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複数アラインメントに対する k-opt アルゴリズム

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複数アラインメント問題は，ゲノム情報処理での代表的な問題である．この問題に対する種々のモデルが提案されており，その中でも最もよく用いられるのが，DNA 同士・アミノ酸同士の間の類似度を採用し，長さ n の d 本の塩基配列・タンパク質配列のアラインメントを d 次元格子グラフのパスとして表現するものである．この定式化では，問題は d 次元格子グラフで各枝が類似度による長さを与えられたときの無閉路有向グラフでの最長路問題となる．この問題は動的計画法により $O(n^d)$ で解けるが，一方 d をパラメタとするとこの問題は NP 困難となり，実際に d が 4 以上となると最適解を求めることが難しかった．Ikeda, Imai [8] は，A* アルゴリズムを用いて，類似度が高い場合には $n = 450$, $d = 8$ でも問題が解けることを示した．しかし，その手法でもさらに d が多くなると最適解を求めるのは難しくなる．本稿では，d が大きい場合に対して，d 本の配列を k 個のグループにわけ，グループ内ではアラインメントを固定して k グループ間の最適アラインメントを求める問題を考え，A* アルゴリズムを用いて $k = 3 \sim 5$ でも十分実用的時間内で k グループのアラインメント問題が解けることを示す．最適化技法の観点からは，これは k-opt 型の近似局所改良反復法とみなせる．

K-Opt Algorithm for Multiple Alignment

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The multiple alignment of the sequences of DNA and proteins is applied to various important fields in bioscience. Due to its importance, there have been proposed many algorithms, and yet new techniques to resolve difficulties in solving large-scale problems are required. This paper proposes a k-group alignment algorithm for multiple alignment as a practical method. It is demonstrated that the k-group alignment can be implemented so as to run in a reasonable time and space under standard computing environments. This is established by generalizing the A* search approach for multiple alignment devised by Ikeda and Imai [8].

1 Introduction

The multiple sequence alignment is the problem to find the alignment of multiple sequences with highest score due to a given scoring criteria between characters. The solution of this problem for multiple sequences of DNA and proteins represents the similarity among them and is applied to various important fields such as the prediction of three dimensional structure of proteins and the inference of phylogenetic tree in molecular biology.

The method based on Dynamic Programming (DP) is a well-known approach for multiple sequence alignment problem. It searches all vertices in the grid-like acyclic graph, and has $O(n^d)$ time and space complexity for d sequences of length at most n. This approach is effective for small dimension of two or three. In fact, with the increase of computing power, 3-dimensional DP became feasible when the length n is not so large. But, it is impractical to apply this method directly to a little larger dimensional problem because n^d becomes enormous then. For large d, approximate algorithms dividing d sequences into two groups and applying 2-dimensional DP between the two groups have been used (Berger, Munson [2], Gotoh [4]). The resultant alignment is improved gradually by iteration of dividing and aligning. Another method for multiple alignment is a tree-based (iterative) algorithm [6], and in it 2-dimensional DP is also used. In connection with these algorithms, Hirosawa et al. [5] employed 3-dimensional DP as the basis for an initial alignment to the subsequent iterative algorithm.

The A* algorithm reduces search space without lack of optimality of the result alignment. There were proposed some methods of reducing

the search space in multiple alignment (Carrillo and Lipman [3], Spouge [9], etc.), but the A* algorithm with upper bounding operation would be the best method to derive an optimal alignment [7, 8] (see also [9]). Araki et al. [1] proposed to use an A* algorithm for 2-dimensional DP in the Berger-Munson iterative algorithm. They use an estimate derived from a score table which can reduce the search space in the 2-dimensional case. The estimate for A* multiple alignment in Ikeda and Imai [8] was demonstrated to be very powerful, and in this respect generalizing the A* algorithm in [8] to k-group alignment is rather natural.

