POLYNOMIAL INVARIANT FOR PHYLOGENETIC NETWORKS

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Abstract. Invariants for complicated objects such as those arising in phylogenetics, whether they are invariants as matrices, polynomials, or other mathematical structures, are important tools for distinguishing and working with such objects. In this paper, we generalize a polynomial invariant on trees, to phylogenetic networks. Networks are becoming increasingly important for their capacity to represent reticulation events, such as hybridization, in evolutionary history. We provide a function from the space of phylogenetic networks to a polynomial ring, and prove that two networks with the same number of leaves and same number of reticulations are isomorphic if and only if they share the same polynomial. While the invariant for trees is a polynomial in \[Z[x_1, \ldots, x_n, y]\] where \(n\) is the number of leaves, the invariant for networks is an element of \[Z[x_1, \ldots, x_n, \lambda_1, \ldots, \lambda_r, y]\], where \(r\) is the number of reticulations in the network. For networks without leaf labels this reduces to a polynomial in \(r + 2\) variables.

1. Introduction

A polynomial invariant able to uniquely distinguish between rooted trees has been recently introduced in [Liu21]. Motivated to analyze and compare tree shapes in a phylogenetic context, this polynomial (which we will refer to as the Liu polynomial) has been used both to define a similarity measure on rooted tree shapes and to estimate parameters and models via their coefficients [LBGC20]. Moreover, its generalization from trees to networks (by analyzing the set of embedded spanning trees in the network) has also been used to study the properties of randomly generated networks [JL21].

We note that the word “invariant” is used here in its traditional sense, and not the one used in algebraic geometry approaches to phylogenetics, in which phylogenetic invariants for an evolutionary model along a tree are the polynomials which vanish on the expected frequencies of base patterns at the leaves [CF87]. Throughout this article, an invariant of a set \(A\) is a function \(f: A \rightarrow B\) with the property that \(x \sim_A y\) if and only if \(f(x) \sim_B f(y)\), where \(B\) is some other set (such as the polynomials), and \(\sim_A\) and \(\sim_B\) are equivalence relations in the respective sets.

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A multitude of (non-polynomial) invariants have been defined for specific subclasses of phylogenetic networks, but these do not completely characterize generic networks. To name just a few, the \( \mu \)-vectors which store the number of paths from nodes to leaves characterizes (among others) tree-child networks [CRV08a] and orchard networks (without stacks) [BESS21]; the set of displayed trees characterizes regular networks [Wil10]; and the induced trinets (minimal subnetworks induced by triples of leaves) characterize (among others) level-2 networks [VIM14] and orchard networks [ST21].

In this paper we show how a polynomial invariant can be defined for rooted phylogenetic networks, generalizing the Liu polynomial invariant for trees. The key results are the characterization of a phylogenetic network by a multilabelled tree (Corollary 3.8), and an associated invariant map from a phylogenetic network on \( X \) to a polynomial in \( |X| + r + 1 \) variables, where \( r \) is the number of reticulations in the network (Corollary 4.4). For a phylogenetic network with \( n \) unlabeled leaves, the map restricts to an invariant polynomial with \( r + 2 \) variables (Proposition 4.5).

2. Definitions

Throughout this paper, \( X \) will denote a non-empty finite set (of taxa). Commonly, we will use \( X = \{x_1, \ldots, x_n\} \), and we will allow ourselves to see each member of \( X \) as an irreducible polynomial in \( \mathbb{Z}[x_1, \ldots, x_n] \); i.e., we will consider the labels of the leaves in our networks to be polynomials of the form \( x_i \) for \( i \in \{1, \ldots, n\} \).

**Definition 2.1.** A rooted binary phylogenetic network \( N = (V,E) \) on \( X \), or simply a phylogenetic network on \( X \), is a rooted directed acyclic graph with no parallel arcs satisfying the following conditions:

(i) any node with out-degree zero (a leaf) has in-degree one, and the set of nodes with out-degree zero, denoted by \( L(N) \), is identified with \( X \) via a bijection \( \varphi : L(N) \rightarrow X \);

(ii) the root is the only node with in-degree zero, and has out-degree two;

(iii) any other node has either in-degree one and out-degree two (a tree node), or in-degree two and out-degree one (called a reticulation node).

We shall consider the leaves and root to be tree nodes.

