Relative Efficiencies of the Fitch-Margoliash, Maximum-Parsimony, Maximum-Likelihood, Minimum-Evolution, and Neighbor-joining Methods of Phylogenetic Tree Construction in Obtaining the Correct Tree[1]

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The relative efficiencies of several tree-making methods for obtaining the correct phylogenetic tree were studied by using computer simulation. The methods examined were the Fitch-Margoliash (FM), maximum-parsimony (MP), maximum-likelihood (ML), minimum-evolution (ME), and neighbor-joining (NJ) methods. We simulated the evolutionary changes of six DNA sequences each with a length of either 300 or 600 nucleotides. Both constant and varying rates of nucleotide substitution were considered. The DNA sequences generated were used to reconstruct phylogenetic trees by applying the five tree-making methods, and the trees obtained were compared with the model (correct) tree. This process was repeated 50 times for each case, and the following results were obtained: (1) The efficiency of obtaining the correct tree for the FM method was considerably lower than those for the other methods. (2) The NJ and ME methods showed a high performance in obtaining the correct tree, and their relative efficiencies were similar to each other. (3) For distance methods (NJ, FM, and ME), the results obtained by using corrected nucleotide substitutions were much better than those obtained by using nucleotide differences when the rate of substitution varied greatly among different branches. (4) The ML method was slightly inferior to the NJ and ME methods when a constant rate of nucleotide substitution was assumed, but it was slightly better than the latter two methods when the evolutionary rate varied drastically among branches. If one considers the computational time involved, the NJ method seems to be a method of choice.

Introduction

The reconstruction of phylogenetic trees is one of the most important and interesting problems of evolutionary study. There are many methods for constructing phylogenetic trees from molecular data. They can be classified into two categories according to the strategy used for finding the best tree. One strategy is to examine all or a large number of possible trees and choose the best one under a certain criterion. We call this the "exhaustive-search" method. The other strategy is to examine local topological relationships of a tree and construct the best tree step-by-step. We call this the "stepwise clustering" method. The maximum-parsimony (MP) method (Eck and Dayhoff 1966; Fitch 1977), the Fitch-Margoliash (FM) method (Fitch and Margoliash 1967), and the maximum-likelihood (ML) method (Felsenstein 1981) belong to the first category,

1. Key words: Fitch-Margoliash method, maximum-parsimony method, maximum-likelihood method, minimum-evolution method, neighbor-joining method.

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whereas the neighbor-joining (NJ) method (Saitou and Nei 1987) and many other distance methods (see Nei 1987 for a review) fall into the second category.

Because of their computational simplicity, the statistical properties of the stepwise clustering methods have been studied extensively (e.g., see Tateno et al. 1982; Saitou and Nei 1987; Sourdis and Krimbas 1987; Sourdis and Nei 1988). However, those of exhaustive-search methods are not well understood. Tateno et al. (1982) and Sourdis and Krimbas (1987) studied the FM method, but they examined only a small number of trees.

When the number of operational taxonomic units (OTUs) is small, we can examine all possible trees. Saitou and Nei (1986) studied the MP method and the FM method for the case of four OTUs. Hasegawa and Yano (1984) and Saitou (1988) examined the MP method and the ML method for the case of four OTUs. Recently, Sourdis and Nei (1988) studied the MP method for the case of six and eight OTUs, examining a subset of possible trees that were close to the model tree, including the model tree itself.

The objective of the present study was to examine the efficiencies of the exhaustive-search methods more extensively by using computer simulation. We studied the following four exhaustive-search methods: the MP method (Eck and Dayhoff 1966; Fitch 1977), the FM method (Fitch and Margoliash 1967), the ML method (Felsenstein 1981), and the minimum-evolution (ME) method proposed by the present study. The NJ method (Saitou and Nei 1987) was also studied as a representative of the stepwise clustering method. In the present study, we used model trees for six OTUs, for which there are 105 possible unrooted trees. All the possible trees were examined for the case of the exhaustive-search methods—except for the ML method, in which only a limited number of trees were examined.

It should be mentioned that two types of errors can occur in the construction of phylogenetic trees: topological errors and branch-length errors (Tateno et al. 1982). Topological errors are more serious than branch-length errors, and we considered only topological errors in the present study. We are mainly concerned with the efficiency of each tree-making method for obtaining the correct tree topology, and the proportion (P_c) of trees in which the correct topology was obtained in 50 replications was used as a measure for comparison of the methods.

