



Tree-like reticulation networks—When do tree-like distances also support reticulate evolution?



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ABSTRACT

Hybrid evolution and horizontal gene transfer (HGT) are processes where evolutionary relationships may more accurately be described by a reticulated network than by a tree. In such a network, there will often be several paths between any two extant species, reflecting the possible pathways that genetic material may have been passed down from a common ancestor to these species. These paths will typically have different lengths but an ‘average distance’ can still be calculated between any two taxa. In this article, we ask whether this average distance is able to distinguish reticulate evolution from pure tree-like evolution. We consider two types of reticulation networks: hybridisation networks and HGT networks. For the former, we establish a general result which shows that average distances between extant taxa can appear tree-like, but only under a single hybridisation event near the root; in all other cases, the two forms of evolution can be distinguished by average distances. For HGT networks, we demonstrate some analogous but more intricate results.

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1. Introduction

Evolutionary relationships between present-day taxa (species, genera etc.) are usually represented by a phylogenetic tree, which shows a branching pattern of speciation from some ancestral taxon to the taxa we observe today [1]. However, reticulate evolution is known to complicate this simple ‘tree model’ due to processes such as the formation of hybrid species [2], and other mechanisms where genetic material is exchanged between species (such as horizontal gene transfer (HGT)) or within a species (recombination, a process we do not consider further in this paper). Consequently, phylogenetic networks that allow ‘vertical’ branching through time as well as ‘horizontal’ reticulation events have increasingly been recognised as providing a more complete picture of much of the evolutionary history of life [3–5].

This transition has brought with it a number of mathematical and computational problems—in particular, how to reconstruct and analyse such networks, and how to distinguish different types of reticulation from tree-like evolution [6,7]. In this note we consider one aspect of the latter topic, namely the question of whether or not, if we knew the average evolutionary distance between each pair of species, we could determine whether the species network could have been a tree, or whether some more complicated reticulate history is required.

In a phylogenetic tree, the evolutionary distance between two present-day species is simply the path length from each species to the other via its most recent common ancestor (here, ‘evolutionary distance’ typically refers to the actual or expected amount of genetic change). However, for networks, there may be many paths linking two present-day species, and the evolutionary distance will be some average of these path lengths. Nevertheless, it is conceivable that in some cases, these distances might still appear to fit a tree exactly. We explore this question for two classes of networks: those relevant to hybrid evolution; and those relevant to HGT. Both are special cases of a more general description of (binary) ‘reticulation’ networks, which we now define.

1.1. Definitions: reticulation networks

Following Ref. [8], a *reticulation network* N on a finite set X is a rooted acyclic digraph (V, A) with the following properties:

- (i) the *root* vertex has in-degree 0 and out-degree 2;
- (ii) X is the set of vertices with out-degree 0 and in-degree 1 (‘leaves’);
- (iii) all remaining vertices are *interior vertices*, and each such vertex either has in-degree 1 and out-degree 2 (a *tree vertex*) or in-degree 2 and out-degree 1 (a *reticulation vertex*);
- (iv) the arc set A of N is the disjoint union of two subsets, the set of ‘reticulation arcs’ A_R and the set of ‘tree arcs’ A_T ; moreover each reticulation arc ends at a reticulation vertex, and each reticulation vertex has at least one incoming reticulation arc;

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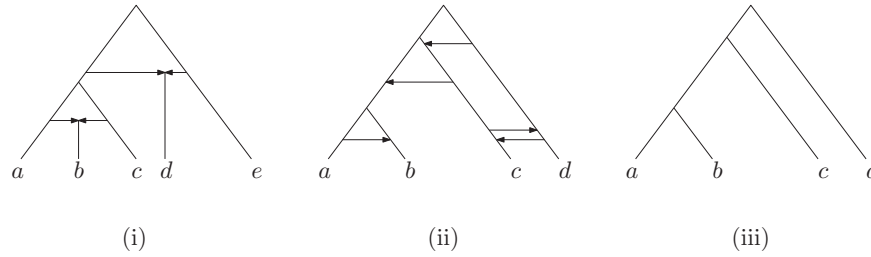


Fig. 1. (i) A hybridisation network on $\{a, b, c, d, e\}$ (usually extant species); (ii) an HGT network on $\{a, b, c, d\}$; and (iii) the tree T_N obtained from the HGT network N in (ii) by deleting all reticulation arcs. Reticulate arcs in (i) and (ii) are drawn as arrows; in each case the reticulate vertices are at the endpoints of the reticulate arcs. Note that (i) has four reticulation arcs and two reticulation vertices, while (ii) has five reticulation arcs and five reticulation vertices.

- (v) every interior vertex has at least one outgoing tree arc; and
- (vi) there is a function $t : V \rightarrow \mathbb{R}$ so that (a) if (u, v) is a tree arc then $t(u) < t(v)$, and (b) if (u, v) is a reticulation arc, then $t(u) = t(v)$.

Condition (vi) embodies the biological requirement that the network has a temporal representation that reflects the order of speciation events, and for which reticulation events involve two species that co-exist at some point in time.

In applications, X typically denotes a set of extant (present day) species. Two types of reticulation networks are particularly relevant in evolutionary biology (for different reasons, as we explain shortly) and these will be the main classes we will consider in this paper. The distinction is in the pair of arcs ending at a reticulation vertex in property (iii). Namely,

- in a *hybridisation network*, both arcs ending in a reticulation vertex are reticulation arcs, and
- in a *horizontal gene transfer network*, exactly one of the arcs ending in a reticulation vertex is a reticulation arc.

A simple example of each type is shown in Fig. 1.