**Definition 2.2.** A rooted binary internally multi-labelled phylogenetic network \( N = (V,E) \) on \( X \), or simply an IMLN on \( X \), is a rooted directed acyclic graph with no parallel arcs satisfying the following conditions:

(i) any node with out-degree zero (a leaf) has in-degree one, and the set of nodes with out-degree zero, denoted by \( L(N) \), is identified with \( X \) via a bijection \( \varphi : L(N) \rightarrow X \);

(ii) the root is the only node with in-degree zero, and it can have out-degree one (in which case we shall say it is an elementary node) or two (a tree node);

(iii) any other node has either in-degree one and out-degree two (again, a tree node), or in-degree two and out-degree one (called a reticulation node), or in-degree one and out-degree one (again, an elementary node); and

(iv) if \( R(N) \) denotes the set of reticulation nodes and \( E(N) \) the set of elementary nodes of \( N \), then there exists \( \ell : R(N) \cup E(N) \rightarrow \{\lambda_1, \ldots, \lambda_r\} \) a labelling function such that its restriction to \( R(N) \) is injective.

We will consider the labels \( \lambda_1, \ldots, \lambda_r \) to be irreducible polynomials in \( \mathbb{Z}[x_1, \ldots, x_n, \lambda_1, \ldots, \lambda_r] \). Notice that Definition 2.2 implies that IMLNs are a recursive structure in the following sense: given any IMLN \( N \), for any \( u \in V(N) \), the subgraph rooted at \( u \) is still an IMLN. This is not the case in general for phylogenetic networks. Moreover, note that a phylogenetic network is an IMLN without elementary nodes and without a labelling on the reticulations.

**Definition 2.3.** A rooted binary internally multi-labelled phylogenetic tree \( T = (V,E) \) on \( X \), or simply IMLT on \( X \), is an IMLN without reticulation nodes.

3. Folding and unfolding

A phylogenetic network cannot in general be characterized by its derived multilabelled tree; this correspondence is valid only for FU-stable networks [HMSW16]. In this section, however, we prove that an IMLN without elementary nodes can be uniquely characterized by an IMLT obtained by a sequence of unfoldings on its reticulation nodes.
Let \( N \) be an IMLN, and \( R(N) \) the set of its reticulation nodes. The relation of being a descendant of another node induces a partial order over \( R(N) \); let \( \mathcal{R}_{\text{min}}(N) \) be the set of the minimal elements of \( R(N) \), i.e. reticulation nodes such that none of their descendants are also reticulation nodes.

**Lemma 3.1.** If \( u \in \mathcal{R}_{\text{min}}(N) \), then the graph rooted at \( u \) is an IMLT.

**Proof.** If \( u \in \mathcal{R}_{\text{min}}(N) \), then there is no path in \( N \) from \( u \) to another reticulation. This means that there are no reticulations in the graph rooted at \( u \); and therefore it is an IMLT.

Let \( N \) be an IMLN, and consider \( u \in \mathcal{R}_{\text{min}}(N) \) (so that \( u \) is labelled by an element in \( \{\lambda_1, \ldots, \lambda_n\} \)). Let \( v_1, v_2 \) be its parents, noting that \( v_1 \neq v_2 \) due to the fact that parallel arcs are excluded. Define \( U(N,u) \) to be the *unfolded IMLN of \( N \) in \( u \), obtained by the following algorithm:

1. Delete edges \((v_1,u)\) and \((v_2,u)\);
2. Duplicate \( N(u) \), the IMLT rooted at \( u \), including all its labels; and
3. Add an edge from \( v_1 \) to one of the resulting copies of \( u \), and an edge from \( v_2 \) to the remaining copy of \( u \).

**Corollary 3.2.** Let \( N \) be an IMLN, and \( u \in \mathcal{R}_{\text{min}}(N) \). Then \( U(N,u) \) is an IMLN.

Let \( N \) be an IMLN. We say that a sequence \((u_1, \ldots, u_k)\) of nodes in \( R(N) \) is *compatible* if, for each \( i \in \{1, \ldots, k-1\} \) there is no path from \( u_i \) to \( u_j \) when \( j > i \); i.e., if it is non decreasing under the partial order induced by the network over \( R(N) \). Given \((u_1, \ldots, u_k)\) a compatible sequence of \( R(N) \), then we can consider the associated sequence \((N, N(u_1), N(u_2), \ldots, N(u_k))\) of IMLNs such that \( N_{u_{i+1}} = U(N_{u_i}, u_{i+1}) \) and \( N_{u_1} = U(N, u_1) \).

