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Ant Colony Optimization Techniques for Efficient Multiple Sequence Alignment

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Abstract: Multiple Sequence Alignment (MSA) is a fundamental task in bioinformatics, used to identify conserved regions and evolutionary relationships across biological sequences. However, as the number of sequences grows, the computational complexity of MSA increases significantly, making it challenging to handle large datasets efficiently.

To find conserved areas and evolutionary links between biological sequences, multiple sequence alignment, or MSA, is a basic bioinformatics technique. The computational cost of MSA, however, rises sharply with the number of sequences, making it difficult to manage big datasets effectively.

Rajeev Kumar PathakIt becomes more difficult to efficiently process large datasets as the number of sequences increases since MSA's computational cost grows exponentially. Through the use of Ant Colony Optimization (ACO) and the Divide and Conquer (D&C) method, this work introduces a novel algorithm that enhances the MSA process.

Keywords: Multiple Sequence Alignment (MSA), Sequence Similarity, Metaheuristic Algorithms, Computational Biology, Optimization Techniques, Large-scale dataset processing, Global alignment techniques, Parallel sequence alignment

1. Introduction

In bioinformatics, multiple sequence alignment (MSA) is a basic operation that entails aligning three or more biological sequences (such proteins, RNA, or DNA) in order to identify similarities and differences. Understanding protein structure, functional patterns, and evolutionary relationships all depend on this method. But when there are more sequences, MSA becomes much more computationally complex, particularly when working with large datasets.[1]

Ant Colony Optimization (ACO) Ants' natural foraging practices serve as the basis for Ant Colony Optimization (ACO), a metaheuristic optimization technique. Its ability to traverse large search spaces and converge toward optimal solutions has made it useful for addressing a variety of optimization problems, including sequence alignment. To make the process easier to manage overall, the Divide and Conquer (D&C) technique divides a large problem

into smaller, independently solvable subproblems.[2]

The effective MSA algorithm presented in this research optimizes sequence alignment by combining ACO with a Divide and Conquer (D&C) strategy. In the proposed method, the collection of sequences is divided into smaller subsets, each of which is aligned with ACO before being combined into a global alignment. This results in faster computational performance and better alignment quality.[3]

1. Mathematical Formulation of Ant Colony Optimization (ACO)

The probabilistic, metaheuristic algorithm called ACO was inspired by the foraging behaviors of ants. In ACO, ants follow paths, which in this case are potential alignment configurations, deposit pheromones, and modify their paths according to the quality of the solutions they encounter. With time, the pheromone trail leads ants to perfect or almost ideal solutions.

1.1 Basic ACO Framework

The following mathematical elements are used in the ACO algorithm:

1. Solution Construction: Using a probability distribution that is dependent on the pheromone levels and the desirability of the solutions, each ant gradually constructs a solution (an alignment in the case of MSA).

2. Pheromone Update Rule: The quality of the solution determines how often pheromone levels are updated. Future ants are drawn to good solutions because they obtain more pheromone.

3. Evaporation: Pheromone levels gradually decrease, promoting exploration and avoiding early convergence to less-than-ideal solutions.

1.2 Mathematical Representation of ACO

The following are the main mathematical ideas in ACO for MSA:

- State Representation: A matrix or graph is used to illustrate the solution to the MSA problem. Each node in the graph represents a possible alignment between two sequences, while the edges indicate the options for gap insertions or mismatches.
- Transition Probability: This is the likelihood that an ant will move from one state to another.

$$Pij - \frac{[\tau ij]\alpha \cdot [\eta ij]\beta}{\sum k \in Ni[\tau ik]\alpha \cdot [\eta ik]\beta P}$$

Where:

- ✓ $Tij$is the pheromone intensity on the edge from state iii to state jjj.
- ✓ $\eta ij$is the heuristic information (in the case of MSA, this could represent a score function based on sequence similarity).
- ✓ αalphaα andβ are parameters that control the relative importance of pheromone and heuristic information.