Hybridisation networks model settings where a new species arises from members of two lineages, a process that occurs in plants, fish, and some animals [2,9], while HGT models the situation where a gene (or genes) is transferred from one species to another (a process that is common in bacteria) [10].

2. Reticulation networks and average distances

2.1. Basic properties of reticulation networks

Firstly, observe that a reticulation network N on X has no reticulation vertices if and only if N is a rooted binary phylogenetic X -tree (as defined, for example, in Ref. [11]).

Moreover, any hybridisation network is necessarily a *tree-child network*; that is, from any interior vertex in N , there is a path to a leaf that avoids any reticulation vertex. Tree-child networks have a number of desirable combinatorial and computational properties (see e.g. Refs. [12,13]).

Hybridisation networks have bounded size once $n = |X|$ is specified, since such a network can have at most $n - 2$ reticulation vertices [14]. To see this, note that in any digraph, the sum of the out-degrees equals the sum of the in-degrees so we obtain:

$$2 + 2t + r = \sum_{v \in V} \text{deg}_{\text{out}}(v) = \sum_{v \in V} \text{deg}_{\text{in}}(v) = n + t + 2r, \quad (1)$$

where t and r refer to the number of tree vertices and hybridisation vertices, respectively. Note that each hybridisation vertex corresponds to two parent tree vertices, and hence $t \geq 2r$ in a hybridisation network. Eq. (1) gives $n = t + 2 - r$, and using $t \geq 2r$ we obtain:

$$r \leq n - 2. \quad (2)$$

A consequence of this bound is that, up to isomorphism, there are only finitely many hybridisation networks for any given n (the

enumeration of hybridisation networks has recently been investigated by McDiarmid et al. [14]).

By contrast, an HGT network with a given leaf set X can have arbitrarily many reticulation vertices, and so there are infinitely many HGT networks for a given X . However, an HGT network N has a useful property that is absent in a hybridisation network: an HGT network always has an associated canonical rooted binary phylogenetic X -tree T that is obtained from N by deleting all the reticulation arcs (and suppressing any resulting vertices that have both in-degree 1 and out-degree 1). We denote this tree with the notation T_N (an example is shown in Fig. 1).

Given any reticulation network N on X , suppose that for each reticulation vertex, we delete exactly one of the in-coming arcs. The resulting graph is a rooted tree with leaf set X and a root that coincides with the root of N . Moreover, if we suppress any resulting vertices that have both in-degree 1 and out-degree 1 we obtain a rooted binary phylogenetic X -tree, T . We say that T is *displayed* by N and we let $\mathcal{T}(N)$ denote the set of all the (at most) 2^r such trees that are displayed by N .

2.2. Tree metrics

Consider any unrooted phylogenetic X -tree $T = (V, E)$ together with a weight function $w : E \rightarrow \mathbb{R}^{>0}$ that assigns strictly positive weights to each edge of the tree. Then (T, w) induces a distance function on X as follows: For each pair of leaves x, y on a tree T , the *tree distance* between them is defined as the sum of the weights of the edges that lie on the (unique) path in T connecting x and y . That is:

$$d_{(T,w)}(x, y) := \sum_{e \in P(T;x,y)} w(e),$$

where if $x = y$ we set $d_{(T,w)}(x, y) = 0$ (the empty path has length zero). The resulting function $d_{(T,w)} : X \times X \rightarrow \mathbb{R}^{\geq 0}$ is a metric on X .

A metric on X that can be represented in this way on some phylogenetic X -tree is said to be a *tree metric*. This holds if and only if the metric satisfies the ‘four-point condition’. This states that for any four (not necessarily distinct) points u, v, w, y from X , two of the three sums $d(u, v) + d(w, y)$; $d(u, w) + d(v, y)$; $d(u, y) + d(v, w)$ are equal, and are greater than or equal to the other one. This classic characterisation of tree metrics dates back to the 1960s (for more recent treatments, see Refs. [11,15]). Moreover, if d is a tree metric on X , then d can be written $d = d_{(T,w)}$ for precisely one choice of the pair (T, w) , where T is a phylogenetic X -tree, and w a strictly positive edge weight function. In the case where T is binary, we will say that d is a *binary tree metric*.

2.3. Average distances on networks

A reticulation network can be thought of as a ‘weighted union’ of the trees displayed by N . We formalise this idea, and extend it to bring in distances, as follows:

For each vertex v in the set V_R of reticulation vertices of N , let $R(v)$ denote the two arcs that end at v . Suppose we are given a reticulation network $N = (V, A)$ on X along with:

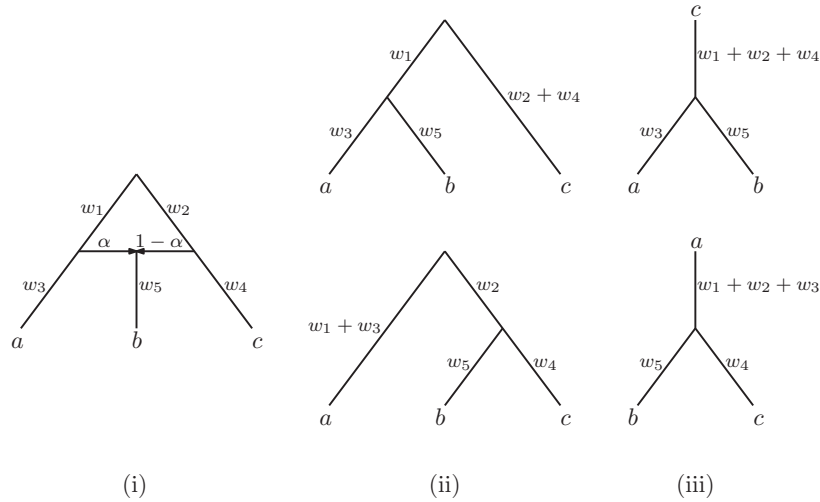


Fig. 2. (i) A simple hybridisation network N with edge weights w , and reticulation values $\alpha, 1 - \alpha$. (ii) The two rooted trees in $\mathcal{T}(N)$ that are displayed by N , together with their associated edge weights. (iii) The unrooted trees from (ii), which have the same topology, even though the trees in (ii) do not. For this example, the network distance between a and b is given by $d_N(a, b) = \alpha(w_3 + w_5) + (1 - \alpha)(w_1 + w_2 + w_3 + w_5)$.