This paper investigates a k-group alignment algorithm for multiple alignment. In the k-group alignment problem, d sequences are given with k disjoint groups of them, each being internally aligned, and a best alignment among these k groups should be found with only inserting a gap simultaneously in the same position for the alignment of each group. First, it is shown that the same approach in [8] can be applied to the group alignment problem. Several ways of applying A* search to this problem are discussed. Then, its connection with the standard iterative improvement algorithm is described. Through computational experiments, it is demonstrated that the k-group alignment can be performed for $k = 3, 4, 5$ in a practical time depending on the problem size, and this produces better alignments. For example, for 21 sequences of length about 450, 5-group alignment can be performed fast enough. About the alignment quality, 3-group alignment yields better solutions compared with 2-group DP almost with a little additional time. 4- and 5-group alignment methods can find better solutions in most cases but require more time. The practicality of k-group alignment is thus shown, and further investigation of elaborating this with other methods and enhancing itself should be done.

2 A* Algorithm for Multiple Alignment

The multiple alignment problem can be solved by finding the shortest path on some directed acyclic graph as follows. Suppose that S_k denotes the k-th sequence of length $n_k = O(n)$ and d denotes the dimension, the number of sequences. Then in the directed acyclic graph $G = (V, E)$ such that $V = \{(x_1, \ldots, x_d) \mid x_i = 0, 1, \ldots, n_i\}$ and $E = \cup_{e \in \{0,1\}^d} \{(v, v + e) \mid v, v + e \in V,\ e \neq 0\}$, a path from the vertex $s = (0, \ldots, 0)$ to the vertex $t = (n_1, \ldots, n_d)$ corresponds to an alignment of sequences. In case of $d > 2$, the sum of all scores for pairwise sequence alignments is used as the score for the multiple sequence alignment in general. This corresponds to defining each edge length in G as the sum of all corresponding edge length in the graphs for pairwise alignments. Let $G_{ij} = (V_{ij}, E_{ij})$ denote the graph for the alignment of S_i and S_j $(i < j)$, that is, $V_{ij} = \{v_{ij} = (x_i, x_j) \mid v = (x_1, \ldots, x_d) \in V\}$ and $E_{ij} = \{(u_{ij}, v_{ij}) \mid (u, v) \in E, u_{ij} \neq v_{ij}\}$. Then the length of edge (u, v) in E is defined as $l(u, v) = \sum_{1 \leq i < j \leq d} l(u_{ij}, v_{ij})$ where $l(u_{ij}, v_{ij})$ denotes the length of edge (u_{ij}, v_{ij}) in graph G_{ij} and is defined from the score table between characters. Thus, the multiple alignment problem can be formulated as a shortest path problem on G. The shortest path on such a graph can be computed by dynamic programming in a direct way, but its complexity $\Theta(n^d)$ is intractably large for large d.

The A* algorithm can find a shortest path without searching the whole graph if good estimates on the shortest path length from each vertex to t are at hand. Ikeda and Imai [8] show a method of obtaining such good estimators by computing pairwise 2-dimensional $\binom{d}{2}$ subproblems for d sequences. For the case $d > 2$ (the 2-group alignment in the sequel can be handled similarly), it uses the following estimator h:

$$h(v) = \sum_{1 \leq i < j \leq d} L^*(v_{ij}, t_{ij}).$$

where $L^*(u, v)$ denote the shortest path length from u to v. This estimator uses the shortest path length in the pairwise alignment problem as the estimate for the length of the path corresponding to the shortest path in the multiple alignment problem by making use of relations of G and G_{ij}. In higher dimensional problem, necessary time and space for solving pairwise problems is negligible. It is clear that each estimate $h(v)$ does not exceed the actual shortest path length from v to t, and moreover h is dual feasible, i.e., for any edge (u, v) in E,

$$\begin{aligned} l(u, v) + h(v) &= \sum_{1 \leq i < j \leq d} (l(u_{ij}, v_{ij}) + L^*(v_{ij}, t_{ij})) \\ &\geq \sum_{1 \leq i < j \leq d} L^*(u_{ij}, t_{ij}) = h(u). \end{aligned}$$

Hence the A* algorithm using this estimator is reduced to the following simple one.

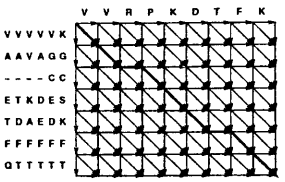
1. For arbitrary pair of i and j satisfying $1 \leq i < j \leq d$, apply DP to graph G_{ij} from vertex t_{ij} and calculate $L^*(v_{ij}, t_{ij})$ for any v_{ij} in V_{ij}.

