Alternative Characterizations of Fitch’s Xenology Relation

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\textbf{Abstract}

According to Walter M. Fitch, two genes are xenologs if they are separated by at least one horizontal gene transfer. This concept is formalized through Fitch relations, which are defined as binary relations that comprise all pairs \((x, y)\) of genes \(x\) and \(y\) for which \(y\) has been horizontally transferred at least once since it diverged from the least common ancestor of \(x\) and \(y\). This definition, in particular, preserves the directional character of the transfer. Fitch relations are characterized by a small set of forbidden induced subgraphs on three vertices and can be recognized in linear time.

In this contribution, we provide two novel characterizations of Fitch relations and present an alternative, short and elegant proof of the characterization theorem established by Geiß et al. in J. Math. Bio 77(5), 2018.

\textbf{Keywords:} Fitch Xenology; Fitch Relation; Phylogenetic Tree; Forbidden Induced Subgraphs; Neighborhoods; Gene Evolution

1 Introduction

Genes are the molecular units of heredity holding the information to build and maintain cells. A gene family covers all homologous genes, that is, genes that share a common ancestor. During evolution, genes are mutated, duplicated, lost and passed to organisms through speciation or horizontal gene transfer (HGT), which is the exchange of genetic material among co-existing species. Therefore, the history of a gene family is equivalently described by a vertex- and edge-labeled rooted phylogenetic tree, called (event-labeled) gene tree, in which the leaves correspond to extant genes and the internal vertices to ancestral genes. The label of a vertex highlights the event at the origin of the divergence leading to the offspring, namely speciation and duplication, while edge-labels express whether an edge corresponds to HGT or not [14].

Homology relations are binary relations between homologous genes and are defined by the particular vertex- and edge-labels of the gene tree [13, 14, 26, 27]. Prominent examples of homology relations are the orthology and paralogy relation that contain all pairs of genes \((x, y)\) where the last common ancestor is a speciation and duplication event, respectively [2, 4, 11, 20, 24, 26, 28–30], RGC-relations that capture the structure of rare genomic changes (RGCs) [5, 10, 12, 21, 42] and the xenology relation that is defined in terms of HGT [9, 14, 16, 18, 19, 22].

Although, homology relations are defined by the true evolutionary history of the genes, which is usually not known with confidence, there are many methods that allow to infer certain types of
homology relations directly from genomic sequence data without requiring any a priori knowledge about the topology of either the gene or the species tree. This includes tools for estimating and resolving orthology assignments at the level of gene pairs, and thus, to derive orthology relations (see e.g. [3, 33–35, 46, 50]); methods to infer RGC-relations (see [21] for an overview) or methods to infer HGT by using certain characteristics of the genome sequences (see [32, 39, 40] for an overview). While best match heuristics have been very successful as approximations of the orthology relation [2, 37], the inference of HGT is still challenging. In particular, the inference of pairwise xenology relationships has not been satisfactorily solved. It is, however, not at all a hopeless task, since genes that are imported by HGT from an ancestor of species A into an ancestor of species B are expected to be more closely related than one could expect from the bulk of the genome [9, 38, 40].

Homology relations are of fundamental importance in many fields of mathematical and computational biology including the reconstruction of evolutionary relationships across species [5, 6, 8, 12, 17, 23, 24, 42], functional genomics and gene organization in species [10, 15, 27, 31, 41, 43, 44, 48, 49], and the identification and testing of proposed mechanisms of genome evolution [1, 36, 49]. It is therefore of central interest to understand whether such inferred relations are “mathematically correct”, i.e., whether there is an event-labeled gene tree that can explain the given relations and thus, provides some evidence about the inferred data.

By way of example, a mathematically correct orthology relation must form a co-graph (graphs that do not contain induced paths on four vertices) [7] and is associated to a unique co-tree, which is equivalent to a not necessarily fully resolved event-labeled gene tree that explains the given orthology relation [4, 20]. Empirically estimated orthology relationships in general violate the co-graph property, suggesting co-graph editing as a means to correct the initial estimate [11, 20, 23, 24, 28–30]. On genome-wide data sets, the cographs and their co-trees can then be used to even infer the evolutionary history of the underlying species [18, 23–25, 28]. Hence, understanding the mathematical structure of orthology helped to obtain deeper insights into the complex processes that drive molecular evolution.