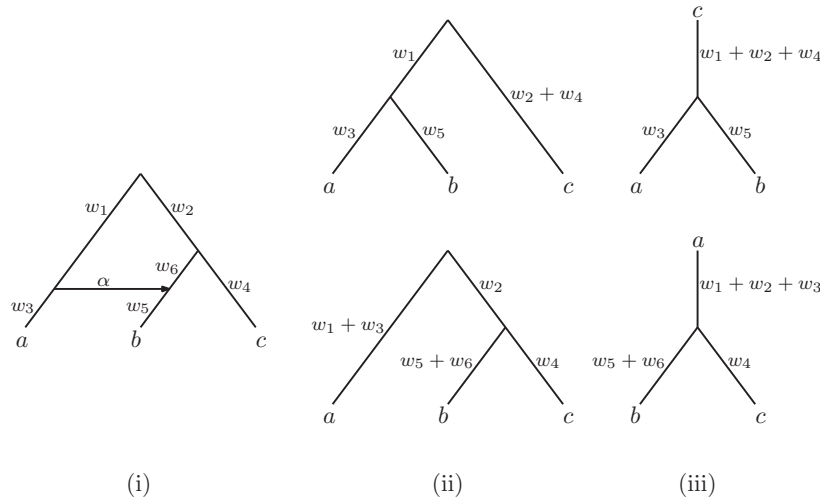


Fig. 3. (i) A simple HGT network with edge weights w , and reticulation value α on the unique reticulation arc, and $1 - \alpha$ on the incident tree arc that has weight w_6 . (ii) The two rooted trees in $\mathcal{T}(N)$ that are displayed by N , together with their associated edge weights. (iii) The unrooted trees from (ii). For this example, the network distance between a and b is given by $d_N(a, b) = \alpha(w_3 + w_5) + (1 - \alpha)(w_1 + w_2 + w_3 + w_5 + w_6)$.

- a weight function $w : A_T \rightarrow \mathbb{R}^{>0}$ that assigns weights to each tree arc; and
- a strictly positive probability distribution β on the set F_N of functions $f : V_R \rightarrow A$ for which $f(v) \in R(v)$.

In evolutionary biology, the weighting w typically describes some measure of genetic change along each tree arc, and each function $f \in F_N$ indicates the line of descent of a particular gene, and so describes a tree T_f in $\mathcal{T}(N)$. Notice that $|F_N| = 2^r$ though it may be possible for different functions f to lead to the same rooted phylogenetic tree (possibly with different tree metrics). For a given $f \in F_N$, its β -value, denoted β_f , can be thought of as the expected proportion of genes that follow the tree T_f . Since $\sum_f \beta_f = 1$, we call β the ‘mixing distribution’ of the network.

For example, suppose we have two arcs a and a' that end at the reticulate vertex v , and a function $\alpha : \{a, a'\} \rightarrow \mathbb{R}^{>0}$ satisfying $\alpha(a) + \alpha(a') = 1$ (such a function α could indicate the proportion of genetic material that is contributed from each of the two parent lineages when a reticulation occurs). When there is just a single reticulation, the mixing distribution β can be identified with the α function for the single reticulation vertex. However, when more than one reticulation vertex is present, one needs to consider how the

different reticulation events might interact. In the simplest case, we might treat the reticulations as (stochastically) independent events (with α now being regarded as assigning probabilities rather than proportions) so that the resulting randomly generated function f would have probability $\beta_f = \prod_{v \in V_R} \alpha(f(v))$. This assumption of independence is very strong and is more than we require here. Indeed, all that we require is that the mixing distribution β of the network satisfies $\beta_f > 0$ for all $f \in F_N$.

We now define the distance induced by a reticulation network with weighted tree arcs and a mixing distribution. For such a triple (N, w, β) we define

$$d = d_{(N, w, \beta)} : X \times X \rightarrow \mathbb{R}^{\geq 0}$$

by

$$d(x, y) = \sum_{f \in F_N} \beta_f d_{(T_f, w_f)}(x, y),$$

where w_f is the edge weight induced by N on T_f . If there are no reticulation vertices in N , so that N is a rooted phylogenetic X -tree T , we take d to be the tree metric $d_{(T, w)}$. We illustrate these ideas for a hybridisation network and an HGT network in Figs. 2 and 3, respectively.

Notice that $d_{(N,w,\beta)}$ is always a metric on X , since it is a convex combination of tree metrics on X .

Some detail is known about the conditions that govern when a metric can be represented on a reticulation network. In Ref. [16], Willson shows how a network can be reconstructed from average distances, given that one knows the underlying network graph already. There are some uniqueness properties, including for the reticulation probabilities (α).

In Ref. [17], Willson shows how one can generate the underlying network graph from the average distances, under some hypotheses. He shows that if there is only one reticulation, it can be done, and he provides an algorithm for this. There are some necessary conditions on the average distance function, and which are sufficient if there is only one reticulation.