2. Apply the Dijkstra method to graph G from vertex s with the length of edge (u, v) modified as $l(u, v) + h(v) - h(s)$ where $h(v) = \sum_{1 \leq i < j \leq d} L^*(v_{ij}, t_{ij})$.

3 Group Alignment and Its Use in Iterative Algorithms

3.1 k-Group Alignment

Since the multiple alignment problem becomes hard to solve when d is large, as a subproblem, the group alignment is considered. Originally, in the group alignment, d sequences are divided into two groups, say d' sequences and $d - d'$ sequences ($0 < d' < d$), and then fixing the alignment in each group, it solves a 2-dimensional alignment problem between two groups. In this 2-dimensional problem, since the alignment in each group is fixed, when a gap is inserted into a group, it is simultaneously inserted in the same position. See Figure 1.



(a)

Thc VVRPKDTFK
Tha VV--KA-FT
Hal VA--ET-FQ
Met VA--TD-FT
Sul VA--DE-FT
Ent VG-CED-FT
Pla KG-CSK-FT

(b)

Figure 1: (a) Aligning seven sequences by a 2-group alignment for {Thc} and others, (b) an obtained alignment

For general $k > 2$ ($k \leq d$), the k-group alignment problem can be defined similarly. In this problem, d sequences are given as k disjoint groups, and each group is associated with some alignment of sequences in the group. In a typical case, for an alignment of d sequences, k aligned groups can be obtained simply by dividing this alignment into k groups (and removing trivial gaps inside each group alignment). Then, the k-group alignment problem finds a best-score alignment of d sequences under a condition that, in each group, each column of its alignment should be fixed. Hence, when a gap is inserted into a sequence in some group, the same gap should be inserted in the same position in every sequence in the group. In this case, a k-dimensional grid-like graph is used to solve the k-group alignment problem, as in the original k-dimensional alignment problem. See an example of $k = 2$ and $d = 7$ in Figure 1. Since the score function is defined to be the sum of scores of all pairs, the A* approach can be directly extended to this k-group alignment problem by virtue of the principle of optimality.

There may be considered several types of A* search algorithm for k-group alignment. We here only mention one. For each pair of groups, compute the score of 2-group alignment between the two groups, and make the summation of those scores as a lower-bound estimator in solving the k-group alignment problem. Here, even when solving 2-group alignment problems between general two groups, we can make use of the scores of all-pairs of sequences computed in the preprocessing stage to solve it by the A* search.

3.2 Iterative improvement

The standard randomized iterative improvement method proposed by Berger and Munson [2] proceeds as follows:

1. Construct an initial alignment by a method;
2. Divide d sequences into two groups randomly;
3. Remove trivial gaps in each group;
4. Solve the 2-group alignment to obtain a new alignment;
5. If the score decreases, update the current alignment to the new one;
6. If a stopping condition is met, stop; otherwise return to step 2;

In the step 2 above, all the sequences are divided into two groups randomly. There is a method of dividing them into a group of one sequence and a group consisting of the other $d-1$ sequences. This partition is called a restricted partition in [6]. Also in that case, instead of using randomization, one sequence for a group of a single element may be changed in a round-robin fashion. There are many other methods (see [6]).

It is rather natural to extend the iterative algorithm in a way that in step 2 it divides the sequences into k groups for $k \geq 2$. We call such iterative algorithm the k-opt iterative algorithm. The A* algorithm for group alignments can be utilized in the k-opt algorithm. There can be

considered two methods of dividing d sequences into k groups, by directly generalizing the above-mentioned existing methods for $k = 2$.

k-random-grouping: This method divides d sequences randomly into k groups so that no group becomes empty. This will be denoted by RA(k) in the computational results below.

k-restricted-grouping: This method divides them into $k - 1$ groups consisting of a single sequence and another group consisting of the other sequences where each sequence in the former $k - 1$ groups is chosen randomly. This will be denoted by RI(k) in the next section.

In [5], the latter method with $k = 2$ was used to derive a best-first iterative improvement algorithm, and it was observed that the restricted-grouping strategy produces favorably nice solutions compared with the random-grouping one. When the A* is used in the iterative method with the restricted-grouping strategy, solving $\binom{k-1}{2}$ subproblems out of $\binom{k}{2}$ can be dispensed with.

