Multi-SpaM: a Maximum-Likelihood approach to Phylogeny Reconstruction based on Multiple Spaced-Word Matches

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Abstract

Motivation: Word-based or 'alignment-free' methods for phylogeny reconstruction are much faster than traditional approaches, but they are generally less accurate. Most of these methods calculate pairwise distances for a set of input sequences, for example from *word frequencies*, from so-called *spaced-word matches* or from the average *length of common substrings*.

Results: In this paper, we propose the first word-based approach to tree reconstruction that is based on multiple sequence comparison and *Maximum Likelihood*. Our algorithm first samples small, gap-free alignments involving four taxa each. For each of these alignments, it then calculates a quartet tree and, finally, the program *Quartet MaxCut* is used to infer a super tree topology for the full set of input taxa from the calculated quartet trees. Experimental results show that trees calculated with our approach are of high quality.

1. Introduction

To gain a better understanding of the evolution of genes or species, reconstructing accurate phylogenetic trees is essential. This can be done using standard methods which rely on *sequence alignments*, either of entire genomes or of sets of orthologous genes or proteins. *Character-based* methods such as *Maximum Parsimony* [14, 20] or *Maximum Likelihood* [15] infer trees based

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on evolutionary substitution events that may have happened since the species evolved from a common ancestor. These methods are generally considered to be accurate, as long as the underlying alignments are of high quality, and as long as suitable substitution models are used. However, for the task of multiple alignment no exact polynomial-time algorithm exists, and even heuristic approaches can be time consuming [46]. Moreover, the most popular heuristic for multiple alignment, the *progressive alignment* [19], has been shown to be relatively unstable: multiple alignments calculated with progressive approaches and trees inferred from these alignments depend on the underlying *guide trees* and even on the order of the input sequences [9]. In addition to these difficulties, exact algorithms for character-based phylogeny approaches are themselves *NP hard* [11, 21].

Distance methods, by contrast, infer phylogenies by estimating evolutionary distances for all pairs of input taxa [16]. Here, pairwise alignments are sufficient which can be faster calculated than multiple alignments, but still require runtime proportional to the product of the lengths of the aligned sequences. There is a loss in accuracy, however, compared to character-based approaches, as all of the information about evolutionary events is reduced to a single number for each pair of taxa, and not more than two sequences are considered simultaneously, as opposed to character-based approaches, where all sequences are examined simultaneously. The final trees are obtained by clustering based on distance matrices, most commonly with *Neighbor Joining* [45]. Since both pairwise and multiple sequence alignments are computationally expensive, they are ill-suited for the increasingly large datasets that are available today due to the next generation sequencing techniques.

In recent years, a number of *alignment-free* approaches to genome-based phylogeny reconstruction have been published which are very fast in comparison to alignment-based methods [49, 57, 5, 7, 40, 42]. Another advantage of these new methods is that they circumvent some well-known problems in genome alignment such as genome rearrangements and duplications. Moreover, alignment-free methods can be applied to incomplete sequence sets and even

to collections of unassembled reads [44, 50, 56, 12]. A disadvantage of these methods is that they are, in general, considerably less accurate than slower, alignment-based methods.

Some 'alignment-free' approaches compare fixed-length *words* of the input sequences to each other, so – despite being called 'alignment-free' – they are using local pairwise 'mini-alignments'. Recently, methods have been proposed that estimate phylogenetic distances based on the relative frequency of mismatches in such local alignments. An example is *co-phylog* [56] which finds short gap-free alignments of a fixed length, consisting of matching nucleotide pairs only, except for the middle position where a mismatch is allowed. Phylogenetic distances are estimated from the fraction of such alignments for which the middle position is a mismatch. As a generalization of this approach, *andi* [26] uses pairs of maximal exact word matches that have the same distance to each other in both sequences; the frequency of mismatches in the segments between those matches is then used to estimate the number of substitutions per position between two input sequences.

co-phylog and *andi* require a minimum length of the flanking word matches in order to reduce the number of random background matches. Threfore, they tend not to perform well on distantly related sequences where long exact matches are less frequent. Moreover, the number of random segment matches grows quadratically with the length of the input sequences while the expected number of homologous matches grows only linearly. Thus, longer exact matches are necessary in these approaches to limit the number of background matches if longer sequences are compared. This, in turn, reduces the number of homologies that are found, and therefore the amount of information that can be used to calculate accurate distances. Other alignment-free approaches are based on the length of maximal common substrings between sequences. These approaches are also very efficient, since common substrings can be rapidly found using suffix trees or related data structures [55, 27]. As a generalization of this approach, some methods use longest common substrings with a certain number of mismatches [30, 54, 53, 33, 3].

