AN ALTERNATING LEAST SQUARES APPROACH TO INFERRING PHYLOGENIES FROM PAIRWISE DISTANCES

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Running Head: Fitting Trees to Distances by Alternating Least Squares

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Abstract. – A computational method is presented for minimizing the weighted sum of squares of the differences between observed and expected pairwise distances between species, where the expectations are generated by an additive tree model. The criteria of Fitch and Margoliash (1967) and Cavalli-Sforza and Edwards (1967) are both weighted least squares, with different weights. The method presented iterates lengths of adjacent branches in the tree three at a time. It can be shown that the weighted sum of squares never increases during the process of iteration, and that the iterates approach a stationary point on the surface of the sum of squares. This iterative approach makes it particularly easy to maintain the constraint that branch lengths never become negative, although negative branch lengths can also be allowed. The method is implemented in a computer program, FITCH, which has been distributed since 1982 as part of the PHYLIP package of programs for inferring phylogenies, and is also implemented in PAUP*. The present method is compared on some simulated data sets to an implementation of the method of De Soete (1983); it is found to be slower than that method but more effective at finding the least squares tree. The relationship of this method to the Neighbor-Joining method is also discussed. [Phylogenies; distance methods; alternating least squares; Fitch-Margoliash method]

Distance matrix methods have long been used for inferring phylogenies, and their popularity has increased in recent years as potential users have become aware of long branch attraction problems that can afflict parsimony methods. They have been particularly widely used with molecular sequences. There are a wide variety of different distance matrix methods, including neighbor-joining (Saitou and Nei, 1987), minimum evolution (Kidd and Sgaramella-Zonta, 1971; Rzhetsky and Nei, 1992), and the least squares methods. The latter are of particular interest because they use a single objective function to solve for branch lengths and to choose among tree topologies, and can thus be related to the least squares method of statistical estimation. Some, though not all, computer simulations (cf. Kuhner and Felsenstein, 1994) have shown least squares methods to perform better than the other distance-based methods.

Fitch and Margoliash (1967) and Cavalli-Sforza and Edwards (1967) proposed different but closely related criteria for fitting trees to distance matrices. Their criteria were both of the form

$$Q = \sum_{i \in T} \sum_{j \in T} w_{ij} (D_{ij} - d_{ij})^2 \tag{1}$$

(T being the set of all tips on the tree) so that they were proposing a least squares fit of observed to expected distances. The statistical model implicit in this criterion is that the distances D_{ij} are distributed independently, with mean d_{ij} and variance the reciprocal of w_{ij}. Fitch and Margoliash took the variance of the distances to be proportional to their expectations d_{ij}, and approximated this by choosing as their weight the squared inverse of the observed distance:

$$w_{ij} = 1/D_{ij}^2, \tag{2}$$

while Cavalli-Sforza and Edwards assumed homogeneity of variances and thus chose $w_{ij} = 1$. In both methods we have an additive tree model: the expectations d_{ij}

are the sums of the branch lengths along a path from species i to species j in an unrooted tree whose tips (terminal nodes) are the species. The branch lengths are to be estimated by minimizing the weighted sum of squares Q. I have reviewed the biological and statistical issues involved in inferring phylogenies from distance matrices (Felsenstein, 1984); references to other distance methods will be found in that paper.

One of the difficulties with least squares methods has been to compute the branch lengths. These can be computed for a given tree topology by solving a set of linear equations (Cavalli-Sforza and Edwards, 1967) one for each branch. However this can be computationally diffcult, and it also does not allow us to constrain the branch lengths to be nonnegative.

The purpose of this paper is to provide details of an alternating least squares method that attempts to minimize Q for a given tree topology. It works for any weighting system for which the weights do not depend on the expected distances d_{ij}, and lends itself particularly well to the maintenance of the constraint that the branch lengths not become negative. The iterations result in a sequence of values of Q that are monotonic non-increasing, and converge to a stationary point on the least squares surface. Combined with an algorithm for searching among tree topologies, this alternating least squares method forms the basis of a computer program, FITCH, which has been distributed as part of the PHYLIP package since early 1982. PHYLIP executables, source code and documentation are available on the World Wide Web at http://evolution.genetics.washington.edu/phylip.html. The algorithm described in this paper also forms the basis for the least squares distance matrix method in David Swofford's program PAUP*.

The alternating least squares method (e.g. Wold, 1966) depends on a transformation that temporarily reduces the dimensionality of the problem. A method will be presented of finding the least squares branch lengths for three branches of the tree at a time, by "pruning" all but those branches from the tree, and solving exactly for the remaining three branch lengths. By doing this successively for different parts of the tree, one approaches asymptotically a least squares solution.

The Method

Notation

Figure 1 illustrates the notation employed. We chose some interior node of the unrooted tree and number it 0. All other nodes are numbered in some order $1, ..., 2n-3$, where n is the number of tips. It is usually most convenient to number the tips 1 through n. The branches of the tree are also numbered: each is assigned the number of the node at the end furthest from node 0. In this Figure tips i, j, and k, and node l, are shown. The length of branch i is denoted v_i.

The tree topology will be specified by constants $x_{ij,k}$, where $x_{ij,k}$ is 1 if branch k lies on the path from node i to node j, and 0 otherwise. In practice, the array $x_{ij,k}$ need not be formed since its elements can be recovered as needed from the data structures representing the tree. The D_{ij} and the w_{ij} are assumed to be given at the beginning of the computation for all pairs of tips i and j.

