ON MOTIFS IN COLORED GRAPHS

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Abstract. One of the most important concepts in biological network analysis is that of network motifs, which are patterns of interconnections that occur in a given network at a frequency higher than expected in a random network. In this work we are interested in searching and inferring network motifs in a class of biological networks that can be represented by vertex-colored graphs. We show the computational complexity for many problems related to colorful topological motifs and present efficient algorithms for special cases. We also present a probabilistic strategy to detect highly frequent motifs in vertex-colored graphs. Experiments on real data sets show that our algorithms are very competitive both in efficiency and in quality of the solutions.

1. INTRODUCTION

Biological networks have been wide and deeply studied in recent years. Their analysis provides comprehension of the underlying biological processes, the function and the structure of their components, and of their evolutionary relationships. Such networks may be reaction graphs, regulatory networks, protein-protein-interaction (PPI) networks or metabolic pathways, all of which can be modeled by vertex-colored graphs where the vertices represent biochemical reactions, genes, proteins or metabolites.

A concept that takes a central role in such network analysis is the so called network motif, i.e., a pattern of interconnections that occur at higher frequency inside a (biological) network than expected in a random network [28].

In this context, we consider two problem variants: Motif search is a graph-theoretic pattern matching problem where a small graph (pattern) is searched in a large graph, such that structure of occurrence of the pattern is preserved, i.e. its topology and connectivity. Motif inference (or motif discovery) receives as input only one (large) graph and the task is to detect (small) subgraphs occurring at high frequency.

The first approaches to motif search in a biological network were proposed in the context of transcriptional regulation networks [26] and later within PPI networks [17]. Since most variations of the motif search problem are computationally hard [9,13,19], several techniques are used in order to overcome that hardness [3,5,16,19]. Searching motifs with a specific topology arose in Shlomi et al. [27] using fixed-parameter tractable algorithms for searching motifs defined as paths within a PPI network. A

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more general solution was implemented in the Qnet tool which searches for motifs defined as trees [10].

Lacroix et al. [19] proposed a new approach for the motif search problem in vertex-colored graphs, where the motif topology is not taken into consideration and only connectivity is required. Such a motif is called colored motif. This motif search problem has received much attention [15,22,23]. A fixed-parameter algorithm was presented by Lacroix et al. [19], extended to infer all colored motifs in metabolic networks and implemented as a tool called MOTUS [25]. Torque [5] is another solution that aims to search a given colorful motif in a PPI network. (A motif is colorful if each color occurs only once.) Later, GraMoFoNe [3] generalized the problem, searching for a given colored motif, not only colorful, in a PPI network.

Most of the previous implementations are concerned only with motif search. Another important task is enumerating all motifs of a given size in a network. MOTUS can solve such a problem only for small motifs (of size up to 7), due to its computational cost.

This paper is an extension of two previous works presented in conferences [1,24] where we showed the hardness of some colorful topological motif problems and presented algorithms for searching and enumerating colorful motifs. Many of those previous results are, for the sake of clarity, also presented in this paper and can be summarized as follows: (i) Given a vertex-colored graph $G$ representing a biological network and a colorful topological motif $M$, finding a simple subgraph of $G$ isomorphic to $M$ is NP-hard (Theorem 1); (ii) Given a vertex-colored graph $G$ and a colorful motif $M$, finding an induced subgraph of $G$ isomorphic to $M$ is NP-hard (Theorem 2); (iii) Given a vertex-colored graph $G$ and a colorful tree $M$, finding $M$ as a simple subgraph of $G$ can be performed in polynomial time (Algorithm 1); (iv) Given a vertex-colored graph $G$ and a colorful tree $M$, an algorithm to enumerate all occurrences of $M$ in $G$ is provided (Algorithm 3); (v) A framework for evaluating high frequency and inferring motifs in colored-vertex graphs is provided (Section 5); and (vi) Experimental evaluation of algorithms for motif search and inference is performed, including analysis of the high frequency evaluation framework and comparison of these algorithms with other similar tools (Section 6).

Besides that, in this paper we improve some of those previous results and present new results, namely:

(i) We propose a new related problem where given a vertex-colored graph $G$, a colorful motif $M$ and an integer $k$, we want to find at least $k$ (vertex) disjoint occurrences of $M$ in $G$. We prove this is an NP-hard problem (Theorem 3);
(ii) We also propose a problem where given two vertex-colored graphs $G$ and $H$ and an integer $k$, we want to find a colorful tree with $k$ vertices which is a subgraph of both $G$ and $H$. We prove this is an NP-hard problem (Theorem 4);
(iii) We show, using a particular data structure, that the algorithm for finding a subgraph of a given vertex-colored graph $G$ isomorphic to a given colorful motif $M$ (Algorithm 1) can be improved from quadratic to linear running time (Theorem 6);
(iv) We present a method (Algorithm 4) that, given a vertex-colored graph $G$ and a colorful tree motif $M$, computes the number of occurrences of $M$ in $G$, showing that it can be performed in linear time (Theorem 10);
(v) We speedup considerably the previous version of our motif inference algorithm (Algorithm 5) by using as subroutine the new algorithm for counting the number of occurrences of motifs (Algorithm 4), updating experimental tests (Section 6) related to this algorithm.

This paper is organized as follows. Section 2 provides basic definitions and notations. Section 3 presents complexity results for problems of interest. Search algorithms such as for finding subgraphs isomorphic to colorful trees, finding a maximum clean graph, finding all colorful motifs in a clean subgraph, and finding the number of occurrences of colorful motifs in a graph are given in Section 4. Next, Section 5 provides a framework to detect subgraphs in a network occurring with high frequency, and also an algorithm for inferring statistically significant colorful motifs in a given vertex-colored graph. Section 6 shows experimental results of implementations of sequential and parallel proposed algorithms. A conclusion is given in Section 7.

2. Preliminaries

Let \( G = (V_G, E_G) \) be a graph such that \( V_G \) is a set of vertices and \( E_G \subseteq \binom{V_G}{2} \) is a set of edges (unordered pairs of vertices). Let \( C \) be a set of colors. A color function \( c : X \rightarrow C \) assigns a color in \( C \) to each element in \( X \). Typically, \( X \) is a subset of the set of vertices of a graph. Thus, a vertex-colored graph is a graph with colored vertices, i.e., a graph \( G \) such that \( c : V_G \rightarrow C \) assigns a color in \( C \) to each vertex in \( V_G \). Sometimes in this text, we refer to a vertex-colored graph simply as a graph.

We denote by \( uv \) an edge \( \{u, v\} \) of a graph and we say that vertices \( u \) and \( v \) are adjacent. A (color-) isomorphism between graphs \( G \) and \( H \) is a bijection \( f \) from \( V_G \) to \( V_H \) such that, for any two elements \( u, v \in V_G \), \( u \) and \( v \) are adjacent in \( G \) if and only if \( f(u) \) and \( f(v) \) are adjacent in \( H \). Furthermore, for each pair \( (v, f(v)) \), where \( v \in V_G \) and \( f(v) \in V_H \), we have \( c(v) = c(f(v)) \). Graphs \( G \) and \( H \) are (color-) isomorphic if there exists an isomorphism between them, and we denote this by \( G \cong H \).

Let \( G \) be a graph, define \( c(X) := \{c(x) : x \in X\} \) for any subset \( X \subseteq V_G \). A subgraph of \( G \) is a graph \( H \) such that \( V_H \subseteq V_G \) and \( E_H \subseteq E_G \). We denote it by \( H \subseteq G \). A subgraph of \( G \) induced by a subset \( X \) of \( V_G \) is the graph \( H \) such that \( V_H = X \) and \( E_H = E_G \cap \binom{X}{2} \). Such a graph \( H \) is also denoted by \( G[X] \).

For a subset \( X \) of \( V_G \) of a graph \( G \), we denote by \( G - X \) the subgraph \( G[V_G \setminus X] \). Similarly, if \( A \) is a subset of \( E_G \), we denote by \( G - A \) the graph \( (V_G, E_G \setminus A) \). Moreover, when it is implicit where adding a vertex and an edge in a graph \( G \) (for instance, adding a vertex \( x \) and an edge \( xy \) in a graph \( G \), with \( y \in V_G \)), then we use the notation \( G + x \). In some cases, we use the notation \( G + xy \) to denote the addition of edge \( xy \) and the vertex \( x \) to \( G \), supposing that \( y \in V_G \). We write \( H \cong G \) if \( H \) is isomorphic to a subgraph of \( G \).

Let \( G \) be a vertex-colored graph and \( M \propto G \). The restriction of color function \( c \) to \( V_M \) is the function \( c_M = (c|V_M) \) and thus \( M \) is also a colored graph. If \( c_M \) is a injection we say that \( M \) is a colorful (sub)graph (informally, each color in \( C \) is assigned to at most one vertex in \( V_M \)). In this setting, we say that such a graph is a colorful motif.

An exact occurrence of a colorful motif \( M \) in a vertex-colored graph \( G \) is a vertex set \( S \) in \( V_G \) such that \( G[S] \) is connected, \( M \propto G[S] \) and \( |V_M| = |S| \). In this paper, we are interested in the following computational problems.
Problem Subgraph-Motif\((G, M)\): given a vertex-colored graph \(G\) and a colorful motif \(M\), does there exist a subgraph of \(G\) isomorphic to \(M\), that is, \(M \prec G\)?

Problem Induced-Subgraph-Motif\((G, M)\): given a vertex-colored graph \(G\) and a colorful motif \(M\), does there exist an induced subgraph of \(G\) isomorphic to \(M\)?

Problem All-Motifs\((G, M)\): given a vertex-colored graph \(G\) and a colorful motif \(M\), enumerate all occurrences of \(M\) in \(G\), such that \(M\) is a subgraph of \(G\).

Problem \(k\)-Disjoint-Motifs\((G, M, k)\): given a vertex-colored graph \(G\), a colorful motif \(M\), and an integer \(k > 0\), do there exist at least \(k\) disjoint occurrences of \(M\) in \(G\), such that \(M\) is a subgraph of \(G\)?

Problem Common-\(k\)-Tree\((G, H, k)\): given two vertex-colored graphs \(G\) and \(H\), and an integer \(k > 0\), does there exist a colorful tree \(T\) with \(k\) vertices such that \(T \prec G\) and \(T \prec H\)?

3. Complexity results

We start this section by proving the computational complexity of Subgraph-Motif.

Theorem 1. Problem Subgraph-Motif is NP-complete.

Proof. We first show that Subgraph-Motif belongs to NP. Given a graph \(G\) and a colorful motif \(M\), the certificate is a graph \(G'\) such that \(G' \prec G\). A verification algorithm can easily check this in polynomial time.