Recently, we proposed to use words with *wildcard characters* – so-called *spaced words* – for alignment-free sequence comparison [29, 28]. Here, a binary pattern of *match* and *don't-care* positions specifies the positions of the *wild-card* characters [38, 36, 23]. In *Filtered Spaced-Word Matches (FSWM)* [32] and *Proteome-based Spaced-word Matches (Prot-SpaM)* [31], alignments of such spaced words are used, where sequence positions must match at the *match* positions while mismatches are allowed at the *don't care positions*. A score is calculated for every such spaced-word match in order to remove – or *filter out* – *background* spaced-word matches; the mismatch frequency of the remaining *homologous* spaced-word matches is then used to estimate the number of substitutions per position that happened since two sequences evolved from their last common ancestor. The filtering step allows us to use patterns with fewer match positions in comparison to above mentioned methods *co-phylog* and *andi*, since the vast majority of the background noise can be eliminated reliably by looking at the *don't-care* positions of the initially found spaced-word matches. As a result, the phylogenetic distances calculated by *FSMW* and *Prot-SpaM* are generally rather accurate, even for large and distantly related sequences.

In this paper, we introduce a novel approach to phylogeny reconstruction called *Multiple Spaced-Word Matches (Multi-SpaM)* that combines the *speed* of the so-called 'alignment-free' methods with the *accuracy* of the *Maximum-Likelihood* approach. While other alignment-free methods are limited to *pairwise* sequence comparison, we generalize the above outlined *spaced-word* approach to *multiple* sequence comparison. For a binary pattern of *match* and *don't care* positions, *Multi-SpaM* identifies *quartet blocks* of four matching spaced words each, *i.e.* gap-free four-way alignments with matching nucleotides at the *match* positions of the underlying binary pattern and possible mismatches at the *don't care* positions, see Figure **??** for an example. For each such quartet block, an optimal *Maximum-Likelihood* tree topology is calculated with the software *RAxML* [51]. The *Quartet MaxCut* algorithm [48] is then used to combine the calculated quartet tree topologies into a super tree. We show that on both simulated and real data, *Multi-SpaM* produces phylogenetic trees of high quality

and often outperforms other alignment-free methods.

2. Material and Method

To describe our method, we first need some formal definitions. A *spaced word* of length ℓ exists in the context of a binary pattern $P \in \{0,1\}^\ell$ of the same length. This pattern marks every position as either a *match position* in case of a 1 or as a *don't care position* in case of a 0. The number of match positions is called the *weight* of the pattern. Given such a pattern P, a *spaced word* w is a word of length ℓ over the alphabet {A,C,G,T,*} such that $w(i) = *$ if and only if $P(i) = 0$, *i.e.* if and only if i is a *don't care* position. The symbol '$*$' is interpreted as a 'wildcard' character. For a DNA Sequence S of length n and a position $0 \leq i \leq n - l + 1$, we say that a *spaced word* w occurs in S at position i – or that $[S, i]$ is an *occurrence* of w – if $S(i + j) = w(j)$ for all match positions j. This follows the definition that we previously used [29, 34].