Models and Methods of Simulation

Two of the model trees used were the same as those of Sourdis and Nei (1988); trees A and B in figure 1 correspond to trees (C) and (A) in their figure 1, respectively. These trees were formed under the assumption of constant rate of nucleotide substitution, and the expected number of nucleotide substitutions per site, from the ancestral sequence to an extant sequence, is denoted by U. U = 0.05 and U = 0.5 were used for both trees. The length of each branch was expressed as multiples of a ($\equiv U/8$) (see fig. 1).

We used two other model trees in which there was a large variation in the rate of nucleotide substitution (trees C and D in fig. 1). For both trees, a = 0.01 and a = 0.05 were used. It should be noted that the heterogeneity of evolutionary rate is more extreme in trees C and D than in the model trees with varying rates used by Saitou and Nei (1987) and Sourdis and Nei (1988).

If we neglect the label of OTUs, there are only six rooted and unlabeled trees for six OTUs. Four of them (trees A–D of fig. 1) are used in our study. Because the labels of OTUs are arbitrary, these are not shown in figure 1. In the actual computer sim-

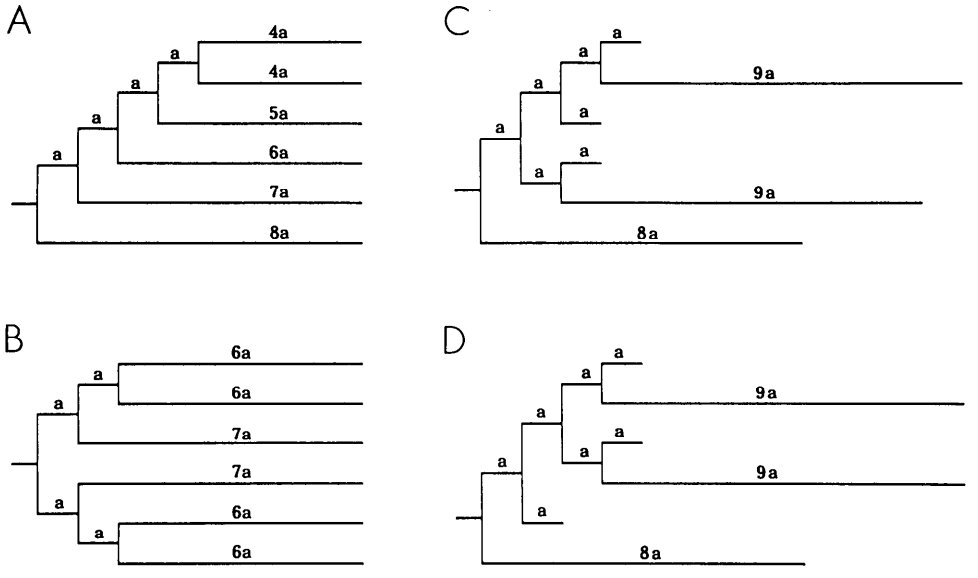

FIG. 1.—Four model trees used for the computer simulation. Constant rate of nucleotide substitution is assumed for trees A and B. U (≡8a) = 0.05 and U = 0.50 were used in the computer simulation. The rate of nucleotide substitution varies with branch for trees C and D. a = 0.01 and a = 0.05 were used for these trees.

ulation, however, OTUs of the model tree were of course labeled to specify the tree topology.

The method of computer simulation used is the same as that of Saitou and Nei (1987). The ancestral sequence of a given number (300 or 600) of nucleotides was generated by using pseudorandom numbers, with equal frequencies for the four nucleotides (A, G, T, and C) being assumed. The evolution of this sequence was simulated by using pseudorandom numbers, according to the predetermined branching pattern of the model tree. Random nucleotide substitutions (the one-parameter model of Jukes and Cantor 1969) were introduced in each branch of the tree by following a Poisson distribution with the mean equal to the expected branch length of the model tree. After the nucleotide sequences for six OTUs were produced, the proportion (p) of the nucleotide differences and Jukes and Cantor's (1969) evolutionary distance (d) were computed for each pair of sequences. The entire process of simulation was repeated 50 times for each case.

In the MP method, a tree that has the smallest number of nucleotide substitutions required for explaining the evolutionary changes of DNA sequences is chosen as the best tree. A simple and quick computer program for counting this number for nucleotide sequence data was developed by applying Fitch's (1971) algorithm. All 105 trees were examined.

