The Structure of Level-k Phylogenetic Networks

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Abstract. Evolution is usually described as a phylogenetic tree, but due to some exchange of genetic material, it can be represented as a *phylogenetic network* which has an underlying tree structure. The notion of *level* was recently introduced as a parameter on realistic kinds of phylogenetic networks to express their complexity and tree-likeness. We study the structure of level-k networks, and how they can be decomposed into level-k generators. We also provide a polynomial time algorithm which takes as input the set of level-k generators and builds the set of level-$(k+1)$ generators. Finally, with a simulation study, we evaluate the proportion of level-k phylogenetic networks among networks generated according to the coalescent model with recombination.

1 Introduction

Networks have been introduced in phylogenetics to generalize the tree model of evolution which can only represent *speciation* events. In a phylogenetic network, additional branches join vertices already connected by a path, hence defining *reticulations*. This enables to represent hybridization [13, 24], recombination [15, 34] or lateral gene transfer events [14, 26]. Phylogenetic networks are a very active field of computational molecular biology and a number of algorithms have been developed recently to reconstruct such objects or parts thereof from various kinds of input: sequences, splits, distances, quartets, rooted or unrooted trees, or networks (see [16, 9] for a comprehensive list of papers).

The fact that networks are generally hard to handle gave rise to many different restrictions on their structure in order to get tractable algorithms. These restrictions are mostly described in terms of combinatorial patterns allowed or forbidden in the various restrictions. We examine here the broad class of networks called *explicit* networks or *reticulate* networks, in which reticulations are interpreted as precise biological events. In this context, a network is a rooted directed acyclic graph whose vertices have degree at most 3 – speciation vertices have indegree 1 and outdegree 2 and reticulation vertices have indegree 2 and outdegree 1. To cover all such explicit phylogenetic networks, the *level-k* hierarchy was introduced in [5]. In this setting, a phylogenetic network is viewed as a blobbed-tree [11], that is a network with tree-like parts and non reticulate ones called *blobs*. The *level* of a network reflects the complexity of its blobs: it is defined as the maximum number of reticulations inside a blob of the network.

Level-1 networks correspond to a class of explicit networks, first studied in 1998 [29] and later named *galled trees* [34, 12], for which many polynomial algorithms have been found [12, 4, 30, 20, 21, 6]. The level-k hierarchy can be seen as a promising framework to generalize these algorithms to all explicit phylogenetic networks.

Although level-k networks have recently attracted a lot of attention in the context of reconstruction from triplets [3, 17, 18, 19, 33] or maximum agreement subnetwork [5], their combinatorial structure has not yet been studied in detail. A notable exception is the work of [17], who introduced combinatorial patterns called *level-k generators* from which *simple* level-k networks [17] can be characterized. Yet, complete lists of generators were not easy to obtain for the first levels of the hierarchy: level-2 generators were only obtained by a case analysis, while the 65 level-3 generators were obtained by a brute force algorithm [22].

In this paper, we generalize these results. In Section 2, we give explicit rules to build, for all k, all level-$(k+1)$ generators from level-k generators. On this basis, we provide an algorithm that builds level-$(k+1)$ generators in time that is polynomial in the number of level-k generators. We use this algorithm to compute the 1993 level-4 generators. These generators can be downloaded as supplementary material from http://www.lirmm.fr/~gambette/ProgGenerators.php. We also provide lower and upper bounds on the number of level-k generators. Section 3 focuses on the structure of level-k networks. We show how they decompose into level-k generators. Finally, in Section 4, we consider the relevance of networks with a small level in the context of the coalescent model with recombination. For this purpose, we measure the proportion of level-k phylogenetic networks among networks generated according to this model.

2 Construction of Level-k Generators

2.1 Definitions

A *phylogenetic tree* is a rooted binary tree with directed arcs and distinctly labeled leaves. A *phylogenetic network* is a generalization of a phylogenetic tree, defined as a directed acyclic graph in which exactly one vertex has indegree 0 and outdegree 2 (the root) and all other vertices have either indegree 1 and outdegree 2 (*split vertices*), indegree 2 and outdegree ≤ 1 (*hybrid vertices*) or indegree 1 and outdegree 0 (*leaves*). The leaves have distinct labels. Note that in this graph, we allow multiple arcs, as is shown by the blob containing r_1 in Fig. 1. Choosing whether to allow this configuration (an "empty" cycle in the network) in the definition of a phylogenetic network is just a technical point (here we allow it to be able, later, to define level-k generators as level-k phylogenetic networks).

A directed acyclic graph is *biconnected* if it contains no vertex whose removal disconnects the graph. A *biconnected component*, or *blob*, of a pylogenetic network, is a maximal biconnected subgraph. An arc is a *cut-arc* if removing it disconnects the graph. For any arc (u, v) of a phylogenetic network N, u is a parent of v, and v a child of u. We say that u is *over* v, or v is *under* u in N, if N contains a directed path from u to v.

A phylogenetic network is called a *level-k phylogenetic network* [5] (or just *level-k network*) if each biconnected component contains at most k hybrid vertices. A level-k network which is not a level-$(k-1)$ network is called a *strict level-k phylogenetic network*. A level-0 phylogenetic network is a phylogenetic tree, and a level-1 network is commonly called a *galled tree*. Many hard problems can be solved in polynomial time on these classes of networks. However, these networks only cover part of the practical networks – see section 4, which motivates the study of upper levels.

Definition 2.1 ([17]). *A* level-k generator *(see Fig. 2) is a biconnected strict level-k network. Vertices of outdegree 0 and arcs of a level-k generator are called its* sides*, they are* empty *if no subtree is hanging from them. We call S_k the set of generators of level at most k, and S_k^* the set of level-k generators.*

Phylogenetic networks have been defined above such that a level-k generator is a level-k phylogenetic network (contrary to [17] we allow phylogenetic networks to contain hybrid vertices of outdegree 0). In particular, level-k generators and level-k networks are not allowed to contain vertices whose indegree and outdegree both equal 1.

