14.5.1. How to score multiple alignments

Although the notion of a multiple alignment is easily extended from two strings to many strings, the *score* or goodness of a multiple alignment is not as easily generalized. To date,

[9] However, this explanation does not cover all the cases of high correlation, for there are pairs of positions with high correlation that do not correspond to base-paired nucleotides in the *tRNA*. Also, positions at which the nucleotide does not mutate are not well handled by this method.

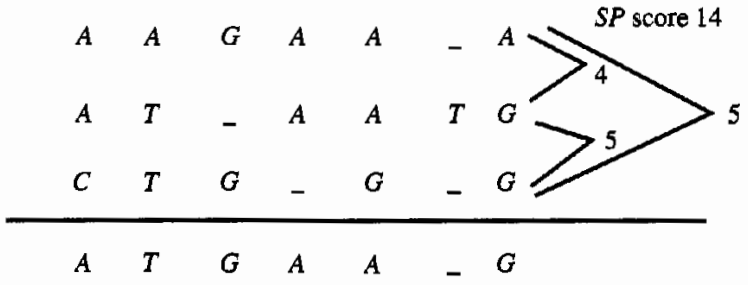


Figure 14.5: Multiple alignment $\mathcal{M}$ of three strings shown above the horizontal line. Using the pairwise scoring scheme of *ms* + *id*, i.e., #(mismatches) + #(spaces opposite a nonspace), the three induced pairwise alignments have scores of 4, 5, and 5 for a total *SP* score of 14. Note that a space opposite a space contributes a zero. Using the plurality rule, the consensus string $S_{\mathcal{M}}$ (defined in Section 14.7.2) for alignment $\mathcal{M}$ is shown below the horizontal line. It has an alignment error of seven.

there is no objective function that has been as well accepted for multiple alignment as (weighted) edit distance or similarity has been for two-string alignment. In fact, as we will see later, some popular methods to find multiple alignments do so without using any explicit objective function. The goodness of those methods is judged by the biological meaning of the alignments that they produce, and so the biological insight of the evaluator is of critical importance.

Not being biologists, we will emphasize multiple alignment methods that *are* guided by explicit objective functions, although we will sketch other methods as well. We will discuss three types of objective functions in this chapter: *sum-of-pairs functions*, *consensus functions*, and *tree functions*. Although these objective functions are quite different, we will explore approximation algorithms for these problems that are highly related. In Part IV of the book we will discuss how multiple alignment problems interact with problems of deducing trees representing evolutionary history.

Definition Given a multiple alignment $\mathcal{M}$, the *induced pairwise alignment* of two strings S_i and S_j is obtained from $\mathcal{M}$ by removing all rows except the two rows for S_i and S_j. That is, the induced alignment is the multiple alignment $\mathcal{M}$ restricted to S_i and S_j. Any two opposing spaces in that induced alignment can be removed if desired.

Definition The *score* of an induced pairwise alignment is determined using any chosen scoring scheme for two-string alignment in the standard manner.

For an example, see Figure 14.5.

Notice that the definition of "score" does not specify whether a score is a weighted distance or a similarity. As before, similarity is more natural for treating local alignment. Most of this chapter concerns global alignment, where the algorithms and consequent theorems all require the score to be a weighted distance.

14.6. Multiple alignment with the sum-of-pairs (*SP*) objective function

The sum-of-pairs (*SP*) score

Definition The *sum of pairs* (*SP*) score of a multiple alignment $\mathcal{M}$ is the sum of the scores of pairwise global alignments induced by $\mathcal{M}$. See Figure 14.5.

Although one can give "handwaving" arguments for the significance of the *SP* score,

it is difficult to give a theoretical justification for it (or any other multiple alignment scoring scheme). However, the *SP* score is easy to work with and has been used by many people studying multiple alignment. The *SP* score was first introduced in [88], and was subsequently used in [33, 334], and [187]. A similar score is used in [153]. The *SP* score is also used in a subtask in the multiple alignment package MACAW [398] developed at the National Institutes of Health (NIH), National Center for Biotechnology Information.

The SP alignment problem Compute a global multiple alignment $\mathcal{M}$ with *minimum* sum-of-pairs score.