Felsenstein's computer program DNAML in his PHYLIP package, version 3.1, was used for the ML method. Because of the long computational time required for this method, we compared a limited number of trees by using the U (user-tree) option of DNAML. For six OTUs, 105 possible trees can be classified into four groups in terms of the topological difference from the model (true) tree. The topological difference was measured by the method of Robinson and Foulds (1981), and it was 0, 2, 4, and 6 for groups 1, 2, 3, and 4, respectively. The numbers of trees for groups 1, 2, 3, and

4 are 1 (true tree), 6, 24, and 74, respectively (see Sourdis and Nei 1988). As a preliminary study, 31 trees that belonged to groups 1, 2, and 3 were examined for the case of model tree B (U = 0.5 with 300 nucleotides) and for the case of model tree C (a = 0.01 and 0.05 with 300 nucleotides). Because the tree with the highest likelihood almost always belonged to either group 1 or group 2, only the seven trees in these two groups were examined for other cases, as Sourdis and Nei (1988) did for the MP method.

Computation of maximum-likelihood values requires various assumptions. We set the options of DNAML such that the likelihood formulation is as close as possible to the simulation scheme used: (1) The C (categories) option was not used because nucleotide substitutions at each site occurred independently and at the same rate in our simulation. (2) The frequencies of the four nucleotides were all set to be 0.25. (3) The transition/transversion ratio was set to be 1.0 by using the T option. Under this assumption, the rate of transitional substitution is twice that of transversional substitution. (Unfortunately, in the current version of DNAML we cannot assume Jukes and Cantor's one-parameter model of nucleotide substitutions, which was used in our simulation.) In any case, the assumptions adopted in the computation were favorable for the ML method.

The other three methods (FM, ME, and NJ) require a distance matrix, and both p and d were used, as Saitou and Nei (1987) did previously. Note that the p distance follows the triangle inequality and is a metric, whereas the d distance is a nonmetric. For the FM and ME methods, all 105 topologies were examined as in the case of the MP method.

The criterion for finding the best tree used in the FM method is the so-called percent standard deviation (PSD), defined as follows:

$$\mathrm{PSD} = \left[\frac{2\sum_{ij}\{(D_{ij} - E_{ij})/D_{ij}\}^2}{n(n-1)}\right]^{1/2} \times 100\,, \qquad (1)$$

for $i < j$, where n is the number of OTUs compared and D_{ij} and E_{ij} are the observed and estimated distances between OTUs i and j, respectively. In the FM method, a tree showing the smallest PSD values is chosen as the best one. A simple and quick computer program was developed for computing the estimated distances by Fitch and Margoliash's (1967) procedure.

Tateno et al. (1982) proposed a measure (S_O) for the comparison between estimated and observed distances:

$$S_O = \left[\frac{2\sum_{ij}(D_{ij} - E_{ij})_2}{n(n-1)}\right]^{1/2}. \qquad (2)$$

S_O is related to PSD, and it can also be used for choosing the best tree (Nei 1987, pp. 301–302). Thus we also used S_O as a criterion for the topology finding. This modification of the FM method is called the FM′ method. Comparison of equations (1) and (2) suggests that PSD and S_O are highly correlated. They did show similar results, as will be seen later.

The ME method is a new method proposed in the present paper. We first compute each branch length of a given tree topology by using Fitch and Margoliash's (1967) procedure for branch-length estimation, and the tree that shows the smallest sum of

Table 1
Proportions (%) of Trees in Which the Correct Topology Was Reconstructed under the Constant-Rate Models

			p				*d*			
Model Tree	MP	ML	FM	FM′	ME	NJ	FM	FM′	ME	NJ
A:										
U = 0.05:										
300 bp.....	34	38	26	26	36	42	26	26	40	40
600 bp.....	76	80	58	58	80	78	58	58	80	82
U = 0.50:										
300 bp.....	60	48	30	32	56	58	22	24	42	46
600 bp.....	84	70	54	50	92	92	40	34	82	82
B:										
U = 0.05:										
300 bp.....	54	62	42	38	68	68	40	38	68	70
600 bp.....	84	88	56	58	82	84	56	56	80	86
U = 0.50:										
300 bp.....	48	56	34	30	70	68	30	28	60	60
600 bp.....	58	76	36	36	76	76	36	28	70	70

NOTE.—Model trees A and B are shown in fig. 1.

branch lengths is chosen as the best one. Thus the criterion used in this method is the sum of branch lengths (SBL) for a given tree:

$$\text{SBL} = \sum_i L_i , \tag{3}$$

where L_i is the estimated length of the ith branch.