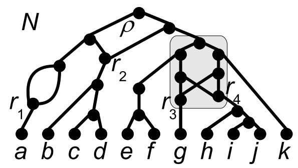


Fig. 1. A level-2 network N with root ρ and leaf set $\{a, b, c, d, e, f, g, h, i, j, k\}$. All unlabeled vertices are split vertices. The gray area is a biconnected component with two hybrid vertices, namely r_3 and r_4. The arc from r_2 to its child is a cut-arc. All arcs are directed downward but orientation is not displayed for the sake of readability, as in the next figures.

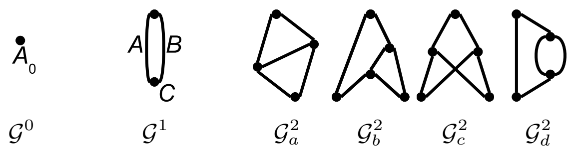


Fig. 2. Level-0 generator $\mathcal{G}^0$, level-1 generator $\mathcal{G}^1$, and level-2 generators: $\mathcal{G}^2_a$, $\mathcal{G}^2_b$, $\mathcal{G}^2_c$ and $\mathcal{G}^2_d$.

2.2 Construction Rules

The level-0, respectively level-1, generator is called $\mathcal{G}^0$, respectively $\mathcal{G}^1$). In [17], the level-2 generators are found by a case analysis which can also be applied to compute the 65 level-3 generators [22]. Here we provide rules to compute all level-$(k+1)$ generators from level-k generators.

Definition 2.2. *Let N be is a level-k generator. We define the following partial order $\succ_N$ on its sides: for two sides X and Y of N, $Y \succ_N X$ if the source of arc Y (or Y itself, if Y is a vertex) can be reached from the target of arc X (or X itself, if X is a vertex).*

The network $R1(N, X, Y)$ is obtained by choosing two sides X and Y of N, such that if $X = Y$ then X is not a hybrid vertex, and hanging a new hybrid vertex under X and Y (see Fig. 3).

The network $R2(N, X, Y)$ is obtained by choosing a side X of N and an arc $Y \not\succ_N X$ of N, and putting an arc from X to Y, which creates a new hybrid vertex "inside" arc Y.

Note that sides X and Y have a symmetric role for rule $R1$ but not for rule $R2$. When we build $R1(N, X, Y)$ from N, we say that we apply rule $R1$ on X and Y (and the same for $R2$). Note also that in the definition of $R1(N, X, X)$, we only allow X to be an arc, or, in the particular case of $N = \mathcal{G}^0$, to be its only node.

Proposition 2.1. *Let N be a level-k generator and X and Y two sides of N such that $N_1 = R1(N, X, Y)$, resp. $N_2 = R2(N, X, Y)$, is well-defined. Then N_1, resp. N_2, is a level-$(k+1)$ generator.*

Proof. We prove in Appendix A that in all cases, the rules provide a level-$(k+1)$ generator. □

We have seen in Proposition 2.1 that we can build level-$(k+1)$ generators from level-k generators, it remains to be proved that any level-$(k+1)$ generator can be obtained in this way.

Proposition 2.2. *For any level-$(k+1)$ generator N, there exists a level-k generator N', and some sides X and Y of N' such that $N = R1(N', X, Y)$ or $N = R2(N', X, Y)$.*

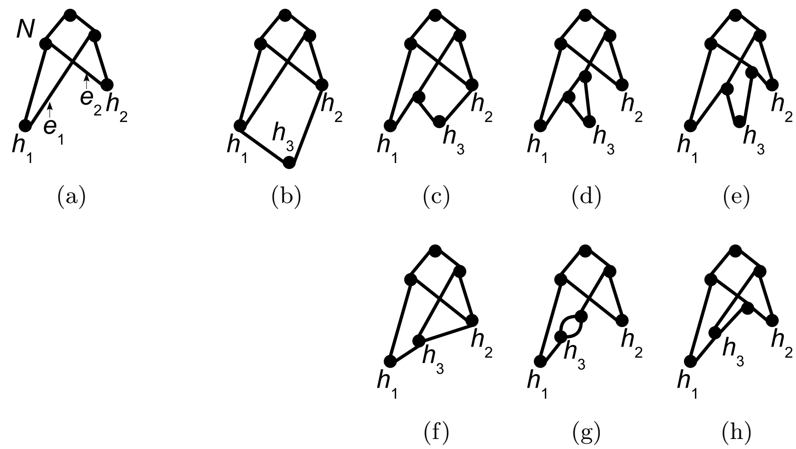


Fig. 3. Results of applying rules $R1$ and $R2$ on a level-2 generator N (a) depending on the type of side (arc or hybrid vertex) where it is applied: $R1(N, h_1, h_2)$ (b), $R1(N, e_1, h_2)$ (c), $R1(N, e_1, e_1)$ (d), $R1(N, e_1, e_2)$ (e), $R2(N, h_2, e_1)$ (f), $R2(N, e_1, e_1)$ (g), $R2(N, e_2, e_1)$ (h). In each case, a new hybrid node, h_3 is created.

Proof. The proof works by "reversing the rules" and finding an appropriate target vertex for the reversed rule. It is detailed in Appendix B. ☐

2.3 Bounding the Number of Generators

The rules we have defined can be used to obtain lower and upper bounds on the number of level-k generators.

Proposition 2.3. *For $k \geq 1$, a level-k generator has at most $3k - 1$ vertices and $4k - 2$ arcs.*

Proof. The unique level-1 generator has two vertices and two arcs. By Proposition 2.2, each level-k generator is obtained by applying rule $R1$ or $R2$ to a level-$(k-1)$ generator, hence by k applications of rules $R1$ or $R2$. We then notice that each application of rule $R1$ or $R2$ just adds at most three vertices and four arcs. The bounds are reached when $R2$ is repeatedly applied on two different arcs as in Fig. 3(e). ☐

Proposition 2.4. *The number g_k of level-k generators is at least 2^{k-1}.*

Proof. The property is true for $k = 0$, so we fix $k \geq 1$. We define an injection G_k between the set of integers $[0..2^{k-1} - 1]$ and a set of level-k generators. The generator $G_k(a)$ is build from the binary representation of a using only rule $R1$. The construction process is illustrated in Fig. 4. Let $a = \sum_{i=0}^{k-2} a_i 2^i \in [0..2^{k-1} - 1]$ such that $a_i \in \{0, 1\}$. We start with the level-1 generator $\mathcal{G}^1$, then for i from 0 to $k - 2$:

- let h_i be the lowest hybrid vertex of the currently built generator G.