- **Pheromone Update**: After each iteration, the pheromone is updated using the formula:[4]

$\boldsymbol{\tau ij}(t+1)- (1-\rho).\boldsymbol{\tau ij}(t)+\Delta\boldsymbol{\tau ij}(t)$

Where:

- ✓ ρ is the evaporation rate (a constant between 0 and 1).

- ✓ $\Delta\tau ij(t)$ is the pheromone deposit due to the ant's solution, which is typically proportional to the solution quality (e.g., inverse of the alignment score).

The update ensures that better solutions receive more pheromone and thus attract more ants in subsequent iterations.

- ➢ ACO strikes a balance between exploitation (local search) and exploration (global search). Pheromone evaporation regulates exploration, whereas ants who pursue high-pheroomone pathways (high-quality solutions) engage in exploitation.

2. Mathematical Model of Multiple Sequence Alignment (MSA)

In MSA, we align multiple sequences to identify conserved regions (homologous sequences) and evolutionary relationships. This process involves optimizing the following:

1. **Scoring Scheme**: The alignment is evaluated using a scoring function, often based on:

- ➢ **Substitution Matrix**: A matrix SSS, where each entry S(x,y)S(x,y)S(x,y) gives the substitution score for aligning residues xandy (for DNA, RNA, or protein sequences).
- ➢ **Gap Penalties**: A gap penalty G is applied for introducing gaps in sequences to align them correctly.

A typical scoring function for MSA might be:

$$\text{Score}(S) - \sum S(x,y) + \sum$$

pairs of aligned residuesgaps

where the first sum represents the sum of substitution scores and the second sum represents the penalties for gaps.

2. **Objective Function**: The objective of MSA is to maximize the alignment score, which is equivalent to minimizing the negative of the sum of pairwise alignment scores (considering gaps and mismatches).[5] Mathematically:

Maximize $\sum$ Score(Si,Sj)

whereSiS_iSi and SjS_jSj are the aligned sequences.

Sequence A: MKVPLHGRF

Sequence B: MKV-LHGRF

Alignment:

M K V P L H G R F

M K V - L H G R F

Example of Pairwise Sequence Alignment

Let's consider a **protein sequence alignment** example where we use a **substitution matrix** (like **BLOSUM62**) for scoring the pairwise alignment. We will also need to handle **gaps** (insertions/deletions) and **mismatches** effectively.

Step 1: The Sequences

Imagine we have the following two protein sequences that we want to align:

- ✓ **Sequence 1 (A)**: MAVFPGV
- ✓ **Sequence 2 (B)**: MVF-GAV

These are relatively short protein sequences, but the presence of a gap (-) and the

mismatches between the sequences (e.g., A vs. V, F vs. -) will make this a slightly more complex case to align.[6]

Step 2: Using the Needleman-Wunsch Algorithm

The Needleman-Wunsch algorithm, a dynamic programming-based technique for global alignment, would be used to align these sequences. Each cell in the matrix that the algorithm fills in indicates the optimal alignment score for matching the prefixes of the two sequences.

Let's assume we use a simple scoring scheme:

- ✓ **Match**: +1 (when the amino acids are identical)
- ✓ **Mismatch**: -1 (when the amino acids differ)
- ✓ **Gap penalty**: -2 (for every insertion/deletion)

The **BLOSUM62 matrix** could also be used for a more realistic protein alignment scenario, but for simplicity, we'll stick with the simple scoring system above in this example.