In this paper, we study conditions under which a tree metric can be represented on a network. We impose no conditions on the distance function.

Network distances arise in a range of models in molecular genetics, for example those in which DNA sequences undergo site mutations along the tree arcs, and for which (i) at speciation events (tree vertices) the sequences on the two outgoing arcs are identical to the sequence at the end of the incoming arc, and (ii) at reticulation vertices, the state at each site is selected from the state at the same position at the end of either one of the two incoming arcs. In such a hybridisation network N on X , the history of the i th position for each species in X traces back according to one of the trees in $\mathcal{T}(N)$. Now, consider the Hamming distance d_H between pairs of species from the set X (so $d_H(x, y)$ is the proportion of sites where taxon x and taxon y differ). For binary sequences, suppose each site mutates at most once in the network (the so-called ‘infinite sites model’ [18]) and at each reticulation vertex the state at each site is (independently) selected to match the state at the site from one of its two incoming arcs with the prescribed α probability values. Then the expected Hamming distance \overline{d}_H on X satisfies $\overline{d}_H = d_{(N,w,\beta)}$, where the weight w of a tree arc (u, v) is the proportion of sites for which a mutation occurs along (u, v) .

2.4. Tree metrics from a network

We will show (Proposition 1) that if each tree in $\mathcal{T}(N)$ is isomorphic to the same (unrooted) phylogenetic X -tree, then the network induces a distance that is tree-like and behaves nicely with respect to the weights. On the other hand, if exactly two different unrooted trees are present in $\mathcal{T}(N)$, then the distance function induced by the network is never tree-like. The proof of this result can be found in Section 6.

Proposition 1.

- (a) Suppose that all the trees in $\mathcal{T}(N)$ are isomorphic as unrooted phylogenetic X -trees to some tree T . Then d_N is a tree metric that is represented by T .
- (b) If the trees in $\mathcal{T}(N)$ can be partitioned into two non-empty isomorphism classes of unrooted trees, then d_N is not a tree metric.

3. Hybridisation networks

In this section, we are interested in whether or not a tree metric can be realised on a hybridisation network and, conversely, whether

a hybridisation network might induce a distance that fits perfectly on some tree. In order to state our main result, Theorem 2, we introduce a further definition: we call a hybridisation network with k hybridisations a k -hybridisation network, and we call a 1-hybridisation network for which the two reticulation arcs have their source vertices adjacent to the root a primitive 1-hybridisation network. The proof of this theorem is given in Section 6.

Theorem 2. Let X be a finite set of taxa, and suppose d is a metric on X , $d : X \times X \rightarrow \mathbb{R}^{\geq 0}$.

- (a) If d is a binary tree metric, then there exists a primitive 1-hybridisation network N and weights w, β such that $d = d_{(N,w,\beta)}$.
- (b) If N is a hybridisation network, and $d = d_{(N,w,\beta)}$ is a tree metric for some $w, \beta > 0$, then N is either a tree, or N is a primitive 1-hybridisation network.

One way to rephrase the key point of this theorem is that a tree metric can be represented on a k -hybridisation network if and only if either $k = 0$ or $k = 1$ and the hybridisation is placed near the root. But there is slightly more here, in that any hybridisation network that is not a tree, and that admits a tree metric must be a primitive 1-hybridisation network. We are also able to count such networks, showing that there are $4(n - 3)$ such primitive 1-hybridisation networks on n taxa for each tree metric (Proposition 3).

3.1. The number of hybridisation networks for each tree metric

There is a unique unrooted tree for each tree metric. Theorem 2 means that for each rooted tree we have at least one primitive 1-hybridisation network. But how many do we in fact have?

Proposition 3. For each tree metric on n leaves, there are $4(n - 3)$ 1-hybridisation networks that realise the metric.

Proof. Each of the $n - 3$ internal edges on the unrooted tree corresponds to a choice for the root, and each one gives four distinct 1-hybridisation networks, since each of the four subtrees nearest the root could be the one descending from the hybridisation (see Fig. 4). Each hybridisation network from a root placed on an external edge can also be obtained from a root placed on an internal edge, and so this adds no new hybridisation networks. □

4. HGT networks

Our main result for hybridisation networks (in the previous section) applies only in one direction for HGT networks. If a single reticulation occurs between the arcs of N that are incident with the root then we obtain a tree metric. However, for HGT networks, it is possible for tree metrics to arise under other scenarios, both for a single reticulation event, and for multiple ones. We now describe two results that demonstrate how this can occur. Recall that T_N is the rooted phylogenetic X -tree associated with an HGT network N , obtained by deleting all the reticulation arcs.

Lemma 4. For any HGT network N , if each reticulation arc is between adjacent tree arcs of T_N , then d_N is tree-like on T_N .

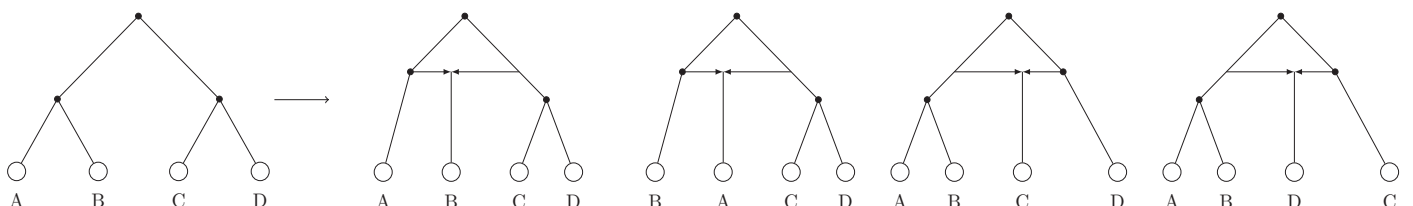


Fig. 4. The four distinct reticulated trees arising from choices of reticulation on a single internal edge of T , shown with the root placed on it on the left hand figure. A, B, C and D represent subtrees of T with the root placed at an internal node.