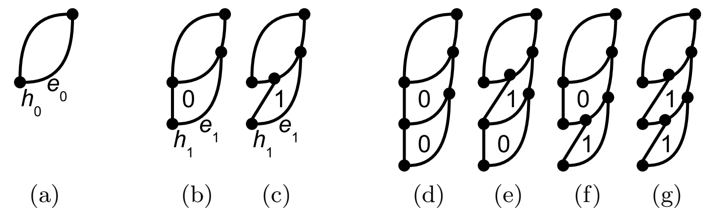


Fig. 4. Construction of 2^{k-1} non-isomorphic level-k generators : we start from generator $\mathcal{G}^1$ (a) and apply $R1(\mathcal{G}^1, e_0, h_0)$ to get $G_2(0)$ (b), $G_2(1) = R1(\mathcal{G}^1, e_0, e_0)$ (c), $G_3(0) = R1(G_2(0), e_1, h_1)$ (d), $G_3(1) = R1(G_2(1), e_1, h_1)$ (e), $G_3(2) = R1(G_2(0), e_1, e_1)$ (f), $G_3(3) = R1(G_2(1), e_1, e_1)$ (g).

- let e_i be the edge from the highest parent of h_i (a simple proof by induction shows that there always exists one parent of h_i under the other).
- change G into $R1(G, e_i, h_i)$ if a_i=1, into $R1(G, e_i, e_i)$ if $a_i = 0$.

This way, we get for $G_k(a)$ a digraph whose structure is a chain of cycles which encodes the binary representation of a. Proposition 2.1 ensures that $G_k(a)$ is a level-k generator. Thus, for each k, we can build a set $\{G_k(a), a \in [0..2^{k-1}-1]\}$ of 2^{k-1} level-k generators. These generators are obviously non isomorphic, since they are each composed by a specific chain of two kinds of cycles. □

Proposition 2.5. *The number g_k of level-k generators is lower than $k!^2 50^k$.*

Proof. The previous proposition ensures that the number of arcs of a level-k generator is less than $4k$, and its number of hybrid vertices is k, so its number of sides is less than $5k$. When applying the k^{th} rule $R1$ or $R2$, we choose a pair of sides, that is hybrid vertices or arcs, so there are less than $(5k)^2$ possibilities. Thus $g_{k+1} \leq 2(5k)^2 g_k < 50k^2 g_k$, so finally $g_k < k!^2 50^k$. □

Note that although these bounds are not tight, they give useful information on level-k generators. The lower bound shows that there is an exponential number of level-k generators, which implies, by the decomposition Theorem of Section 3, a great complexity inside the blobs of a network of high level. The upper bound for g_{k+1} from g_k and the fact that $g_3 = 65$ [22] shows that it seems realistic to generate automatically level-4 and 5 generators at least.

2.4 The Generator Construction Algorithm

We now study how to use rules $R1$ and $R2$ in practice to build level-$(k+1)$ generators knowing the set of level-k generators. Note that different sequences of rules may produce isomorphic level-k generators. Hence, isomorphic level-k generators have to be removed in the process.

Theorem 2.1. *There exists a polynomial algorithm which takes as input the set S_k^* of all level-k generators and outputs the set of all level-$(k+1)$ generators.*

Proof. The algorithm, `BuildGenerators`, detailed below, works by simply trying to apply rules $R1$ and $R2$ on any generator in S_k^*, then removing the isomorphic ones. To prove the polynomial complexity, the main point is the fact that the isomorphism test which is Graph Isomorphism-complete on general digraphs [35], can be done, in our case, in polynomial time [25, 27] in the size of the graph which is polynomial in $|S_k^*|$ by propositions 2.3 and 2.4. The proof is also detailed in Appendix D. □

Algorithm 1: *BuildGenerators* builds the set S of level-$(k+1)$ generators from the set S_k^* of level-k generators in polynomial time.

```
BuildGenerators(S_k^*: set of level-k generators)
S ← ∅
forall level-k generators g in S_k^* do
    forall pairs (X,Y) of sides of g do
        if rule R1 can be applied on sides X and Y then
            g' ← R1(g,X,Y)
            forall level-(k+1) generators h in S do
                if g' is not isomorphic to h then S ← S ∪ {g'}
        if rule R2 can be applied on sides X and Y then
            g' ← R2(g,X,Y)
            forall level-(k+1) generators h in S do
                if g' is not isomorphic to h then S ← S ∪ {g'}
return S
```

Though graph isomorphism is decidable in polynomial time for graphs of bounded maximum degree, there exists no implementation of this algorithm, which seems difficult to use in practice [23]. Instead, to actually build all level-4 generators from the 65 level-3 generators, we used an exponential time backtracking algorithm which tests isomorphism by trying to identify corresponding vertices by going through both input graphs at the same time. Among the 8501 level-4 generators built by applying rule $R1$ or $R2$, a total of 1993 are non-isomorphic. The list of these generators, the program to build them, its source and implementation notes are available at http://www.lirmm.fr/~gambette/ProgGenerators.php. Note that the sequence 1,4,65,1993 is not present in the On-Line Encyclopedia of Integer Sequences [31].

3 Generating Level-k Phylogenetic Networks

The concept of generator was introduced in [17] to build restrictions of level-k phylogenetic networks, called *simple*, which contain no cut-arc except the *trivial* ones leading to leaves. We give an explicit composition theorem which shows how generators can be used to build any level-k network, and exhibits the link with the blobbed-tree structure of phylogenetic networks.