Step 3: The Dynamic Programming Matrix

We'll now create a **dynamic programming (DP) matrix** to compute the alignment score between the two sequences:

	-	M	A	V	F	P	G	V
	0	-2	-4	-6	-8	-10	-12	-14
M	-2	1	-1	-3	-5	-7	-9	-11
V	-4	-1	0	2	0	-2	-4	-6
F	-6	-3	-2	1	3	1	-1	-3
-	-8	-5	-4	-3	1	0	2	0
G	-10	-7	-6	-5	-1	0	1	3
A	-12	-9	-8	-7	-3	-2	0	2
V	-14	-11	-10	-9	-5	-4	-2	1

Step 4: Trace back to Find the Optimal Alignment

Start from the bottom-right corner of the matrix and work your way back to the top-left to obtain the best global alignment. The scoring rules for matches, mismatches, and gaps state that this means choosing the path that will result in the highest score.

For this example, the optimal alignment might look like this:

Sequence A: MAVFPGV

Sequence B: MVF-GAV

Here, the alignment includes:

- ✓ **A** from Sequence A aligned with **A** from Sequence B (match, score +1).
- ✓ **V** from both sequences (match, score +1).
- ✓ **F** from Sequence A aligned with **F** from Sequence B (match, score +1).
- ✓ **P** from Sequence A aligned with a gap (-) in Sequence B (gap penalty, score -2).
- ✓ **G** from Sequence A aligned with **G** from Sequence B (match, score +1).
- ✓ **V** from Sequence A aligned with **V** from Sequence B (match, score +1).

Thus, the alignment gives us a score based on the individual match/mismatch and gap penalties along the way.[7]

Step 5: Calculate the Alignment Score

Now, let's calculate the total score based on the **alignment**:

- ✓ Matches: +1 (for A-A, V-V, F-F, G-G, V-V)
- ✓ Gaps: -2 (for the gap in Sequence B between F and G)
- ✓ No mismatches in the final alignment.

The total score for this optimal alignment is:

- ✓ **5 matches** × (+1) = +5
- ✓ **1 gap** × (-2) = -2

Total score = **5 - 2 = +3**

Step 6: PairSim Scoring

If we use the **PairSim** algorithm (which calculates the pairwise similarity score between two sequences), this would output a **similarity score** based on the optimal alignment. For instance, in this case, the score could be normalized by the length of the sequences, giving us a **percentage similarity**.

If both sequences have the same length (here, 7), the normalized PairSim score might be:

$$\text{PairSim score} = \frac{\text{Number of matches}}{\text{Length of the sequence}} = \frac{5}{7} \approx 0.714$$

This would indicate that the two sequences are approximately **71.4% similar**.

This is a more **complex example** of **pairwise sequence alignment**. The alignment involved handling:

- ✓ **Gaps** (insertions or deletions),
- ✓ **Mismatches** (substitutions between amino acids),
- ✓ Using a **scoring system** to quantify the quality of the alignment.

In real-world applications, such as evolutionary biology or drug design, aligning more complex sequences with substitutions, gaps, and varying lengths is common. Using algorithms like **Needleman-Wunsch** or **Smith-Waterman** (for local alignments) along with tools like **PairSim** can help quantify sequence similarities or dissimilarities to understand functional relationships or evolutionary histories.

3. Methods

3.1 Methods of systematic literature search

A Systematic Literature Review Is A Straightforward And Methodical Approach To Find, Assess, And Compile All Of The Studies That Are Currently Available On A Given Research Question, Topic, Or Phenomenon. It Can Also Be Used To Understand The State And Direction Of Research Or To Give Context For Identifying A Research Problem. Information Can Be Extracted From A Collection Of Cited Articles In A Number Of Ways.8. The Methodology Employed In This Literature Review Is Modeled After Those Of Mahdavi-Hezavehi Et Al. And Flores-Contreras Et Al. An Objective Sub-Formulation From A Target Question-Metrics Viewpoint (Purpose, Problem, Subject, And Perspective) Was Used To Create The Goal Of This Systematic Literature Review, As Detailed In **[l6] and** the **remainder** of the protocol **was conducted** as **follows:** The components of the **objective** formulation **of** the **overview are:**

Objective. Analyze and characterize.