Pruning the Tree

Our immediate objective will be, starting with some arbitrarily specified branch lengths, to minimize the sum of squares Q with respect to the lengths of the three branches incident on an interior node (e.g. node 0), holding all other branch lengths constant. We can reduce the size of the problem by "pruning" the tree, removing tips i and j and replacing the interior node k by a new tip, in such a way that the sum of squares for this new tree as a function of the lengths of its remaining branches differs from the sum of squares Q by a calculable constant. This constant does not depend on the three branch lengths whose lengths we are to improve. By pruning the tree repeatedly we can finally reduce it to a three-species tree whose central node is node 0. The least squares branch lengths for this tree can be easily computed, and the value of Q for the full tree differs from that of this three-species tree by a known constant. This means that we can pick any interior node, and then find for the three branches incident upon it the branch lengths that minimize Q, and the value of Q. We can move around the tree, doing this succesively for different interior nodes. The result is the required iterative method for minimizing the sum of squares Q.

Considered as a function of the unknown branch lengths v_i, the sum of squares is

$$Q = \sum_{p \in T} \sum_{q \in T} w_{pq} (D_{pq} - \sum_{u \in B} x_{pq,u} v_u)^2. \tag{3}$$

where T is the set of all tips and B the set of all branches of the tree. This is a quadratic form in the v_i. It could be minimized by differentiating with respect to the v_i and equating the derivatives to zero to obtain normal equations for the v_i, but this will not be the approach taken here. We want to replace the problem of minimizing Q with respect to the full set of the v_i by a smaller problem. Consider the phylogeny in Figure 1. For the moment we revert to the simpler equation (1), keeping in mind that the d_{ij} are sums of branch lengths. Suppose that we were to regard the lengths of the branches connected to two adjacent tips, i and j, as constants, and consider minimizing Q with respect to all branch lengths except i and j. Let S be the set of all remaining tips after i and j have been removed, and after the node at which they join, k, has been added to the set of tips. If k were actually a tip it would have distances D_{kl} to all other tips still on the tree. In this smaller tree which lacks segments i and j but has k, the sum of squares is

$$R = \sum_{p \in S} \sum_{q \in S} w_{pq} (D_{pq} - d_{pq})^2. \tag{4}$$

the d_{pq} being the sums of branch lengths between p and q in this pruned tree. We would like to find a constant K, a set of distances D_{kl}, and a set of weights w_{kl} such that $Q = R + K$, where K does not depend on the lengths of any of the branches except i and j. If we can do this, then the quadratic form R will be minimized at the same values of the remaining branch lengths as will Q.

We can find K, the D_{kl}, and the w_{kl}, by a process of equating coefficients in R to corresponding terms in Q. We consider Q and R as functions of the branch lengths v_u. We first note that, for all pairs of tips (p, q) in S, neither of which can be equal to i, j or k, the terms in R are identical to those in Q. This leaves us with only terms involving i or j in Q or k in R. Taking the difference $Q - R$, we can write this as

$$Q - R = \sum_{l \in S} w_{il} (D_{il} - d_{il})^2 + \sum_{l \in S} w_{jl} (D_{jl} - d_{jl})^2 + w_{ij} (D_{ij} - d_{ij})^2 - \sum_{l \in S} w_{kl} (D_{kl} - d_{kl})^2 \tag{5}$$

Recall that the lengths of the branches other than i and j remain in (5) only in the quantities d_{kl}. Recall also that

$$\begin{aligned} d_{il} &= d_{kl} + v_i \\ d_{jl} &= d_{kl} + v_j. \end{aligned} \tag{6}$$

Inserting the expressions in (6) into (5) and collecting the terms in d_{kl} and d_{kl}^2, we find that the coefficient of d_{kl}^2 in $Q - R$ is

$$w_{kl} - w_{il} - w_{jl}$$

and the coefficient of d_{kl} is

$$-2w_{kl}D_{kl} + 2w_{il}D_{il} + 2w_{jl}D_{jl} - 2w_{il}v_i - 2w_{jl}v_j.$$

If the difference between Q and R is to be a constant independent of the lengths of the branches remaining on the pruned tree, then the above coefficients must all be zero. Equating them to zero we can solve for the w_{kl} and the D_{kl}:

$$w_{kl} = w_{il} + w_{jl} \tag{7}$$

and

$$D_{kl} = \frac{w_{il}(D_{il} - v_i) + w_{jl}(D_{jl} - v_j)}{w_{il} + w_{jl}}. \tag{8}$$

Since the terms in d_{kl} have been eliminated from $Q - R$, we have now eliminated all terms that could contain any of the branch lengths other than v_i and v_j. The difference between Q and R must then be a constant not containing the lengths of any branches other than i and j. We can find it by collecting all the terms in (5) that do not have d_{kl} in them:

$$Q - R = \sum_{l \in S} w_{il}(D_{il} - v_i)^2 + \sum_{l \in S} w_{jl}(D_{jl} - v_j)^2 + w_{ij}D_{ij}{}^2 - \sum_{l \in S} w_{kl}D_{kl}^2 \tag{9}$$

and using (7) and (8) this can be reduced after some algebra to

$$Q - R = w_{ij}D_{ij}^2 + \sum_{l \in S} w_{il}w_{jl}\left[(D_{il} - v_i) - (D_{jl} - v_j)\right]^2 \Big/ (w_{il} + w_{jl}), \tag{10}$$

which can never be negative.

Equations (7), (8) and (10) give us the means of reducing the size of the problem from n tips to $n-1$ tips, dropping two branch lengths from the minimization problem. We now show how this can be used to construct an alternating least squares method for minimizing Q.

Iteration of Branch Lengths

We consider only the case of an unrooted bifurcating tree, which has each interior node connected to three neighbors. As can be seen in Figure 1, if one proceeds outwards from an internal node such as node 0 in any of the three possible directions, one finds a rooted bifurcating tree. In any rooted bifurcating tree having two or more tips there are always at least two tips branching from the same interior node: in Figure 1 tips i and j are two of these, but three other such pairs are also visible. Equations (7), (8) and (10) permit us to reduce the size of the problem by removing two tips and creating one new one, while not affecting the values of the least squares estimates of the lengths of the remaining branches. We can continue this process, repeatedly applying these equations until only three tips are left, all connected directly to the designated interior node (node 0). Let us designate these three nodes as a, b, and c.