The principle of minimum evolution was proposed by Cavalli-Sforza and Edwards (1967), who considered a Steiner tree. With the use of Fitch and Margoliash's procedure for branch-length estimation, computation becomes much simpler than the Cavalli-Sforza and Edwards method. The ME method also seems to be related to Dayhoff's (1978) method (see Blanken et al. 1982).

Although this principle bears some resemblance to that of maximum parsimony, the ME method is much more similar to the NJ method. This is because the principle of minimum evolution is also adopted in the NJ method (see Saitou and Nei 1987) and because both the ME and NJ methods are distance methods. Our simulation results have shown that the ME method and the NJ method are indeed quite similar. However, the ME method is an exhaustive-search method and examines all possible trees to choose the best one. In contrast, the NJ method is a stepwise clustering method, and the computational time is much shorter in this method than in the ME method.

Results and Discussion

Model Trees with Constant Evolutionary Rate

Table 1 shows the proportion (P_c, in %) of trees in which the correct tree topology was obtained. When model tree A (U = 0.05) is used, there is a clear dichotomy in terms of the efficiency, measured by P_c. That is, the FM and FM′ methods are less efficient than the other four methods. However, the efficiencies of these two methods

are higher for the case of 600 nucleotides than for that of 300 nucleotides. Therefore these methods may eventually become as useful as the other methods if long nucleotide sequences are compared.

This low efficiency of the FM and FM′ methods is also observed for the case of U = 0.50, but the other four methods show some differences in P_c. The ME and NJ methods perform quite well when the *p* distance is used, and the MP method is also good. Compared with these three methods, the ML method is less efficient, but it is better than the FM and FM′ methods. It is interesting that all four distance methods (FM, FM′, ME, and NJ) show slightly better performances when *p* distances are used than when *d* distances are used. This is consistent with the previous results of Saitou and Nei (1987) and Sourdis and Krimbas (1987).

The results for model tree B are similar to those for model tree A; the FM and FM′ methods are both less efficient than the other four methods. For the case of U = 0.05, the ML method is better than the MP method, but the ML method is slightly less efficient than the ME and NJ methods when 300 nucleotides are compared. As in the case of model tree A, for the FM, FM′, ME, and NJ methods *p* distances give slightly better results than do *d* distances.

In conclusion, the FM and FM′ methods are consistently less efficient than the other four methods. Saitou and Nei (1986) studied the efficiencies of several tree-making methods, including the MP, FM, and NJ methods, for the case of four OTUs and showed that the FM method was worst among the three (the transformed-distance method used in their study is identical with the NJ method for the case of four OTUs). Thus the present finding supports Saitou and Nei's (1986) conclusion. The results for model trees A and B also suggest that the time-consuming ML method is no better than the other, much quicker methods. Furthermore, the close similarity between the ME and NJ methods indicates that the algorithm for the NJ method gives the minimum-evolution tree most of the time.

Model Trees with Varying Evolutionary Rate

The results obtained for model trees C and D are presented in table 2, and they are in several respects quite different from those for model trees A and B. First, for all four distance matrix methods, *d* distances give much better results than do *p* distances. This clear-cut difference has already been noted by Saitou (1988) for the case of a four-sequence tree, and the present study confirms this. Li et al. (1988) performed a similar study for four sequences, but they used only *p* distances, and they concluded that the NJ method is less efficient than Lake's (1987) method. However, their conclusion might have been drastically different had they used *d* distances.

Low efficiencies of the FM and FM′ methods are also observed for trees C and D, whereas the ML method is the best in all cases. However, the P_c values of the NJ and ME methods (when *d* distances were used) are close to those of the ML method. On the other hand, the MP method is good only for a = 0.01; for a = 0.05, the MP method is much less efficient than the ME and NJ methods. These results clearly show the superiority of the principle of minimum evolution when a correctly estimated evolutionary distance is used. This is probably because, with the *d* distances, an additive tree is approached as the number of nucleotides used increases. These results also indicate that the principle of maximum parsimony is different from the principle of minimum evolution, contrary to their superficial similarity.