We provide a reduction from the 3-sat problem, which is NP-complete \[14\]. Given an arbitrary Boolean formula \(\Phi\) in conjunctive normal form (CNF) with \(m\) clauses \(C_1, \ldots, C_m\) as an instance of 3-sat, we construct \(G\) with \(3m\) vertices, where each vertex represents a literal of a clause in \(\Phi\). Vertices \(u\) and \(v\) in \(G\) have the same color if and only if \(u\) and \(v\) come from the same clause in \(\Phi\). An edge \(uv \in E_G\) if and only if literals representing vertices \(u\) and \(v\) are not opposite. We construct \(M\) as a colorful clique with \(m\) vertices, whose colors are the \(m\) distinct colors of vertices in \(G\) (Fig 1). Clearly, this transformation can be done in polynomial time.

We argue that \(\Phi\) is satisfiable if and only if \(M \prec G\). Suppose that \(\Phi\) is satisfiable. Then there exists a truth assignment to the variables satisfying all clauses. Let \(S\) be a set of vertices in \(G\) corresponding to \(m\) literals with values “true”, one for each clause, that satisfies \(\Phi\). By definition of \(G\), it follows that \(G[S] \cong M\) and thus \(M \prec G\). On the other hand, suppose that \(M \prec G\). Then there exists a subgraph \(H \subseteq G\) such that \(H \cong M\). It means that \(H\) is a colorful clique and it follows that the set of vertices in \(H\) represents a set \(S\) of \(m\) non-opposite literals in \(\Phi\), one per clause. Consequently, a truth assignment to literals in \(S\) satisfies \(\Phi\). \(\square\)

Now we show the computational complexity for the related problem Induced-Subgraph-Motif.

Theorem 2. Problem Induced-Subgraph-Motif is NP-complete, even when the given colorful motif is a tree.

Proof. As for Subgraph-Motif (Theorem \[1\]), we can show easily that Induced-Subgraph-Motif belongs to NP.

To show NP-hardness, we present a reduction from 3-sat, similar to Theorem \[1\]. Given an arbitrary Boolean formula \(\Phi\) in CNF with \(m\) clauses \(C_1, \ldots, C_m\) as an
instance of 3-sat, we construct a graph $G$ with $3m + 1$ vertices, where each one of the first $3m$ vertices represents a literal of a clause in $\Phi$. The last extra vertex is called core. Vertices $u$ and $v$ in $G$ have the same color if and only if $u$ and $v$ come from the same clause in $\Phi$. The core vertex has a color different from any other vertex, and it is adjacent to each one of the $3m$ remaining vertices. Moreover, two vertices representing literals are adjacent if and only if the literals representing them are opposite. Furthermore, $M$ is a colorful star with $m + 1$ vertices, whose colors are in $V_G$ and the color of its center is the color of the core (Fig 2). Notice that such a transformation can be performed in polynomial time.

Now, we show that $\Phi$ is satisfiable if and only if there exists an induced subgraph of $G$ isomorphic to star $M$. Suppose that $\Phi$ is satisfiable. Then, there exists a truth assignment to the variables satisfying all clauses. Let $S$ be a set of vertices corresponding to $m$ literals with values "true", belonging to different clauses, that satisfy $\Phi$. By definition of $G$, we have $G[S \cup \{\text{core}\}] \cong M$ and thus $G[S \cup \{\text{core}\}]$ is an induced subgraph of $G$ isomorphic to $M$. Conversely, suppose that there exists an induced subgraph $H$ of $G$ isomorphic to $M$. Since the color of the core is unique in $G$ and $M$, the color of the center of $H$ has the same color as the vertex core in $M$ and the set of vertices in $V_H - \{\text{core}\}$ represents a set $S$ of $m$ not opposite literals in $\Phi$, one per clause. Hence, a truth assignment to literals in $S$ satisfies $\Phi$. □

In the following we prove the computational complexity for $k$-DISJOINT-MOTIFS using a reduction from a well-known computational problem LONGEST-COMMON-SUBSEQUENCE. Before doing that we need a few definitions. Given a finite set of sequences $R = \{S_1, \ldots, S_p\}$, we denote by $s_{i,j}$ the $j$th symbol in sequence $S_i$. We say that a $p$-tuple of integers $\mathbf{j} = (j_1, \ldots, j_p)$ is a column in $R$ if $s_{i,j_i} = s_{i+1,j_i+1}$ for each $1 \leq i < p$. We also say that columns $\mathbf{j} = (j_1, \ldots, j_p)$ and $\mathbf{k} = (k_1, \ldots, k_p)$ are crossing in $R$ when there exists some $i$, $1 \leq i < p$, such that $j_i \leq k_i$ and $j_{i+1} \geq k_{i+1}$ (or $j_i \geq k_i$ and $j_{i+1} \leq k_{i+1}$). Otherwise, they are non-crossing, i.e., when $j_i < k_i$ (or $j_i > k_i$) for all $i$, $1 \leq i \leq p$. Given a set $C$ of columns in $R$, we say that $C$ is
a common subsequence in $R$ if $j$ and $k$ are non-crossing for each pair of distinct columns $j$ and $k$ in $C$. Fig. 3 presents a graphical example of a common subsequence.

Now we can present the following decision problem, which has been shown to be NP-complete [20]:

Problem Longest-Common-Subsequence($R, k$): given a set $R$ of sequences and an integer $k$, does there exist a common subsequence $C$ in $R$ such that $|C| \geq k$?

We are ready to demonstrate the following result.

Theorem 3. Problem $k$-Disjoint-Motifs is NP-complete.

Proof. It is easy to show that $k$-Disjoint-Motifs belongs to NP. Given a graph $G$, a colorful motif $M$, an integer $k$, and a set of $k' \geq k$ subgraphs of $G$, it can be checked, in polynomial time, if each of those $k'$ subgraphs is isomorphic to $M$ and no two of them share a vertex.

To complete the NP-hardness proof, we present a reduction from Longest-Common-Subsequence. Given a set of sequences $R = \{S_1, \ldots, S_p\}$ and an integer $k$ as an input of Longest-Common-Subsequence, let us construct a graph $G$ and a colorful motif $M$ such that there exists a common subsequence in $R$ of length at least $k$ if and only if there exist at least $k$ disjoint occurrences of $M$ in $G$. 
(Graph $G$). We describe the graph $G$ constructed from $R$. The set of vertices $V_G$ is partitioned into sequence vertices $V^S$, core vertices $V^C$, and transversal vertices $V^X$. Two vertices in different subsets have different colors. The set of edges $E_G$ is partitioned into core edges $E^C$ and transversal edges $E^X$.

((Sequence vertices $V^S$)). Each symbol $s_{i,j}$ in a sequence of $R$ is represented by a vertex $v^S_{i,j}$ in $V^S$. All vertices from the sequence $S_i$ have color $c_i$, and $c_i \neq c_{i'}$ for $i \neq i'$.

((Core vertices $V^C$ and core edges $E^C$)). The set of core vertices is partitioned into $p - 1$ disjoint sets $V^C_1, ..., V^C_{p-1}$. Each pair of vertices $(v^C_{i,j}, v^C_{i,j+1})$ such that $s_{i,j} = s_{i+1,j'}$ is represented by a core vertex $v^C_{i,j}$ in $V^C_i$, where $z = (v^C_{i,j}, v^C_{i,j+1})$. All vertices in $V^C_i$ have color $d_i$, and $d_i \neq d_{i'}$ for $i \neq i'$. The set $E^C$ is partitioned into $p - 1$ disjoint sets $E^C_1, ..., E^C_{p-1}$, and for each core vertex $v^C_{i,j}$, representing a pair $z = (v^C_{i,j}, v^C_{i,j+1})$, we have edges $(v^C_{i,j}, v^C_{i,j+1})$ and $(v^C_{i,j}, v^C_{i,j+1})$ in $E^C$. We say that a pair of core vertices $(v^C_{i,j}, v^C_{i,j})$ in $V^C_i$, with $y = (v^C_{i,j}, v^C_{i,j+1})$ and $z = (v^C_{i,j}, v^C_{i,j+1})$, is opposing in $i$ if $j < k$ and $j' > k'$.

((Transversal vertices $V^X$ and transversal edges $E^X$)). Sets $V^X$ and $E^X$ are partitioned into $p - 1$ sets $V^X_1, ..., V^X_{p-1}$ and $E^X_1, ..., E^X_{p-1}$, respectively. Two vertices in distinct sets $V^X_i$ and $V^X_j$ have different colors. Let $\chi_i$ be the set of pairs of (core) vertices opposing in $i$ and suppose that $V^C_i = \{v^C_{i,j}, ..., v^C_{i,p}\}$ with $\ell = |V^C_i|$. Now, observe that we relabel arbitrarily the superscripts of vertices in $V^C_i$ with integer numbers. For each $x$ in $\chi_i$, we list a sequence $x^1, x^2, ..., x^\ell$ such that $x^j = x^j$ if the pair of core vertices $v^C_{i,j}$ and $v^C_{i,j}$ is opposing in $i$. Then, we add all the $\ell - 1$ distinct elements of this sequence to the set of transversal vertices $V^X_i$. Each core vertex $v^C_{i,j}$ in $V^X_i$ is adjacent to the transversal vertex $x^j$ in $V^X_i$. Furthermore, two transversal vertices in $V^X_i$ have the same color if and only if they come from a pair of core vertices opposing in $i$. This completes the description of $G$.

(Motif $M$). The colorful tree $M$ consists of a path of length $2p - 1$, where vertices have colors $c_1, d_1, c_2, d_2, ..., c_{p-1}, d_{p-1}, c_p$. Moreover, each vertex of color $d_i$ is adjacent to $|\chi_i|$ vertices whose colors are those used for coloring the transversal vertices in $V^X_i$. See Fig 4.

Graphs $G$ and $M$ can be obtained in polynomial time with respect to the size of $R$. Then we must show that there exist at least $k$ disjoint occurrences of $M$ in $G$ if and only if there exists a common subsequence in $R$ of length at least $k$.

Consider a vertex set $K$ such that $K \subseteq V_G$. Denote by $K^Z$ the set $K \cap V^Z$, for $Z \in \{S, C, X\}$.

Notice that there exists an obvious correspondence between columns in $R$ and occurrences of $M$ in $G$, i.e., for each column $j = (j_1, ..., j_p)$ in $R$, there exists an occurrence $J$ in $G$ such that $J^S = \{v^S_{j_1}, \ldots, v^S_{j_p}\}$. Therefore, to show that there exists a common subsequence in $R$ of length at least $k$ if and only if there exist at least $k$ disjoint occurrences of $M$ in $G$, it is enough to prove that two columns $j = (j_1, \ldots, j_p)$ and $k = (k_1, \ldots, k_p)$ in $R$ are non-crossing if and only if subgraphs $J$ and $K$ are disjoint in $G$.

