonesian ............... | 38.2 | 6.5 | 4.1 | 4.2 | 3.2 | 4.0 | 2.0 | 3.0 | 5.7 | 2.2 | 5.7 | 45.0 | |
| 13. Asian (C-V) .............. | 50.4 | 13.1 | 8.1 | 7.3 | 5.6 | 5.3 | 6.0 | 7.7 | 9.8 | 1.5 | 1.9 | 1.5 | 36.3 |

NOTE.—Diagonal and off-diagonal entries are average heterozygosities and $D_A$ distances, respectively. C-V: Chinese and Vietnamese.

1 million years (MY) ago and *H. sapiens* is believed to have evolved worldwide with the effects of gene flow and natural selection. Yet this theory asserts that several regional characters such as the shovel-shaped incisors in East Asians and the prominent brow ridge in Australian aborigines have remained unchanged for more than 1 MY, from the time of their ancestral species *H. erectus.* By contrast, the latter theory proposes that *H. sapiens* originated in Africa 100,000–200,000 years ago and that all present human populations outside sub-Saharan Africa are primarily descendants of a population that moved out of Africa about 100,000 years ago.

As mentioned earlier, genetic data have suggested that sub-Saharan Africans are genetically most divergent



FIG. 5.—Phylogenetic tree for *Alu* insertion frequency data. The tree was constructed by using $D_A$ distances in table 4. The number for each interior branch is the bootstrap value. C-V: Chinese and Vietnamese.

from the rest of the world populations, and the time of divergence between Africans and non-Africans has been estimated to be at most about 200,000 years ago but probably 115,000 years ago under the assumption of constant rate of evolution (Nei and Roychoudhury 1974, 1982; Cann, Stoneking, and Wilson 1987; Vigilant et al. 1991; Goldstein et al. 1995; Horai et al. 1995; Nei 1995). The present study does not give estimates of the time of divergence because $D_A$ is not linear with time, but it shows that even without the assumption of constant rate, Africans are considered to be the group that first split from the rest of the human populations. This conclusion is quite strong, because all five different sets of genetic data give the same results.

These results are consistent with the out-of-Africa theory, because the most divergent and first-established population is likely to stay in the place of origin and new populations would be formed when they move out of the original place. Genetic estimates of the time of divergence between Africans and non-Africans are also consistent with the out-of-Africa theory (see Nei 1995 for review). Recent archeological studies have suggested that some modern humans who produced sophisticated tools were already living in sub-Saharan Africa about 90,000 years ago (Brooks et al. 1995; Yellen et al. 1995). If this new finding holds up, an advanced tool-making culture evolved much earlier in Africa than in Europe (about 40,000 years ago), and the African origin of modern humans is supported.

Some authors (e.g., Wolpoff 1989) have contended that the first splitting of African populations is also consistent with the multiregional theory, because if the extent of gene flow among different populations has been sufficiently large, the genetic depth of the first split of human populations would look shallow. However, if this is the case, it seems very difficult to maintain regional
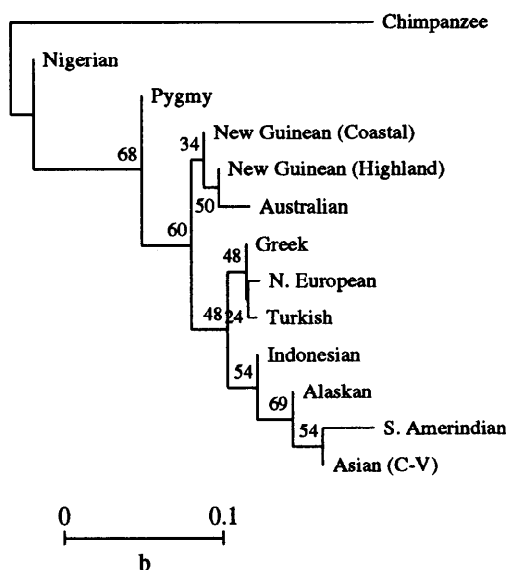
continuity of certain morphological characters for more than 1 MY. To maintain this regional continuity, the extent of gene flow must be very low. If so, the age of the latest common ancestor of human mtDNAs should be much higher than the observed value of about 143,000 years (Takahata 1993; Horai et al. 1995; see Nei 1995 for a recent review of this subject).