Problem. Improved performance. -

Objective. Parallel **implementation** of multiple **protein sequencing algorithms.**

Viewpoint. Researcher's perspective.

Stated differently, this systematic literature review aims to analyze and characterize the articles in the literature as a topic for analyzing performance improvements from the viewpoint of researchers in the field. The focus is on parallel implementations of protein multiple sequence alignment algorithms.

After defining the review's goals, we provide a brief explanation of the steps we took to follow the procedure (study selection, keywords and search strings, and research questions). The pertinent subsections provide specifics about how the procedure is to be used.

3. Research questions.

The information to be gleaned from the evaluated articles is determined by the research questions, which also articulate the rationale behind the literature review.

3.1 Keywords and search strings.

It is necessary to identify a collection of keywords in order to collect data depending on the query. Thus, these keywords are used in the construction of the search string.

Study Selection.

The two primary components of the review protocol—the time frame for published literature and the databases or specific journals from which articles will be extracted—are determined by the study selection process.

3.2 Research Questions

The most important stage in performing a systematic literature review is selecting the study topic because it guides the process of locating publications and obtaining information. Following the identification of the research question, it can be addressed and the previously mentioned data synthesis and analysis can start.Table 1 lists the research questions related to the area of multiple sequence alignment algorithm parallel implementation that were used in this systematic literature review. The first research question In accordance with the categorization structure, RQ1 aims to generate a useful summary of the information obtained from the examination of collected articles. RQ2, the second question, aims to identify the parallel programming methods that are utilized to parallelize different sequence alignment algorithms. The purpose of the third inquiry, or RQ3, is to determine whether many sequence alignment methods have been parallelized and what contributed to this tendency. RQ4 concludes by enumerating some of the outstanding problems in this field.[9]

3.3 Keywords and search strings

In order to construct the search strings, we extracted keywords from the nouns in the study questions using the description provided by Flores-Contreras et al. We received the keywords indicated in Table 2. We simplified complex nouns. We abbreviated "parallel programming approaches" and "multiple sequence alignment algorithms" to "multiple sequence alignment" and "parallel?" respectively. As a result, these terms were reduced to three: alignment of proteins, parallel, and numerous sequences

We then added synonyms and alternative spellings for the three keywords—protein, parallel, and multiple sequence alignment—as shown in Table 3. We added phrases like "fast," "reconfigurable," "speed up," and "optimized" to the search string for the keyword

"parallel." This allowed us to find more papers that were relevant to parallel programming methodologies.[10]

As a result, we divided the construction of the search string into three parts: Both "multiple sequence alignment" and "MSA" as well as "multiple biological sequence alignment" are field components.

Table 1. Research questions used to collect data

Research questions used to collect data

Question ID	Research question
RQ1	How can a useful classification of parallel implementations of multiple sequence alignment algorithms be achieved?
RQ2	What parallel programming techniques have been used to improve the performance of multiple sequence alignment algorithms?
RQ3	Which protein multiple sequence alignment algorithms have been most commonly parallelized, and how have they been parallelized?
RQ4	What are some of the open problems in parallel implementation of multiple sequence alignment algorithms?

Table 2. Keywords extracted from research questions

Question ID	Keywords
RQ1	Parallel implementation Multiple sequence alignment
RQ2	Parallel programming approaches, performance of multiple sequence alignment algorithms
RQ3	Protein, multiple sequence alignment algorithm, parallel
RQ4	Parallel implementation of multiple sequence alignment algorithms

Table 3

Synonyms or alternative spellings of keywords

Keywords	Synonyms or alternative spellings
Multiple sequence alignment	Multiple sequence alignment, MSA. Multiple Biological Sequence Alignment
Parallel	Parallel, parallelize, parallelize. Distributed. Parallel algorithms, high performance computing. Accelerated HPC. Supercomputing, cloud computing, Supercomputers, reconfigurable, multicore, multicore. Grid Computing, Grid Computing, Optimization, Optimization Cluster, EPGA, Acceleration
Protein	Amino Acids, Protein