**Lemma 3.3.** Let \( N \) be a IMLN and \( u_1, u_2 \in \mathcal{R}_{\text{min}}(N) \). Then,

\[ U(U(N,u_1),u_2) = U(U(N,u_2),u_1) \]

**Proof.** It is straightforward by Lemma 3.1 and the steps of the unfolding algorithm. If \( u_1 \in \mathcal{R}_{\text{min}}(N) \), then \( u_2 \in \mathcal{R}_{\text{min}}(U(N,u_1)) \); otherwise there would be a reticulation node \( u' \) in \( R(U(N,u_1)) \) and a path from \( u_2 \) to \( u' \) in \( U(N,u_1) \). Then the same path from \( u_2 \) to \( u' \) is also in \( N \) which is a contradiction, since \( u_2 \in \mathcal{R}_{\text{min}}(N) \). Then, by Lemma 3.1, the graph rooted at \( u_2 \) in \( U(N,u_1) \) is an IMLT. Since \( u_2 \) is not a vertex in any of the copies of the IMLT rooted at \( u_1 \) in the construction of \( U(N,u_1) \), there is no intersection between the copies from \( u_1 \) and the copies from \( u_2 \). Since the same argument holds if we start by \( u_2 \), then the result is achieved.

The previous result combined with the fact that if \((N, N(u_1), N(u_2), \ldots, N(u_k))\) is such a sequence, then \( u_{i+1} \in \mathcal{R}_{\text{min}}(N_{u_i}) \), easily leads to the following result.

**Corollary 3.4.** Let \( N \) be an IMLN, \((u_1, u_2, \ldots, u_k)\) a compatible sequence of elements of \( R(N) \) and \((N, N(u_1), N(u_2), \ldots, N(u_k))\) its associated sequence of IMLNs, such that \( N_{u_{i+1}} = U(N_{u_i}, u_{i+1}) \). Let \( \sigma : [k] \to [k] \) be a permutation such that \( u_{\sigma(i)} < u_{\sigma(j)} \) if \( u_i < u_j \), and \((N, N'_{u_{\sigma(1)}}, N'_{u_{\sigma(2)}}, \ldots, N'_{u_{\sigma(k)}})\) be a sequence such that \( N'_{u_{\sigma(i)+1}} = U(N'_{u_{\sigma(i)}}, u_{\sigma(i+1)}) \). Then, \( N'_{u_{\sigma(k)}} = N_{u_k} \).

Therefore, given a compatible sequence \((u_1, u_2, \ldots, u_r)\) of all the elements of \( R(N) \), and its associated sequence \((N, N_{u_1}, N_{u_2}, \ldots, N_{u_r})\), we define the *unfolding* of an IMLN \( N \), denoted by \( U(N) \), by means of the equation \( U(N) = N_{u_r} \). We may refer to such a sequence as a *sequence of unfoldings*. See Figure 1 for an example of a sequence of unfoldings for a phylogenetic network.

We can, given an IMLN \( N \), also define a partial order over the set of elementary nodes \( E(N) \) by saying that for any two \( u, v \in E(N) \), \( u \preceq v \) if and only if there exist \( u', v' \in E(N) \) with \( \ell(u) = \ell(u') \) and \( \ell(v) = \ell(v') \) and a directed path from \( v' \) to \( u' \). We call the set of elementary nodes that are maximal under this order \( E_{\text{max}}(N) \).

**Lemma 3.5.** Let \((N, N_{u_1}, N_{u_2}, \ldots, N_{u_s})\) be a sequence of unfoldings. For any \( N_{u_i} \) in it, there are exactly \( s = \deg_{\text{in}}(u_i) \) nodes in \( E(N) \), say \( v_1, \ldots, v_s \) such that the IMLT \( N_{u_i}(v_s), \ldots, N_{u_i}(v_s) \) are isomorphic.