Suppose that $j$ and $k$ are non-crossing. Then, $j_i \neq k_i$ for each $i$ which implies that $v^S_{j_i} \neq v^S_{k_i}$ for each $i$ and therefore, $J^S \cap K^S = \emptyset$. By construction, all core vertices in $J$ are adjacent to two vertices in $J^S$ and all core vertices in $K$ are adjacent to two vertices in $K^S$, which implies, since $J^S \cap K^S = \emptyset$, that $J^C \cap K^C = \emptyset$. Below, until the end of this paragraph, we are going to show that an arbitrary
vertex \( u \in J^X \) does not belong to \( K^X \), which implies that \( J^X \cap K^X = \emptyset \). By construction, since \( u \in J^X \), it follows that \( u \) must be connected to vertex \( v_i^y \in J^C \), where \( y = (v_i^m, v_i^{j+1}) \) for some \( i \). Since \( J^C \cap K^C = \emptyset \) and \( u \) is connected to \( v_i^y \in J^C \), if \( u \) is a leaf, then \( u \) is not connected to any vertex in \( K^C \) which implies that \( u \not\in K^X \). Then, assume that \( u \) is not a leaf. It follows by construction that \( u \) is connected to another vertex \( v_i^x \in V^C \), where \( z = (v_i^n, v_i^{n+1}) \neq y \), for some pair \( m, n \).

This implies that \( v_i^x \) and \( v_i^y \) are opposing in \( i \), and thus \( j_i < m \) and \( j_{i+1} > n \), or \( j_i > m \) and \( j_{i+1} < n \). Therefore, since \( j \) and \( k \) are non-crossing columns in \( R \), we have that \( s_i, m \not\in k \) or \( s_{i+1}, n \not\in k \) and consequently, \( v_i^m \not\in K \) or \( v_i^{n+1} \not\in K \). However, by construction, if an arbitrary core vertex \( v \) belongs to an occurrence, then each vertex \( u \) adjacent to \( v \) also belongs to this occurrence, which implies that \( u \not\in K \).

Since \( J^S \cap K^S = J^C \cap K^C = J^X \cap K^X = \emptyset \), we conclude that \( J \) and \( K \) are disjoint in \( G \).

Conversely, suppose that \( j \) and \( k \) are crossing in \( R \). Then, for some integer \( i \), we have that \( j_i \leq k_i \) and \( j_{i+1} \geq k_{i+1} \) or \( j_i \geq k_i \) and \( j_{i+1} \leq k_{i+1} \). W.l.o.g., suppose that \( j_i \leq k_i \) and \( j_{i+1} \geq k_{i+1} \). If \( j_i = k_i \) or \( j_{i+1} = k_{i+1} \), we have that \( v_i^k \) or \( v_i^{j+1} \) belongs to \( J^S \cap K^S \), which implies that \( J \) and \( K \) are not disjoint. Then, we assume that \( j_i < k_i \) and \( j_{i+1} > k_{i+1} \). By construction, we have \( v_i^y = (v_i^m, v_i^{j+1}) \) in \( J^C \) and \( v_i^z = (v_i^k, v_i^{j+1}) \) in \( K^C \) opposing in \( i \) and there exists a vertex \( u \) in \( V^X \) adjacent to both \( v_i^y \) and \( v_i^z \). Since all vertices adjacent to \( v_i^y \) belong to \( J \) and all vertices adjacent to \( v_i^z \) belong to \( K \), it follows that \( u \in J \cap K \), which implies that \( J \) and \( K \) are not disjoint.

Finally, we show NP-hardness of \textbf{Common-}k-\textbf{Tree}.

\textbf{Theorem 4.} \textbf{Problem \textbf{Common-}k-\textbf{Tree} is NP-complete even if \( G \) is a tree with maximum degree three.}

\textbf{Proof.} Fellows et al. [12] showed that the following problem is NP-complete: Given a vertex-colored tree \( G \) with maximum degree three and a set of colors \( C \), decide if there exists a colorful tree \( T \prec G \) such that \( |V_T| = |C| \). This problem can be formulated...
as a particular instance of $\text{Common-}k\text{-Tree}(G, G, |C|)$. Thus, $\text{Common-}k\text{-Tree}$ is NP-complete even if $G$ is a tree with maximum degree three. □

4. Search algorithms

In this section we present algorithms for searching various types of motifs in vertex-colored graphs. The following section presents an efficient algorithm such that we are given a vertex-colored graph $G$ and a colorful tree $T$ and we want to find a subgraph of $G$ isomorphic to $T$. We also want to find all occurrences of a colorful motif $T$ in a vertex-colored graph $G$. Next, we first introduce a data structure simpler than $G$, called maximum clean graph, from which it is possible to obtain all subgraphs. We can then obtain more smoothly all colorful motifs in a clean subgraph later. Finally, we describe a linear time algorithm for computing the number of occurrences of colorful motifs in a vertex-colored graph without enumerating all occurrences.

4.1. Finding subgraphs isomorphic to a colorful tree. If the given colorful motif $M$ for the Subgraph-Motif problem is a tree, we can solve the problem efficiently. In the following we present a linear time algorithm for Subgraph-Motif for this particular type of instances.

Let $G$ be a graph and $T$ be a tree which is a colorful motif. Algorithm $\text{tcg}$ starts by identifying an arbitrary leaf $x$ of $T$ in Step 1. Step 2 collects in a set $A$ all vertices of $G$ with the color of $x$. Then, if $T$ is a graph with only one vertex, Algorithm $\text{tcg}$ verifies whether the set $A$ is empty, returning yes or no in Step 3. Otherwise, Step 4 takes $y$ as the only vertex adjacent to $x$ in $T$. Then, Step 5 builds a set $B$ with all vertices in $G$ with the color of $y$ and with no neighbors with the color of $x$. Finally, Step 6 is a recursive call to $\text{tcg}$ removing vertices in $A$ and $B$ from $G$ and the leaf $x$ from $T$. We suppose that graphs $G$ and $T$ are given to Algorithm $\text{tcg}$ as adjacency lists.

#### Algorithm 1 $\text{tcg}(G,T)$

**Input:** graph $G$, colorful tree $T$  
**Output:** YES/NO, whether $T \prec G$ or not  
1: let $x$ be some leaf of $T$  
2: let $A := \{a \in V_G : c(a) = c(x)\}$  
3: if $T$ is trivial, i.e., it has one single vertex then  
4: \quad \text{return } A \neq \emptyset  
5: let $y$ be the vertex such that $xy \in E_T$  
6: let $B := \{b \in V_G : c(b) = c(y) \text{ and for all } b' \in V_G \text{ such that } b'b' \in E_G, \text{ then } c(b') \neq c(x)\}$  
7: \text{return } \text{tcg}(G - (A \cup B), T - \{x\})

The following shows the correctness of Algorithm $\text{tcg}$.

**Theorem 5.** Given a vertex-colored graph $G$ and a colorful tree $T$, Algorithm $\text{tcg}$ returns correctly whether $G$ has a subgraph isomorphic to $T$ or not.

**Proof.** Denote by $G_i$ and $T_i$ the input graphs in the $i$th recursive call to Algorithm $\text{tcg}$, for $i \geq 1$, where $G_1 = G$ and $T_1 = T$. (Since there is no change of colors in the graphs $G$ and $T$ for any call of the algorithm, we drop those indices
for the color function.) To establish the correctness, consider a sequence of calls 1, 2, \ldots, |V_T| of Algorithm TCG. Then, it is enough to show that

\[ T_{i-1} \prec G_{i-1} \text{ if and only if } T_i \prec G_i, \]

for any recursive call \( i > 1 \). If \(|V_T| = 1\), for some recursive call \( i \), Algorithm TCG runs the three first steps and returns correctly whether \( G \) has a subgraph isomorphic to \( T \).

Suppose first that \( T_{i-1} \prec G_{i-1} \) at the \((i-1)\)st call of Algorithm TCG. Since we have a subsequent call \( i \), there exists a subgraph \( G' \) of \( G_{i-1} \) such that \( T_{i-1} \cong G' \). Still, there exists a vertex \( a \) in \( G_{i-1} \) such that \( c(a) = c(x) \) for a chosen leaf \( x \) in \( T_{i-1} \). \( a \) is adjacent to a vertex \( b \) in \( G' \) with color \( c(b) = c(y) \), and \( xy \) is an edge in \( T_{i-1} \). Then, \( G' - \{a\} \subseteq G_i \) and \( T_i = T_{i-1} - \{x\} \cong G' - \{a\} \). Thus, \( T_i \prec G_i \).

Now, suppose that \( T_i \prec G_i \) at the \( i \)th call of Algorithm TCG. Then, there exists a subgraph \( G' \) of \( G_i \) such that \( T_i \cong G' \). Let \( b \) be a vertex in \( G' \) and \( y \) be a vertex in \( T_i \) such that \( c(b) = c(y) \) and \( y \) is adjacent to the removed vertex \( x \) in \( T_{i-1} \). Since \( b \) was not removed in \( G_i \), there should exist at least one vertex \( a \) in \( G_{i-1} \) such that \( c(a) = c(x) \) and \( ab \in E_{G_{i-1}} \). Thus, the subgraph \( G'' = G' + ab \) is a subgraph of \( G_{i-1} \) and \( G'' \cong T_{i-1} \). Hence, \( T_{i-1} \prec G_{i-1} \).

A quick inspection of the pseudocode of Algorithm TCG gives us a quadratic running time in the size of \( G \). Nevertheless, some preprocessing and a more careful analysis lead to a linear running time as the following result states.

**Theorem 6.** Given a vertex-colored graph \( G \) and a colorful tree \( T \), Algorithm TCG returns correctly whether \( G \) has a subgraph isomorphic to \( T \) in time linear in the size of \( G \).

**Proof.** We start by preprocessing graphs \( G \) and \( T \) in linear time. Visiting all vertices in \( V_T \) we have a list of leaves \( L_T \) of \( T \). Moreover, for each color \( \gamma \) in \( C \) we create a list of vertices \( C_\gamma \) in \( V_G \), such that each vertex \( v \) in \( C_\gamma \) satisfies \( c(v) = \gamma \).

Steps 1–4 can then be performed in \( O(1) \) time: Step 1 can be done by obtaining a vertex from \( L_T \) and Step 2 by obtaining vertices from \( C_\gamma \), not removed from \( V_G \) such that \( \gamma = c(x) \). Steps 3 and 4 are trivial.

The overall time spent in Step 5 across all recursive calls is linear in the size of \( G \). Notice that whenever we remove \( x \) from \( T \), we have to add \( y \) to \( L_T \) if \( y \) becomes a leaf.