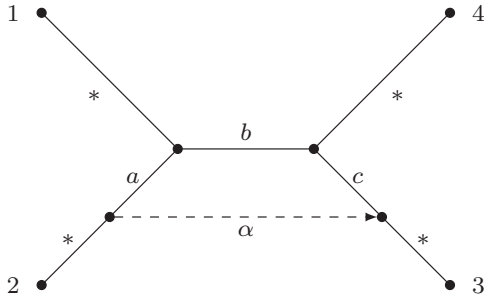


Fig. 5. The generic case of a single HGT from a tree arc to a non-adjacent tree arc. The rooting of the tree has been suppressed to simplify the analysis; however, there are six locations where the root can be placed to subdivide the tree arcs shown (the arc labelled * leading to leaf 3 cannot contain the root, as this would create a directed cycle in the network, but any other tree arc can). Here * denotes weights that occur in each quartet sum in the four-point condition and that hence can be ignored.

Proof. If N has the property described, then every tree in $\mathcal{T}(N)$ is isomorphic as a rooted phylogenetic X -tree to T_N , and so these two trees are also isomorphic as unrooted trees. The lemma now follows from Proposition 1(a). \square

Our main result for this section is the following.

Theorem 5.

- (a) If an HGT network N has a single reticulation arc, then d_N is tree-like if and only if that arc is either (i) from one arc to an adjacent arc or (ii) between a root arc and one of the two children of the other root arc. Moreover, this holds for any (positive) parameters on N , and the only tree that harbours a representation for d_N is T_N .
- (b) There exist 2-reticulated HGT networks N that can be represented on T_N and (for other parameter settings) on a tree that is different from T_N , even when the mixing distribution treats the two reticulations independently.

Proof. Part (a): For the ‘if’ part, condition (i) suffices by Lemma 4. For case (ii), we note that although the two trees in $\mathcal{T}(N)$ are no longer isomorphic to the same rooted phylogenetic X -tree, they are isomorphic to the same unrooted phylogenetic X -tree, so Proposition 1 applies.

For the ‘only if’ direction, suppose that neither condition (i) nor (ii) is satisfied. That is, the reticulation arc is not between adjacent arcs and not from a root arc to one of the two children of the other arc. There is a quartet then in which the reticulation is between non-adjacent and non-root arcs, in which case, if we suppress the location of the root, it corresponds to the scenario shown in Fig. 5, up to permutation of the leaves.

Let us abbreviate the sums of distances arising in the four-point condition as $S_1 = d(1, 2) + d(3, 4)$, $S_2 = d(1, 3) + d(2, 4)$ and $S_3 = d(1, 4) + d(2, 3)$. Ignoring the terms that appear in every sum (shown as * in Fig. 5), the quartet distance sums in the case shown in Fig. 5 are:

$$S_1 = a + [\alpha(a + b) + (1 - \alpha)c],$$

$$S_2 = [(1 - \alpha)(b + c) + \alpha a] + (a + b),$$

$$S_3 = b + [(1 - \alpha)(a + b + c)].$$

Noting that $S_1 < S_2$ since $\alpha < 1$, for these quartets to satisfy the four-point condition we must have $S_2 = S_3$. However, this implies that either $a = 0$ or $\alpha = 0$, which is a contradiction.

Part (b): It suffices to provide an example. Consider the 2-reticulated network shown in Fig. 6, where HGT events represented by the branches labelled α and α' occur independently (the independence model, as described in Section 2.3).

We have the following quartet distances involved in the four-point condition, with S_i being as defined in (a):

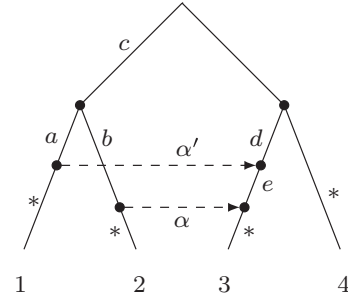


Fig. 6. A 2-reticulated HGT network N that can be represented on a tree that is different from T_N . Here, we assume that $a, b, c, d, e > 0$ and $0 < \alpha, \alpha' < 1$.

$$\begin{aligned} S_1 &= [a + b] + [(1 - \alpha)(1 - \alpha')(d + e) + \alpha(b + c) \\ &\quad + (1 - \alpha)\alpha'(a + c + e)] \\ &= (1 + (1 - \alpha)\alpha')a + (1 + \alpha)b + (\alpha + (1 - \alpha)\alpha')c \\ &\quad + (1 - \alpha)(1 - \alpha')d + (1 - \alpha)e, \\ S_2 &= [(1 - \alpha)(1 - \alpha')(a + c + d + e) + (1 - \alpha)\alpha'e \\ &\quad + \alpha(a + b)] + [b + c] = (1 - (1 - \alpha)\alpha')a + (1 + \alpha)b \\ &\quad + (1 + (1 - \alpha)(1 - \alpha'))c + (1 - \alpha)(1 - \alpha')d + (1 - \alpha)e, \\ S_3 &= [a + c] + [(1 - \alpha)\alpha'(a + b + e) + (1 - \alpha)(1 - \alpha')(b + c \\ &\quad + d + e)] = (1 + (1 - \alpha)\alpha')a + (1 - \alpha)b \\ &\quad + (1 + (1 - \alpha)(1 - \alpha'))c + (1 - \alpha)(1 - \alpha')d + (1 - \alpha)e. \end{aligned}$$

In the underlying tree (T_N) of the network we have S_1 as the smaller of these, so that $S_1 \leq S_2 = S_3$. The equality of S_2 and S_3 requires $\alpha b = (1 - \alpha)\alpha'a$, and $S_1 \leq S_2$ implies $\alpha'a \leq (1 - \alpha')c$. Together we require $\alpha b = (1 - \alpha)\alpha'a \leq (1 - \alpha)(1 - \alpha')c$,

which is certainly possible for some regions of the parameter space.