Given an IMLN, \( u \in \mathcal{R}_{\text{min}}(N) \) and \( U(N,u) \), we would like to consider \( N \) to be the result of a folding operation over \( U(N,u) \): \( N = F(U(N,u),u) \), for some suitable \( F \). Given an ILMN \( N \) and an unfolding sequence \((N, N_{u_1}, N_{u_2}, \ldots, N_{u_r})\), we say that each of its members is a (phylogenetic) pseudo-network —in particular, they are IMLNs. Equivalently, we can define a pseudo-network recursively as follows: let \( N \) be an IMLN; it is a pseudo-network if it satisfies the following three conditions:
Let \( \text{Lemma 3.6.} \) IMLN of \( N \) be an IMLN and, \( \text{Corollary 3.7.} \) Let \( N \) be an IMLN without elementary nodes. Then

\[
\text{Figure 1. Top two figures: A phylogenetic network } N \text{ on } \{x_1, x_2, x_3, x_4\}, \text{ and its associated IMN with labeling function over } R(N) \text{ given by } \ell(u_i) = \lambda_i \text{ for } i = 1, 2, 3.
\]

The three figures below are the sequence of unfoldings \( (N_{u_2}, N_{u_3}, N_{u_1}) \) associated to the compatible sequence of reticulations \((u_2, u_3, u_1)\). Following the introduced terminology, \( N_{u_2} = U(N, u_2) \), \( N_{u_3} = U(U(N, u_2), u_3) \) and \( N_{u_1} = U(U(U(N, u_2), u_3), u_1) = U(N) \). Note that \( u_2, u_3 \in R_{\text{min}}(N) \) and \( u_1 \notin R_{\text{min}}(N) \), there is a path from \( u_1 \) to \( u_2 \) in \( N \).

(i) no reticulation node descends from an elementary node;
(ii) for any \( u \in E_{\text{max}}(N) \) there exists \( v \in E_{\text{max}}(N) \) such that \( \ell(u) = \ell(v) \) and \( N(u) = N(v) \) as IMLTs; and
(iii) for any \( u \in E_{\text{max}}(N) \), the IMN obtained by the process of
(1) considering the node \( v \in E_{\text{max}}(N) \) such that \( \ell(u) = \ell(v) \) and \( N(u) = N(v) \), and its parent(s) \( v^1 \) (and \( v^2 \));
(2) deleting the IMLT subtree rooted at \( v \), as well as the edge(s) \( (v^1, v) \) (and \( (v^2, v) \)); and
(3) adding arc(s) \( (v^1, u) \) (and \( (v^2, v) \)),
is also a pseudo-network.

The IMN obtained by the process described in (iii) is denoted by \( F(N, u) \), and called the **folded IMN** of \( N \) in \( u \). Notice that if \( u, v \in E_{\text{max}} \) are such that \( \ell(u) = \ell(v) \), then \( F(N, u) = F(N, v) \).

**Lemma 3.6.** Let \( N \) be an IMN and \( u \in R_{\text{min}} \). Then,

\[
F(U(N, u), u) = N.
\]

Given \( N \) an IMN and \( (N, N_{u_1}, N_{u_2}, \ldots, N_{u_r}) \) a sequence of unfoldings, by Lemma 3.6 we have that \( N_{u_i} = F(N_{u_{i+1}}, u_{i+1}) \) and that \( N = F(N_{u_1}, u_1) \). Therefore, we derive the following result.

**Corollary 3.7.** Let \( N \) be an IMN and \( (N, N_{u_1}, N_{u_2}, \ldots, N_{u_r}) \) any sequence of unfoldings. Then

\[
N = F(F(\ldots F(U(N, u_r), \ldots), u_2)u_1).
\]

If \( N \) is a pseudo-network we know that it is the product of a sequence of unfoldings performed over an IMN, \( N' \). We can then rewrite Corollary 3.7, by defining a function \( F \) from the set of pseudo-networks to the set of IMNs by \( F(N) := N' \). Hence,

**Corollary 3.8.** Let \( N \) be an IMN without elementary nodes. Then

\[
N = F(U(N)).
\]

4. A POLYNOMIAL INVARIANT FOR PHYLOGENETIC NETWORKS

Given a phylogenetic network \( N \) on \( X \), one can obtain a (rooted) tree by removing one incident arc to each reticulation node. An unrooted tree on \( X \) derived in this way where the direction of arcs are suppressed is called an **embedded spanning tree** of \( N \). Those trees characterize tree-child phylogenetic networks \([FM18]\), but not general ones. In \([JL21]\), the Liu polynomial is generalized to phylogenetic networks by their sets of embedded spanning trees. Roughly speaking, the polynomial of the network
is the product of the polynomials of the embedded spanning trees (considering trees with multiplicity). Consequently, this extension is an invariant for tree-child networks.