The problem is now reduced to minimizing the sum of squares

$$Q = K + w_{ab}(D_{ab} - v_a - v_b)^2 + w_{bc}(D_{bc} - v_b - v_c)^2 + w_{ac}(D_{ac} - v_a - v_c)^2 \qquad (11)$$

As Farris (1972) has pointed out, the minimimum of (11) is achieved when the rightmost three terms become zero when the branch lengths v_a, v_b, and v_c are chosen to satisfy the equations:

$$\begin{aligned} D_{ab} &= v_a + v_b, \\ D_{bc} &= v_b + v_c, \\ D_{ac} &= v_a + v_c, \end{aligned} \qquad (12)$$

these being the normal equations that are derived by differentiating Q with respect to the branch lengths and equating the derivatives to zero. The solution (Farris, 1972) is

$$\begin{aligned} v_a &= (D_{ab} + D_{ac} - D_{bc})/2, \\ v_b &= (D_{ab} + D_{bc} - D_{ac})/2, \\ v_c &= (D_{ac} + D_{bc} - D_{ab})/2. \end{aligned} \tag{13}$$

Having used equations (7) and (8) to compute the quantities w_{ab}, w_{bc}, w_{ac}, D_{ab}, D_{bc}, and D_{ac}, we have minimized the sum of squares Q with respect to the three branch lengths v_a, v_b, and v_c, holding all the other branch lengths constant. It follows that Q is nonincreasing during each such step.

If we follow the strategy of moving through the tree, taking each interior node of the tree in turn, pruning the tree to reduce the problem to minimization of Q with respect to the three branch lengths incident on that node, and finding the optimum lengths for those three branches, the successive values of the sums of squares Q cannot increase: they will form a monotonic nonincreasing sequence bounded below by zero. The sequence of values of Q must thus converge; it seems a reasonable expectation that the sequence of values of the branch lengths will also converge.

The iteration at each stage sets the derivatives of Q with respect to three of the branch lengths to zero. When further iteration through all interior nodes of the tree produces no change, it follows that the derivatives of Q with respect to all branch lengths are zero, so that we have reached a stationary point. We cannot guarantee from this that the stationary point is a minimum. However, a glance at equation (1) shows that if the w_{ij} are nonnegative the quadratic form Q cannot ever be negative; this is sufficient to guarantee that no saddle-points or maxima can exist. We have not ruled out that there could be directions in which the sum of squares might be unchanging. This is the case where the quadratic form is positive semi-definite. In such a case the algorithm will reach one of the tied points along the line (or plane) of equally good solutions. We can in any case guarantee that we have minimized the quadratic form (1).

An alternative scheme would of course be to set up and solve the linear equations which are the normal equations for the least squares problem. The present method amounts to an iterative approach to solving these equations, differing from conventional iterative approaches such as Gauss-Seidel iteration by changing the variables three at a time instead of one at a time. It also avoids directly setting up the equations, and takes advantage of the sparseness of the coefficient matrix in a natural way. As we shall see, it also enables us to maintain the constraint that branch lengths not be negative, if that is desired.

The Algorithm

The strategy used in the program FITCH starts with the first three species. These are connected into an unrooted tree (only one topology is possible) and equations (13) used to solve for least squares branch lengths. The program then considers where the fourth species can be added to the tree. There are three possible places that a new internal node could be added, with the fourth species connected to it, and these are on the interiors of the three branches. Each of these is tried in turn, and the least squares branch lengths found for each of these topologies. The program accepts that placement of the species that results in the smallest sum of squares.

The algorithm continues in this fashion, adding each species to all possible places in the tree, and picking the placement which minimizes the sum of squares after iterative computation of the least squares branch lengths. It is convenient to start the iteration for each topology by calculating the lengths of the branches incident upon the newly introduced node, since that provides us with starting values for their lengths. The iteration for each topology procedes by traversing the tree outwards from that node, optimizing branch lengths for each internal node encountered. Although it would perhaps be best to repeat the traversal until the sum of squares stopped changing, I have found for the data sets that I have encountered that four passes through the tree is quite sufficient.

After each species is added to the tree, except the fourth species, a series of local rearrangements is carried out to see if the tree can be improved. A local rearrangement switches the order of adjacent branches in the tree. In the present version of the program all local rearrangements are tried after each species is added. If any local rearrangements improve the tree, as judged by the sum of squares, the rearrangement process is continued until no further improvement by local rearrangement is possible.

The user has the option of specifying that, after the last species is added to the tree, the last bout of rearrangements should be global. In that case, each subtree is removed from the tree and reinserted in all possible places, the best of these being chosen, and the process continued until no further improvement results. Swofford and Olsen (1990) have described this rearrangement strategy as Subtree Pruning and Regrafting. At that point we have a tree that cannot be improved upon by moving any single group. This does not guarantee us that we have found the best topology, but it gives us some reassurance that we have at least made a serious attempt to find it.

In PAUP* the strategy for searching among tree topologies is different and will not be described here.

Avoiding Negative Branch Lengths

I have argued elsewhere (Felsenstein, 1984; 1986) that it is appropriate to find the least squares tree among all those having no negative branch lengths. The present algorithm does not avoid negative branch lengths, as it is entirely possible for one or more of the solutions to equations (13) to be negative. However, it is quite easy to alter the algorithm to avoid negative branch lengths; this is what is done by default in FITCH, allowing negative branch lengths being an option.

When we are finding the optimum values of the three branch lengths around an interior node, suppose that we require that (11) be minimized but without allowing any of v_a, v_b, or v_c to become negative. If the solution to (13) does not find any negative branch length then there is no problem. If one or more of the branch lengths is negative, in principle we should examine all seven possible patterns of zero branch lengths in which one, two, or all three of the branch lengths are zero.