Step 6 is the most tricky one. First of all, we visit vertices in \( A \) and mark their neighbors. Then we add to set \( B \) each unmarked vertex in \( C_\gamma \) such that \( \gamma = c(y) \) (according to Step 5). Finally, neighbors of vertices in \( A \) are unmarked. Notice that in Step 6 a vertex can be analyzed many times across the recursive calls. However, if a vertex is removed at some point, it happens at most once. On the other hand, a vertex is analyzed and not removed at most a number of times equal to the number of adjacent edges. This means that the vertex is adjacent to at least one vertex in \( A \). Hence, the overall time spent in Step 6, considering all the recursive calls, is linear in the size of \( G \).

Therefore, the overall running time of Algorithm TCG is linear in the size of the instance \( G \). \( \square \)

In general, we are not only interested in whether there exists a subgraph of \( G \) isomorphic to a colorful motif \( T \), but also in finding one or all different such subgraphs. Actually, finding one subgraph of a graph \( G \) isomorphic to a colorful
motif $T$ can be achieved by a minor change of Algorithm TCG. Namely, we maintain a copy $G'$ of $G$ and a pointer array $p$ representing the predecessors of each vertex $v$ in $V_{G'}$, responsible for $v$ to become a candidate belonging to some subgraph of $G$ isomorphic to $T$. Initially $p[v]$ is nil for all $v \in V_{G'}$. During Step 5, for each non-removed vertex $v$ in $V_G$ with $c(v) = c(y)$ the list $p[v]$ is updated, containing each vertex $w$ in $V_{G'}$ adjacent to $v$ such that $c(w) = c(x)$. Hence, a motif can easily be found after the execution of Algorithm TCG using the array $p$. The second task, i.e., finding all occurrences of a colorful motif $T$ in $G$, is realized by algorithms described in the following.

4.2. Maximum clean subgraphs. Finding all occurrences of a colorful motif $T$ in a vertex-colored graph $G$ can be a highly time consuming task, since the number of occurrences of some $T$ in $G$ may in fact be exponential in the worst case. See an example in Fig. 5. Hence, finding all isomorphic subgraphs of $T$ in $G$ can be prohibitive in the general case. Nevertheless, it is useful to have a structure simpler than $G$ from which it is possible to obtain all subgraphs of $G$ isomorphic to $T$. This structure is described below.

**Lemma 1.** Given a graph $G$ and a colorful tree $T$, let $H$ be a graph obtained by Algorithm MCG. Let $v \in V_T$ be a leaf and let $V = \{ w \in V_H : c(w) = c(v) \}$ be the set of vertices in $H$ with color $c(v)$. Then, $\text{MCG}(H, T - \{v\})$ returns $H - V$.

**Proof.** Since $T - v$ is a colorful motif, lines 2–4 of Algorithm MCG remove all vertices in $H$ with color $c(v)$, i.e., the vertices in $V$. $\square$
Algorithm 2 MCG(G, T)

Input: graph G, colorful tree T
Output: maximum clean subgraph H of G regarding T

1: \( H := G \)
2: for each \( v \in V_H \) do
3: \( \text{if } c(v) \text{ is not a color in } T \) then
4: \( H := H - \{v\} \)
5: for each \( vv' \in E_H \) do
6: \( \text{if there exists no edge } vv' \text{ in } T \) such that \( c(v) = c(w) \) and \( c(v') = c(w') \) then
7: \( H := H - \{vv'\} \)
8: while there exist \( v \in V_H \) and \( w, w' \in V_T \) such that \( c(v) = c(w) \) and \( wv' \in E_T \), but there
\( \text{do not exist } v' \in V_H \) such that \( vv' \in E_H \) and \( c(v') = c(w') \) do
9: \( H := H - \{v\} \)
10: return H

Lemma 2. Given a graph G and a colorful tree T, let H be a graph obtained by Algorithm MCG. Then, any vertex x in H is a vertex of a subgraph \( \tilde{G} \) in H isomorphic to T.

Proof. We prove the lemma by induction on \( |V_T| \).

Suppose that \( |V_T| \leq 1 \). If \( |V_T| = 0 \), after lines 1–4, H has no vertex and the proof is completed. If \( |V_T| = 1 \), after lines 1–4, the proof is also completed since each vertex in H is isomorphic to T.

If \( |V_T| > 1 \) then T has at least two different leaves. Let x be an arbitrary vertex in H. Since T is a colorful motif, it follows that, for one of these leaves, say v, we have \( c(x) \neq c(v) \). Let w be the neighbor of v in T. From Lemma 1, we have that \( \text{MCG}(H, T - \{v\}) = H - V \), where \( V = \{u \in V_H : c(u) = c(v)\} \). Since \( c(x) \neq c(v) \), then \( x \in V_H - V \). By the induction hypothesis, x is a vertex of a subgraph \( \tilde{G}' \cong T - \{v\} \) in \( H - V \). Let \( w' \) be a vertex in \( \tilde{G}' \) such that \( c(w') = c(w) \). Since \( w' \in V_H \), \( w' \) must have a neighbor \( v' \in V_H \) with color \( c(v) \), otherwise \( w' \) would have been removed in line 9 of Algorithm MCG(G, T). It follows that \( \tilde{G} = \tilde{G}' + \{v'w'\} \cong T \), \( \tilde{G} \) is a subgraph of H, and \( x \in V_{\tilde{G}} \).

Lemma 3. Given a vertex-colored graph G and a colorful tree T, let H be a vertex-colored graph obtained by Algorithm MCG. Then, any edge xy in H is an edge of a subgraph G of H isomorphic to T.

Proof. From Lemma 2 there exist subgraphs \( \tilde{G}_x \cong T \) and \( \tilde{G}_y \cong T \) in H containing vertices x and y, respectively. Then, there exist vertices \( x' \) and \( y' \) with colors \( c(x) \) and \( c(y) \), respectively, and \( xy' \) is an edge in \( \tilde{G}_x \) and \( x'y \) is an edge in \( \tilde{G}_y \). Let \( G'_x \) be the component of \( \tilde{G}_x - \{xy'\} \) containing vertex x, and \( G'_y \) be the component of \( \tilde{G}_y - \{x'y\} \) containing vertex y. The graph \( \tilde{G} \) which is the union of \( G'_x \) and \( G'_y \) plus the edge \( xy \) is a subgraph of H isomorphic to T.

Lemma 4. At the start of each iteration of the loop of lines 3, 4 in Algorithm MCG, all vertices in \( V_G \setminus V_H \) and all edges in \( E_G \setminus E_H \) do not belong to any subgraph of G isomorphic to T.

Proof. Since, in any subgraph of G isomorphic to T, the color of a vertex must be a color of a vertex in T and the colors of endpoints of an edge must be the colors of endpoints of an edge in T, we have that steps 4 and 7 remove only vertices and edges.
that do not belong to any subgraph of $G$ isomorphic to $T$. Thus, the statement is true prior to the first iteration of the loop of lines 8–9.

Suppose that the statement is true before an iteration of the loop of lines 8–9. Let $v$ in $V_H$ and $w, w'$ in $V_T$ be vertices such that $c(v) = c(w), ww' \in E_T$, and there does not exist $v'$ in $V_H$ such that $vw' \in E_H$ and $c(v') = c(w')$. Since, by hypothesis, all vertices in $V_G \setminus V_H$ and all edges in $E_G \setminus E_H$ do not belong to any subgraph of $G$ isomorphic to $T$, we have that vertex $v$ and edges incident to it do not belong to any subgraph of $G$ isomorphic to $T$ either. Since they are precisely the vertices and edges removed in $H$, the statement is still true before the next iteration. □

**Theorem 7.** Given a vertex-colored graph $G$ and a colorful tree $T$, let $H$ be a graph given by Algorithm $mcg$. Then, $H$ is the maximum clean subgraph of $G$ regarding $T$.

**Proof.** From Lemmas 4 and 6, since all vertices and edges belong to a subgraph of $G$ isomorphic to $T$, we have that $H$ is a clean subgraph. On the other hand, as a consequence of Lemma 4, all vertices and edges removed by Algorithm $mcg$ are not in any subgraph of $T$ in $G$, and thus $H$ is the maximum clean subgraph of $G$ regarding $T$.

We show now the running time of Algorithm $mcg$.

**Theorem 8.** Given a vertex-colored graph $G$ and a colorful tree $T$, Algorithm $mcg$ runs in time cubic in the number of vertices of $G$.

**Proof.** Consider that $G$ and $T$ are given by their adjacency matrices and the set of colors is given by an ordered array.

Line 1 of Algorithm $mcg$ can be implemented in $O(V_G^2)$ time since $H$ is a copy of $G$. Notice that we adopt the notation of 2 where inside asymptotic notation, the symbol $V$ denotes $|V|$ and the symbol $E$ denotes $|E|$. The color of each vertex in $H$ can be checked in $O(1)$ time, and each vertex can be removed in $O(V_G)$ time, which implies that lines 2–4 can be performed in $|V_G| \cdot (O(1) + O(V_G)) = O(V_G^2)$ time. We can verify whether colors of the extremities of an edge in $H$ are also the colors of the extremities of an edge in $T$ in $O(1)$ time, and, if necessary, its deletion can also be done in $O(1)$ time which implies, since we have $O(V_G^2)$ edges, that Step 3 can be performed in $O(V_G^2) \cdot (O(1) + O(1)) = O(V_G^2)$ time. In Step 4, deciding whether a vertex should be removed or not, can be done spending $O(V_G^2)$ time, and the removal can be done in $O(V_G)$ time, when necessary, implying that the total time spent on Step 4 is $|V_G| \cdot (O(V_G^2) + O(V_G)) = O(V_G^3)$. Therefore, the running time of Algorithm $mcg$ is $O(V_G^3)$. □

4.3. Finding all occurrences of a colorful motif in a clean subgraph. We present now Algorithm $ALL-COLORFUL$ for finding all the colorful motifs in a given maximum clean subgraph. Despite this task being superpolynomial, sometimes it is useful to find all motifs in order to choose those we are interested in. The input of the algorithm is the maximum clean subgraph $H$, as defined in the previous section. The colorful tree $T$ is implicit. If $V_H = \emptyset$, then there does not exist any subgraph of $H$ isomorphic to $T$ (except if $V_T = \emptyset$). Thus, we assume that $H$ has at least one vertex.

Initially, we can show the following easy result.