14.6.1. An exact solution to the *SP* alignment problem

As might be expected, the *SP* problem can be solved exactly (optimally) via dynamic programming [334]. Unfortunately, if there are k strings and each is of length n say, dynamic programming takes $\Theta(n^k)$ time and hence is practical for only a small number of strings. Moreover, the exact *SP* alignment problem has been proven to be NP-complete [454]. We will therefore develop the dynamic programming recurrences only for the case of three strings. Extension to any larger number of strings is straightforward, although it proves to be impractical for even five strings whose lengths are in the low hundreds (typical of proteins). We will also develop an accelerant to the basic dynamic programming solution that somewhat increases the number of strings that can be optimally aligned.

Definition Let S_1, S_2, and S_3 denote three strings of lengths n_1, n_2, and n_3, respectively, and let $D(i, j, k)$ be the optimal *SP* score for aligning $S_1[1..i]$, $S_2[1..j]$, and $S_3[1..k]$. The score for a match, mismatch, or space is specified by the variables *smatch*, *smis*, and *sspace*, respectively.

The dynamic programming table D used to align three strings forms a three-dimensional cube. Each cell (i, j, k) that is not on a boundary of the table (i.e., that has no index equal to zero) has seven neighbors that must be consulted to determine $D(i, j, k)$. The general recurrences for computing the cost of a nonboundary cell are similar in spirit to the recurrences for two strings but are a bit more involved. The recurrences are encoded in the following pseudocode:

Recurrences for a nonboundary cell (i, j)

```
for i := 1 to n1 do
    for j := 1 to n2 do
        for k := 1 to n3 do
            begin
            if (S1(i) = S2(j)) then cij := smatch
            else cij := smis;
            if (S1(i) = S3(k)) then cik := smatch
            else cik := smis;
            if (S2(j) = S3(k)) then cjk := smatch
            else cjk := smis;

            d1 := D(i−1, j−1, k−1) + cij + cik + cjk;
            d2 := D(i−1, j−1 ,k) + cij + 2*sspace;
            d3 := D(i−1, j, k−1) + cik + 2*sspace;
```

```
d4 := D(i, j−1, k−1) + cjk + 2*sspace;
d5 := D(i−1, j, k) + 2*sspace;
d6 := D(i, j−1, k) + 2*sspace;
d7 := D(i, j, k−1) + 2*sspace;

D(i, j, k) := Min[d1, d2, d3, d4, d5, d6, d7];
end;
```

What remains is to specify how to compute the D values for the boundary cells on the three initial faces of the table (i.e., when $i = 0$, $j = 0$, or $k = 0$). To do this, let $D_{1,2}(i, j)$ denote the familiar pairwise distance between substrings $S_1[1..i]$ and $S_2[1..j]$, and let $D_{1,3}(i, k)$ and $D_{2,3}(j, k)$ denote the analogous pairwise distances involving pairs S_1, S_3 and S_2, S_3. These distances are computed in the standard way. Then,

$$D(i, j, 0) = D_{1,2}(i, j) + (i + j) * sspace,$$

$$D(i, 0, k) = D_{1,3}(i, k) + (i + k) * sspace,$$

$$D(0, j, k) = D_{2,3}(j, k) + (j + k) * sspace,$$

and

$$D(0, 0, 0) = 0.$$

The correctness of the recurrences and the fact that they can be evaluated in $O(n_1 n_2 n_3)$ time is left to the reader. The recurrences can be generalized to incorporate alphabet-weighted scores, and this is also left to the reader.

A speedup for the exact solution

Carillo and Lipman [88] suggested a way to reduce some of the work needed in computing the optimal *SP* multiple alignment in practice. An extension of their idea was implemented in the program called MSA [303], but that extension was not described in the paper. Here we will explain the idea in the MSA version of the speedup. Additional refinements (particularly space reductions) to MSA are reported in [197]. We again restrict attention to aligning three strings.

The program for multiple alignment shown in the previous section is an example of using recurrences in a *backward* direction. At the time that the algorithm sets $D(i, j, k)$, the program looks backward to retrieve the D values of the seven (at most) cells that influence the value of $D(i, j, k)$.

In the alternative approach of forward dynamic programming (see also Section 12.6.1), when $D(i, j, k)$ is set, $D(i, j, k)$ is then sent *forward* to the seven (at most) cells whose D value can be influenced by cell (i, j, k). Another way to say this is to view optimal alignment as a shortest path problem in the weighted edit graph corresponding to a multiple alignment table. In that view, when the shortest path from the source s (cell $(0, 0, 0)$) to a node v (cell (i, j, k)) has been computed, the best-yet distance from s to any out-neighbor of v is then updated. In more detail, let $D(v)$ be the shortest distance from s to v, let (v, w) be a directed edge of weight $p(v, w)$, and let $p(w)$ be the shortest distance yet found from s to w. After $D(v)$ is computed, $p(w)$ is immediately updated to be $\min[p(w), D(v)+p(v, w)]$. Moreover, the true shortest distance from s to w, $D(w)$, must be $p(w)$ after $p(w)$ has been updated from every node v having an edge pointing into w. One more detail is needed. The forward dynamic programming implementation will keep a *queue* of nodes (cells)