A pair $([S, i], [S', i'])$ of occurrences of the same spaced word w is called a *spaced-word match*. For a substitution matrix assigning a *scores*(X, Y) to every pair (X, Y) of nucleotides, we define the *score* of a spaced word match $([S, i], [S', i'])$ as

$$\sum_{P(k)=0} s(S(i + k), S'(i' + k))$$

That is, if we align the two occurrences of w to each other, the score of the spaced-word match is the sum of the scores of the nucleotides aligned to each other at the *don't-care* positions of P. In *Multi-SpaM*, we are using the nucleotide substitution matrix below that has been proposed by Chiaromonte *et al.* [10]:

	A	C	G	T
A	91	−114	−31	−123
C		100	−125	−31
G			100	−114
T				91

Multi-SpaM starts with generating a binary pattern P with user-defined length ℓ and weight w. By default, we use values $\ell = 110$ and $w = 10$, *i.e.* by default the pattern has 10 *match positions* and 100 *don't-care* positions, but other values for ℓ and w can be chosen by the user. Given these parameters, P is calculated by running our previously developed software tool *rasbhari* [24].

As a basis for phylogeny reconstruction, we are using four-way alignments consisting of occurrences of the same spaced word with respect to P in four different sequences. We call such an alignment a *quartet P-block* or a *P-block*, for short. A P-block is thus a gap-free alignment of length ℓ where in the k-th column identical nucleotides are aligned if k is a *match* position in P, while mismatches are possible if k is a *don't-care* position, see Figure **??** for an example. Note that the number of such P-blocks can be very large: if there are n occurrences of a spaced-word w in n different sequences, then this gives rise to $\binom{n}{4}$ different P-blocks. Thus, instead of using all possible P-blocks, *Multi-SpaM* randomly samples a limited number of P-blocks to keep the program runtime under control.

Moreover, for phylogeny reconstruction, we want to use P-blocks that are likely to represent true homologies. Therefore, we introduce the following definition: a P-block – *i.e.* a set of four occurrences of the same spaced word w – is called a *homologous P-block* if it contains at least *one* occurrence $[S_i, p]$ of w such that all remaining three occurrences of w have positive scores when compared to $[S_i, p]$. To sample a list of homologous P-blocks, we randomly select spaced-word occurrences with respect to P from the input sequences and their reverse complements. For each selected $[S_i, p]$, we then randomly select occurrences of the same spaced word from sequences $S_j \neq S_i$, until we have found three occurrences of w from three different sequences that all have positive scores with $[S_i, p]$.

To find spaced-word matches efficiently we first sort the list of all occurrences of spaced words with respect to P in lexicographic order. This way, we obtain a list of spaced-word occurrences where all occurrences of the same spaced word w are appearing as a contiguous block. Once we have sampled a homologous

P-block as described, we remove the four occurrences of w from our list of spaced-word occurrences, so no two of the sampled P-blocks can contain the same occurrence of a spaced word. The algorithm continues to sample P-blocks until no further P-blocks can be found, or until a given number of P-blocks is reached. By default, *Multi-SpaM* uses a maximal number of $M = 1,000,000$ P-blocks, but this parameter can be adjusted by the user.

For each of the sampled quartet P-blocks, we infer an unrooted tree topology. This most basic *unrooted* phylogenetic unit is called a *quartet* topology; there are three possible different quartet topologies for a set of four taxa. To identify the best of these three topologies, we use the *Maximum Likelihood* program *RAxML* [51]. We note that *RAxML* is a general *Maximum-Likelihood* software, its use in our context is fairly degenerated, as we only use it to infer optimal quartet topologies.

After having the optimal tree topology for each of the sampled quartet P-blocks, we need to amalgamate them into a single tree spanning the entire taxa set. This task is denoted the *Supertree Task* [6] and is known to be *NP hard*, even for the special case where the input is limited to quartets topologies, as in our case [52]. Nevertheless there are several heuristics for this task, with *MRP* [4, 41] the most popular. Here we chose to use *Quartet MaxCut* [47, 48] that proved to be faster and more accurate for this kind of input [2]. In brief, *Quartet MaxCut* partitions recursively the taxa set where each such partition corresponds to a split in the final tree. In each such recursive step, a graph over the taxa set is built where the set of quartets induces the edge set in that graph. The idea is to partition the vertex set (the taxa) such that the minimum quartets are violated. This is achieved by a *semidefinite-programming*-like algorithm that embeds the graph on the unit sphere and applies a random hyperplane through the sphere.

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