Definition 3.1. *Given a set S_k of generators of level at most k, and a phylogenetic network N, we define the following rules, illustrated in Fig. 5:*
- $MergeRoot_k(G_0, G_1)$ *is obtained by hanging generators G_0 and $G_1 \in S_k$ under a root.*
- $Attach_k(v, G, N)$ *is the network obtained by adding an arc from hybrid vertex $v \in N$ of outdegree 0 to a copy of a generator $G \in S_k$.*
- $Attach_k(a, G, N)$ *is the network obtained by subdividing arc a (i.e. adding a vertex of indegree 1 and outdegree 1 inside a) and adding an arc from the created vertex to a copy of $G \in S_k$.*

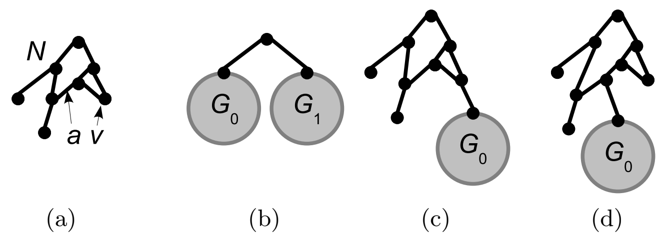


Fig. 5. Rules for building a level-k network from generators of level at most k: a phylogenetic network N (a); the network obtained by applying $MergeRoot_k(G_0, G_1)$ (b), $Attach_k(v, G_0, N)$ (c), and $Attach_k(a, G_0, N)$ (d).

Note that rule $MergeRoot_k$ can be used only once, and that it is used for level-k networks that are disconnected when removing their root.

Theorem 3.1. *N is a level-k network iff there exists a sequence of $r \in \mathbb{N}$ locations (arcs or hybrid vertices) $(\ell_j)_{j\in[1,r]}$ and a sequence of generators $(G_j)_{j\in[0,r]}$ in S_k, such that:*

$$N = Attach_k(\ell_r, G_r, Attach_k(\ldots Attach_k(\ell_2, G_2, Attach_k(\ell_1, G_1, G_0)) \ldots)),$$
$$\textit{or } N = Attach_k(\ell_r, G_r, Attach_k(\ldots Attach_k(\ell_2, G_2, MergeRoot_k(G_1, G_0)) \ldots)).$$

Proof. The proof works by induction and is detailed in Appendix C. □

Theorem 3.1 characterizes level-k networks by a sequence of rules on a finite set of generators. In this form, the characterization does not yield canonicity: two different sequences of rule applications may lead to the same phylogenetic network (typically, by just changing the order in which rules are applied).

However, this characterization is deeply based on a canonical tree decomposition of level-k networks which by lack of space cannot be detailed here, but is illustrated in Figure 6. It enters

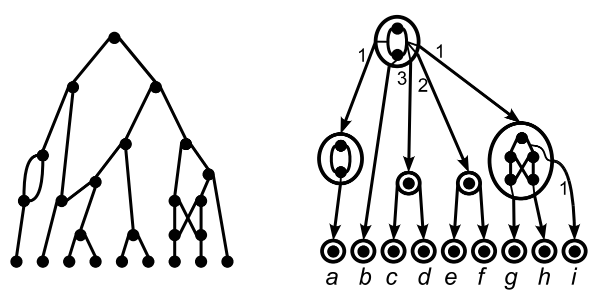


Fig. 6. A level-2 phylogenetic network and its canonical decomposition tree: each node of the tree contains a generator of level $\leq k$; each arc of the tree is linked to a side of the generator at the source node, and labeled by an integer showing in which order it is attached to the side, if the side is an arc.

the framework of graph grammars [7, 10]. Such a canonical representation is a first step towards counting or efficient exhaustive generation of level-k phylogenetic networks, which would extend currently known results on the number of unicyclic networks and galled trees [32].

4 Level-k Networks and the Coalescent Model with Recombination

In [2], Arenas *et al* conducted a simulation study to generate a number of realistic phylogenetic networks, according to the coalescent model with recombination, and measure the proportion of these networks contained in different subclasses of phylogenetic networks, among which trees and galled trees, i.e. level-0 and level-1 phylogenetic networks. We extend their study by computing the level of a sample of phylogenetic networks generated by the program Recodon [1]. The Java implementation of a simple biconnected component decomposition algorithm to compute the level is also available at http://www.lirmm.fr/~gambette/ProgGenerators.php.

For small levels, the results we obtained are shown in Table 1, and an insight on upper levels is given in Fig. 7.

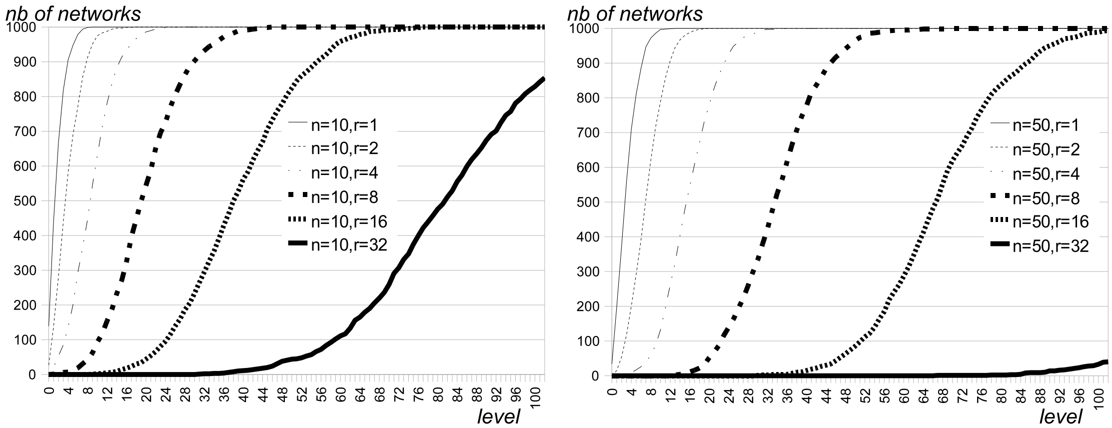
n	r	Tree	Level-1	Level-2	Level-3	Level-4	Level-5
10	0	1000	1000	1000	1000	1000	1000
10	1	139	440	667	818	906	948
10	2	27	137	281	440	582	691
10	4	1	21	53	85	136	201
10	8	0	1	1	6	7	12
10	16, 32	0	0	0	0	0	0
50	0	1000	1000	1000	1000	1000	1000
50	1	34	198	373	557	709	811
50	2	0	15	54	117	200	292
50	4	0	1	1	2	9	17
50	8, 16, 32	0	0	0	0	0	0

Table 1. Number of simulated networks falling in each class as a function of the recombination rate $r = 0$, 1, 2, 4, 8, 16, and 32 for sample size $n = 10$ or $n = 50$.