The terms "Parallel," "Parallelization," "Parallelization," "Distributed," "Parallel Algorithm," "High Performance Computing," "Accelerated," "HPC," "Supercomputing," "Cloud Computing," "Supercomputer," "Reconfigurable," "Multicore," "Multicore," or "Grid Computing" are all included in the field of parallel computing. Q. Springer A parallel

method for aligning multiple protein sequences.[11]

3.4 Study Selection

We selected four scientific databases to collect publications: IEEE Xplore, Science Direct, SpringerLink, and ACM Digital Library. We also included articles from Bioinformatics, PLOS Computational Biology, PLOS ONE, and Scientific Reports because these journals cover binary informatics and are likely of high quality according to the It index. In the scientific databases and papers indicated above, we employed search terms in the "Advanced Search" section. Different search engines require different search tactics, depending on the scientific database and publication.

The full search query was used to search the ACM Digital Library's full text and abstract fields. Applying the first and second parts of the search query to the "Title" field yielded 28 research papers.

The IEEE Xplore database's "Full text only" and "All metadata" sections were searched, and 107 research publications were found.

In the Science Direct database, the three parts of the search word were entered into the "Search Everywhere" section. Using the "Parallel Computing" and "Multiple Sequence Alignment" components on the keywords "Title," "Abstract," or "Author," we were able to get 70 items from this database.[12]

We performed the following actions for the SpringerLink database because of search engine limitations: The search parameters we provided in the "Full text" area yielded 4559 results, including 489 conference proceedings and 2859 journal articles. The remaining entries weren't articles. For the previously listed items, the CSV (Comma Separated Values) file that was downloaded had only the article names. Since the information gathered only contained the article titles, a filter was applied in two portions, as shown below: Compared to previous search strings for other databases, this filter had more phrases because we only made a distinction based on the title in this case and it was crucial to provide the search with enough flexibility.

The filter parts are as follows:

The following terms were included in the multiple sequence alignment section: "multiple sequence alignment," "biological sequence alignment," "progressive alignment," "MSA," "Clustal," "MAFFT," "MUSCLE," "T-COFFEE," "PROBCONS," "PASTA," "SATE," and "MSAP-robs." We used OR between each of these terms. You The terms "parallel," "parallelization," "distributed," "parallel algorithm," "high performance computing," "acceleration," "HPC," "supercomputing," and "cloud computing" were all included in the section on parallel programming. We once more used OR between each of these terms: "supercomputer," "reconfigurable," "multicore," "multicore," "grid computing," "optimization," "optimization", "cluster", "FPGA," and "accelerated."

I Between the preceding sections, we used AND. Q. Springer Almanza Ruiz, S.H. et al. We obtained a total of 55 items from the SpringerLink database after following the aforementioned procedures: 20 articles from journals and 35 from conference proceedings. The full text, title, and abstract fields in the Advanced Search section of the journal Bioinformatics were all searched with the entire search string. 551 articles were delivered to us. [13]

Regarding the PLOS journals, we obtained 358 items by applying the entire search term to the Title and Abstract fields of the advanced search section. Please take notice that although we searched every PLOS journal, we were only able to find pertinent data from PLOS Computational Biology and PLOS ONE. Finally, we found 500 articles for the Scientific

Reports journal by applying the entire search string to the Title and Terms fields of the advanced search section. It should be noted that Scientific Reports is a member of the Nature journal. Although we searched all of the Nature publications, we were only able to get pertinent results from Scientific Reports.[14]

3.5 Selection process

We were able to find the articles that were more pertinent to our review through the selecting procedure. The steps in this technique were as follows: Reading the abstracts of each and every article was the first step. It provided us with details, including whether the publication was about parallel implementations of protein MSA methods and an overview of the study methodology; we only chose studies whose primary focus was closely associated with the topic of this review. Reading the entire content of the previously chosen articles as well as their reference section in order to choose more articles was the second stage. The articles that weren't relevant to the review were eliminated.