4.1. Extending the Liu polynomial. There are some natural extensions of the Liu polynomial to more general phylogenetic networks that come to mind. The first one is to completely unfold a phylogenetic network and, from any elementary node $u$ labelled $\lambda_i$, for some $i \in \{1, \ldots, r\}$, grow an arc to a new node $v$, label $v$ as $\lambda_i$, and finally forget the labelling of $u$. Thus, the unfolded IMLT becomes a multi-labelled tree over leaves $\{x_1, \ldots, x_n, \lambda_1, \ldots, \lambda_r\}$. See an example of that decomposition in Figure 2 from the phylogenetic network $N$ depicted in Figure 1. By means of Corollary 3.5 in [Liu21], this extension of the polynomial is immediately seen to uniquely characterize a phylogenetic network.

We will here deal with a natural extension that reflects the reticulation process in the sheer morphology of the polynomial, rather than in the name of the variables. Let $N$ be an IMLN as defined in the previous sections. Then, consider $p : V(N) \to \mathbb{Z}[x_1, \ldots, x_n, \lambda_1, \ldots, \lambda_r, y]$ to be defined recursively as follows: let $u \in V(N)$, then:

- if $u$ is a leaf, $p(u) = \varphi(u)$;
- if $u$ is an internal tree node whose two children are $v_1, v_2$, $p(u) = y + p(v_1)p(v_2)$; and
- otherwise, i.e. if $u$ has only one child $v$ and is associated to $\lambda_i = \ell(v)$, then $p(u) = \lambda_i p(v)$.

Then, let $\rho_N$ be the root of $N$; we define $p(N)$ to be $p(\rho_N)$.

For example, the polynomial associated to the phylogenetic network represented in Figure 1 is

$$
p(N) = y + y^2 + y^3 + \lambda_1\lambda_2x_2y^3 + \lambda_3x_3x_4y^2 +
+ \lambda_1\lambda_2x_1x_2y + \lambda_1\lambda_2x_1x_2y^2 + \lambda_1\lambda_2\lambda_3x_2x_3x_4y^2 +
+ \lambda_1^2\lambda_2^2\lambda_3^2x_3x_4^2y + \lambda_1^2\lambda_2^2\lambda_3^2x_3x_4^2y^2 +
+ \lambda_1^2\lambda_2^2\lambda_3^2x_3x_4^2y + \lambda_1^2\lambda_2^2\lambda_3^2x_3x_4^2y^2 +
+ \lambda_1^2\lambda_2^2\lambda_3^2x_3x_4^2y + \lambda_1^2\lambda_2^2\lambda_3^2x_3x_4^2y +
+ \lambda_1^2\lambda_2^2\lambda_3^2x_3x_4^2y^2 + \lambda_1^2\lambda_2^2\lambda_3^2x_3x_4^2y.
$$

**Proposition 4.1.** Let $N$ be an IMLN. Then, for any $u \in V(N)$, $p(u) \in \mathbb{Z}[x_1, \ldots, x_n, \lambda_1, \ldots, \lambda_r, y]$ is an irreducible polynomial if and only if $u$ is a tree node.

**Proof.** If $u$ is not a tree node the polynomial will not be irreducible, since then there would exist $v \in V(N)$ as the only descendant of $u$, and $p(u) = \ell(u)p(v)$.

It then remains only to see that if $u$ is a tree node, $p(u)$ is irreducible. In this case, either $u$ is a leaf and then $p(u) = \varphi(u) = x_i$ for some $i \in \{1, \ldots, n\}$ and so irreducible, or $u$ has two children and $p(u) = y + \Delta p(v)p(w)$, where $\Delta$ is a product of different $\lambda_i$ from $\lambda_1, \ldots, \lambda_r$ and $v, w$ are the first descendants from $u$ at each side that are tree nodes (possibly the same). Now consider the polynomial $p'(u)$ obtained from $p(u)$ by changing every variable $x_1, \ldots, x_n, \lambda_1, \ldots, \lambda_r$ for, say, $x_1$. Then, it is easy to see that $p'(u)$ satisfies Eisenstein’s irreducibility criterion in $\mathbb{Z}[y][x_1]$, and then $p(u)$ is irreducible when seen as a polynomial in $\mathbb{Z}[y][x_1, \ldots, x_n, \lambda_1, \ldots, \lambda_r]$. But, since $y$ does not divide $p(u)$, then $p(u)$ is irreducible in $\mathbb{Z}[x_1, \ldots, x_n, \lambda_1, \ldots, \lambda_r, y]$. \hfill $\Box$