4 Computational Results

In order to investigate the actual efficiency of this approach, experiments aligning actual sequences of proteins have been performed. Our implementation is designed to evaluate several strategies in a system, and is coded in such a general setting. This causes in some places redundant computation which can be avoided by cleverly using the information obtained in the preprocessing stage. This point should be remarked especially in observing timing results in the computational results. For example, the following points can be improved further from the current code.

(a) When the A* is used in the iterative improvement method with the restricted-grouping, solving $\binom{k-1}{2}$ subproblems out of $\binom{k}{2}$ can be dispensed with. However, in the current implementation, these subproblems are solved from scratch every time.

(b) Even when solving 2-group alignment problems between general two groups, we can make use of the scores of all-pairs of sequences computed in the preprocessing stage to solve it by A* search. However, we do not incorporate this in the code. Instead, a kind of lazy 2-dimensional DP algorithm is implemented. Here, "lazy" execution may be done in many ways. For example, in the beginning only a fraction of DP table is computed, say in a constant-bandwidth region, and values of other elements are computed when necessary.

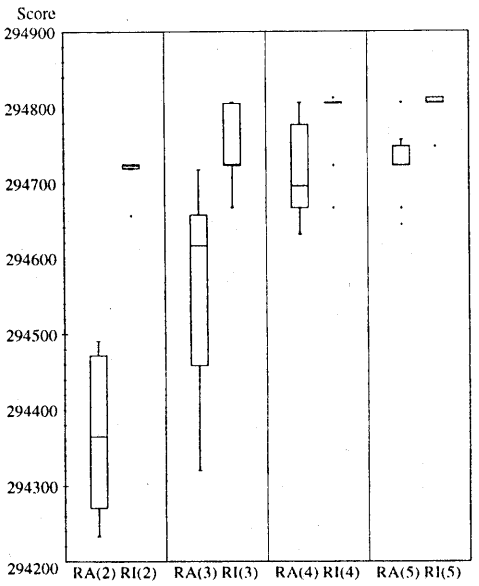
(c) In the experiment, the linear gap system is used instead of the affine gap system (see below). To obtain more meaningful alignments, the affine gap system is regarded as a better one, and performing the experiment with the affine gap system is important. For k-dimensional DP alignments, it is known that with the affine gap system it takes more time compared with the case of the linear gap system as k increases. This point should be actually tested, which is left as future work. Concerning the performance of our A* approach, we suspect that changing the alignment cost system from the simple pairwise sum of 2-alignments to the weighted sum may affect more.

Concerning the score matrix, the PAM-250 matrix has been used in assigning edge length with each sign of score reversed. The linear gap system ax for the gap of length x is used (extending the current system to the affine gap system $ax + b$ for the gap of length x would be practically important work). With regard to the gap penalty, the minimum value in the PAM-250 matrix, $a = -8$, has been adopted. All the experiments were done on SPARCStation 20 with 128 megabytes memory.

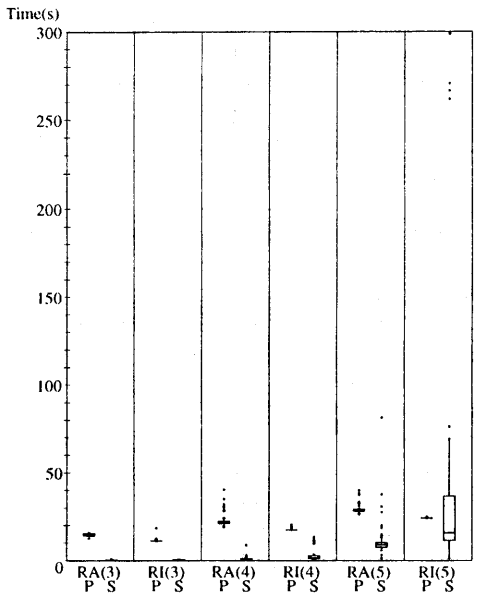
4.1 Case with High Similarity

In this experiment, elongation factor TU (EF-TU) and elongation factor 1α (EF-1α) are used as in [8]. The number of sequences is 21, and the length of each sequence is about 450. The best alignment found by the experiment is 294813 with length 482.