However, there are alternative solutions, as required by the theorem. For instance, it is possible to have S_3 as the shortest of the three quartet distances, so that $S_3 \leq S_2 = S_1$. This is possible so long as

$$(1 - \alpha)(1 - \alpha')c = (1 - \alpha)\alpha'a \leq \alpha b.$$

Just to be explicit, this is possible whenever, for example, $\alpha \geq \frac{1}{2}$, $b \geq a$ and $\alpha'a = (1 - \alpha')c$. The unrooted tree that realises this metric has taxa 1 and 4 together and taxa 2 and 3 together (14|23), and is not T_N . This completes the proof of Theorem 5. \square

5. Discussion and further questions

The four point condition provides a very precise characterisation, in terms of pairwise distances between taxa, of the circumstances under which a metric is able to be displayed on a tree (see Section 2.2). It is so successful that it is tempting to assume that once a metric satisfies this condition then we have a tree, and that this is the end of the story. However the results in this paper show that such ‘tree metrics’ can also be realised by hybridisation and HGT networks. Any surprise at this conclusion may be partly due to the biconditional statement of the four point condition; namely that a metric is a tree metric if and only if it satisfies the condition. Superficially this appears to leave little room to manoeuvre. However, as we show, being realised on a tree does not preclude the possibility that the metric can also be realised on a reticulation network.

The practical implication of this wriggle-room is that phylogenies displaying tree metrics may in fact involve hybridisation or horizontal gene transfer in their histories. However, the results in this paper also show that ‘all hell is not about to break loose’: for the network to be a hybridisation network, strict restrictions apply (Theorem 2). In particular, there can be at most one hybridisation event and it must be adjacent to the root. However, such restrictions do not hold for

HGT networks (Theorem 5). In this case there is some control when the network contains a single reticulation, but surprisingly, it is also possible to have a tree metric displayed on an HGT network with more than one reticulation.

While it is biologically unlikely for a single network to contain both hybridisation and HGT events, our results suggest several intriguing questions for further study. For instance:

1. It would be interesting to determine how far Theorem 5(b) extends. For example, is the following true? For any two binary phylogenetic X -trees T_1 and T_2 (where X can be of any size), is there an HGT network for which $T_N = T_1$ and yet where d_N is representable on T_2 (where the mixing distribution is given by the independence model)?
2. How do our results change if we allow some leaves to be missing (due to extinction or sampling omission)?
3. Let $\rho(d)$ denote the minimum number of hybridisations required to represent d on a hybridisation or an HGT network. What conditions characterise those metrics d with $\rho(d) = 1$? What about $\rho(d) = k$ for any $k \geq 1$?

6. Materials and methods

6.1. Proof of Proposition 1

In the following, for a subset q of X of size 4 (a ‘quartet’), we use $T|q$ to denote the phylogenetic tree with leaf set q that is induced by the X -tree T on q . Moreover, if $q = \{x, y, w, z\}$, we write $T|q = xy|wz$ if the path in T connecting x and y is vertex-disjoint from the path in T connecting w and z .

Recall the statement of Proposition 1:

Proposition 1.

- (a) Suppose that all the trees in $\mathcal{T}(N)$ are isomorphic as unrooted phylogenetic X -trees to some tree T . Then d_N is a tree metric that is represented by T .
- (b) If the trees in $\mathcal{T}(N)$ can be partitioned into two non-empty isomorphism classes of unrooted trees, then d_N is not a tree metric.

Both parts of Proposition 1 follow from the respective parts of the following Lemma 6, noting that in part (b), if two trees are non-isomorphic as unrooted trees, then they must resolve at least one quartet differently.

Lemma 6. Let $(T_1, w_1), (T_2, w_2), \dots, (T_k, w_k)$ be a sequence of phylogenetic X -trees with associated strictly positive edge weights.

- (a) If $T_i = T$ for all i , then for any values $\beta_i \in \mathbb{R}^{\geq 0}$, we have:

$$\sum_{i=1}^k \beta_i d_{(T, w_i)} = d_{(T, w)}$$

for the positive edge weights $w = \sum_i \beta_i w_i$ on T .

- (b) Suppose that there is a quartet $q \subseteq X$, for which $|\{T_i|q, i = 1, \dots, k\}| = 2$. Then for any values $\beta_i \in \mathbb{R}^{> 0}$, we have:

$$\sum_{i=1}^k \beta_i d_{(T_i, w_i)} \neq d_{(T, w)}$$

for any phylogenetic X -tree T having non-negative edge weights w .