This contribution is concerned with the mathematical structure of xenology, which is intimately related to HGT. The horizontal transfer of genomic material can be annotated in the gene tree by assigning a label to the edge that points from the horizontal transfer event to the next event in the history of the copy. The concept of xenology, as introduced by Walter M. Fitch [14], calls two homologous genes xenologs, if their history, since their common ancestor, involves a horizontal transfer for at least one of them [14, 26]. In other words, two genes \( x \) and \( y \) are xenologs if the unique path between \( x \) and \( y \) in the underlying event-labeled gene tree contains a transfer edge. The class of such relations has been characterized by Hellmuth et al. [19] and coincides with the class of complete multipartite graphs. Note that HGT is intrinsically a directional event, i.e., there is a clear distinction between the horizontally transferred “copy” and the “original” that continues to be vertically transferred. Preserving the directionality of horizontal transfer, Geiß et al. [16] formalized this concept and introduced Fitch relations, which comprise all pairs of genes \( (x, y) \) for which the unique path from the last common ancestor \( \text{lca}(x, y) \) to \( y \) in the gene tree contains a transfer edge. It has been shown by Geiß et al. [16] that Fitch relations are characterized by the absence of eight forbidden subgraphs on three vertices and can be recognized in linear time.

In order to understand Fitch relations in more detail, we provide in this contribution two additional characterizations, the first one is based on neighborhoods and the second one is based on forbidden subrelations on three vertices. The proof of the characterization theorem in [16, Thm. 2] is quite involved and very technical, and it includes plenty of case studies. We will use the new characterization to provide a novel, simpler and elegant proof of this theorem.

2 Preliminaries

**Basics.** For a finite set \( L \) we put \( [L \times L]_{\text{irr}} := (L \times L) \setminus \{ (x, x) : x \in L \} \) and \( (L^k) := \{ L' \subseteq L : |L'| = k \} \).

All binary relations considered here are irreflexive, and we omit to mention it each time and simply call them relations. A relation \( \mathcal{X} \) on \( L \) is a subset \( \mathcal{X} \subseteq [L \times L]_{\text{irr}} \).

We consider directed graphs \( G = (V, E) \) with finite vertex set \( V \) and edge set \( E \subseteq [V \times V]_{\text{irr}} \) and
thus, $G$ does not contain loops or multiple edges. For a directed graph $G = (V, E)$ and a subset $W \subseteq V$ let $G[W] = (W, F)$ denote the induced subgraph of $G$ that has edge set $F \subseteq E$ such that every edge $(x, y) \in E$ with $x, y \in W$ is also contained in $F$.

Every relation $\mathcal{X}$ on $L$ can be represented by a directed graph $G = (V, E)$ with vertex set $V = L$ and edge set $E = \mathcal{X}$. In what follows, we therefore will interchangeably speak of $\mathcal{X}$ as graph or relation and use the standard graph terminology such as “induced subgraph of $\mathcal{X}$”.

**Trees.** A rooted tree $T = (V, E)$ (on $L$) is an undirected connected cycle-free graph with finite leaf set $L$ and one distinguished vertex $\rho_r$ that is called the root of $T$. The set of inner vertices of $T$ is denoted by $V^0 := V \setminus L$. An edge $(v, w)$ is called inner edge if $v, w \in V^0$ and outer edge otherwise.

In what follows, we always consider phylogenetic trees $T$ (on $L$), that is, rooted trees on $L$ such that the root $\rho_r$ has at least degree 2 and every other inner vertex $v \in V^0 \setminus \{\rho_r\}$ has at least degree 3. Note that phylogenetic trees $T$ on $L$ always satisfy $|L| \geq 2$, since $\rho_r$ has at least degree 2.

Given a rooted tree $T = (V, E)$, we call $u \in V$ an ancestor of $v \in V$, $u \preceq_T v$, if $u$ lies on the unique path from $\rho_r$ to $v$. We write $u \prec_T v$ for $u \preceq_T v$ and $u \neq v$. If neither $u \preceq_T v$ nor $v \prec_T u$, the vertices $u$ and $v$ are incomparable and comparable otherwise. We always write $(v, w) \in E$ to indicate that $v \prec_T w$. In the latter case, the unique vertex $v$ is called parent of $w$, denoted by $\text{par}(w)$. For a non-empty subset $Y \subseteq V$ of vertices, the last common ancestor of $Y$, denoted by $\text{lca}_T(Y)$, is the unique $\preceq_T$-maximal vertex of $T$ that is an ancestor of every vertex in $Y$. We will make use of the simplified notation $\text{lca}_T(x, y) := \text{lca}_T(\{x, y\})$ for $Y = \{x, y\}$. We will omit the explicit reference to $T$ for $\prec_T$ and $\text{lca}_T$ whenever it is clear which tree is considered.