**Theorem 4.2.** Let $N$ be an IMLT. Then, the polynomial $p(N)$ uniquely characterizes $N$ modulo paths of elementary nodes.
Proof. We proceed by complete induction over the (maximum) depth of \( N, d \). If \( d = 0 \), then \( N \) is only a leaf and therefore the statement trivially holds. If \( d = 1 \) there are two possibilities: either the root has only one child or it has two children; in either case, the children of the root must be leaves. In the first case the root is also an elementary node, say it is labelled by \( \lambda_1 \), and so the polynomial of \( N \) would be \( \lambda_1q_1 \), where \( q_1 \) is the label of the only child of the root.

Hence, assume this statement to hold up to a depth of \( d \), and let us make the case for an IMLT with depth \( d + 1 \), and let \( N_1, N_2 \) be two IMLT attaining that maximum depth, and let \( u_i \) be the first tree node descending from the root (which may or may not be the root itself), for \( i \in \{1, 2\} \).

Assume that \( p(N_1) = p(N_2) \). By construction, \( p(N_i) = \Lambda_i p(u_i) \) for \( i \in \{1, 2\} \), where \( \{p(u_i) \neq \langle \lambda_1, \ldots, \lambda_r \rangle \} \). Therefore, \( p(u_1) = p(u_2) \) must hold. Let \( v_i, w_i \) be the first tree nodes descendant from \( u_i \) at each side, for \( i \in \{1, 2\} \). Therefore, \( p(u_i) = y + \Lambda_i' p(v_i) p(w_i) \) for \( i \in \{1, 2\} \), where by construction neither \( p(v_i) \) nor \( p(w_i) \) have any independent term. Therefore, since \( y \) is an irreducible polynomial, we deduce that \( \Lambda_i' p(v_i) p(w_i) = \Lambda_i'' p(v_2) p(w_2) \) and hence again that \( p(v_1) p(w_1) = p(v_2) p(w_2) \). Both polynomials are irreducible by means of Proposition 4.1, and so either \( p(v_1) = p(v_2) \) and \( p(w_1) = p(w_2) \) or the other way around. In either case, since the IMLT subtrees rooted at \( v_1, v_2, w_1, w_2 \) have depths smaller than \( d + 1 \), we can apply the inductive hypothesis and conclude that \( N_1 \) and \( N_2 \) must be equal.

□

Given a sequence of unfoldings \( (N, N_{u_1}, N_{u_2}, \ldots, N_{u_r}) \), only \( N_{u_r} = U(N) \) is an IMLT. However, as we shall now see, the polynomial uniquely characterizes the sequence itself.

Proposition 4.3. Let \( N \) be a phylogenetic network, and \( (N, N_{u_1}, N_{u_2}, \ldots, N_{u_r}) \) be a sequence of unfoldings. Then, \( p(N) = p(N_{u_1}) \) and, for any \( i \in \{1, \ldots, r - 1\} \), \( p(N_{u_{i+1}}) = p(N_{u_i}) \).

Proof. Let \( N' \) be an IMLN, and \( u \in R_{\operatorname{min}}(N') \). If we are able to show that \( p(N') = p(U(N', u)) \), then the proposition will hold. Let \( v^1, v^2 \) be the parents of \( u \), in \( U(N', u) \) each of them will be the parent of at least one elementary node \( u_i \), \( i \in \{1, 2\} \), which will be the root of a copy of the IMLT \( N'(u) \), and by construction \( p(u_1) = p(u_2) = p(u) = p(N'(u)) \). Now, by the definition of the polynomial, \( p(u_i) \), \( i \in \{1, 2\} \), will be the same in \( N' \) and in \( U(N', u) \). Therefore, \( p(N') = p(U(N', u)) \).

□

Corollary 4.4. Let \( N_1, N_2 \) be two phylogenetic networks. Then, \( p(N_1) = p(N_2) \) implies that \( N_1 = N_2 \).