We observe that phylogenetic networks with a small level, like restricted phylogenetic networks formerly studied (*regular*, *tree-sibling* and *tree-child*, see [2]), cover a small portion of the networks corresponding to the coalescent model with high recombination rates. Still, the proportion of level-2 phylogenetic networks for 10 leaves is greater than the proportion of tree-child networks, but to get similar proportions on 50 leaves we have to consider level-3 networks.

In fact, our results show that level-k phylogenetic networks do not have a blobbed-tree structure in the context of the coalescent model. Instead, most of the simulated networks have all their hybrid vertices inside one same blob. This phenomenon even appears with a small recombination rate, as shown in Fig. 8. Thus, for this context, new structures and algorithmic techniques have to be found.

The coalescent model is not suitable to describe all cases of reticulate evolution. For example, a simple model of horizontal gene transfer based on inserting transfer events according to a Poisson distribution, respecting time constraints was given in [8]. The use of phylogenetic networks of bounded level may be more appropriate for this model, or others [28].



(a) (b)

Fig. 7. Level-k phylogenetic networks and the coalescent model with recombination: for recombination rates r =1, 2, 4, 8, 16, 32, the number of phylogenetic networks of level-k is shown, for simulations on 10 leaves (a) and on 50 leaves (b).

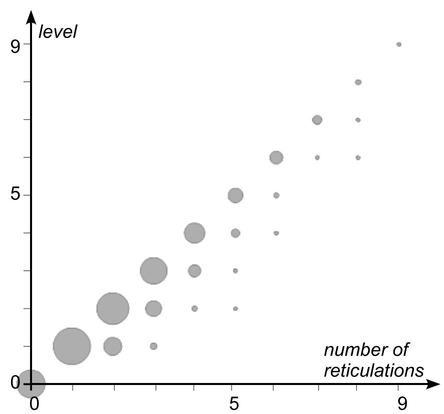


Fig. 8. Number of reticulations and level of the simulated networks for $n = 10$ and $r = 1$. The size of the dot at position (x, y) reflects the number of strict level-x networks with y hybrid vertices.

Acknowledgments

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Appendix A

We recall Proposition 2.1 and prove it.

Proposition 4.1. *Let N be a level-k generator and X and Y two sides of N such that $N_1 = R1(N, X, Y)$, resp. $N_2 = R2(N, X, Y)$, is well-defined. Then N_1, resp. N_2, is a level-$(k + 1)$ generator.*

Proof. Since N_1 and N_2 are well-defined, sides X and Y meet the requirements imposed in Definition 2.2 for $R1$ and $R2$. These definitions ensure that the acyclicity of the graph is preserved. Thus, we just have to show that for any type of sides (arc or hybrid vertex) X and Y (as detailed in Fig. 3), applying rule $R1$ or $R2$ always adds split vertices and exactly one hybrid vertex, with outdegree ≤ 1.

We first check what happens when applying rule $R1$ to get $R1(N, X, Y)$:
- if $N = \mathcal{G}^0$, then applying $R1$ gives the level-1 generator $\mathcal{G}^1$.
- if X and Y are distinct hybrid vertices, they have outdegree 0 as they are sides of N, so applying $R1$ will just give them outdegree 1, and create a new hybrid vertex of outdegree 0 (Fig. 3(b)).
- if X is a hybrid vertex, and Y is an arc, then applying $R1$ gives X outdegree 1, adds a new hybrid vertex of outdegree 0 and creates a new split vertex "inside" Y (whose parent is the upper extremity of Y, and whose children are the lower extremity of Y and the new hybrid vertices created), as shown in Fig. 3(c). By symmetry we also get a valid generator if X is an arc and Y is a hybrid vertex.
- if X and Y are both arcs (possibly the same as in Fig. 3(d)) then applying $R1$ creates two split vertices, one inside X and the other inside Y (Fig. 3(e)).

In all cases $R1(N, X, Y)$ is obtained from N by adding a hybrid vertex and possibly some split vertices. Thus $R1(N, X, Y)$ meets the definition of a strict level-$(k+1)$ network. As the transformation preserves biconnectivity, then $R1(N, X, Y)$ is a level-$(k + 1)$ generator.

We now check what happens when applying rule $R2$ to get $R2(N, X, Y)$:
- if X is a hybrid vertex, and Y is an arc, then applying $R2$ gives X outdegree 1, and creates a new hybrid vertex of outdegree 1 "inside" Y (whose parents are X and the upper extremity of Y, and whose child is the lower extremity of Y), as shown in Fig. 3(f).
- if X and Y are both arcs (possibly the same, see Fig. 3(g)) then applying $R1$ creates a split vertex inside X and a hybrid vertex of outdegree 0 inside Y (Fig. 3(h)).

In all cases $R2(N, X, Y)$ is obtained from N by adding a hybrid vertex and possibly some split vertices so, similarly as above, $R2(N, X, Y)$ is a level-$(k + 1)$ generator. □

Appendix B

The proof of Proposition 2.2 directly follows from Lemma 4.1 detailed below. But we first need to define the removal of a hybrid vertex by reversing rules $R1$ and $R2$.