Table 2
Proportions (%) of Trees in Which the Correct Topology Was Reconstructed under the Varying-Rate Models

			p				*d*			
MODEL TREE	MP	ML	FM	FM′	ME	NJ	FM	FM′	ME	NJ
C:										
a = 0.01:										
300 bp.....	64	78	26	38	56	56	34	34	72	72
600 bp.....	90	98	42	50	80	80	68	78	92	92
a = 0.05:										
300 bp.....	24	92	0	0	2	2	22	16	68	68
600 bp.....	20	100	0	0	0	0	60	60	96	96
D:										
a = 0.01:										
300 bp.....	68	80	44	52	64	64	64	50	74	74
600 bp.....	94	96	56	68	90	90	88	70	92	92
a = 0.05:										
300 bp.....	26	96	0	0	6	4	30	24	78	78
600 bp.....	46	100	0	0	10	6	64	42	100	100

Trees Obtained by Different Tree-making Methods

We have seen that the FM and FM′ methods are quite similar in their efficiency for obtaining the correct tree, as were the ME and NJ methods. These similarities occur because the same tree is often chosen by these tree-making methods. Close relationships of the trees chosen were also observed for other methods. An example is presented in table 3.

All methods chose an erroneous tree in replication 1; the MP, ML, ME, and NJ

Table 3
Trees Chosen by Different Methods in the Case of Model Tree B with U = 0.50 and 300 Nucleotides

			p				*d*			
REPLICATION NUMBER	MP	ML	FM	FM′	ME	NJ	FM	FM′	ME	NJ
1.....	5	5	4	4	5	5	4	12	5	5
2.....	5	5	11	11	1	1	1	1	5	5
3.....	1	1	1	1	1	1	1	10	1	1
4.....	1	1	1	1	1	1	1	1	1	1
5.....	1	1	1	1	1	1	1	1	1	1
6.....	1	1	1	1	1	1	1	1	1	1
7.....	2	3	2	2	1	1	2	2	1	1
8.....	1	1	1	1	1	1	1	1	1	1
9.....	8	4	4	4	8	8	4	4	8	8
10.....	3, 9	9	10	10	3	3	8	8	9	9

NOTE.—The identification numbers of the trees chosen by each method for the case of model tree B with U = 0.50 and 300 nucleotides are shown. Tree 1 is the correct (model) tree, trees 2–7 are different from tree 1 with a topological difference of two, and trees 8–12 are different from tree 1 with a topological difference of four.

Table 4
Average Topological Differences between the Trees Obtained by Different Tree-making Methods and the True Tree

	True	FM-*d*	ME-*d*	FM-*p*	ME-*p*	ML	MP
True		2.28	0.76	5.68	3.96	0.16	2.18
FM-*d*	2.08		2.04	5.00	3.84	2.24	2.50
ME-*d*	0.96	1.76		5.36	3.56	0.80	1.68
FM-*p*	1.80	0.72	1.64		3.24	5.60	4.56
ME-*p*	0.68	1.88	0.36	1.72		3.96	2.94
ML	1.00	1.84	0.64	1.72	0.76		2.18
MP	1.23	2.17	0.93	1.91	1.01	0.87	

NOTE.—Figures below the diagonal are for case (a) model tree B (U = 0.50 and 300 nucleotides were compared), and those above the diagonal are for case (b) model tree C (a = 0.05 and 300 nucleotides were compared). The model tree is designated as "True."

methods chose tree 5, whereas the FM and FM′ methods chose tree 4 or tree 12. In the case of replication 7, the ME and NJ methods chose the correct tree (tree 1) for both *p* and *d* distances, but the other methods chose either tree 2 or tree 3. A more interesting situation is observed in replication 10, in which trees 3 and 9 were equally parsimonious when the MP method was used. The ML method chose tree 9, as did the ME and NJ methods when *d* distances were used. However, tree 3 was chosen by the ME and NJ methods when *p* distances were used. The FM and FM′ methods chose neither tree 3 nor tree 9 (see table 3).

The relationship of the trees obtained by different tree-making methods can be represented by a matrix of average topological differences for all replications. Two examples are shown in table 4. One [table 4(a)] is from the case of model tree B with U = 0.50 and 300 nucleotides, and the other [table 4(b)] is from the case of model tree C with a = 0.05 and 300 nucleotides. In both matrices, the FM′ and NJ methods were omitted because these were very close to the FM and ME methods, respectively.