Since neither Proposition 4.1 nor Theorem 4.2 make any use of the different labels of the leaves of an IMLN, the arguments could be translated, mutatis mutandis, to rooted unlabelled phylogenetic networks; i.e. phylogenetic networks whose leaves are not labelled (although internal labels would still be necessary), modelled by labelling all leaves using a single variable \( x \), to give a polynomial in \( \mathbb{Z}[x, \lambda_1, \ldots, \lambda_r, y] \). This leads to the following proposition:

Proposition 4.5. Let \( N_1, N_2 \) be two unlabelled phylogenetic networks. Then, \( p(N_1) = p(N_2) \) implies that \( N_1 = N_2 \).

We end this section with two final remarks, the first concerning the interpretation of the coefficients and the second about the reconstruction of the unfolding of a network from the polynomial.

Remark 1. The interpretation of the coefficients of the polynomial \( p(N) \) can be extended from Lemma 2.4 in [LBGC20] by slightly modifying the definition of primary subtrees to the IMLT \( T = U(N) \). Consider in this case that a primary subtree \( S \) of \( T \) is a rooted subtree of \( T \) such that \( S \) shares the same root vertex with \( T \) and any leaf vertex of \( T \) is either a leaf vertex of \( S \) or a descendant of a leaf vertex of \( S \) which can not come from an elementary node. Then, if we represent \( p(N) \) as \( \sum c(\gamma_1, \ldots, \gamma_r, \alpha_1, \ldots, \alpha_n, \beta) \lambda_1^{\gamma_1} \cdots \lambda_r^{\gamma_r} x_1^{\alpha_1} \cdots x_n^{\alpha_n} y^\beta \), each one of its coefficients counts the number of primary subtrees of \( U(N) \) satisfying that:

- \( \gamma_i \) (from \( i = 1, \ldots, r \)) is the number of vertices labelled by \( \lambda_i \) of these subtrees;
- \( \alpha_i \) (from \( i = 1, \ldots, n \)) is the number of leaf vertices labelled by \( x_i \) of these subtrees which are also leaves in \( U(N) \); and
- \( \beta \) is the number of leaf vertices of these subtrees which are internal vertices in \( U(N) \).

See Figure 3 for the interpretation of some terms of the polynomial \( p(N) \) of the phylogenetic network \( N \) depicted in Figure 1. Notice that these primary subtrees can then be folded into a sort of “primary network”.

Remark 2. In order to reconstruct the Newick string of the IMLT \( U(N) \) from \( p(N) \), roughly speaking, we proceed as follows: Start by subtracting \( y \) from \( p(N) \) and then factor \( p(N) - y = q_1 \cdot q_2 \). Then the Newick string to consider is \( (q_1, q_2) \). From now on, whenever it is possible to subtract \( y \)
from a polynomial $q$, do so. When it is not possible, and the factorization involves only two members, $q = q_1 \cdot q_2$, then proceed as before and replace $q$ by $(q_1, q_2)$. Otherwise, there could be conflicts in terms of decide how to group members in a factorization of type

$$\prod_{j \in J \subseteq \{1, \ldots, r\}} \lambda_j \prod_{k} q_k,$$

where $q_k$ are polynomials. But, there will always in the queue of factorizations pending to be grouped, a pair of them where a “minimum” monomial of type $\lambda_i \cdot q_s$ is common in both; this allows to determine that there is an arc from an elementary node labelled by $\lambda_i$ to the subtree determined by the polynomial $q_s$. In terms of the Newick string, it could be replaced by $(\lambda_i(q_s))$.

5. Discussion and Conclusion

In this paper a new polynomial invariant for (binary) phylogenetic networks is introduced. This generalises results in both [LBGC20] for phylogenetic trees and in [JL21] for phylogenetic networks where their set of embedded spanning trees (like tree-child) characterizes it. The polynomial presented here is an invariant for general phylogenetic networks; it is not exclusive to specific subclasses of phylogenetic networks, as common with other invariants.

Establishing a polynomial invariant for phylogenetic networks opens the doors to several interesting opportunities for exploration, such as new ways to define metrics on networks, fast methods to distinguish networks, and possibly ways to extract important features of a network by examining the polynomial. To this end, it may be helpful to understand whether a particular polynomial is derived from a network (clearly not all irreducible polynomials give networks).