It would be possible to minimize (11) for each and pick the best solution, but I have followed a simpler and less exact strategy. Any of the branch lengths that have become negative are set to zero, and the resulting values taken as a starting point. Each of the three branch lengths is then considered in turn. Fixing the values of the other two, the least squares solution for v_a is

$$\hat{v}_a = \left[w_{ab}(D_{ab} - v_b) + w_{ac}(D_{ac} - v_c)\right] \Big/ (w_{ab} + w_{ac}) \tag{14}$$

If the minimum occurs at a negative branch length, then the nonnegative value of that branch length which minimizes (11) will be zero, since (11) is a quadratic in each of its three variables, and has positive curvature in each. Thus, when a branch length computed from (14) becomes negative, it is instead set to zero. The branch lengths v_a, v_b, and v_c are determined in this way in turn, until there is no further change. Although this procedure is not guaranteed always to find the best nonnegative values of the branch lengths, it seems to do quite well in practice.

Complexity of the Computation

In calculating how much effort is involved in the computation, we must consider how many topologies of each given size are examined, and how much effort is involved in optimizing the branch lengths for each topology. We start with three species, adding the fourth in each of three possible places. When we add the n-th species, there are $2n - 5$ possible places to add it. There are also, for $n > 4$, $2n - 6$ local rearrangements that will be tried, assuming that none of them causes the tree to be altered, which in turn would require additional rounds of rearrangement. The addition of the n-th species will thus cause $4n - 11$ evaluations of the least squares branch lengths.

The evaluation of branch lengths on a tree requires four passes through the tree. Each of these prunes the tree $n - 3$ times if it possesses n tips, and each time it is pruned the distances of the central node to all other nodes are updated. When there are m nodes remaining on the tree as it is being pruned, this updating requires on the order of $(2m - 3)$ operations.

Putting all of this together, one can show that estimating a tree and its branch lengths requires on the order of n^4 operations. This means that the algorithm will be fairly slow for large numbers of tips. In a case having 20 species, FITCH required 46.9 seconds to execute on my DECstation 5000/125, a machine whose SPECfp92 rating is approximately 25 (so that it is approximately as fast as a 486DX/66). A similar case having 40 species (the 20 species were a random sample from these 40) took 730 seconds. This is 15.565 times longer, very close to the expected multiplier of $2^4 = 16$.

A Numerical Example

We can get some feel for the progress of the iteration by watching its progress on an example. Consider the data set of Sarich (1969), which is reproduced in Table 1.

Running FITCH on this data set without allowing negative branch lengths, using the Fitch-Margoliash criterion, gives the tree shown in Figure 2, which is rooted using the monkey as an outgroup. The weighted sum of squares for this tree is 0.06996. Figure 3 shows the progress of branch length iteration when we take this tree topology and initially set all branch lengths equal to 1. The graph shows 8 passes through the tree: the branch lengths essentially cease changing after the first four.

It should not be unexpected that the iteration succeeds rapidly. For moderately clean data, the iteration computes the internal branch lengths using (13), which in effect uses weighted averages of the distances between members of the three different subtrees defined by an internal node. Once the tree approaches its final branch lengths, if there is not serious internal conflict in the data these weighted averages depend rather little on the details of the branch lengths within the subtrees, and hence the branch lengths rapidly stabilize. It is the very independence of evolution in the different subtrees, the very treeness of the data, that ensures the success of this alternating least squares approach.

An Example with Sequence Data

To give a better feel for how the present algorithm copes with real data, we will analyze the metazoan data set of Turbeville et. al. (1994). These have 16 species, many of them chordates or their near relatives. These data can be retrieved from the alignments section of the EMBL database as alignment DS16914. Both their analysis and this one used only a subset of better-aligned sites. FITCH took 17.6 seconds to analyze these distances on a 486DX/33 running the Linux operating system. On a Digital Alphastation 400 4/233 it took 1.46 seconds. The tree (shown in Figure 4) is identical to the tree produced by Turbeville et. al. using the same program. It may be worth noting that in the study by Turbeville et. al., the trees produced by FITCH were similar to those produced by neighbor-joining and by likelihood, and trees produced by parsimony analysis of a combined molecular and morphological data set, and these trees all supported the existence of the Chordata. These differed from the tree produced by parsimony on the molecular data. The utility of a comparison based on a single case, is, however, limited.

Comparison with De Soete's method

An approach to searching over additive trees, constraining the estimated branch lengths to be nonnegative, is the innovative method of De Soete (1983), which starts with the observed distances and then gradually brings them closer to additive tree distances while searching for the least squares fit. This is quite different from the present approach, which searches in the space of additive trees: De Soete's method approaches that space from outside. It is therefore possible that it may carry out a far more effective search for the tree topology that leads to the minimum sum of squares.

The most widely used implementation of De Soete's method is probably *lsadt*, a C program by Michael Maciukenas which is distributed in Stephen Smith's Genetic Data Environment (GDE) package of programs for DNA analysis. To test whether the present method had any advantages over *lsadt*, I have simulated the evolution of 20 DNA sequences on a tree. The tree was generated by a branching process, with the rate of branching of a lineage being 1 per unit time. Branching was continued until the 21st lineage was just about to be produced, and then the process was stopped.

DNA sequences of 300 bases in length were stochastically evolved along the resulting trees. Each branch had an expected rate of change per unit time of either

0.066667 or 0.2, these values being chosen with equal probability. Thus the resulting trees were not ultrametric when the branch lengths relevant to base change are considered. All sites changed with equal probabilities, and a Kimura 2-parameter model (Kimura, 1980) was used, with instantaneous transition/transversion ratio equal to 2.