Definition 4.1. *Let N be a level-$(k + 1)$ phylogenetic network, and v a vertex of N, that is not a child of the root, except for the case where $N = \mathcal{G}^1$. We define the $R1R2$-removal of v, which provides a level-k network N', in the following way. The vertex v is first removed from the graph with all its adjacent arcs; then, in several cases, arcs are added to the network to maintain connectivity.*

If v has outdegree 0, then five cases arise (see Fig. 9 (a)-(e)):

(a) *the parents of v are distinct hybrid vertices X and Y, then by deleting v, vertices X and Y get outdegree 0, and no other vertex is changed, as shown in Fig. 9(a). If we call N' the network obtained after deletion then we note $N = R1(N', X, Y)$,*
(b) *a parent of v, say Y, is a hybrid vertex and the other, X, is a split vertex, then, as shown in Fig. 9(b), by deleting v, X, and joining the parent of X to the second child of X (other than v) thanks to arc e_X, we get a network N' such that $N = R1(N', Y, e_X)$,*
(c) *the parents of v are split vertices X and Y such that X is neither a child nor the parent of Y, cf. Fig. 9(c). Then, by deleting v, X, Y, and joining the parent of X to the second child of X (other than v) thanks to an arc e_X, and the parent of Y to the second child of Y (other than v) thanks to an arc e_Y, we get a network N' such that $N = R1(N', e_X, e_Y)$.*
(d) *the parents of v are split vertices X and Y where X is the parent of Y, cf. Fig. 9(d). Then, by deleting v, X, Y, and joining the parent of X to the second child of Y (other than v) thanks to an arc e_{XY}, we get a network N' such that $N = R1(N', e_{XY}, e_{XY})$.*
(e) *v is the only child of the root, then N has to be $\mathcal{G}^1$, as shown in Fig. 9(e), we remove v and its two incoming arcs to get the level-0 generator $N' = \mathcal{G}^0$ with one vertex A_0 and $N = R1(N', A_0, A_0)$.*

If v has outdegree 1, then three cases arise:

(f) *at least one parent of v, say Y, is a hybrid vertex. Then by deleting v, and joining X to the child of v thanks to arc e_X, vertex Y gets outdegree 0 and the degree of no other vertex is changed, as shown in Fig. 9(f), we get a network N' such that $N = R2(N', Y, e_X)$,*
(g) *both parents of v are different split vertices X and Y, then, as shown in Fig. 9(g), by deleting v, Y, and joining the parent of Y to the second child of Y (other than v) thanks to arc e_Y, and joining X to the child of v thanks to arc e_X, we get a network N' such that $N = R2(N', e_Y, e_X)$.*
(h) *v only has one parent which is the split vertex X, then, as shown in Fig. 9(h), by deleting v, X, and joining the parent of X to the child of v thanks to arc e_X, we get a network N' such that $N = R2(N', e_X, e_X)$.*

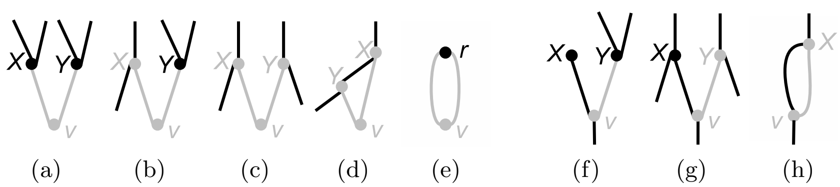

Fig. 9. Different possible cases to "reverse" rules $R1$ and $R2$, depending on whether the rule has created an outdegree 0 hybrid vertex (a-e) which corresponds to rule $R1$ or an outdegree 1 hybrid vertex (f-h) which corresponds to rule $R2$: gray arcs and vertices are to be deleted to reverse the rule.

Note that, in each case, the nodes of N' meet the degree requirements stated in the definition of a phylogenetic network. Reusing the names $R1$ and $R2$ above is an abuse of notation as we have no guarantee yet, when we write $N = Ri(N', X, Y)$ in this definition of reversed rules, that N' is a generator, nor that X and Y are sides. However, we will only use them in such proper cases.

Lemma 4.1. *Let N be a level-$(k+1)$ generator. There exists a vertex v of N such that the $R1R2$-removal of v from N gives a level-k generator.*

Proof. We prove it by induction.

Base case:

Call A_0 the only vertex of $\mathcal{G}^0$, A and B the two arcs of $\mathcal{G}^1$, and C its hybrid vertex. Then we can check the base cases for $k \leq 1$ (see Fig. 2) as $\mathcal{G}^1 = R1(\mathcal{G}^0, A_0, A_0)$ (vertex C is $R1R2$-removed, we are in case (e)), and for $k = 1$ we remove hybrid vertices which are not children of the root: $\mathcal{G}_a^2 = R1(\mathcal{G}^1, B, C)$ (b), $\mathcal{G}_b^2 = R1(\mathcal{G}^1, B, B)$ (d), $\mathcal{G}_c^2 = R1(\mathcal{G}^1, A, B)$ (c) and $\mathcal{G}_d^2 = R2(\mathcal{G}^1, B, B)$ (h).

Inductive step:

We now fix $k \geq 2$. We suppose that the expected property is true for any level-j generator, with $j < k$ and prove it for level k. So consider a level-$(k+1)$ generator N. It contains at least three hybrid vertices, so at least one of the three, say v, is not a child of the root. Then, we $R1R2$-remove it, and obtain a level-k *network* N'.

We now have to prove that either N' is a level-k *generator*, or we can directly choose another hybrid vertex v'' whose $R1R2$-removal gives a level-k generator.

If N' is biconnected, then by definition of a generator, it is a level-k generator.

Assume N' is not biconnected as illustrated in Fig. 10. Let N'' be a biconnected component of N' which does not contain the root. Note that by definition 4.1, the $R1R2$-removal of v from N did not create any leaf in N', therefore N'' is a non-trivial biconnected component, so it hosts at least one hybrid vertex. Either N'' is a level-j generator with $0 < j < k$, then we call it G''.