Networks showing the relationships between the tree-making methods can then be made using the NJ method from the distance matrices in table 4 (see fig. 2) This kind of graphic representation is useful for grasping the relationships among different tree-making methods. There are three clusters in the tree shown in figure 2A: [ME-*d*, ME-*p*], [FM-*d*, FM-*p*], and [ML, MP]. These clusters indicate that the trees obtained by members of each cluster are highly correlated. For the case of figure 2B, the trees from *d* distances and *p* distances are no longer highly correlated. In this case, ME-*p* and FM-*p* make a cluster, and the ML method is no longer associated with the MP method. The ML method is closest to the true tree, followed by the ME-*d* method. These comparisons clearly show that the relationships of the trees obtained by different tree-making methods can be quite different, depending on the data used. We should therefore be cautious in selecting a tree-making method for reconstruction of phylogenetic trees.

A Numerical Example

Saitou (1988) applied his maximum-likelihood algorithm and the NJ method to five mitochondrial DNA sequences of humans and apes (data from Hixson and Brown 1986), and both methods chose a tree in which two chimpanzee species (common chimpanzees and pygmy chimpanzees) were clustered and in which humans and gor-

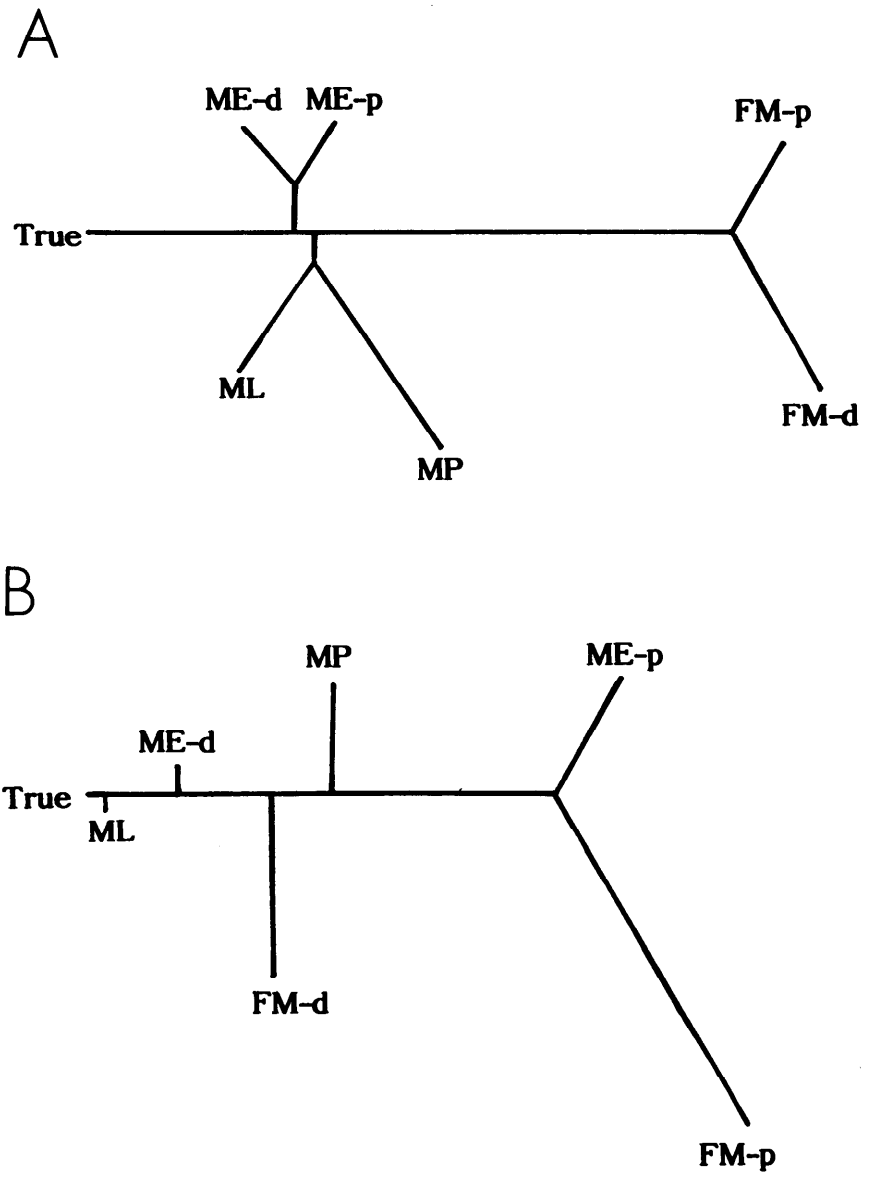

FIG. 2.—Two relationships (A and B) of trees obtained by different tree-making methods and the true tree, in terms of the topological difference matrices for cases (a) and (b) of table 4, respectively. These unrooted trees were constructed by the NJ method.

illas were clustered. Both the *p* and *d* distances obtained from the same data set are shown in table 5.