**Lemma 5.** Let $T$ be a colorful tree such that $|V_T| = 1$. Then, for each $v \in V_H$, we have $\{v\} \in G$, $\{v\}$ is a subgraph of $H$ and $\{v\} \cong T$. 
Algorithm 3 \textsc{All-Colorful}(H)

\textbf{Input:} maximum clean subgraph $H$

\textbf{Output:} set $\mathcal{G}$ of all subgraphs of $H$ isomorphic to a colorful motif

\begin{algorithmic}[1]
\State \If{$|\{c(a) : a \in V_H\}| = 1$} \State $\mathcal{G} := \{(a,\emptyset) : a \in V_H\}$ \EndIf
\State $\mathcal{G} := \emptyset$
\State $H := \text{\textsc{All-Colorful}}(H - A)$
\For{each vertex $a$ in $A$}
\State $B := \{b \in V_H : c(b) = \beta \text{ and } ab \in E_H\}$
\For{each vertex $a$ in $B$}
\State $\mathcal{G} := \mathcal{G} \cup \{G + \{ab\}\}$
\EndFor
\EndFor
\State \Return $\mathcal{G}$
\end{algorithmic}

\textbf{Proof.} Since $H$ is a clean subgraph regarding $T$, the lemma holds immediately. $\Box$

The following result shows that $\text{\textsc{All-Colorful}}(H)$ returns the set of all subgraphs of $H$ isomorphic to $T$.

\textbf{Theorem 9.} Let $G$ be a vertex-colored graph and $T$ be a colorful tree. Suppose that $H$ is the maximum clean subgraph returned by $\text{mcg}(G, T)$, $\mathcal{G}$ is the set of graphs returned by $\text{\textsc{All-Colorful}}(H)$, and $\mathcal{G}'$ is a subgraph of $H$. Then, $\mathcal{G}' \in \mathcal{G}$ if and only if $\mathcal{G}' \cong T$.

\textbf{Proof.} We prove the theorem by induction on the number of vertices in $T$. If $|V_T| = 1$, then, since $\text{mcg}(G, T)$ returns $H$, all vertices in $H$ have the same color. Thus, from lines 1–2 of the Algorithm \text{\textsc{All-Colorful}}, we have that $a \in \mathcal{G}$ for each $a \in V_H$. On the other hand, from Lemma \ref{lem:1} $\{a\} \cong T$ for each $a \in V_H$. Therefore the theorem holds if $|V_T| = 1$.

Suppose that $|V_T| > 1$ and $\mathcal{G}' \in \mathcal{G}$. Then, $A \neq \emptyset$ and $B \neq \emptyset$. Since $T$ is a tree and $|V_T| > 1$, $T$ has at least a vertex $v$ of degree one and since $H$ is a clean subgraph there exists a vertex $v'$ in $V_H$ such that $c(v') = c(v)$, i.e., $A \neq \emptyset$. Likewise, each vertex $w$ in $V_T$ adjacent to $v$ implies that $c(w) = \beta$, for some color $\beta$. Hence, as $H$ is a clean subgraph, then $B \neq \emptyset$. It follows that, by line 11, $\mathcal{G}' = \tilde{G} + \{ab'\}$, where $b' \in B$ is a vertex in $\tilde{G}$ in $H$ and $a \in A$. Let $u, u'$ be vertices in $V_T$ such that $c(u) = c(a)$ and $c(u') = c(b')$. Since $H$ is a clean subgraph regarding $T$, it follows that $u$ and $u'$ are neighbors in $T$. Further, since $H$ is the maximum clean subgraph regarding $T$ and every neighbor of a vertex with color $c(u)$ has color $c(u')$, then $u$ is a leaf in $T$. From Lemma \ref{lem:2} $H - A$ is the maximum clean subgraph regarding $T - \{u\}$. By the induction step, $\tilde{G}$ is a subgraph of $H - A$ isomorphic to $T - \{u\}$. Thus, $\mathcal{G}' = \tilde{G} + \{ab'\}$ is isomorphic to $T = T - \{u\} + \{uu'\}$. Consequently, if $\mathcal{G}' \in \mathcal{G}$, then $\mathcal{G}'$ is isomorphic to $T$, for $|V_T| > 1$.

Conversely, suppose that $\mathcal{G}'$ is a subgraph of $H$ isomorphic to $T$. Let $a$ and $b'$ be vertices in $\mathcal{G}'$. By the inductive hypothesis, $\text{\textsc{All-Colorful}}(H - A)$ returns $\tilde{G} = \mathcal{G}' - \{a\}$. Then, by line 11 we have $\tilde{G}' = \tilde{G} + \{ab'\} \in \mathcal{G}$. $\Box$

For a vertex-colored graph $G$ and a colorful motif $T$, we denote the \textit{number of occurrences of $T$ in $G$} by $\eta(G, T)$. Clearly, an algorithm for enumerating motifs has running time $\Omega(\eta(G, T))$ and, since $\eta(G, T)$ can be superpolynomial, an algorithm
for enumerating motifs has exponential running time in the worst case. On the other hand, considering all recursive calls, the time consumed by All-Colorful is dominated by Step 11 and, since each edge can be added to at most \( \eta(G,T) \) subgraphs, the total running time of the algorithm is \( O(E_G \cdot \eta(G,T)) \), which implies that the exponential part lies only in the output size. With this observation, we have the following immediate result.

**Corollary 1.** If \( \eta(G,T) \) is polynomial in the size of \( G \), then Algorithm All-Colorful also runs in polynomial time in the size of \( G \).

### 4.4. Finding the number of occurrences of a colorful motif in a graph.

Let \( G \) be a graph and \( T \) be a colorful tree. Notice that \( \eta(G,T) \) can be easily obtained by finding all occurrences of \( T \) in \( G \). As seen in the previous section, this procedure runs in exponential time in the worst case, since the number of occurrences of \( T \) in \( G \) may be exponential. Thus, we describe now a linear time algorithm for computing \( \eta(G,T) \) without enumerating all occurrences.

Choose an arbitrary vertex \( r \) in \( T \) and, from now on, deal with \( T \) as a rooted tree with root \( r \). Let \( u \) and \( v \) be vertices in \( T \). If \( u \) belongs to a path from \( r \) to \( v \), we say that \( v \) is a descendant of \( u \) in \( T \). We define \( T_u \) as the rooted subtree of \( T \) with root \( u \) plus all descendants of \( u \). We also define the direct descendants of \( u \) as the set of vertices \( A_u := \{ v \in T_u : uv \in E_T \} \). Moreover, \( L(i) \) denotes a list of vertices of \( G \) with color \( i \). Now, for the next definitions, we consider a vertex \( u \) in \( T \) and a vertex \( z \) in \( V_G \) such that \( c(u) = c(z) \). We then define a set of vertices \( \text{adj}_z := \{ y \in V_G : yz \in E_G \text{ and } c(y) = c(w) \text{ for some vertex } w \in A_u \} \), and an integer \( N_z := |\{ S : S \text{ is an occurrence of } T_u \text{ in } G \text{ and } z \in S \}| \).

Thus, considering \( u \in V_T \) and \( z \in V_G \) such that \( c(u) = c(z) \), we have

\[
N_z = \begin{cases} 
1 & \text{if } u \text{ is a leaf in } T, \\
\prod_{v \in A_u} \left( \sum_{y \in \text{adj}_z : c(y) = c(v)} N_y \right) & \text{otherwise}.
\end{cases}
\]

Let \( v_1 (= r), v_2, \ldots, v_{|V_T|} \) be a list of visited vertices of \( T \) in a breadth-first search with an arbitrary source \( r \). Assume that \( c(v_j) = i \). We use the reverse order of this list to compute \( N_z \), according to (1), for each vertex \( z \) in \( L(i) \) and color \( i \). After \( N_z \) has been computed for each \( z \) in \( V_G \), we return \( \sum_{z \in L(1)} N_z \). See Fig 6.

![Figure 6. A colorful tree \( T \) rooted at the white vertex. In \( G \), each vertex \( z \) is labeled with \( N_z \). Notice that \( N_z \) represents the number of occurrences of \( T_u \) in \( G \), where \( c(u) = c(z) \).](image-url)
The procedure to compute $\eta(G,T)$ is summarized in Algorithm $\eta$.

**Algorithm 4 $\eta(G,T)$**

**Input:** graph $G$, colorful tree $T$

**Output:** number of occurrences of $T$ in $G$

1: let $r$ be an arbitrary vertex in $T$
2: let $v_1, v_2, \ldots, v_{|V_T|}$ be a list of visited vertices of $T$ in a breadth-first traversal with source $r$
3: let $c(v_i) := i$
4: for each color $i = |V_T|\downto 1$
5: for each $z$ in $L(i)$ do
6: if $v_i$ is a leaf in $T$ then
7: $N_z = 1$
8: else
9: $N_z = \prod_{v \in A_z} \left( \sum_{y \in \text{adj}_z : c(y) = c(v)} N_y \right)$
10: return $\sum_{z \in L(1)} N_z$

Since vertices in $V_G$ are processed according to their colors given by Step 2, the values $N_y$, for all $y$ in $\text{adj}_z$, are previously computed before the computation of $N_z$. This implies, using sum and product rules, that $N_z$ is correctly computed.

The last step of Algorithm $\eta(G,T)$ takes into account that each occurrence of $T$ in $G$ has a vertex of color 1, there are $|L(1)|$ vertices in $G$ with color 1, and, for each vertex $z$ with color 1, $N_z$ is the number of occurrences of $T_{v_i} \cong T$ in $G$ with vertex $z$. Therefore, the number of occurrences of $T$ in $G$ is given by the sum of all $N_z$ such that $z \in L(1)$, according to Step 8.

**Theorem 10.** Let $G$ be a vertex-colored graph and $T$ a colorful tree such that $|V_T| \leq |V_G|$. The number $\eta(G,T)$ can be computed in $O(V_G + E_G)$ time.

**Proof.** The list of vertices to be processed can be established in a preprocessing step in $O(V_T)$ time. Taking into account the computation of $N_z$ for all vertices in $V_G$, Algorithm $\eta(G,T)$ performs at most $|E_G|$ products and $|E_G|$ sums, spending thus $O(E_G)$ time. Then, we compute $\sum_{z \in L(1)} N_z$ in $O(V_G)$ time. Therefore, the running time for calculating $\eta(G,T)$ is $O(V_G + E_G)$. $\square$

Note that, with some preprocessing and especially for small motifs, Algorithm 4 can also be used when one is interested only in colors, not in topology, i.e., ignoring structural similarity. In order to do so, given a set of colors, we generate and count all colorful trees containing exactly those colors, summing the output values.