**Clusters and Hierarchies.** An arbitrary subset $\mathcal{H} \subseteq \mathcal{P}(L)$ of the powerset of a finite set $L$ that satisfies $P \cap Q \in \{P, Q, \emptyset\}$ for all $P, Q \in \mathcal{H}$ is called hierarchy-like. A hierarchy on a finite set $L$ is a subset $\mathcal{H} \subseteq \mathcal{P}(L)$ that is hierarchy-like and additionally satisfies $L \in \mathcal{H}$ and $\{x\} \in \mathcal{H}$ for all $x \in L$.

Given a phylogenetic tree $T = (V, E)$, we can define for each vertex $v \in V$ the set of descendant leaves as $C_T(v) := \{x \in L : v \preceq x\}$, called a cluster of $T$. A cluster $C_T(v)$ is trivial if $C_T(v) = L$ or $C_T(v) = \{v\}$ and non-trivial otherwise. The cluster set of $T$ is then $\mathcal{C}(T) := \{C_T(v) : v \in V\}$. It is well-known that $\mathcal{C}(T)$ forms a hierarchy and that there is a one-to-one correspondence between (isomorphism classes of) rooted trees and their cluster sets [45, 47], as summarized as follows.

**Theorem 1** ([45, Thm. 3.5.2]). For a given subset $\mathcal{H} \subseteq \mathcal{P}(L)$, there is a phylogenetic tree $T$ on $L$ with $\mathcal{H} = \mathcal{C}(T)$ if and only if $\mathcal{H}$ is a hierarchy on $L$.

Moreover, if there is such a phylogenetic tree $T$ on $L$, then, up to isomorphism, $T$ is unique.

We say that a phylogenetic tree $T'$ is a coarsement of a phylogenetic tree $T$, in symbols $T' \prec T$, if $\mathcal{C}(T') \subseteq \mathcal{C}(T)$.

**The Fitch Relation.** We follow the notation in [16] and consider edge-labeled trees as defined as follows.

**Definition 1.** An edge-labeled tree $(T, \lambda)$ (on $L$) is a phylogenetic tree $T = (V, E)$ on $L$ together with a map $\lambda : E \rightarrow \{0, 1\}$, called edge-labeling.

For simplicity, we will speak of 0-edges and 1-edges of $T$ depending on their labeling.

The concept of xenologs as defined by Fitch [14] was refined and formalized by Geiß et al. [16] to preserve the directional character of gene transfer.

**Definition 2.** Given an edge-labeled tree $(T, \lambda)$ on $L$ we set $(x, y) \in \mathcal{X}_{(T, \lambda)}$, for distinct $x, y \in L$ whenever the (unique) path from $\text{lca}_T(x, y)$ to $y$ contains at least one 1-edge.

By construction, $\mathcal{X}_{(T, \lambda)}$ is a binary irreflexive relation on $L$; and therefore, it can be regarded as a directed graph. It is easy to check that $\mathcal{X}_{(T, \lambda)}$ is in general neither symmetric nor antisymmetric. See Fig. 1 for an illustrative example.
Figure 1: An edge-labeled tree \((T, \lambda)\) (left) and the resulting relation \(X_{(T, \lambda)}\) (middle) is shown. The unique least-resolved tree \((T^*, \lambda^*)\) that explains \(X_{(T, \lambda)}\) is shown in the right panel. All 1- and 0-edges are highlighted by drawn-through and dashed lines, respectively.

Definition 3. An edge-labeled tree \((T, \lambda)\) on \(L\) explains a given relation \(X\) on \(L\), whenever there is a 1-edge on the path from \(\lca(x, y)\) to \(y\) if and only if \((x, y) \in X\), i.e., \(X = X_{(T, \lambda)}\).

A relation \(X\) that can be explained by an edge-labeled tree is called Fitch relation.

The enumeration of all induced subgraphs of size three of a relation \(X\) is shown in Fig. 2: up to isomorphism there are 16 subgraphs \(A_1 - A_8\), called allowed triangles, and \(F_1 - F_8\), called forbidden triangles. It has been shown by Geiß et al. [16] that Fitch relations can be characterized in terms of such triangles.

Theorem 2 ([16, Thm. 2]). A relation \(X\) is a Fitch relation if and only if \(X\) does not contain one of the forbidden triangles \(F_1 - F_8\) as an induced subgraph and hence, all induced subgraphs on three vertices are isomorphic to one of the allowed triangles \(A_1 - A_8\).