whose final D value has not yet been set. The algorithm will set the D value of the node v at the head of the queue and remove that node. When it does, it updates $p(w)$ for each out-neighbor of v, and if w is not yet in the queue, it adds w to the end of the queue. It is easy to verify that when a node comes to the head of the queue, all the nodes that point to it in the graph have already been removed from the queue.

For the problem of aligning three strings of n characters each, the edit graph has roughly n^3 nodes and $7n^3$ edges, and the backward dynamic programming computation will do some work for each of these edges. However, if one can identify a large number of nodes as not possibly being on any optimal (shortest) path from $(0, 0, 0)$ to (n, n, n), then work can be avoided for each edge out of those excluded nodes. The Carillo and Lipman speedup excludes some nodes before the main dynamic programming computation is begun, and forwards or backwards dynamic programming is equally convenient. In the extension used in MSA, nodes are excluded *during* the main algorithm, and forward dynamic programming is critical.

Definition Let $d_{1,2}(i, j)$ be the edit distance between suffixes $S_1[i..n]$ and $S_2[j..n]$ of strings S_1 and S_2. Define $d_{1,3}(i, k)$ and $d_{2,3}(j, k)$ analogously.

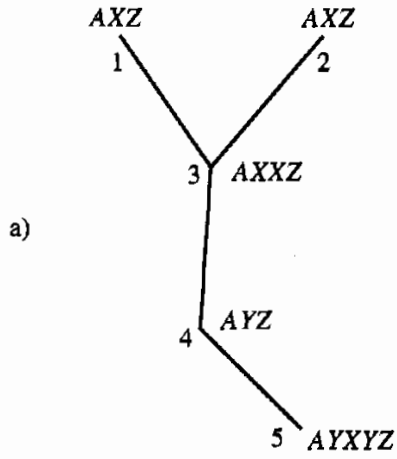
Certainly, all these d values can be computed in $O(n^2)$ time by reversing the strings and computing three pairwise distances. Also, the shortest path from node (i, j, k) to node (n, n, n) in the edit graph for S_1, S_2, and S_3 must have distance at least $d_{1,2}(i, j) + d_{1,3}(i, k) + d_{2,3}(j, k)$.

Now suppose that some multiple alignment of S_1, S_2, and S_3 is known (perhaps from a bounded-error heuristic of the type to be discussed later) and that the alignment has *SP* score of z. The idea of the heuristic speedup is:

Key idea Recall that $D(i, j, k)$ is the optimal *SP* score for aligning $S_1[1..i]$, $S_2[1..j]$, and $S_3[1..k]$. *If* $D(i, j, k) + d_{1,2}(i, j) + d_{1,3}(i, k) + d_{2,3}(j, k)$ is greater than z, then node (i, j, k) cannot be on any optimal path and so (in a forward computation) $D(i, j, k)$ need not be sent forward to any cell.

When this heuristic idea applies to a cell (i, j, k), it saves the work of not sending a D value forward to the (seven) out-neighbors of cell (i, j, k). But more important than the speedup observed at cell (i, j, k) is that when the heuristic applies at many cells, additional cells downstream may be automatically excluded from consideration because they never get placed in the queue. In this way, the heuristic may prune off a large part of the alignment graph and dramatically reduce the number of cells whose D value is set when determining $D(n, n, n)$. This is a standard heuristic in shortest path computations. Notice that the computation remains exact and will find the optimal alignment.

If no initial z value is known, then the heuristic can be implemented as an A^* heuristic [230, 379] so that the most "promising" alignments are computed first. In that approach, during the running of the algorithm the best alignment computed so far provides the (falling) z value. It can be proven [230] that this approach prunes out as much of the alignment graph as the Carillo and Lipman method does, but usually it will exclude much more. It is reported in [303] that MSA can align six strings of lengths around 200 characters in a "practical" amount of time. It doesn't seem likely, however, that this approach will make it practical to optimaly align tens or hundreds of strings, unless one begins with an extremely good value for z. Even then, it is not clear that MSA would be practical.



a)

b)

3	A	X	X	_	Z
1	A	X	_	_	Z
2	A	_	X	_	Z
4	A	Y	_	_	Z
5	A	Y	X	X	Z

Figure 14.6: a. A tree with its nodes labeled by a (multi)set of strings. b. A multiple alignment of those strings that is consistent with the tree. The pairwise scoring scheme scores a zero for each match and a one for each mismatch or space opposite a character. The reader can verify that each of the four induced alignments specified by an edge of the tree has a score equal to its respective optimal distance. However, the induced alignment of two strings which do not label adjacent nodes may have a score greater than their optimal pairwise distance.