100 trees and data sets were produced by this simulation. Of these, 30 had at least two identical DNA sequences on them. The resulting distances in those cases caused *lsadt* to terminate with an error message. The remaining 70 distance matrices were analyzed with both programs. The sum of squares of the fit from the *lsadt* results is not provided by that program – it was computed by using the estimated trees that emerged from *lsadt* as user trees whose branch lengths were not to be re-estimated in a run of FITCH. In analysis of the distance matrices by FITCH the unweighted least squares method of Cavalli-Sforza and Edwards (1967) was used, as *lsadt* was also using that unweighted criterion.

In every case *lsadt* was at least 10 times faster than FITCH. Of the 70 distance matrices on which both methods were run, for 4 of them the results were identical to five decimal places. In all 66 of the ones in which the results were different, FITCH gave a lower sum of squares. In many of these the differences were small, indicating that some accuracy may have been sacrificed for speed in *lsadt*. But in 25 of the 66 cases, the *lsadt* sum of squares was more than 1% greater, and in 5 cases it was more than 10% greater. When the trees were examined more closely, there were found to be 24 cases in which the tree topologies estimated by *lsadt* and FITCH differed. In none of these was the difference due to rearrangement of zero-length branches. In 11 of these 24 cases the sum of squares differed by more than 1%, in 2 cases by more than 10%. It is interesting to note that this means that in 14 cases two trees of the same topology differed in sum of squares by more than 1%, and in 3 cases by more than 10%.

The results suggest that the present method can sometimes find better tree topologies, and/or better branch lengths, than De Soete's method, in spite of the constraint on its search algorithm to stay within the space of additive trees. It is not clear how other implementations of DeSoete's method would perform in these comparisons.

Relationship to the Neighbor-Joining method

The Neighbor-Joining method of Saitou and Nei (1987) has become popular owing to its speed: its execution time is proportional to the cube of the number of species. Simulation studies (Kuhner and Felsenstein, 1994) show it to be nearly as effective as the Fitch-Margoliash method in recovering the true phylogeny. It estimates the lengths of branches to two tips that are "neighbors" on the tree, then removes these and replaces them with a new tip. Distances are calculated from the new tip to all other tips currently on the tree. Saitou and Nei show that the step that estimates the branch lengths of two neighbors makes a least squares estimate, by the unweighted criterion of Cavalli-Sforza and Edwards (1967) of the branch lengths v_i and v_j, for a tree which has i and j as neighbors but has all the other tips that remain on the tree branching from a multifurcating node. Figure 5 shows the tree topology to which this least squares estimate applies. Neighbor-Joining may thus be regarded as an approximation to the least squares algorithm of Cavalli-Sforza and Edwards (1967). It differs from the present method in that, having settled on branch lengths v_i and v_j, it never returns to that part of the tree to re-estimate them.

Saitou and Nei's algorithm "prunes" the tree. Its recalculation of distances from node k to each remaining tip closely parallels the current method: it uses

$$D_{kl} = (D_{il} + D_{jl} - D_{ij})/2. \tag{15}$$

Note that since, in Neighbor-Joining as in the current method, for two neighbors on the tree $D_{ij} = v_i + v_j$, we can substitute this into equation (15) and rearrange it into the case of equation (8) for which $w_{il} = w_{jl} = 1$, as is true for the Cavalli-Sforza and Edwards criterion.

In the Neighbor-Joining method, the values of v_i and v_j are determined when the tree is as shown in Figure 5. Being identical to the values one would get from Cavalli-Sforza and Edwards's unweighted least squares criterion, they are also identical to the values that our algorithm would give to v_i and v_j if iteration was done with the tree structure of Figure 5. The relative success of the Neighbor-Joining algorithm in approximating the least squares solution to a completely resolved tree suggests that the estimate is not very sensitive to the details of the resolution of the multifurcation in Figure 5. David Swofford (personal communication) has pointed out that this may also be the reason for the rapidity with which the present algorithm converges, as shown in Figure 3. As v_i and v_j are not very sensitive to the other details of the structure of the tree, they reach reasonable values very rapidly.

We may thus regard the Neighbor-Joining method as a quick and fairly accurate approximation to the unweighted least squares method.

Relationship to the Minimum Evolution method

The Minimum Evolution distance matrix method (Kidd and Sgaramella-Zonta, 1971; Rzketsky and Nei, 1992) searches among tree topologies. For each tree topology it evaluates branch lengths by least-squares fitting. The topology is evaluated, not by the overall sum of squares, but by the sum of the lengths of these branches. The present algorithms can thus be used to do the branch length calculations in a minimum-evolution method. It is thus not hard to modify a program that infers phylogenies by least squares to make one that infers them by minimum evolution. This will be done as an option in future versions of FITCH.

Summary

This paper does not describe a new distance matrix method. Instead, it introduces a new computational framework for the long-existing least squares family of distance matrix methods. This framework, by making effective use of the structure of the tree, allows us to maintain a constraint of nonnegativity of branch lengths. The algorithm "prunes" the sum of squares on the tree in a natural way. This process may be helpful for other tree calculations that use least squares. It can serve as the basis for either least squares estimation of the tree or minimum-evolution estimation, and has some relationship to the neighbor-joining method. When used in connection with local rearrangement of the tree, it seems more effective, if slower, than De Soete's innovative algorithm for searching the space of trees. It forms the basis of the least-squares distance matrix calculations in the FITCH program of PHYLIP, and in PAUP*. Having been in distribution in the former since 1982, it has been used to compute most of the least squares trees that have been published in the systematics and the molecular evoluition literature.

Acknowledgments

I wish to thank David Swofford for illuminating comments in discussion of these matters, and John Huelsenbeck, Peter Beerli, and Mary Kuhner for corrections of the manuscript and helpful suggestions. This work was supported in part by task agreement number DE-AT06-76EV71005 of contract number DE-AM06-76RLO2225 between the U. S. Department of Energy and the University of Washington, by National Science Foundation Grant numbers BSR-8614807, DEB-9207558, and BIR-9527687, and by National Institutes of Health grants 2 R55 GM41716-04 and 1 R01 GM51929-01.