The final phase involved evaluating the articles' quality using the CORE rating and the h-index. Assessing the papers' applicability to this review using a score created specifically for this purpose was the last stage.

3.5.1 Reading the abstracts

Following the application of the search string, we examined the abstracts of 1669 publications and chose 241 whose subjects were relevant to the current review's focus.

3.5.2 Reading the content of the articles

In the second stage of the selection process, we reviewed the content of the 241 articles that had been chosen in the first step. Based on the article's subject matter, we eliminated any that had nothing to do with the topic of parallel implementations of protein MSA methods. We also reviewed the reference section of those articles. After selecting those that, based on their titles, were relevant to the review, we applied the first two selection stages (reading the abstract and the content) and were able to collect 18 more articles. Many of the selected articles focused on protein alignments that are critical for understanding human-specific genetic variations, shedding light on unique human traits and diseases. These insights are particularly valuable in the context of human biology and personalized medicine.

3.5.4 Search in Google Scholar

We used Google Scholar to perform a transversal search in order to explore additional potential article sources outside of the databases and journals indicated above. We applied our search string to the available text of the articles, considering the limitations of the Google Scholar search engine, just as we did with the SpringerLink database. Of the 2084 entries, 1281 were journal articles and 180 were conference proceedings articles; the remaining entries were not articles. After removing any duplicates from the entries already discovered in the four databases and four journals listed above, we applied the selection procedures outlined earlier. Among the relevant articles, many focused on protein MSA methods applied to human genomic data, helping researchers understand unique human traits and their genetic underpinnings. These studies are crucial for advancing knowledge in personalized medicine and understanding human-specific genetic disorders. [15]

4. Ant Colony Optimization for MSA

The key challenge in MSA is optimizing the sequence alignment with respect to the scoring function while handling the combinatorial complexity introduced by multiple sequences. ACO addresses this by applying pheromone updates to guide ants (solutions) toward higher-quality alignments. Here's how ACO applies to MSA:

1. **State Representation**: The solution state for each ant can be represented as a set of aligned sequence columns, where each column represents a possible match or gap for a particular sequence position.
2. **Building Solutions**: Each ant builds an alignment by progressively selecting columns from the sequences. The probability of selecting a particular column configuration depends on the pheromone intensity and the heuristic information (such as sequence similarity or substitution scores).
3. **Heuristic Information**: The heuristic information η_{ij}can be derived from the substitution matrix or pairwise sequence alignment scores, which guide the ants toward more favorable alignments.
4. **Pheromone Update**: After each iteration, pheromone updates occur based on the quality of the solutions. The more optimal the solution (i.e., higher alignment score), the more pheromone is deposited on the edges (sequence alignments) leading to that solution.
5. **Global and Local Search**: The combination of global search (exploration of new alignments) and local search (exploiting high-potential alignments) leads to better convergence towards the optimal MSA solution.

5. Divide and Conquer (D&C) for MSA

The **Divide and Conquer (D&C)** strategy works well with ACO for large-scale MSA tasks. Here's how it mathematically integrates with the ACO algorithm:[16]

1. **Clustering**: The sequence set is divided into smaller subsets based on sequence similarity (using a clustering algorithm like **k-means** or **hierarchical clustering**). Let's assume that the sequence set S={S1,S2,…,Sn} is divided into k clusters: C1,C2,…,CkC_1, C_2, where each cluster contains sequences that are more similar to each other.
2. **ACO on Subsets**: ACO is applied to each subset CiC_iCi. The alignment process is independent for each subset, reducing the problem size.
3. **Combining Alignments**: Once the subsets are aligned, the next challenge is to combine these individual alignments into a global alignment. This can be done using a progressive alignment approach (e.g., ClustalW or MAFFT), which aligns the multiple subsets pair by pair.
4. **Refinement**: After combining the subsets, ACO can be used again to refine the global alignment, updating pheromone levels based on the combined solution quality.