14.6.2. A bounded-error approximation method for *SP* alignment

Because the exact solution to the *SP* alignment problem is feasible for only a small number of strings, most practical (heuristic) multiple alignment methods do not insist on finding the optimal *SP* alignment. However, little is usually known about how much the alignments produced by those heuristics can deviate from the optimal *SP* alignment. In contrast, in this section we discuss one of the few methods where such error analysis has been possible. We develop here a *bounded-error approximation* method, from [201], for *SP* alignment. That is, the method is provably fast (runs in polynomial worst-case time) and yet produces alignments whose *SP* score is guaranteed to be less than twice the score of the optimal *SP* alignment. This will be the first of several bounded-error approximation methods presented in this book, which will include additional methods for different multiple alignment problems.

An initial key idea: alignments *consistent* with a tree

The *SP* approximation we present, the improvements of it, and several other methods for other problems all use a key idea that relates multiple alignments to trees.[10] We develop that idea in general before continuing with *SP* alignment in particular. Recall that for two strings, $D(S_i, S_j)$ is the (optimal) weighted edit distance between S_i and S_j.

Definition Let $\mathcal{S}$ be a set of strings, and let T be a tree where each node is labeled with a distinct string from $\mathcal{S}$. Then, a multiple alignment $\mathcal{M}$ of $\mathcal{S}$ is called *consistent* with T if the induced pairwise alignment of S_i and S_j has score $D(S_i, S_j)$ for each pair of strings (S_i, S_j) that label adjacent nodes in T. For an example, see Figure 14.6.

Theorem 14.6.1. *For any set of strings $\mathcal{S}$ and for any tree T whose nodes are labeled by distinct strings of $\mathcal{S}$, we can efficiently find a multiple alignment $\mathcal{M}(T)$ of $\mathcal{S}$ that is consistent with T.*

[10] This connection between trees and multiple alignments should not be confused with the connection between *evolutionary* trees and multiple alignments, to be discussed in Sections 14.8 and 14.10.2.

Note that the role of T in this theorem is very special and the induced alignment of two strings S_i and S_j that do not label adjacent nodes will generally have a score greater than $D(S_i, S_j)$.

PROOF OF THEOREM 14.6.1 We will construct the multiple alignment $\mathcal{M}(T)$ one string at a time and show inductively that Theorem 14.6.1 holds after each new string is added to the alignment. To start, choose any two strings S_i and S_j that label adjacent nodes in T and form a two-string alignment of S_i and S_j with distance $D(S_i, S_j)$. The theorem trivially holds at this point. Assume that the theorem holds after some arbitrary number of strings have been added to the multiple alignment. To continue, select any string S' not yet included in the alignment such that S' labels a node adjacent to a node whose label, S_i say, is already in the alignment. In that existing multiple alignment, some spaces may have been inserted into S_i, and we use $\overline{S}_i$ to denote the string S_i with those spaces included. Note that for every string in the existing multiple alignment, each character of that string is in a distinct column with exactly one character of $\overline{S}_i$.

Next, optimally align string S' with $\overline{S}_i$ using a scoring scheme for two-string alignment, with the added rule that two opposing spaces have zero cost (they are considered a match). It is immediately apparent that the score of that resulting pairwise alignment is exactly $D(S_i, S')$. Now we will add S' to the existing multiple alignment so that the induced alignment of S_i and S' has score $D(S_i, S')$ and so that all the induced scores of the strings already in the alignment remain unchanged. Let $\overline{S}'$ be the string S' with any spaces inserted by the alignment of S' and $\overline{S}_i$. If the pairwise alignment of S' and $\overline{S}_i$ does not insert any new spaces into $\overline{S}_i$, then append $\overline{S}'$ to the existing multiple alignment. The result is a multiple alignment with one more string, where the induced score of S_i and S' is $D(S_i, S')$ and where all the induced scores from the previous multiple alignment remain unchanged.