Otherwise, it is not even a level-k phylogenetic network because of the degree requirements. We claim that in this case N'' contains exactly one vertex having both indegree and outdegree 1. Indeed, N'' is only connected by cut-arcs to the rest of N'. As N is biconnected, the presence of biconnected components in N' results from the $R1R2$-removal of v. Since v has indegree 2, N' contains at most two cut-arcs incident to vertices of N'', as shown in Fig. 10(b). One of those cut-arcs leads to the root of N'', as N'' does not contain the root of N', so there is at most one cut-arc hanging from N'' in N'. This is the only problematic point which impedes N'' from being a generator, as it reflects the presence of an indegree and outdegree 1 vertex when considering N'' by itself. In this case, we consider the level-j generator G'' obtained by deleting this vertex, and connecting its parent to its child in N''.

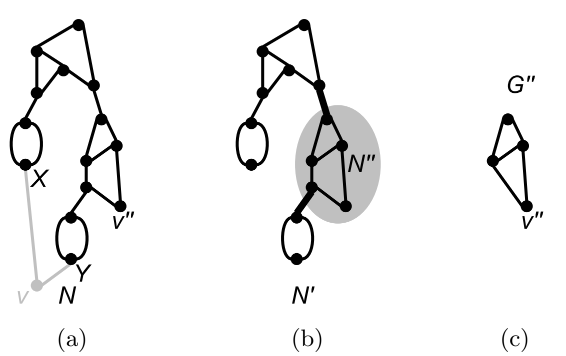

Fig. 10. If removing v disconnects the graph, then we find another vertex v'' which can be $R1R2$-removed to get a level-$(k-1)$ generator.

In both cases, we apply the induction hypothesis on this level-j generator G'': as $j > 0$, it contains a hybrid vertex v'', that can be $R1R2$-removed. Even if the $R1R2$-removal of v'' from G'' gives a valid level-$(j-1)$ generator, where biconnectivity is preserved, it remains to prove that the $R1R2$-removal of v'' from N gives a valid level-$(k-1)$ generator. Fig. 10 shows that this is not always straightforward: in the depicted case, one parent of v'' in G'' is a split vertex and the other one is a hybrid vertex, which corresponds to case (b) for the $R1R2$-removal of v'', whereas in N, both parents of v'' are split vertices, which corresponds to case (c). Hence we have to show which case of the $R1R2$-removal applies in N depending on the one applying in the context of G''.

Let X'' and Y'' be the parents of v'' in G'' (we name X'' and Y'' similarly to X and Y in the definition of $R1R2$-removal). We recall that each case is illustrated in Fig. 9.

We first consider case (a). If v'' also has outdegree 0 in N then:

- if both parents of v'' in N are hybrid vertices, then $R1R2$-remove v'' from N according to case (a).
- if exactly one of the parents of v'' in N is not a hybrid vertex, then $R1R2$-remove v'' from N according to case (b).
- if no parent of v'' in N is a hybrid vertex, then $R1R2$-remove v'' from N according to case (c).

Otherwise, v'' has outdegree 1 in N then $R1R2$-remove v'' according to case (f). Note that it is impossible that neither X'' nor Y'' is the parent of v'' in N: as there is already an arc under v'' in N, there can only be one other arc (leading to v, or cut-arc in N') hanging from N'' (thus creating a split vertex under one of v'''s parent and over v'') in N.

We now consider case (b). If v'' also has outdegree 0 in N then the same applies as in case (a). Otherwise, v'' has outdegree 1 in N:

- if one of the parents of v'' in N is a hybrid vertex, then $R1R2$-remove v'' in N according to case (f).
- otherwise $R1R2$-remove v'' from N according to case (g).

We now consider case (c). If v'' also has outdegree 0 in N then:

- if exactly one of the parents of v'' in N is a hybrid vertex, then $R1R2$-remove v'' from N according to case (b).
- otherwise $R1R2$-remove v'' from N according to case (c).

Otherwise, v'' has outdegree 1 in N, the same applies as in case (b).

We now consider case (d). If v'' also has outdegree 0 in N then, if X'' and Y'' are still the parents of v'' in N then $R1R2$-remove v'' from N according to case (d), otherwise:

- if exactly one of the parents of v'' in N is a hybrid vertex, then $R1R2$-remove v'' from N according to case (b).
- otherwise $R1R2$-remove v'' from N according to case (c).

Otherwise, v'' has outdegree 1 in N, the same applies as in case (b).

We now consider case (e). If v'' also has outdegree 0 in N then, the case where v'' still has only one parent in N cannot happen (otherwise N would not be biconnected), so:

- if exactly one of the parents of v'' in N is a hybrid vertex, then $R1R2$-remove v'' from N according to case (b).
- otherwise $R1R2$-remove v'' from N according to case (c).

Otherwise, v'' has outdegree 1 in N. If v'' still has only one parent in N then $R1R2$-remove v'' according to case h, otherwise the same applies as in case (b).

We now consider cases (f) and (g):

- if one of the parents of v'' in N is a hybrid vertex, then $R1R2$-remove v'' in N according to case (f).
- otherwise $R1R2$-remove v'' from N according to case (g).

We finally consider case (h): if v'' still has only one parent in N then $R1R2$-remove v'' according to case (h), otherwise the same applies as in case (f).

We can also check in all these cases that $R1R2$-removing v'' from N maintained the biconnectivity ensured when $R1R2$-removing v'' from G''.

In any case, we have found a hybrid vertex which can be removed to get a level-k generator, therefore the proposition is true. □

Appendix C

We recall Theorem 3.1 and prove it.

Theorem 4.1. *N is a level-k network iff there exists a sequence of $r \in \mathbb{N}$ locations (arcs or hybrid vertices) $(\ell_j)_{j\in[1,r]}$ and a sequence of generators $(G_j)_{j\in[0,r]}$ in S_k, such that:*

$$N = Attach_k(\ell_r, G_r, Attach_k(\dots Attach_k(\ell_2, G_2, Attach_k(\ell_1, G_1, G_0)) \dots)),$$
$$\text{or } N = Attach_k(\ell_r, G_r, Attach_k(\dots Attach_k(\ell_2, G_2, MergeRoot_k(G_1, G_0)) \dots)).$$

Proof. $\Leftarrow$: This implication is trivially proved by induction, as any sequence of the above rules repeatedly attaches one or two new biconnected components (each containing at most k hybrid vertices) by cut-arcs to the structure already built.