Proof. Part (a): By the definitions and the interchange of the order of summation we have, for any $x, y \in X$:

$$\sum_i \beta_i d_{(T, w_i)}(x, y) = \sum_i \beta_i \sum_{e \in P(T; x, y)} w_i(e) = \sum_{e \in P(T; x, y)} w(e) = d_{(T, w)}(x, y).$$

Part (b): Suppose that $q = \{x, y, w, z\}$ satisfies the condition stated, with $T_j|q = xy|wz$ for all $j \in J \subseteq [k] = \{1, \dots, k\}$, and $T_j|q = xz|wy$ for all $j \in [k] - J$, for some non-empty proper subset J of $[k]$. Let $d_1 = \sum_{j \in J} \beta_j d_{(T_j, w_j)}$, $d_2 = \sum_{j \in [k] - J} \beta_j d_{(T_j, w_j)}$ and $d = d_1 + d_2 = \sum_{j \in [k]} \beta_j d_{(T_j, w_j)}$. By Part (a) and the four-point condition, we have:

$$d_1(x, y) + d_1(w, z) < d_1(x, w) + d_1(y, z) = d_1(x, z) + d_1(y, w);$$

and

$$d_2(x, z) + d_2(w, y) < d_2(x, y) + d_2(w, z) = d_2(x, w) + d_2(y, z).$$

It follows that:

$$\begin{aligned} d(x, w) + d(y, z) &= (d_1(x, w) + d_1(y, z)) + (d_2(x, w) + d_2(y, z)) \\ &> (d_1(x, y) + d_1(w, z)) + (d_2(x, y) + d_2(w, z)) \\ &= d(x, y) + d(w, z). \end{aligned}$$

Similarly $d(x, w) + d(y, z) > d(x, z) + d(y, w)$. Therefore,

$$d(x, w) + d(y, z) > \max\{d(x, y) + d(w, z), d(x, z) + d(y, w)\},$$

violating the four-point condition. Thus d has no realisation on any unrooted phylogenetic tree (binary or not) with non-negative edge weights. \square

6.2. Proof of Theorem 2

Recall the statement of Theorem 2:

Theorem 2. Let X be a finite set of taxa, and suppose d is a metric on X , $d : X \times X \rightarrow \mathbb{R}^{\geq 0}$.

- (a) If d is a binary tree metric, then there exists a primitive 1-hybridisation network N and weights w, β such that $d = d_{(N, w, \beta)}$.
- (b) If N is a hybridisation network, and $d = d_{(N, w, \beta)}$ is a tree metric for some $w, \beta > 0$, then N is either a tree, or N is a primitive 1-hybridisation network.

The proof of Theorem 2 relies on first establishing some preliminary results.

Lemma 7. The two binary phylogenetic X -trees displayed by a 1-hybridisation network are isomorphic as unrooted trees if and only if the tree is a primitive 1-hybridisation network.

Proof. The ‘if’ part is clear. Conversely, suppose that a 1-hybridisation network is not primitive. Then if (u, v) and (u', v) denote the two reticulation arcs, there is a vertex w of N that has a leaf z as a descendant (following a path of tree edges) that is not a descendant of u, u' or v . Select leaves x, x' and y that are descendants (following a path of tree arcs) of u, u' and v , respectively. Then for the two induced phylogenetic X -trees obtained from N , one tree resolves the quartet $\{x, x', y, z\}$ as $xy|x'z$, while the other tree resolves the quartet as $x'y|xz$ (as above, the vertical bar here refers to the path that connects the pair of taxa on the left being vertex-disjoint from the path connecting the pair of taxa on the right). It follows that these two induced X -trees are not isomorphic as unrooted trees [11]. \square

Corollary 8. In a 1-hybridisation network, with an edge weighting w and a mixing distribution β , the induced distance function $d_{(N, w, \beta)}$ is equal to $d_{(T, w)}$ for a phylogenetic X -tree if and only if the hybridisation is between the two edges that are incident with the root.

Proof. By combining Proposition 1 and Lemma 7. \square

We have dealt with the case in which a network has a single hybridisation and shown that for it to satisfy the four-point condition (i.e. be a tree metric) the hybridisation must be in a particular position, namely next to the root (the network must be a primitive 1-hybridisation network). This is because both quartet trees must

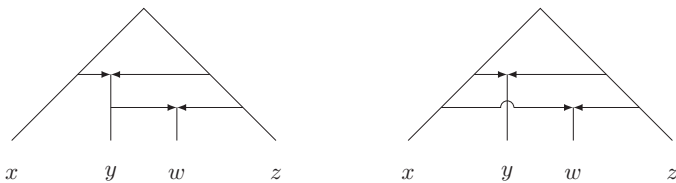


Fig. 7. The distinct quartets with two hybridisations, up to labelling.

agree, and the only way for this to occur is if the hybridisation is in this position.

To deal with the case where the network has more than one hybridisation, we require a further result.

Lemma 9. *If a hybridisation network N has four leaves and two hybridisation vertices then if we consider the (at most four) trees in $\mathcal{T}(N)$ and ignore their rooting, they produce exactly two unrooted quartet trees.*

Proof. This is an elementary check, as follows. There are only two 2-hybridisation networks on four leaves, up to symmetry, namely those in Fig. 7.

Resolving these hybridisations into the alternative unrooted quartet trees, we find that the first hybridisation network only yields the unrooted quartet trees $xy|wz$ and $xz|yw$, and the second yields $xy|wz$ and $xw|yz$. \square

We can now prove Theorem 2.