There are several other reasons to believe that the multiregional theory or gene flow hypothesis is suspect. First, if the alleles from different populations are mixed extensively by migration and the extent of gene flow is the primary factor to determine the distance values between populations, the tree constructed would not reflect the real evolutionary history of the populations and the root located would be unreliable; the location of the root is expected to vary from gene to gene. However, our analysis shows that the five different sets of data give the same root and similar phylogenetic trees. Mitochondrial DNA data also gives essentially the same root (Hedges et al. 1991), though it is not statistically significant. This suggests that our tree is not unreasonable and the effect of gene flow is probably unimportant for our purpose.

Second, if we assume that the effects of mutation, migration, and genetic drift are balanced between two populations with either the infinite-allele or the stepwise-mutation model, the equilibrium value of Nei's genetic distance ($D$) is approximately given by $D = v/(v + m)$ when $D$ is small, where $v$ and $m$ are the mutation rate and the migration rate, respectively (Nei 1975, 1987). Using allozyme data, Nei and Roychoudhury (1974) obtained $D = 0.01$ between Europeans and Northeast Asians and $D = 0.02$ between Europeans and Africans. Therefore, if we assume $v = 2 \times 10^{-6}$ per locus per generation following Nei (1975, p. 194), we obtain $m = (1 - 2) \times 10^{-4}$ per generation. This is a quite small value but still seems to be too high for the migration rate among Europeans, Africans, and Asians, because in the prehistoric age with primitive culture, it should have been very difficult to move around among different continents or subcontinents.

Third, the Australian and Highland New Guinean populations, which have been isolated from Asian populations for about 50,000 years, show smaller genetic distances from Asians and Europeans than sub-Saharan African populations do. Since very little migration seems to have occurred between Australopapuans and other populations for the last 40,000–50,000 years, the greater distances between Africans and other populations suggest that Africans have been isolated for a longer period of time. A similar argument can be made with respect to Amerindian populations, which also have been isolated for 12,000–40,000 years. Goldstein et al. (1995) examined the relationship between their genetic distance ($[\delta\mu]^2$) and divergence time using information on several well-dated non-genetic evolutionary events during the last 100,000 years. Their results support the view that African populations have genetically diverged from other populations without much migration.

Fourth, if the evolution of large mammals gives any hint about the evolution of *Homo sapiens,* it suggests that evolution of a species with worldwide gene flow is rare. Most species of large mammals are distributed within a continent or a relatively small part of it, unless they are introduced into new territories by humans. This suggests that even within one continent various types of territorial barriers exist and these barriers generate many different species in the course of evolution. *H. sapiens* seem to be the only species which has a worldwide distribution and whose genetic distances among different geographical populations are very small. Since *H. sapiens* do not necessarily have a high degree of mobility compared with other large mammals such as tigers, lions, and wolves, the close genetic relationships of different human populations suggest that they dispersed in recent years.

Of course, if we consider human populations which are geographically closely located, there is almost always a substantial amount of gene flow. However, we are interested in tracing the evolutionary pathways of human populations by using distantly related populations from the entire world and thus avoiding the effect of local gene flow as much as possible.

Nevertheless, it should be noted that the present study does not resolve all the controversy over the origin of *H. sapiens*. For example, the multiregional theory is based on cranial and dental characters of human remains, and it is still unclear whether the out-of-Africa theory is capable of explaining all paleontological and archeological data or not (see Nei 1995). Therefore, it is necessary to conduct a more detailed study from various points of view to resolve this controversy.

## Acknowledgments

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