Three exhaustive search methods requiring distance matrices (FM, FM′, and ME) were then applied to the distance matrix of table 5, and four different trees given in table 6 were examined. Common chimpanzees and pygmy chimpanzees are clustered in trees 1, 2, and 3, whereas pygmy chimpanzees and humans are clustered in tree 4.

Because the results for *p* distances were identical with those for *d* distances, only the results for *d* distances are presented in table 6. Values of PSD, S_O, and SBL were

Table 5
Pairwise Distances Measured by the Jukes-Cantor Formula for Five Hominoid Species

	Common Chimpanzee	Pygmy Chimpanzee	Gorilla	Human
Pygmy chimpanzee	0.0118 (0.0117)			
Gorilla	0.0427 (0.0415)	0.0416 (0.0405)		
Human	0.0382 (0.0373)	0.0327 (0.0319)	0.0371 (0.0362)	
Orangutan	0.0953 (0.0895)	0.0916 (0.0863)	0.0965 (0.0905)	0.0928 (0.0873)

NOTE.—The values in parentheses are *p* distances. Gaps (insertions and additions of nucleotides) were excluded from the analysis, and a total of 939 nucleotides were compared. Nucleotide sequence data are from Hixson and Brown (1986). Scientific binomens for the five species compared are as follows: *Homo sapiens* (humans), *Pan troglodytes* (common chimpanzees), *Pan paniscus* (pygmy chimpanzees), *Gorilla gorilla* (gorillas), and *Pongo pigmaeus* (orangutans).

computed for each tree, and those for the best tree were set to be zero. Tree 2 was chosen by the FM and FM′ methods, whereas tree 3 was chosen by the ME method. The values for other trees are differences from the value of the best tree. It is interesting that all three methods gave rather large values when tree 4 was considered. Humans and pygmy chimpanzees, instead of common chimpanzees and pygmy chimpanzees, were clustered in this tree, and this unusual clustering apparently caused large values for PSD, S_O, and SBL.

The comparison of these four trees was also made for the MP and ML methods. The MP method chose trees 1 and 3 as equally parsimonious. This is consistent with Hixson and Brown's (1986) result. We need a rather large number (eight) of additional nucleotide substitutions for tree 4, compared with trees 1 and 3. As for the ML method, we used Felsenstein's DNAML program (observed nucleotide frequencies of A = 0.333, C = 0.257, G = 0.191, and T = 0.219 and a transition/transversion ratio = 5.0 were used), and tree 3 gave the highest likelihood value among four trees (see table 6). This is consistent with the result of Saitou (1988), who used a different maximum-likelihood algorithm.

The above results suggest that common chimpanzees and pygmy chimpanzees should be clustered. Among trees 1, 2, and 3, in all of which two chimpanzee species

Table 6
Analyses of Primate DNA Sequence Data by the Five Tree-making Methods

Method	Tree 1 (((PC)H)G)	Tree 2 (((PC)G)H)	Tree 3 ((PC)(HG))	Tree 4 (((PH)C)G)
FM	+0.60	0	+0.47	+25.33
FM′	+0.35	0	+0.16	+4.45
ME	+0.35	+1.10	0	+6.59
MP	0	+1	0	+8
ML	−2.98	−3.97	0	−33.86

NOTE.—The branching patterns of trees 1–4 are represented by designating a cluster of two species (or neighboring species) *i* and *j* as (*ij*). P, C, H, and G = pygmy chimpanzees, common chimpanzees, humans, and gorillas, respectively. The remaining species (orangutans) is used as an outgroup species. The values for the FM, FM′, and ME methods (*d* distances were used) are PSD, S_O, and SBL, respectively (see text for these quantities). Those for the MP method are the required number of nucleotide substitutions, and those for the ML method are the log likelihood values. The values for the best tree are set to be zero, and the other values represent the differences from the values of the best tree. The values for the FM′ and ME methods should be multiplied by 1/1,000.

were clustered, tree 3 was chosen as the best tree when we used the ME, NJ, and ML methods. However, tree 1 was chosen in an analysis of a larger data set, in which Hixson and Brown's (1986) data were combined with Brown et al.'s (1982) data (Saitou and Nei 1986). It has been shown that mitochondrial DNA sequence data of >2,000 nucleotides are probably necessary for determining the branching order of the mitochondrial DNA tree (a gene tree) for humans, chimpanzees, and gorillas (Saitou and Nei 1986). It is thus clear that no method is reliable unless there are more data.