Proof of Theorem 2. *Part (a):* Suppose d is a binary tree metric. Writing $d = d_{(T,w)}$ for a binary tree T , select any interior vertex v of T , and consider the three edges e_1, e_2, e_3 that are incident with v , their corresponding weights w_1, w_2 and w_3 and the rooted subtrees T_1, T_2 and

T_3 that these edges are attached to, at the opposite end from v , as in Fig. 8(i). Let N be the primitive 1-hybridisation network that is obtained as follows: first, consider the rooted binary tree T consisting of a root vertex attached by edges e_1 and e_2 to the roots of T_1 and T_2 . Next, place reticulation arcs from (a point on each of) e_1 and e_2 to a reticulation vertex, and place a tree arc from this vertex to the root of T_3 . Select any strictly positive value of x with $x < \min\{w_1, w_2\}$, and assign edge weights to N as follows. To the two edges that are incident with the root vertex assign weight x ; to the tree arcs that are incident with T_1 and T_2 assign weights $w_1 - x$ and $w_2 - x$, respectively, and to the tree arcs incident with T_3 , assign the weight w_3 . To the reticulation arcs assign a uniform hybridisation distribution ($\alpha_1 = \alpha_2 = \frac{1}{2}$) (see Fig. 8(ii)). Then it can be checked that N , together with this arc weighting and hybridisation distribution, gives a distance function that coincides exactly with d .

Part (b): Suppose a network N has more than one hybridisation. We first consider the case where there is at least one reticulation arc (u, v) whose source vertex (u) lies below a non-root vertex w that has two outgoing tree arcs. Let (u', v) be the other reticulation arc of N that ends at reticulation vertex v . We will construct a quartet of leaves that give rise to a non-primitive 1-hybridisation network.

Let a, b and c be three leaves obtained by following tree arcs from u, v and u' , respectively (every internal vertex has at least one outgoing tree arc by the definition of a reticulation network, part (v)). Choose a fourth leaf d that is reached by an arc from the root that does not pass through u or u' . This can always be done; consider two cases. First, if w lies above u' as well as u , then paths from the root that do not go through w will also not go through u or u' . Alternatively, there is a path from the root through w that goes down the “other” tree arc from w (the one not leading to u) that will not pass through u' . The restrictions of N to $\{a, b, c, d\}$ in these two cases are

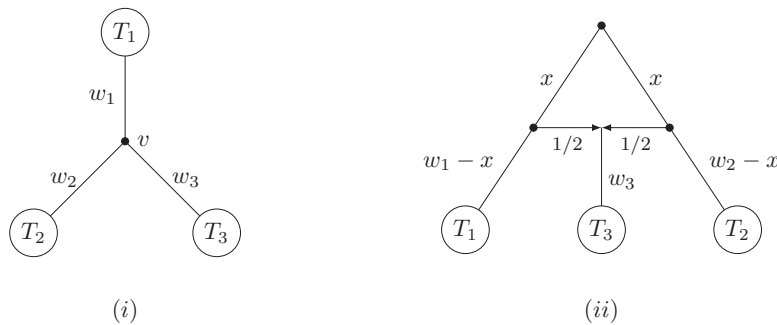


Fig. 8. T_i represents rooted subtrees of T . As in the proof of Theorem 2 Part (a), we have an unrooted tree and a primitive 1-reticulated network with the same average distance function.

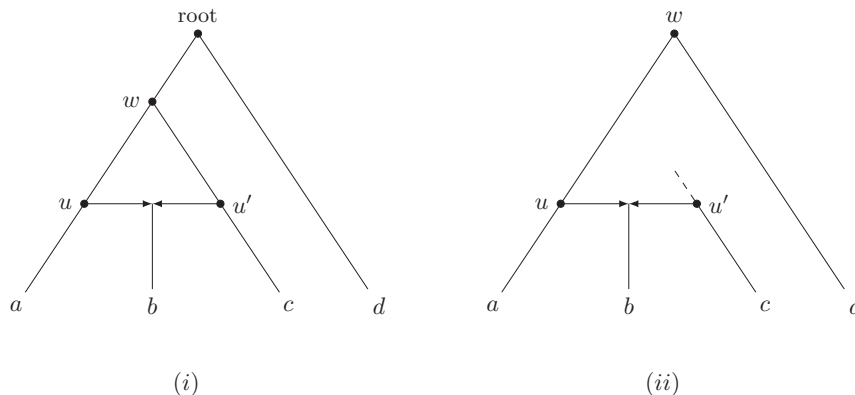


Fig. 9. The two cases arising in Part (b) of the proof of Theorem 2, showing the restriction of N to $\{a, b, c, d\}$. (i) shows the case that w is above both u and u' , and (ii) shows the case w is above u but not u' .

shown in Fig. 9. In either case, the restriction is a 1-hybridisation network that is not primitive, and so, by Corollary 8, does not induce a tree metric on $\{a, b, c, d\}$. Thus $d = d_{(N,w,\beta)}$ cannot be a tree metric on X .

Thus, we may suppose that if N has more than one hybridization, then none of the source vertices of any reticulation arc lies below any non-root vertex that has two outgoing tree arcs. That is, the source vertices of all reticulation arcs lie below either the root, or another source vertex of a reticulation arc (if a non-root vertex does not have two outgoing tree arcs, then it must have a reticulation arc). Another way to view this is that as one proceeds along any path from the root to a leaf, once one encounters a tree vertex one never encounters another reticulation vertex. This forces the reticulation vertices to be near the root, and for there to be a quartet in which at least two hybridizations appear (see Fig. 7). Therefore, since $r \leq n - 2$ (Inequality (2)), exactly two hybridizations occur.

Such a quartet can be chosen simply by a suitable choice of leaves. By Lemma 9 and Proposition 1 this implies that d_N restricted to this quartet is not a tree metric on that quartet, which violates the assumption that d_N is a tree metric on all of X . Thus, N must be a 1-hybridization network. Lemma 7 and Proposition 1(b) now imply that N must also be primitive. This completes the proof. \square

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