5. **Inference**

In this section, we consider the problem of detecting subgraphs in a network $G$ occurring with high frequency. The concept “high frequency” here depends not only on the number of occurrences of a given subgraph but also on the number of occurrences that the subgraph appears in a random graph. In previous work, Schbath et al. [25] define a null model, based on Erdős [11], assuming that edges are independent and distributed according to a Bernoulli distribution with the same parameter $p \in (0,1]$ for each edge. In addition, we add vertex colors to the random graph model and, since we are also considering topological structure to define motifs, our null model uses a parameter function that depends on the colors of vertices that are adjacent in the graph.
5.1. Evaluating high frequency. We propose a null model as a generalization of the Erdős-Rényi model, where the occurrence of colors is considered. This approach takes into account that some pairs of colors in vertex-colored graphs representing biological networks are more likely to be connected than others.

Let \( a \) and \( b \) be colors in \( C \) and let \( G \) be a vertex-colored graph. Let \( |a| := |\{ v \in V_G : c(v) = a \}| \) and \( |(a,b)| := |\{(u,v) \in E_G : c(u) = a \text{ and } c(v) = b \}| \). We define a parameter function (which implicitly depends on \( G \))

\[
p(a,b) = \frac{|(a,b)|}{|a| \cdot |b|},
\]

for each pair of colors \( a, b \in C \). Notice that, for a colorful graph, \(|a| = |b| = 1\) and \(|(a,b)| \in \{0,1\}\), which implies that \(p(a,b)\) is either 0 or 1.

A random vertex-colored graph \( R \) defined from a vertex-colored graph \( G \) is such that \( V_R = V_G \), with same color function and each edge \((x,y)\) occurs in \( E_R \) independently with probability \( 0 \leq p(c(x),c(y)) \leq 1 \). Thus, the probability of a particular graph \( R \) in a sample space \( \mathcal{G} = \{ \text{vertex-colored graph } R : V_R = V_G \} \) is

\[
\Pr\{R\} = \prod_{(x,y) \in E_R} p(c(x),c(y)) \cdot \prod_{(x,y) \notin E_R} (1 - p(c(x),c(y))).
\]

A candidate for occurrence of a motif \( T \) in \( R \) (or \( G \)), or simply candidate, is a vertex set \( S \subseteq V_R \) such that \( c(S) = c(V_T) \) and \(|S| = |V_T|\). We denote by \( S \) the set of candidates of \( T \) in \( R \). We have the following collection of straightforward results on candidates for occurrence of a motif in a random graph.

**Lemma 6.** Let \( R \) be a random vertex-colored graph, \( T \) a colorful tree, and \( S \) a set of candidates for occurrence of \( T \) in \( R \). For a candidate \( S \in \mathcal{S} \), define the indicator random variable \( X_S \) which states whether a candidate \( S \) is an occurrence of \( T \) and let \( \mu \) be the expected value of \( X_S \) for any candidate \( S \in \mathcal{S} \). Let \( X \) be a random variable representing the number of occurrences of \( T \) in \( R \). Then we have the following results:

(i) The number of candidates for occurrence of \( T \) in \( R \) is \(|S| = \prod_{t \in c(T)} |t|\), and it can be obtained in \( O(V_G) \) time.

(ii) The expectation \( \mathbb{E}[X_S] = \prod_{(u,v) \in E_T} p(c(u),c(v)) \) can be obtained in \( O(V_T) \) time.

(iii) The expected number of occurrences of \( T \) in \( R \) is given by \( \mathbb{E}[X] = |S| \cdot \mu \), and it can be obtained in \( O(V_G) \) time.

(iv) The variance of the number of occurrences of \( T \) in \( R \) is given by \( \text{Var}[X] = \\sum_{I \subseteq c(T)} m(I) \cdot \mu^2 / f(I) \), where the value \( m(I) \) denotes the number of ordered pairs of candidates \( S_i \) and \( S_j \) in \( V_R \) such that \( c(S_i \cap S_j) = I \), \( f(I) = \prod_{(u,v) \in E_T \cap I} \Pr\{ (c(u),c(v)) \} \), and \( T[I] \) is the subgraph of \( T \) induced by vertices whose colors are in \( I \). Moreover, \( \text{Var}[X] \) can be obtained in \( O(2^{V_T} \cdot V_T) \) time.

**Proof.** (Proof for (i)). For a vertex \( t \) in \( T \), recall that \(|t|\) is the number of vertices in \( V_G (= V_R) \) with color \( t \). Since \( T \) is a colorful graph, the number of candidates of \( T \) in \( R \) is obtained directly by the product of the multiplicity of each color in \( V_G \) and therefore the assertion follows. Moreover, that number can be computed in \( O(V_G) \) time.
(Proof for (ii)). For a candidate $S$ in $\mathcal{S}$, we introduce an indicator random variable

$$X_S = \begin{cases} 1, & \text{if candidate } S \text{ is an occurrence of } T, \\ 0, & \text{otherwise.} \end{cases}$$

Notice that a necessary and sufficient condition for a candidate $S$ being an occurrence of a colorful tree $T$ in $R$ is that vertices of $S$ with color $c(u)$ and $c(v)$ are adjacent in $R$, for each $(u, v) \in E_T$. Thus, the probability of $S$ being an occurrence of $T$ in $R$ is

$$(2) \ \Pr\{X_S = 1\} = \prod_{(u,v) \in E_T} p(c(u), c(v)).$$

Since $X_S$ is an indicator random variable, we have

$$E[X_S] = 1 \cdot \Pr\{X_S = 1\} + 0 \cdot \Pr\{X_S = 0\},$$

and thus, by equality (2), the assertion follows. Notice also that $|E_T| = O(V_T)$ and therefore $E[X_S]$ can easily be computed in $O(V_T)$ time.

(Proof for (iii)). Notice from (ii) that $E[X_S]$ does not depend on the candidate $S$. That is, if $S$ and $S'$ are two candidates of $T$ in $R$, then $E[X_S] = E[X_{S'}]$. Thus, for simplicity, we denote $E[X_S]$ by $\mu$, for any $X_S$ and $S$.

Consider another random variable $X$ denoting the number of occurrences of a motif $T$ in $R$. That is,

$$X = \sum_{S \in \mathcal{S}} X_S.$$

It is immediate that

$$E[X] = E\left[\sum_{S \in \mathcal{S}} X_S\right] = \sum_{S \in \mathcal{S}} E[X_S] = \sum_{S \in \mathcal{S}} \mu = |S| \cdot \mu,$$

where the second equality follows from linearity of expectation. By (i), $|S|$ can easily be computed in $O(V_G)$ time and, by (ii), $\mu$ can be computed in $O(V_T)$ time. It follows that $|S| \cdot \mu$ can be computed in $O(V_G) + O(V_T) = O(V_G)$ time.

(Proof for (iv)). Recall that

$$(3) \ Var[X] = E[X^2] - E^2[X] = E[X^2] - (|S|\mu)^2,$$

where the last equality is valid from (iii). Notice that

$$(4) \ X^2 = \left(\sum_{S \in \mathcal{S}} X_S\right)^2 = \sum_{S_i, S_j \in \mathcal{S}} X_{S_i}X_{S_j}.$$

Since

$$X_{S_i}X_{S_j} = \begin{cases} 1, & \text{if } X_{S_i} = 1 \text{ and } X_{S_j} = 1, \\ 0, & \text{otherwise}, \end{cases}$$

we have

$$(5) \ E[X_{S_i}X_{S_j}] = \Pr\{X_{S_i}X_{S_j} = 1\} = \Pr\{X_{S_i} = 1 \text{ and } X_{S_j} = 1\}.$$

In addition, considering a set of colors $I$, we define

$$\Pr\{I\} = \sum_{S_i, S_j \in \mathcal{S}: c(S_i \cap S_j) = I} \Pr\{X_{S_i} = 1 \text{ and } X_{S_j} = 1\}.$$

It follows, using (4), (5) and linearity of expectation, that
Let $\eta$ denote the number of occurrences of a colorful tree $T$ in a graph $G$. If $\eta(G, T) > \mathbb{E}[X]$, then

$$
\Pr\{X \geq \eta(G, T)\} \leq \frac{\text{Var}[X]}{(\eta(G, T) - \mathbb{E}[X])^2}.
$$

**Theorem 11.** Let $G$ be a vertex-colored graph, $T$ a colorful tree and $X$ a random variable describing the number of occurrences of $T$ in $G$. If $\eta(G, T) > \mathbb{E}[X]$, then

$$
\Pr\{X \geq \eta(G, T)\} \leq \frac{\text{Var}[X]}{(\eta(G, T) - \mathbb{E}[X])^2}.
$$
Proof. After computing \( \eta(G, T) \), and \( E[X] \) and \( \text{Var}[X] \) according to Lemma 6 (iii) and Lemma 6 (iv), we use Chebyshev’s inequality and the result follows immediately. Such an inequality is used for evaluating an upper bound for the chance of a random graph having at least \( \eta(G, T) \) occurrences of \( T \) in \( G \), suggesting that \( T \) is an actual motif in \( G \) if \( \text{Var}[X]/(\eta(G, T) - E[X])^2 \) is small.

Moreover, we define a quality measure indicating how expected is the number \( \eta(G, T) \). For a real number \( y \), we say that a colorful tree \( T \) is a \( y \)-motif of a vertex-colored graph \( G \) if

\[
y = \max \left\{ 0, 1 - \frac{\text{Var}[X]}{(\eta(G, T) - E[X])^2} \right\}.
\]

Observe that the running time to compute \( y \) is exponential, as Lemma 6 (iv) states. However, we are usually interested in small values of \( |V_T| \). Typically for these cases we can obtain \( y \) very quickly as we will see in Section 6.

5.2. Inferring motifs. Given the previous theoretical framework, we present now an algorithm for inferring statistically significant colorful motifs in a given vertex-colored graph. This algorithm is an enhanced version of that in [24], improved with significant speedup.

First, consider a vertex-colored graph \( G \) representing a biological network. A consistent colorful tree with respect to \( G \) is a colorful tree \( T \) such that \( (u, v) \in E_T \) only if there exists \( (u', v') \in E_G \) such that \( c(u) = c(u') \) and \( c(v) = c(v') \), for each pair of vertices \( u, v \) in \( T \). Intuitively, a consistent colorful tree does not contain a pair of adjacent vertices whose colors never occur on adjacent vertices in the given network. Considering only consistent colorful trees when inferring motifs is a (constant-factor) optimization, since in biological networks usually some pairs of colors are never adjacent.