However, if the pairwise alignment does insert a new space in $\overline{S}_i$ between characters l and $l+1$, say, then insert a space between characters l and $l+1$ in every string in the *existing* multiple alignment. This will create new column(s) in the existing multiple alignment in which every character is a space, but otherwise retains the columns of the existing multiple alignment. (For example, suppose that the first four strings from Figure 14.6 have been multiply aligned and that $\overline{S}_4$ at that point is AY_Z. The alignment of S_5 to $\overline{S}_4$ inserts an additional space into $\overline{S}_4$ at the fourth position. The first four rows of Figure 14.6 show the resulting multiple alignment after that space is replicated in strings S_1, S_2, and S_3.) The result of adding a column containing only spaces is a multiple alignment in which the score of all pairwise induced alignments is the same as before any spaces were inserted. Then appending $\overline{S}'$ to that multiple alignment creates a multiple alignment of one more string, where the statement of the theorem holds. The existence of $\mathcal{M}(T)$ then follows by induction.

The time needed to compute $\mathcal{M}(T)$ is dominated by the time to compute $k-1$ pairwise alignments. If each string has length n, then each pairwise alignment takes time $O(n^2)$ and the time to construct $\mathcal{M}(T)$ is $O(kn^2)$. □

Although Theorem 14.6.1 has become a part of "folklore", it may have been first explicitly stated in [153]. We can now return to the approximation method for the *SP* alignment problem.

The center star method for *SP* alignment

We will describe the method in terms of an alphabet-weighted scoring scheme for two-string alignment, and let $s(x, y)$ be the score contributed when a character x (possibly a space) is aligned opposite a character y (possibly a space).

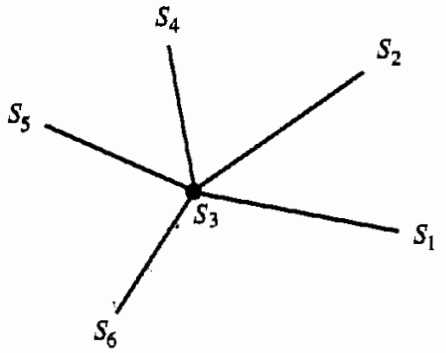


Figure 14.7: A generic center star for six strings, where the center string S_c is S_3.

Definition A scoring scheme satisfies the *triangle inequality* if for any three characters x, y, and z, $s(x, z) \leq s(x, y) + s(y, z)$.

Triangle inequality makes good intuitive sense in the context of edit distance. It says that the "cost" of transforming x to z directly is no more than the cost of first transforming x to an intermediate character y, and then transforming y to z. It should be noted, however, that not all scoring matrices in use in computational biology satisfy the triangle inequality.

Definition Given a set of k strings $\mathcal{S}$, define a *center* string $S_c \in \mathcal{S}$ as a string in $\mathcal{S}$ that minimizes $\sum_{S_j \in \mathcal{S}} D(S_c, S_j)$, and let M denote that minimum sum. Define the *center star* to be a star tree of k nodes, with the center node labeled S_c and with each of the $k-1$ remaining nodes labeled by a distinct string in $\mathcal{S} - S_c$. For an example, see Figure 14.7.

Definition Define the multiple alignment $\mathcal{M}_c$ of the set of strings $\mathcal{S}$ to be the multiple alignment *consistent* with the center star.

Definition Define $d(S_i, S_j)$ as the score of the pairwise alignment of strings S_i and S_j *induced* by $\mathcal{M}_c$. Denote the score of an alignment $\mathcal{M}$ as $d(\mathcal{M})$.

Clearly, $d(S_i, S_j) \geq D(S_i, S_j)$, and $d(\mathcal{M}_c) = \sum_{i<j} d(S_i, S_j)$. Also, since $\mathcal{M}_c$ is consistent with the star tree, and string S_c is at the center of the star, $d(S_i, S_c) = D(S_i, S_c)$ for each string S_i. We will show that $d(\mathcal{M}_c)$ is at most twice the score of the optimal *SP* multiple alignment of $\mathcal{S}$, provided that the scoring scheme used for pairwise alignment satisfies the triangle inequality.