$\Rightarrow$: We prove by induction on the number p of vertices of a level-k phylogenetic network N, that for any $k \in \mathbb{N}$, N can be obtained by repeated applications of the *Attach* rule after a possible initial application of the $MergeRoot$ rule.

Base case: if $p = 1$ then the only possible network is $\mathcal{G}^0$, which corresponds to not applying any rule (take $r = 0$ in the theorem) to the level-0 generator $\mathcal{G}^0$.

Inductive step: now suppose that all networks with strictly less than p vertices verify the desired property, let N be a network with p vertices.

A. If N contains a leaf l, then:

i) either l has at least one grand-parent, say u, then:
  - either the parent of u is a split vertex, then delete l, its parent, and connect the grand-parent u to the sibling v of u. The obtained network N' has less than p vertices, so the induction hypothesis applies, and the observation that

$$N = Attach_k((u, v), \mathcal{G}^0, N'),$$

  shows that N has the desired property.

- either the parent of l is a hybrid vertex h, then delete l, and (h, l). The obtained network N' has less than p vertices, so the induction hypothesis applies, and since $N = Attach_k(h, \mathcal{G}^0, N')$, the desired property is true for N.

ii) Otherwise, l has no grand-parent, that is its parent is the root. Then the network N' obtained by considering the sibling u of l and the subnetwork rooted at u has less than p vertices, so we can apply the induction hypothesis, from which we have:

- either

$$\begin{aligned} N' = \; & Attach_k(\ell_r, G_r, Attach_k(\ldots \\ & Attach_k(\ell_2, G_2, Attach_k(\ell_1, G_1, G_0))\ldots)), \end{aligned} \tag{1}$$

  then

$$\begin{aligned} N = \; & Attach_k(\ell_r, G_r, Attach_k(\ldots \\ & Attach_k(\ell_2, G_2, Attach_k(\ell_1, G_1, MergeRoot_k(\mathcal{G}^0, G_0)))\ldots)) \end{aligned}$$

- or

$$\begin{aligned} N' = \; & Attach_k(\ell_r, G_r, Attach_k(\ldots \\ & Attach_k(\ell_2, G_2, MergeRoot_k(G_1, G_0))\ldots)). \end{aligned} \tag{2}$$

  then

$$\begin{aligned} N = \; & Attach_k(\ell_r, G_r, Attach_k(\ldots \\ & Attach_k(\ell_2, G_2, Attach_k(\ell', G_1, MergeRoot(G_0, \mathcal{G}^0)))\ldots)), \end{aligned}$$

  where ℓ' is the arc from the root to G_0 in $MergeRoot(G_0, \mathcal{G}^0)$.

B. If N contains no leaf, it only contains a root, split and hybrid vertices.

i) either N is biconnected, then it is a generator, and it has k hybrid vertices or less (as N is level-k), so the expected property is true.

ii) otherwise, N is not biconnected, and N has a hybrid vertex of outdegree 0. Consider its biconnected component tree. Consider a leaf of this tree, that is one of the "lowest" biconnected components. Let C be this biconnected component, i.e. C is a level-k generator. We treat C exactly like leaf l in equations (1) and (2) above, by replacing $\mathcal{G}^0$ by C in the decomposition formulas.

□

Appendix D

We recall Theorem 2.1 and prove it.

Theorem 4.2. *There exists a polynomial algorithm which takes as input the set S_k^* of all level-k generators and outputs the set S_{k+1}^* of all level-$(k+1)$ generators.*

Proof. The algorithm, `BuildGenerators`, is described in Algorithm 1.

By the proof of Proposition 2.5, rules $R1$ and $R2$ are applied at most $50k^2|S_k^*|$ times in the algorithm. Proposition 2.4 ensures that $|S_k^*| \geq 2^{k-1}$, so $k = o(|S_k^*|)$, and by Proposition 2.3 the size of a generator is also polynomial in $|S_k^*|$. Hence, the number of isomorphism tests is polynomial in $|S_k^*|$.

To prove that this algorithm is polynomial in the size of the input S_k^*, we prove that the isomorphism test can be done in polynomial time in k.

Digraph isomorphism is Graph Isomorphism-complete [35], which implies that no polynomial algorithm is currently known for this problem in the general case. However, here we restrict to instances where the digraphs have maximal degree 3 in particular, and isomorphism for graphs of bounded maximum degree can be determined in polynomial time [25]. We now show how to polynomially reduce the problem of isomorphism for digraphs of maximum degree 3 and maximum outdegree and indegree 2 to the problem of isomorphism for graphs of bounded degree.

For each digraph D whose vertices all have degree at most 3, outdegree and indegree at most 2, and possibly multiple arcs, we use the gadget introduced by Miller [27] to build a graph $G(D)$ in the following way:

- all vertices of D are vertices of $G(D)$,
- every arc (u, v) of D is transformed into a P_4 graph $u - u' - v' - v$, completed with a P_2 attached to u' and a P_3 to v'.

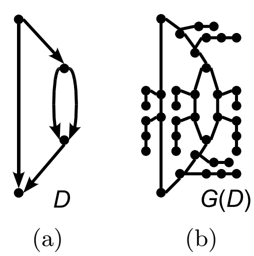

Fig. 11. A digraph D (a) and the associated undirected graph $G(D)$ (b) by a transformation introduced by Miller [27] which preserves isomorphism and bounded degree.

This construction, illustrated in Fig. 11, is done in a time that is polynomial to the size of the digraph and provides an undirected graph of maximum degree 3. It ensures that D_1 is isomorphic to D_2 iff $G(D_1)$ is isomorphic to $G(D_2)$. □