Since the direct inference for large motifs is highly time expensive, the algorithm begins inferring motifs of some smaller initial size \( s \), using the results as a base for increasing the size of motifs inferred up to a large size \( g \). This is performed in two major steps. Given a set of colors of interest \( C \subseteq \mathbb{C} \) (e.g. colors representing a set of relevant reactions that one wants to investigate in a metabolic network), a graph \( G \), an initial size \( s \), a goal size \( g \), a threshold \( t \), and a score \( y \), with \( 0 \leq y \leq 1 \), the first main step generates all possible subsets \( \Gamma \subseteq \mathbb{C} \), with \( |\Gamma| = s \). Then, for each \( \Gamma \), it obtains every consistent colorful tree \( T \) of size \( s \) such that \( c(T) = \Gamma \), producing a set \( \mathcal{T} \) of trees that occur at least \( t \) times in \( G \) and which are \( y \)-motifs. In the second step, it obtains several consistent colorful trees \( T' \) from every \( T \in \mathcal{T} \) by adding a new vertex to \( T \), searching \( T' \) in \( G \), and obtaining a new set \( \mathcal{T}' \) containing \( y \)-motifs that occur at least \( t \) times in \( G \). At the end of this step, parameter \( s \) is incremented and the second step is repeated for \( \mathcal{T}' \) as \( \mathcal{T} \). Algorithm 5 (Motif-Inference) presents a pseudocode implementing these steps.

The duplicate removal in line 14 avoids repeated motifs in \( \mathcal{T} \), since they may arise in the second step of the algorithm. For each \( T \in \mathcal{T} \), a signature is generated, which is unique for each colorful tree. Signatures are used together with a hash table to detect and discard identical motifs.

Lines 1 and 2 of Motif-Inference spend \( O(s \cdot (\binom{|C|}{s})) = O(s|C|^s) \) time. For finding occurrences of a motif in a graph we use the Algorithm 3. This is a significant improvement over the inference algorithm version in [24], which used Algorithm 3 since finding the number of occurrences of some motif in \( G \) is much faster than
Algorithm 5 Motif-Inference($C, G, s, g, t, y$)

**Input:** set of colors $C$, graph $G$, initial size $s$, goal size $g$, threshold $t$, score $y$ (with $0 \leq y \leq 1$)

**Output:** a set $T$ of trees occurring at least $t$ times in $G$ and which are $y$-motifs

1. Let $\mathcal{P}(C)$ be the powerset of $C$ and $\mathcal{P}_s(C) \subseteq \mathcal{P}(C)$ such that $\Gamma \in \mathcal{P}(C)$ if $\Gamma \in \mathcal{P}(C)$ and $|\Gamma| = s$
2. $T \leftarrow \emptyset$
3. **for each** $\Gamma$ in $\mathcal{P}_s(C)$ **do**
   4. **for each** consistent colorful tree $T$ of size $s$ such that $c(V_T) = \Gamma$ **do**
      5. **if** $\eta(G, T) \geq t$ **and** $T$ is a $y$-motif **then**
         6. $T \leftarrow T \cup \{T\}$
   7. **while** $s < g$ **do**
      8. $T' \leftarrow \emptyset$
      9. **for each** $T \in T$, $u \in V_T$ **and** $a \in C \setminus c(V_T)$ **do**
         10. $v \leftarrow$ a new vertex with color $a$
         11. $T' \assign T \cup \{uv\}$
         12. **if** $T'$ is a consistent colorful tree, $\eta(G, T') \geq t$ **and** $T'$ is a $y$-motif **then**
              13. $T \leftarrow T \cup \{T'\}$
         14. **Remove-Duplicates**($T'$)
      15. $T \leftarrow T'$
      16. $s \leftarrow s + 1$
17. **return** $T$

finding the occurrences themselves. Given a graph $G$ with $|V_G|$ vertices and $|E_G|$ edges and a colorful tree $T$, the number of occurrences of $T$ in $G$ is given by $\eta(G, T)$ in $O(V_G + E_G)$ time. Thus, the running time of lines 3-6 is $O(|C|^s \cdot s^{s-2} \cdot (V_G + E_G))$, since ${|C| \choose s} = O(|C|^s)$ and, from Cayley’s formula, the number of colorful trees with $s$ distinct colors is $s^{s-2}$. Besides that, the algorithm spends $O(|C|^g g^{g-2} \cdot (V_G + E_G))$ time in the loop of lines 7-16 in the worst case. Therefore, the running time for Motif-Inference is bounded by lines 7-16, since $g > s \geq 1$.

We note that the inference algorithm returns a set $T$ of $y$-motifs in $G$. After that, the All-Colorful algorithm (Algorithm 3) can be used to find all occurrences in $G$ of some $T \in T$ of interest.

We also observe that only few changes in the algorithm are needed for building a parallel version. First, it must divide $\mathcal{P}_s(C)$ (line 3) among $p$ processors, assigning a set $\Gamma_i$ to each processor $i \in \{1, \ldots, p\}$. Thus, each processor will finish the major first step of the motif inference (lines 3-6) having a pruned set $T_i$. Then, it must rebalance the sets $T_i$ among processors, e.g., by sending all $T_i$ to a master processor and spreading them equally among processors or by some load balancing heuristic. After the loops in lines 3-6 and lines 9-13, the algorithm gathers at the master processor a set $T = T_1 \cup \cdots \cup T_p$, scattering it again before lines 7 and 15.

6. Experimental results

We implemented Algorithms 2 to 5 and a parallel version of Algorithm 5 comparing their results to publicly available tools. We also implemented some methods described in this paper to evaluate them in practice (e.g., Formula (3) to calculate approximately the variance of the number of occurrences for some colorful tree). Sequential experiments were made on a single Intel i7 3.40GHz processor (one thread), while parallel experiments were run on a cluster with 40 Intel Xeon Quad-core 2.4GHz machines, 2 threads per core, totaling up to 320 simultaneous processes.
In the following paragraphs seven sets of experiments are presented. The first set evaluates the variance calculated by Formula (3), and the second evaluates the method for accessing motif exceptionality. Following, the performance of Algorithm 4 is measured for counting occurrences of non-topological motifs as described above. Next, regarding the inference of larger motifs, the quality of the incremental heuristic of Algorithm Motif-Inference \((s < g)\) is compared against the inference directly for the goal size \((s = g)\). The following set of experiments correlate the number of motifs in the output and the running time of the Algorithm Motif-Inference to some of its parameters. Algorithm 5 is compared then to the tool MOTUS (inference), and finally Algorithm 3 is compared to the tool Torque (search).

In all the experiments except for the last we used the metabolic network of \(E. coli\) strain K-12 from BioCyc [6]. We removed big molecule reactions, pathway holes and some highly connected compounds, such as \(H_2O\), \(H^+\) and \(CO_2\), resulting in a reaction graph with 1576 vertices and 3657 edges. Vertices representing reactions associated to enzymes whose EC numbers coincide in the first three positions were colored with the same color. We selected as colors of interest the 30 most frequent colors, since high frequency colors should challenge our algorithms in workload and memory consumption most. All remaining vertices, about half of the total number, were colored by the same “dummy” color.

We address the accuracy of the sample variance (8) for a colorful tree compared to the calculated variance (3) (see Fig 7), corroborating the correctness of (3). The sample variance was obtained generating random graphs based on the real network. They have the same vertices as the base graph, and an edge is added connecting vertices \(u\) and \(v\) according to the frequency of edges connecting vertices with colors \(c(u)\) and \(c(v)\) in the base graph. This model captures the fact that some reactions and their context are more frequent and/or better conserved during evolution than others [8,21]. We have made experiments with motifs of size 6 and 9, observing that the sample variance always converged to the calculated variance after generating a large amount of random graphs \((\approx 5 \times 10^6)\). Moreover, the calculated variance is obtained in a fraction of a second whereas the sample variance takes from minutes to hours to be calculated.

For comparison against the null model, we ran the motif inference algorithm for the \(E. coli\) metabolic network, using different combinations of parameters, and for random graphs generated as described above. As can be seen in Fig 8 for \(y = 0.999\) as score for \(y\)-motifs, the cuts made by the algorithm on random graphs drop significantly the amount of motifs found when they grow in size, which is the opposite behavior of inference for real networks. The same occurs for \(y = 0.99\), in different scale (not shown). This indicates that the method proposed in this work is able to evaluate the exceptionality of motifs, i.e., the occurrence of a motif more frequently than expected at random. When using the method for inferring relevant motifs in practice, however, a higher \(y\) value or some other filter must be used, resulting in output sets of reasonable sizes.

Regarding counting occurrences ignoring structural similarity as mentioned above, we performed experiments for this together with Algorithm 5. Given sets of 6, 7, 8, 9 and 10 colors of high frequency, we inferred motifs with these colors ignoring topology and the process took 0.01, 0.15, 0.3, 0.4 and 0.7 seconds, respectively, on a single machine.
We performed tests regarding differences between two methods: incremental inference with $s = 5$, $g = 7$ and straight generation of all motifs of size $g \in \{6, 7\}$. As we can see in Fig 7, there is little difference in the amount of motifs found, particularly when setting $y$ to reasonable values. Apart from being much faster, the incremental method misses few motifs compared to generating all possibilities. Both tests were performed in a parallel environment, the first (incremental) method taking 8 seconds on average and the second about 208 seconds for $g = 7$.

Note that in the experiments for motif inference, the number of found motifs can grow exponentially. Even when starting with a small set of motifs, the incremental steps may lead to a large result set. In the parallel environment, for motifs of size up to 12, the algorithm took 250 seconds for $y = 0.9999$ and $t = 30$, and 1137 seconds for $y = 0.999$ and $t = 40$, finding 167,673 and 262,819 topological motifs, respectively. Ignoring topology in the output, i.e., grouping results by color, the most frequent set of colors occurred more than $10^5$ times for both $y$ values. Importantly, when limiting the number of motifs kept in memory to a few thousand, we managed to infer motifs up to size 17 in about 160 seconds in parallel.

We compared Algorithm 5 with MOTUS [18,19] in inference mode, using default parameters (Table 1). While our algorithm calculates $y$-motif scores and discards those below a given $y$ value, the only way of accessing motif exceptionality in MOTUS is generating random networks, by default 100, calculating and displaying a $p$-value. We grouped results output by Algorithm 5 by color sets, allowing the analysis of motifs disregarding their topologies, additionally displaying results sorted by $y$-motif score. Our incremental method is fast even for greater sizes (see Table 1), while maintaining the quality of results close to those obtained when generating and counting all possibilities (non-incremental), as shown in Fig 9. Furthermore, the speedup is significant for tests performed in parallel. We observe that MOTUS is considerably slower, which is reasonable, since the set of colored motifs is a superset of colorful motifs. However, to the best of our knowledge there is no other tool available for colorful motif inference which may be used for comparison.
Table 1. Running time for motif inference ($s = 4, y = 0.999$).

| Motif size | MOTUS   | Algorithm 6 |
|------------|---------|-------------|
|            | sequential | parallel    |
| 4          | 200 s    | 3.9 s       | 2.1 s       |
| 5          | 2100 s   | 4.0 s       | 2.3 s       |
| 6          | 22000 s  | 4.8 s       | 3.2 s       |
| 7          | 220000 s | 13.4 s      | 4.1 s       |

We also ran experiments described in [5] to compare Algorithm 6 (All-Colorful) to Torque regarding searching known protein complexes, counting how many of them occur at least once in a PPI network (a match). To evaluate the quality of the matches found we used the Functional Coherence method with the same parameters as in [5]. A set of proteins (match) found to be functionally coherent by the GO TermFinder tool [4] with respect to the Gene Ontology (GO) annotation [2] has a good quality and is likely to be used in prediction of GO annotations for these proteins, when not available. The network data was obtained from the Torque website, for which we had to add some missing protein sequences, while query motifs used on its experiments were found in the GraMoFoNe website [3]. The threshold was set to $10^{-7}$ for BLAST e-values and to 0.0 for protein interaction probabilities, meaning we connect pairs of vertices representing proteins with interaction probability greater than zero. The queries were processed by our algorithm in parallel, and about 97% of them finished in no more than 3 seconds. Table 2 presents the total number of
Motif inference comparison for some $y$ values, $C = \{1, \ldots, 30\}, s = 5, g = 7, t = 10$

Figure 9. Motif counting for incremental method (with $s = 5$ and $g = 7$) compared to non-incremental (directly for sizes 6 and 7). We infer motifs considering the 30 higher frequency colors and set $t = 10$.