As an initial alignment, we adopted a solution of the A algorithm in [7, 8] with parameter 81/80. The score of this initial alignment is 294201. By using a tree-based DP, better initial solutions can be obtained, but those solutions are processed by group DP, while the solution by the A algorithm is not. Starting with the solution by the A algorithm, the alignment score is improved fast initially. We tested 10 series of 100 iterations with using different random numbers. Both of the k-random-grouping RA(k) and k-restricted-grouping RI(k) are examined. Some of computational results are given in Figure 2. Box plots are used, and a box plot comprises these elements: 1) a box with 1a) a central line showing the median, 1b) a lower line showing the first quartile, 1c) an upper line showing the



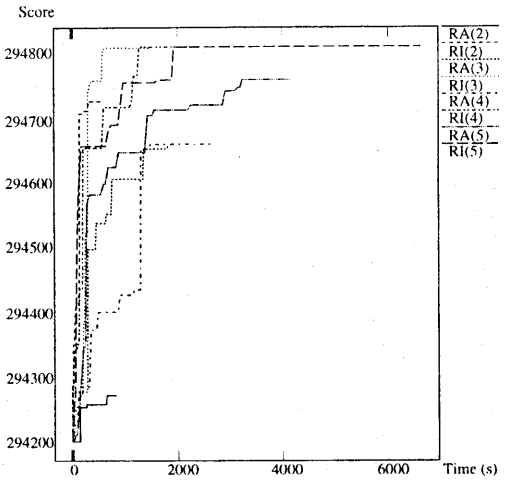
(a)



(b)

third quartile; 2) 2 lines extending from the central box of max- imal length 3/2 the interquartile range but not extending past the range of the data; 3) outliers, points that lie outside the extent of the previous elements.

For smaller k such as 2 and 3, the k-random-



(c)

Figure 2: Computational results for EF-1α: (a) A box plot of final scores after 100 iterations (10 series), (b) A box plot of running times for the estimator precomputation ("P" in short; in the left side) and A* search ("S" and in the right side) per step in the computation of typical series in (c), (c) Score improvement processes of typical series such that its final score attains the median score among the first three trials for each k and strategy.

grouping method RA(k) tends to produce worse solutions than the k-restricted-grouping method RI(k). For large k such as 4 and 5, this tendency becomes less clear. In these experiments, the 3-restricted grouping method may be said to be the best one in regard to both computation time and solution quality. About the solution quality, the 3-restricted grouping produces better solutions than 2-restricted grouping, as seen in Figure 2(a) and even if we use the scores of the 2-restricted grouping with 200 iterations, results are almost same. Here, it should be stressed that this speed of 3-group alignment is achieved by the use of A*. It is also observed that, even for $k = 4, 5$, k-alignment problem can be solved in a reasonable time even for this rather large-scale problem. Concerning the effect of A*, for $k = 3$ (and 4), its estimates are nice enough to reduce the search space drastically. In fact, the A* search takes much less time than the precomputation of estimator in this case (Figure 2(b)). For $k = 5$, the estimates become less

effective, and sometimes the A* search space becomes large, although it is still manageable.

4.2 Observations on Computational Results

Although the above-mentioned results are still preliminary ones, the following may be observed.

(a) We have tested two methods of dividing d sequences into k groups. It is observed that the k-restricted-grouping method tends to produce much better-score alignments than the k-random-grouping method on the average.

(b) As k becomes larger, the k-opt algorithm takes more time, but produces on the average better alignment results. Although for $k = 4, 5$ it takes definitely larger time compared with 2- and 3-group alignments, by these results it is verified that 4-(and 5- when the similarity is high)group alignments can be practically used to polish up an almost final alignment further.

(c) The A* search greatly reduces the running time required by DP. In fact, in these experiments, 3-group alignments can be solved in time by a small constant factor of the running time of standard 2-dimensional DP. Furthermore, their solution quality is definitely better than that of 2-group methods.

(d) Related to a widely known fact concerning k-opt local search algorithms for combinatorial problems, there are so many local optima in such combinatorial problems, and there do exist many local optima for 2-group alignments which are not local optima for 3- and higher order group alignments. By increasing k, it takes more time to check the local optimality of current solution, but this is not a big problem since 3- and higher order group alignments can produce better solution than 2-grouping method in reasonable additional time and checking the local optimality is less important in this respect.

Thus, this paper proposed the use of k-group alignment for $k \geq 3$, and showed its power via computational experiments. Yet, as is noted above, there still are many points which can be improved further in the current code, and developing a refined system would be very interesting as future work.

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