When the number of nucleotides used is large, the NJ, ME, and ML methods show more or less the same efficiency, as seen from the comparison of the results for 300 and 600 nucleotides. However, there is a substantial difference in the computational time between the ML method and the other two methods. The computational time for the MP method is also considerably shorter than that for the ML method. Besides, the ML, ME, and MP methods are exhaustive search methods and require a prohibitively long computational time when the number of DNA sequences compared is large. Therefore, if we consider these practical aspects, the NJ method seems to be superior to the other methods.

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LITERATURE CITED

BLANKEN, R. L., L. C. KLOTZ, and A. G. HINNEBUSCH. 1982. Computer comparison of new and existing criteria for constructing evolutionary trees from sequence data. J. Mol. Evol. **19**:9–19.

BROWN, W. M., E. M. PRAGER, A. WANG, and A. C. WILSON. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. J. Mol. Evol. **18**:225–239.

CAVALLI-SFORZA, L. L., and A. W. F. EDWARDS. 1967. Phylogenetic analysis: models and estimation procedures. Am. J. Hum. Genet. **19**:233–257.

DAYHOFF, M. O. 1978. Ribosomal and other RNAs. P. 308 *in* M. O. DAYHOFF, ed. Atlas of protein sequence and structure. National Biomedical Research Foundation, Washington, D.C.

ECK, R. V., and M. O. DAYHOFF. 1966. Pp. 161–169 *in* M. O. DAYHOFF, ed. Atlas of protein sequence and structure. National Biomedical Research Foundation, Silver Spring, Md.

FELSENSTEIN, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. **17**:368–376.

FITCH, W. M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. Syst. Zool. **20**:406–416.

———. 1977. On the problem of discovering the most parsimonious tree. Am. Nat. **111**:223–257.

FITCH, W. M., and E. MARGOLIASH. 1967. Construction of phylogenetic trees. Science **155**: 279–284.

HASEGAWA, M., and T. YANO. 1984. Maximum likelihood method of phylogenetic inference from DNA sequence data. Bull. Biometric Soc. Japan **5**:1–7.

HIXSON, J. E., and W. M. BROWN. 1986. A comparison of the small ribosomal RNA genes from the mitochondrial DNA of the great apes and humans: sequence, structure, evolution, and phylogenetic implications. Mol. Biol. Evol. **3**:1–18.

JUKES, T. H., and C. R. CANTOR. 1969. Evolution of protein molecules. Pp. 21–132 *in* H. N. MUNRO, ed. Mammalian protein metabolism. Academic Press, New York.

LAKE, J. A. 1987. A rate-independent technique for analysis of nucleic acid sequences: evolutionary parsimony. Mol. Biol. Evol. **4**:167–191.

LI, W.-H., K. H. WOLFE, J. SOURDIS, and P. M. SHARP. 1988. Reconstruction of phylogenetic trees and estimation of divergence times under nonconstant rates of evolution. Cold Spring Harbor Symp. Quant. Biol. **52**:847–856.

NEI, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

ROBINSON, D. F., and L. R. FOULDS. 1981. Comparison of phylogenetic trees. Math. Biosci. **53**:131–147.

SAITOU, N. 1988. Property and efficiency of the maximum likelihood method for molecular phylogeny. J. Mol. Evol. **27**:261–273.

SAITOU, N., and M. NEI. 1986. The number of nucleotides required to determine the branching order of three species, with special reference to the human-chimpanzee-gorilla divergence. J. Mol. Evol. **24**:189–204.

———. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406–425.

SOURDIS, J., and C. KRIMBAS. 1987. Accuracy of phylogenetic trees estimated from DNA sequence data. Mol. Biol. Evol. **4**:159–166.

SOURDIS, J., and M. NEI. 1988. Relative efficiencies of the maximum parsimony and distance-matrix methods in obtaining the correct phylogenetic tree. Mol. Biol. Evol. **5**:298–311.

TATENO, Y., M. NEI, and F. TAJIMA. 1982. Accuracy of estimated phylogenetic trees from molecular data. I. Distantly related species. J. Mol. Evol. **18**:387–404.

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