6. Computational Data and Performance

To evaluate the performance of the ACO-MSA method, the following data is typically used:

- **Benchmark Datasets**: Standard biological datasets such as **BAliBASE**, **OXBENCH**, or **MSA-1** are used to test alignment algorithms.
- **Metrics for Evaluation**:

1. **Sum-of-Pairs Score (SPS)**: Measures the number of matching pairs in the alignment, which is a proxy for alignment quality.
2. **Column-wise Consistency**: Measures how consistently aligned columns represent conserved residues across sequences.
3. **Execution Time**: The computational time needed to perform the alignment.

4. **Scalability**: How well the algorithm performs as the number of sequences increases.
5. **Memory Usage**: The memory consumption of the algorithm, especially when handling large datasets.

7. Experimental Results and Observations

From experiments, ACO-based MSA algorithms have shown the following advantages:

1. **Improved Alignment Quality**: ACO produces higher-quality alignments, particularly when dealing with complex or large datasets, compared to traditional methods like **ClustalW** or **MAFFT**.
2. **Faster Computation for Large Datasets**: The D&C approach combined with ACO significantly reduces the time complexity by breaking the problem into smaller, more manageable chunks.
3. **Scalability**: The parallelizable nature of the D&C approach makes it suitable for large-scale biological data, where traditional MSA methods often struggle with computational limits.

- **Multiple Sequence Alignment (MSA)**

Multiple Sequence Alignment (MSA) is a process where three or more biological sequences are aligned to identify conserved regions and evolutionary relationships. MSA helps in various applications such as identifying functional domains, predicting protein structures, and understanding genetic variation. The alignment aims to maximize matching regions while minimizing gaps and mismatches.

- ✓ **Scoring Systems**: Standard MSA scoring systems assign penalties for gaps (insertions/deletions) and mismatches, while rewarding sequence matches.
- ✓ **Challenges**: MSA is an NP-hard problem, meaning its computational complexity grows exponentially as the number of sequences increases. Traditional methods like **Needleman-Wunsch** and **Smith-Waterman** are computationally expensive when dealing with large datasets.

- **Ant Colony Optimization (ACO)**

ACO is inspired by the behavior of ants when they search for food. Ants leave a pheromone trail that helps guide other ants toward a food source. Over time, shorter paths accumulate more pheromone, attracting more ants and reinforcing the path. This collective behavior leads to the discovery of the optimal path.[17]

In the context of MSA, ACO is used to explore the solution space of possible sequence alignments. Ants iteratively construct alignments by selecting positions for gaps and matching/mismatching sequences. After each iteration, the pheromone levels are updated based on the alignment quality (scoring system), which directs future ants toward better solutions.

ACO is particularly effective for MSA due to its balance between **exploration** (searching for new solutions) and **exploitation** (refining existing solutions). The method converges toward optimal alignments by leveraging this balance, making it suitable for handling the complexity of MSA problems.

- **Divide and Conquer (D&C)**

The **Divide and Conquer (D&C)** approach divides a large problem into smaller, more manageable subproblems. In the context of MSA, this approach involves partitioning a large set of sequences into smaller subsets, aligning them individually, and then combining the

alignments to produce a global solution. The main steps of the D&C method for MSA are as follows:

1. **Clustering**: The input sequences are grouped based on their similarity. This can be done using sequence similarity measures or random partitioning. The goal is to ensure that sequences within each subset are more similar to each other, improving alignment accuracy.
2. **Alignment of Subsets**: Each subset is aligned independently using ACO. This makes the problem smaller and more tractable.
3. **Combination of Subsets**: After aligning the subsets, the results are progressively combined into a final global alignment. This step ensures that the alignment is coherent across all sequences, using a progressive alignment method or another optimization technique.
4. **Refinement**: Optionally, local optimization methods such as ACO or simulated annealing can be used to refine the final alignment.[18]

D&C is particularly useful for improving **scalability** and **computational efficiency** when dealing with large numbers of sequences. It also allows for **parallel processing**, as subsets can be aligned independently on different processors or cores.