Lemma 14.6.1. *Assume that the two-string scoring scheme satisfies the triangle inequality. Then, for any strings S_i and S_j in $\mathcal{S}$, $d(S_i, S_j) \leq d(S_i, S_c) + d(S_c, S_j) = D(S_i, S_c) + D(S_c, S_j)$.*

PROOF Consider any single column in the multiple alignment $\mathcal{M}_c$, and let x, y, and z be the three characters in this column from the three strings S_i, S_c, and S_j, respectively. By the triangle inequality, $s(x, z) \leq s(x, y) + s(y, z)$, and so the claimed inequality follows by the definition of d. The claimed equality follows because the pairwise alignment of S_i and S_c induced by $\mathcal{M}_c$ is an optimal alignment of S_i and S_c, and this is true also for the alignment of S_c and S_j. □

Definition Let $\mathcal{M}^*$ be the optimal multiple alignment of the k strings of $\mathcal{S}$. Let $d^*(S_i, S_j)$ be the score of the pairwise alignment of strings S_i and S_j induced by $\mathcal{M}^*$. Then $d(\mathcal{M}^*) = \sum_{i<j} d^*(S_i, S_j)$.

We can now state and prove the main theorem of this section.

Theorem 14.6.2. $d(\mathcal{M}_c)/d(\mathcal{M}^*) \leq 2(k-1)/k < 2$.

PROOF First, define $v(\mathcal{M}_c) \equiv \sum_{(i,j)} d(S_i, S_j)$ and $v(\mathcal{M}^*) \equiv \sum_{(i,j)} d^*(S_i, S_j)$, where the pair (i, j) is an *ordered* pair in each case. Clearly, $v(\mathcal{M}_c) = 2d(\mathcal{M}_c)$ and $v(\mathcal{M}^*) = 2d(\mathcal{M}^*)$, and so the ratios $d(\mathcal{M}_c)/d(\mathcal{M}^*)$ and $v(\mathcal{M}_c)/v(\mathcal{M}^*)$ are equal. It is more convenient to work with the second ratio. Recall that the minimum sum of distances, M, is defined as $\sum_j D(S_c, S_j)$. Now $v(\mathcal{M}_c) = \sum_{(i,j)} d(S_i, S_j) \leq \sum_{(i,j)} [D(S_i, S_c) + D(S_c, S_j)]$, by Lemma 14.6.1. For any fixed j, $D(S_c, S_j)$ (which equals $D(S_j, S_c)$) shows up in this expression exactly $2(k-1)$ times. So $v(\mathcal{M}_c) \leq 2(k-1) \times \sum_j D(S_c, S_j) = 2(k-1)M$.

From the other side, $v(\mathcal{M}^*) = \sum_{(i,j)} d^*(S_i, S_j) \geq \sum_{(i,j)} D(S_i, S_j) = \sum_i \sum_j D(S_i, S_j)$ $\geq k \times \sum_j D(S_c, S_j) = kM$ (by the choice of S_c). So $d(\mathcal{M}_c)/d(\mathcal{M}^*) = v(\mathcal{M}_c)/v(\mathcal{M}^*) \leq$ $2(k-1)M/kM = 2(k-1)/k = 2 - 2/k < 2$. □

Note that for $k = 3$ the guaranteed upper bound is 4/3. That is, for three strings, the multiple alignment produced by the center star method will never be more that 34% more than the optimal *SP* score. Translated into lower bounds this says that for $k = 3$, $d(\mathcal{M}^*) \geq .75d(\mathcal{M}_c)$. For $k = 4$ the upper bound is only 1.5, and for $k = 6$ (a problem size considered to be too large for efficient exact solution with strings of length 200) the bound is still only 1.67.

Corollary 14.6.1.
$kM \leq \sum_{i<j} D(S_i, S_j) \leq d(\mathcal{M}^*) \leq d(\mathcal{M}_c) \leq [2(k-1)/k] \sum_{i<j} D(S_i, S_j)$.

In practice one can better measure the goodness of $\mathcal{M}_c$ by the ratio $d(\mathcal{M}_c)/\sum_{i<j} D(S_i, S_j)$. By Corollary 14.6.1 this ratio is always less than two, but the analysis used there is worst case, so one can expect the ratio to often be considerably less than two. Similarly, one should expect that $d(\mathcal{M}_c)/d(\mathcal{M}^*)$ will often be considerably less than two, since typically $\sum_{(i,j)} D(S_i, S_j)$ will be considerably larger than kM; that $d(\mathcal{M}^*)$ will not generally be close to $\sum_{i<j} D(S_i, S_j)$ for any strings except those that are very similar; and that $D(S_i, S_j)$ will be less than $D(S_i, S_c) + D(S_c, S_j)$ for most typical strings.

Corollary 14.6.1 is also useful in the MSA speedup discussed in Section 14.6.1 for the exact solution to *SP* alignment, since that method requires knowing an efficiently computed upper bound z on the optimal *SP* alignment score.