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TABLE 1

The immunological distance data set of Sarich (1969) symmetrized. Dog: *Canis familiaris*, bear: *Ursus americanus*, raccoon: *Procyon lotor*, weasel: *Mustela vison*, seal: *Phoca vitulina richardii*, sea lion: *Zalophus californicus*, cat: *Felis domestica*, monkey: *Aotus trivirgatus*. These data were used to construct the tree given in Figure 2.

	dog	bear	raccoon	weasel	seal	sea lion	cat	monkey
dog	0	32	48	51	50	48	98	148
bear	32	0	26	34	29	33	84	136
raccoon	48	26	0	42	44	44	92	152
weasel	51	34	42	0	44	38	86	142
seal	50	29	44	44	0	24	89	142
sea lion	48	33	44	38	24	0	90	142
cat	98	84	92	86	89	90	0	148
monkey	148	136	152	142	142	142	148	0

FIGURE CAPTIONS

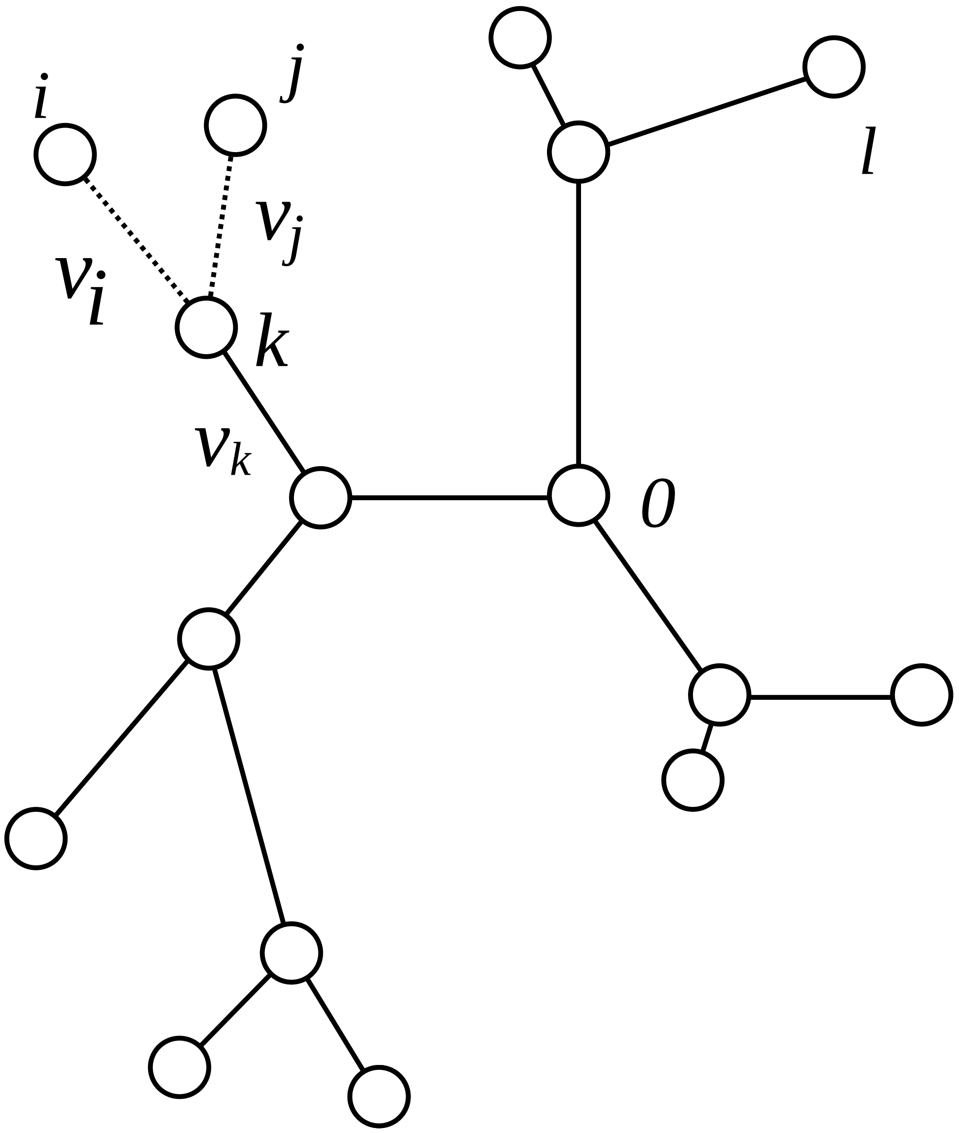
FIGURE 1. The tree used to illustrate the pattern of computations in the text.

FIGURE 2. The least squares estimate for the Sarich (1969) data set, using the Fitch-Margoliash criterion and the algorithm described in this paper. Horizontal distance is proportional to branch length. The tree has been rooted with monkey as the outgroup.

FIGURE 3. Change of branch lengths in eight passes through a tree (that of Figure 2) starting with arbitrary initial values. Note that the lengths essentially reach their final values after four passes. As each iteration consists of successive changes around each of the six interior nodes, we divide each time interval into sixths and show each change at the point that it occurs.

FIGURE 4. The tree produced by FITCH on the 16s rRNA data set of Turbeville et. al. (1994), when distances are computed by the Kimura 2-parameter method with transition/traversion ratio 2.0. The alignment and selection of sites used was that recommended by these authors.