Furthermore, the computation of $p(N)$ here may be performed reticulation-by-reticulation for some network classes, e.g. orchard networks [ESS19]. That is, suppose that $N$ is an IMLN derived from an orchard network and $N = N_0, N_1, \ldots, N_k$ is a complete cherry reduction sequence of $N$ (that is $N_k$ is a single node). We can perform an assignment of polynomials to all leaves in every intermediate IMLN $N_j$. Finally, $p(N)$ is the polynomial assigned to the single node in $N_k$. Start by assigning $p(u) = \varphi(u)$, for every leaf $u$ in $N_0$. Then, let $\{v_1, v_2\}$ be the two leaves involved in the reduction to move from $N_j$ to $N_{j+1}$ and let $p(v_i)$ be the polynomial assigned to $v_i$ in $N_j$ for $i = 1, 2$. Then,

- if $\{v_1, v_2\}$ is a cherry, then we assign to the resulting leaf in $N_{j+1}$ the polynomial $y + p(v_1)p(v_2)$.
- if $\{v_1, v_2\}$ is a reticulated cherry (being $v_2$ the child of the reticulation labelled by $\lambda_i$), then we assign to the resulting leaf in $N_{j+1}$ coming from the parent of $v_1$ the polynomial $y + \lambda_i p(v_1)p(v_2)$, and to the resulting leaf in $N_{j+1}$ coming from the parent of $v_2$, the polynomial $\lambda_i p(v_2)$.

It would be interesting to investigate more optimisations for general or for specific subclasses of phylogenetic networks.

It is also interesting to think about ways to reduce the complexity of the polynomial assigned to a network; even at the expense of a loss the uniqueness of this assignment. One possibility, could be, for instance, if we define a polynomial for a phylogenetic network over the IMLT into which is transformed the network following a similar approach that allow the computation of its extended Newick format [CRV08b]. That is, for every reticulation, split it (also copying its label) in two copies, the first such copy with one of its parent and its child, and the other copy with the other parent and no children. See two examples of this decomposition in Figure 4 from the phylogenetic network $N$ depicted in Figure...
Clearly, this transformation process is not unique, and different IMLTs can be obtained from the same network; but different networks result in different sets of IMLTs. Notice that this process can be understood as a way to prune irrelevant subtrees of the IMLT $U(N)$ defined in Section 3, with the goal to keep enough information to code the network. Roughly speaking, to recover the network from these IMLTs one should only merge every pair of nodes labelled by the same $\lambda_i$. Applying the definition of the polynomial $p$ defined in Section 4.1 to these IMLTs, we obtain, for the example depicted in Figure 4 (a), the polynomial

$$p(N) = y + y^2 + y^3 + \lambda_1 y^3 + \lambda_1 \lambda_3 x_4 y^2 +$$
$$+ \lambda_1 \lambda_2 \lambda_3 x_2 x_3 y^2 + \lambda_1 \lambda_2 \lambda_3^2 x_2 x_3 x_4 y +$$
$$+ \lambda_1 \lambda_2 x_1 y + \lambda_1 \lambda_2 x_1 y^2 + \lambda_1 \lambda_2 \lambda_3 x_1 x_4 y +$$
$$+ \lambda_1^2 \lambda_2 x_1 y^2 + \lambda_2^2 \lambda_3 x_1 x_4 y +$$
$$+ \lambda_1^2 \lambda_2^2 \lambda_3 x_1 x_2 x_3 y + \lambda_1^2 \lambda_2^2 \lambda_3^2 x_1 x_2 x_3 x_4,$$

where (some of) the terms are notably simpler than in the original.

All the results presented in the paper are proved in the binary scenario, where the tree nodes have in-degree one and out-degree two, and reticulation nodes have in-degree two and out-degree one; but, we consider that it would not be overly complicated to generalise these for the non-binary case where tree nodes could have out-degree bigger than two and reticulations could have in-degree bigger than two.

There are potentially many further questions arising that relate to phylogenetic networks more broadly. For instance, do embedded spanning trees characterize general phylogenetic networks (with labelled reticulations)? That is, if we keep the labels on elementary nodes (which come from reticulation vertices) of the embedded spanning trees, can we extend the results in [FM18] from tree-child networks to more general networks?

With all this, it is to be hoped that the results here can stimulate these and many other investigations.

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