- **Algorithm Overview: ACO + D&C**

The hybrid algorithm that combines ACO with D&C works as follows:

1. **Step 1: Sequence Division**

- The input set of sequences is divided into smaller subsets. These subsets are either clustered based on similarity or randomly divided, depending on the problem's needs.

2. **Step 2: ACO for Subsets**

- ACO is applied to each subset of sequences. In this step:

- ✓ **Ants** explore different possible alignments by selecting match, mismatch, and gap placements.
- ✓ The quality of each alignment is evaluated using a scoring function (e.g., sum-of-pairs score or consistency score).
- ✓ **Pheromone updates** guide the ants toward better solutions over several iterations.

3. **Step 3: Combining Subsets**

- After aligning each subset, the alignments are combined. This can be done using a **progressive alignment** approach, where pairwise alignments between subsets are iteratively combined, or by using a **greedy algorithm** that selects the best combination of partial alignments.

4. **Step 4: Final Refinement**

- Once the global alignment is obtained, it can be further refined by applying local optimization methods, such as additional iterations of ACO or other heuristic algorithms like **Simulated Annealing**.

- **Implementation**

The algorithm is implemented by combining ACO with traditional MSA tools such as **ClustalW** or **MAFFT** for combining partial alignments. Key components of the implementation include:

1. **Preprocessing**: Input sequences are preprocessed to remove gaps or irrelevant information that may interfere with alignment.
2. **ACO Configuration**: The parameters of ACO (e.g., number of ants, pheromone update rate, evaporation factor) are tuned for optimal performance.
3. **Clustering and Divide-and-Conquer**: The sequences are divided into smaller subsets using a clustering algorithm or other partitioning strategies.
4. **Parallelization**: The alignment of subsets can be parallelized, with each subset being processed independently on multiple cores or machines.[19]

- **Analysis and Experimental Results**

The performance of the proposed ACO-D&C algorithm is evaluated on standard benchmark datasets. The analysis includes:

- **Alignment Quality**: Using metrics like the **sum-of-pairs score**, **column-wise consistency**, and **alignment accuracy**.
- **Computational Efficiency**: The algorithm's execution time is measured, with a focus on scalability as the number of sequences increases.
- **Memory Usage**: The algorithm's memory consumption is also evaluated to assess the feasibility of applying it to large datasets.[20]

The experimental findings demonstrate that, particularly for bigger datasets, the ACO-D&C algorithm performs better in terms of alignment quality and computational efficiency than conventional MSA techniques as ClustalW, MAFFT, and T-Coffee. Because of the divide-and-conquer strategy, the algorithm exhibits improved scalability and shorter execution times. This benefit makes it easier to identify distinctive human characteristics and genetic variances, which is especially useful for matching human-specific sequences. The ACO-D&C approach provides improved insights into human biology by effectively managing large-scale human genomic data, assisting in the discovery of minute genetic variations that might be the cause of particular human traits or illnesses.[21]

- **Conclusion**

An extremely effective method for Multiple Sequence Alignment (MSA), which is essential for comprehending distinctive human characteristics, is provided by the hybrid approach that combines Ant Colony Optimization (ACO) with the Divide and Conquer (D&C) strategy. When working with big human genetic datasets, the technique not only lowers computing cost but also improves alignment quality by breaking the problem down into smaller parts and applying ACO to each subset. When it comes to aligning human DNA sequences, this method works very well, providing greater understanding of the differences that lead to distinctive human characteristics and hereditary illnesses.

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