FIGURE 5. Tree form used in the computation of the lengths of branches i and j in the Neighbor-Joining method.


i
j
v_i
v_j
k
v_k
l
0

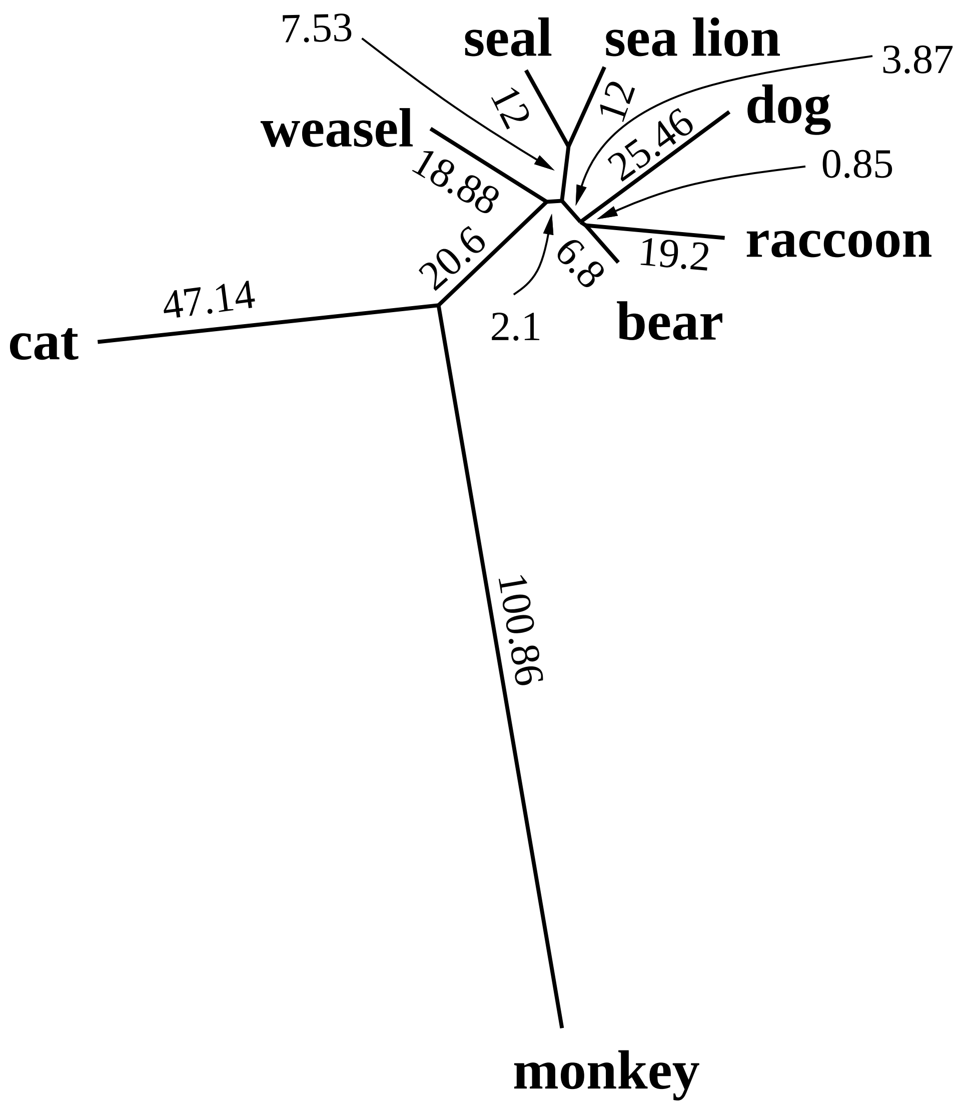

7.53
seal
sea lion
3.87
12
12
dog
weasel
25.46
18.88
0.85
raccoon
20.6
6.8
19.2
47.14
cat
2.1
bear
100.86
monkey