Torque matches (novel and previously known) reported in their paper compared with the counts found by the Algorithm 3. The table also presents how many matches found by the latter are functionally coherent. Compared to [5], the number of matches found to be functionally coherent is very satisfactory.

Table 2. Protein complex (motif) search in PPI networks.

| Network | Complex | Matches          | Functional Coherence |
|---------|---------|------------------|----------------------|
|         |         | Torque | Alg. 3 | Torque | Alg. 3 |
| Yeast   | Bovine  | 4      | 4       | 4      | 3      |
|         | Mouse   | 18     | 19      | 16     | 12     |
|         | Rat     | 26     | 22      | 19     | 8      |
| Fly     | Bovine  | 0      | 1       | 0      | 1      |
|         | Mouse   | 13     | 21      | 0      | 7      |
|         | Rat     | 35     | 35      | 17     | 8      |
| Human   | Bovine  | 4      | 7       | 2      | 5      |
|         | Mouse   | 58     | 113     | 32     | 66     |
|         | Rat     | 49     | 111     | 32     | 55     |
7. Conclusion

In this work we studied the search and inference of different constraints of topological colored motifs in vertex-colored graphs. Such studies could help us in the understanding of the computational complexity for many related problems.

Considering a motif as a colorful tree $T$ and an occurrence as a subset $S$ of vertices of a vertex-colored graph $G$ such that $|V_T| = |S|$ and $T \cong G[S]$, we gave a simple linear time algorithm for the searching problem. We also presented a method for enumerating all occurrences of $T$ in $G$ and described a linear time algorithm to compute the number of occurrences of $T$ in $G$. For the inference problem, we developed a variant of the Erdős model, where we take into account the colors of vertices as a parameter to calculate the probability of a given colorful tree $T$ being a motif. The comparison between the number of occurrences of a colorful tree $T$ in a given subgraph and the expectation of the number of occurrences of $T$ in a random graph obtained by the presented method can decide whether $T$ is a motif in $G$ or not, which takes time exponential in the size of $V_T$, but is very fast in practice if $|V_T| \leq 20$.

When there exists a high rate of noise in a biological network, especially due to missing/wrong data, we can allow finding occurrences of motifs with gaps, meaning that some vertices can be part of an occurrence of a motif in the network, not in the motif itself. Thus, it is important to allow a flexible search of motifs when we cannot find exact matches, only approximate. An approximate occurrence of a motif is referred to as a gap among some vertices of the given network [19]. Regarding highly frequent motifs with gaps, notice first that we can obtain the number of subgraphs of a given vertex-colored graph that are isomorphic to a colorful motif with gaps by making few changes to an algorithm given in [1]. Then, considering induced subgraphs, given a random vertex-colored graph $G$ and a colorful tree motif $T$ with gaps, a naive method to calculate the expected value and variance of the number of occurrences is quite slow. In this case, we can compute those measures using the following strategy. Let $\mathcal{G}$ be the set of all random vertex-colored graphs defined from $G$. The expected value and variance of the number of occurrences of $T$ in $G$ are

$$\begin{align*}
E[X] &= \sum_{R \in \mathcal{G}} X(R) \cdot \Pr\{R\}, \\
\text{Var}[X] &= \sum_{R \in \mathcal{G}} (X(R) - E[X])^2 \cdot \Pr\{R\},
\end{align*}$$

where $R$ is a random graph generated, according to the previous section, and $X(R)$ and $\Pr\{R\}$ denote the number of occurrences of $T$ in $R$ and the probability of $R$ to be generated, respectively. Then, the main idea is generating explicitly all graphs in $\mathcal{G}$ and counting the number of occurrences of each of them. However, notice that $|\mathcal{G}|$ can be very large. Thus, we generate randomly a smaller set $\mathcal{G}$ of random vertex-colored graphs, representing a sample from $\mathcal{G}$, and then calculate

$$E[X] \approx \frac{1}{|\mathcal{G}|} \sum_{R \in \mathcal{G}} X(R) \quad \text{and}$$
\[ \text{Var}[X] \simeq \frac{1}{|G|} \sum_{R \in G} (X(R) - E[X])^2. \]

This approach can also be used when gaps are not considered and \(|VT|\) is large, especially for calculating \(\text{Var}[X]\). However, these ideas should be extended further.

Our concept of occurrence for topological motifs extends to weights of occurrences, gaps in the networks, and deletions in motifs. The results of such extensions can be used for proposing more general problems, where the structure of the motifs is not considered.

References

1. E. Araújo and M. A. Stefanes, Some results on topological colored motifs in metabolic networks, Proc. of BIBE, 2013, pp. 1–5.
2. Michael Ashburner et al., Gene ontology: Tool for the unification of biology, Nat. Genet. 25 (2000), no. 1, 25–29.
3. Guillaume Blin, Florian Sikora, and Stéphane Vialette, GraMoFoNe: a cytoscape plugin for querying motifs without topology in protein-protein interactions networks, Proc. of BICoB, 2010, pp. 38–43.
4. Elizabeth I. Boyle et al., GO::TermFinder–open source software for accessing Gene Ontology information and finding significantly enriched Gene Ontology terms associated with a list of genes, Bioinformatics 20 (2004), no. 18, 3710–3715.
5. S. Bruckner et al., Topology-free querying of protein interaction networks, J. Comput. Biol. 17 (2010), no. 3, 237–252.
6. R. Caspi et al., The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases, Nucleic Acids Res. 44 (2016), no. D1, D471–80.
7. Thomas H. Cormen, Charles E. Leiserson, Ronald L. Rivest, and Clifford Stein, Introduction to algorithms, third edition, 3rd ed., The MIT Press, 2009.
8. Juan Javier Díaz-Mejía, Ernesto Pérez-Rueda, and Lorenzo Segovia, A network perspective on the evolution of metabolism by gene duplication, Genome Biology 8 (2007), no. 2, R26.
9. Riccardo Dondi, Guillaume Fertin, and Stéphane Vialette, Complexity issues in vertex-colored graph pattern matching, J. Discrete Algorithms 9 (2011), no. 1, 82–99.
10. B. Dost et al., QNet: A tool for querying protein interaction networks, J. Comput. Biol. 15 (2008), no. 7, 913–925.
11. Paul Erdős, Some remarks on the theory of graphs, B. Am. Math. Soc. 53 (1947), no. 4, 292–294.
12. Michael R. Fellows, Guillaume Fertin, Danny Hermelin, and Stéphane Vialette, Sharp tractability borderlines for finding connected motifs in vertex-colored graphs, Proc. of ICALP, LNCS, vol. 4596, 2007, pp. 340–351.
13. ________, Upper and lower bounds for finding connected motifs in vertex-colored graphs, J. Comput. Syst. Sci. 77 (2011), no. 4, 799–811.
14. Michael R. Garey and David S. Johnson, Computers and intractability: A guide to the theory of np-completeness, W. H. Freeman and Company, Murray Hill, New Jersey, 1979.
15. Sylvain Guillemot and Florian Sikora, Finding and counting vertex-colored subtrees, Algorithmica 65 (2013), 828–844.
16. Zahra Razaghi Moghadam Kashani et al., Kavosh: a new algorithm for finding network motifs, BMC Bioinformatics 318 (2009), no. 10.
17. B. P. Kelley et al., Conserved pathways within bacteria and yeast as revealed by global protein network alignment, P. Natl. Acad. Sci. USA 100 (2003), no. 20, 11394–11399.
18. V. Lacroix, C. G. Fernandes, and M.-F. Sagot, Reaction motifs in metabolic networks, Proc. of WABI, LNB, vol. 3692, 2005, pp. 178–191.
19. ________, Motif search in graphs: Application to metabolic networks, IEEE/ACM Trans. Comput. Biol. Bioinform. 3 (2006), no. 4, 360–368.
20. D. Maier, The complexity of some problems on subsequences and supersequences, J. ACM 25 (1978), no. 2, 322–336.
21. Thomas Pfeiffer, Orkun S Soyer, and Sebastian Bonhoeffer, The evolution of connectivity in metabolic networks, PLOS Biology 3 (2005), no. 7.
22. Ron Pinter, Hadas Shachnai, and Meirav Zehavi, *Deterministic parameterized algorithms for the graph motif problem*, Discrete Appl. Math. **213** (2016), 162–178.
23. Ron Pinter and Meirav Zehavi, *Algorithms for topology-free and alignment network queries*, J. Discrete Algorithms **27** (2014), 29–53.
24. Diego P. Rubert, Elói Araujo, and Marco A. Stefanes, *SIMBio: Searching and inferring colorful motifs in biological networks*, Proc. of BIBE, 2015, pp. 1–6.
25. Sophie Schbath, Vincent Lacroix, and Marie-France Sagot, *Assessing the exceptionality of coloured motifs in networks*, EURASIP J. Bioinform Syst. Biol. Article ID 616234 (2009), 9 pages.
26. S. S. Shen-Orr, R. Milo, S. Mangan, and U. Alon, *Network motifs in the transcriptional regulation network of Escherichia coli*, Nat. Genet. **31** (2002), no. 1, 64–68.
27. Tomer Shlomi, Daniel Segal, Eytan Ruppin, and Roded Sharan, *QPath: a method for querying pathways in a protein-protein interaction network*, BMC Bioinformatics **7**:199 (2006).
28. Sebastian Wernicke and Florian Rasche, *FANMOD: a tool for fast network motif detection*, Bioinformatics **22** (2006), no. 9, 1152.