Figure 2

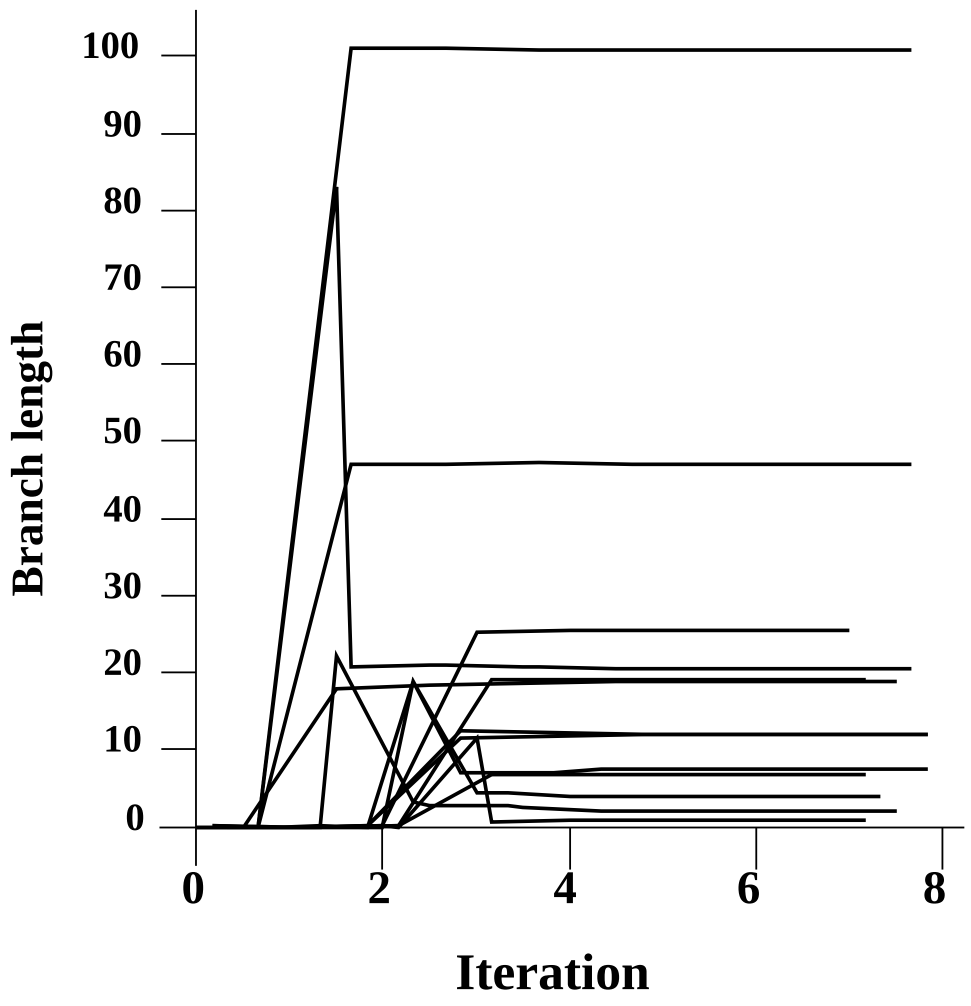


Figure 3

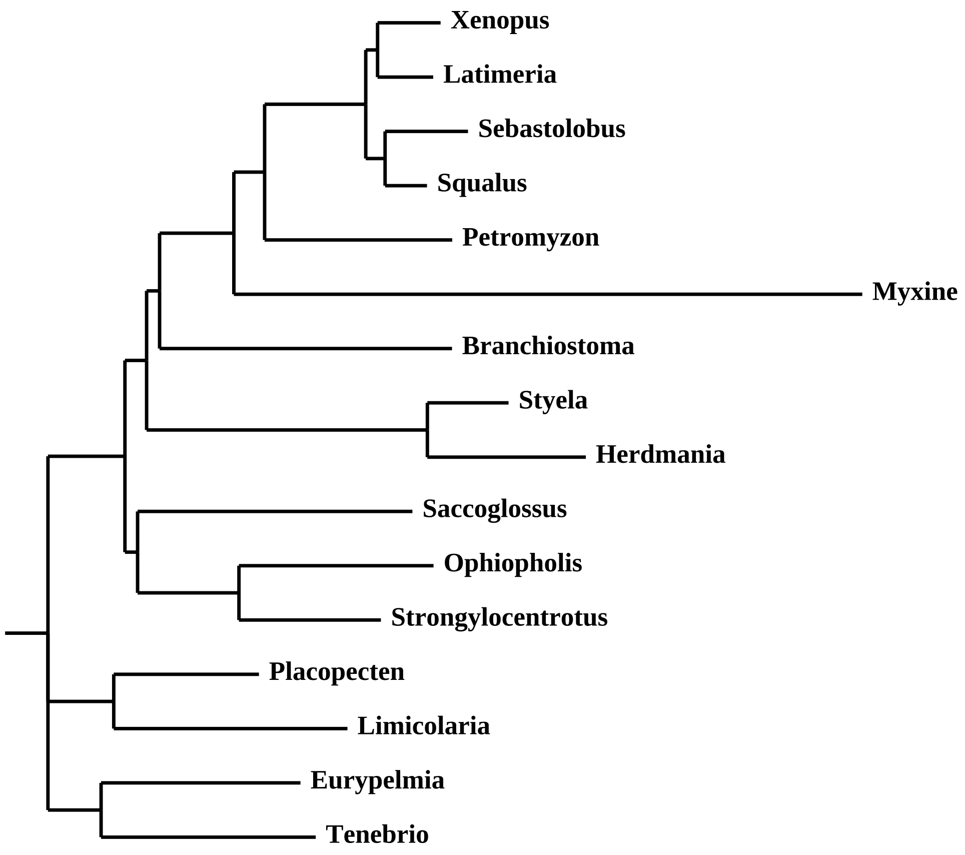

Xenopus
Latimeria
Sebastolobus
Squalus
Petromyzon
Myxine
Branchiostoma
Styela
Herdmania
Saccoglossus
Ophiopholis
Strongylocentrotus
Placopecten
Limicolaria
Eurypelmia
Tenebrio

Figure 4

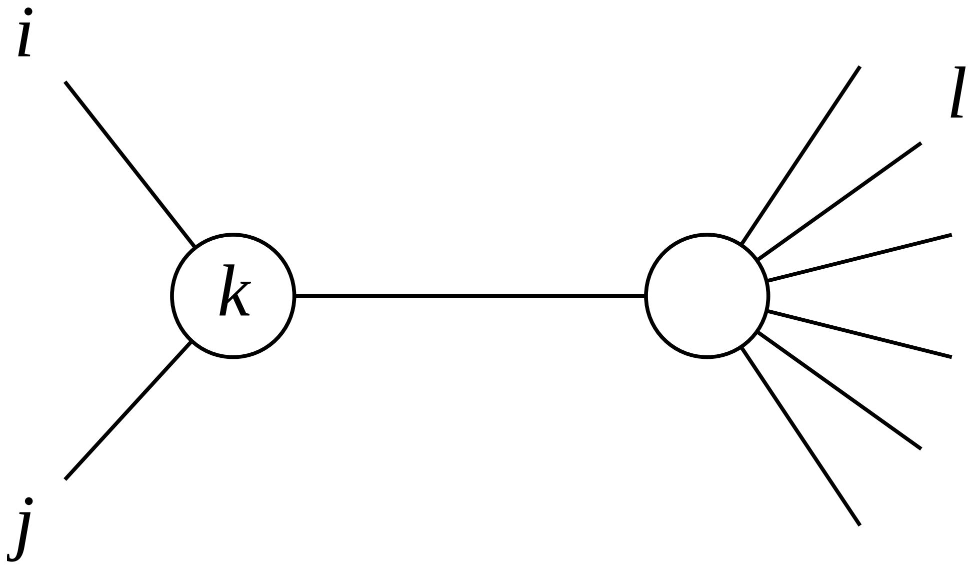


Figure 5