\((T, \lambda)\) is least-resolved w.r.t. a relation \(X\), if \((T, \lambda)\) explains \(X\) and there is no coarsement \(T' < T\) and no labeling \(\lambda'\) such that the edge-labeled tree \((T', \lambda')\) still explains \(X\). Geiß et al. [16] characterized such least-resolved trees and, even more, showed that they are unique.

Theorem 3 ([16, Thm. 1 and Lemma 11]). Let \(X\) be a Fitch relation and \((T, \lambda)\) be an edge-labeled tree that explains \(X\). The following two statements are equivalent:

1. \((T, \lambda)\) is least-resolved w.r.t. \(X\).
2. (a) Every inner edge of \((T, \lambda)\) is a 1-edge and 
   (b) for every inner edge \((\text{par}(v), v)\) there is an outer 0-edge \((v, x)\) in \((T, \lambda)\).

Moreover, the least-resolved tree w.r.t. the Fitch relation \(X\) is, up to isomorphism, unique.

It is worth to be mentioned that deciding whether a relation \(X\) on \(L\) is a Fitch relation and, in the positive case, to construct the unique least-resolved tree \((T, \lambda)\) that explains \(X\) can be done in \(O(|L| + |X|)\) time, cf. [16, Section 6].

3 Alternative Characterizations

3.1 Characterization via Neighborhoods

Theorem 2 provides a characterization in terms of forbidden and allowed triangles. In what follows, we will present a new characterization in terms of neighborhoods.

Definition 4. Let \(X\) be a relation on \(L\). The (complementary) neighborhood \(N[y]\) of \(y \in L\) w.r.t. \(X\) is defined as follows:

\[ N[y] := \{ x \in L \setminus \{ y \} : (x, y) \notin X \} \cup \{ y \}. \]

Moreover, we define the set of neighborhoods w.r.t. \(X\) as follows:

\[ N[X] := \{ N[y] : y \in L \}. \]
Essentially, $N[y]$ covers $y$ and all incoming neighbors of $y$ in the complement of $X$. To give some intuition, why we defined $N[y]$ and $N[X]$ we refer first to Theorem 3 and consider the unique least-resolved tree $(T, \lambda)$ that explains the Fitch relation $X$. Theorem 3 implies that each inner edge $(\text{par}(v), v)$ of $(T, \lambda)$ must be a 1-edge and $v$ must be incident to an outer 0-edge $(v, y)$. Now, consider the cluster $C_1(v)$. Since $(v, y)$ is a 0-edge and $y$ is a leaf, we can observe that for every leaf $x \in C_1(v)$ with $x \neq y$ it must hold $(x, y) \notin X$. Moreover, since $(\text{par}(v), v)$ is a 1-edge, we have $(x, y) \in X$ for all leaves $x \notin C_1(v)$. Hence, the latter two arguments, together with $y \in N[y]$, imply that $N[y]$ provides precisely all elements of this particular cluster $C_1(v)$. For an example, consider the least-resolved tree $(T^*, \lambda^*)$ for $X = X(T, \lambda)$ in Fig. 1. There is only one inner 1-edge $(u, v)$ in $(T^*, \lambda^*)$. The vertex $v$ is incident to the outer 0-edge $(v, 5)$. Obviously, $(6, 5) \notin X$ and $N[5] = \{5, 6\} = C_1(v)$. In particular, $N[X]$ contains all non-trivial clusters of $T^*$.

In what follows, we show that such neighborhoods can be used to characterize Fitch relations. Essentially, we prove that $X$ is a Fitch relation if and only if $N[X]$ is hierarchy-like and the elements in $N[X]$ are “well-behaved” as defined as follows.

**Definition 5.** Let $X$ be a relation on $L$. Then, we say that $X$ satisfies

- the hierarchy-like-condition (HLC), if $N[X]$ is hierarchy-like; and
- the inequality-condition (IC), if for every neighborhood $N \in N[X]$ and every $y \in N$, we have $|N[y]| \leq |N|$.

To see the intuition behind the inequality-condition, consider the relation $X = \{(c, b)\}$ on $L = \{a, b, c\}$ which corresponds to the forbidden subgraph $F_1$ in Fig. 2. Hence, $X$ is not a Fitch relation. In this example, $N[a] = N[c] = \{a, b, c\}$ and $N[b] = \{a, b\}$. Thus, $N[X] = \{\{a, b, c\}, \{a, b\}\}$ is hierarchy-like, although $X$ cannot be explained by any tree. Hence, hierarchy-likeness of $N[X]$ is not sufficient to characterize Fitch-relations. However, for $N := N[b] \in N[X]$ and $a \in N$ we have $|N[a]| > |N|$ and thus, $X$ does not satisfy IC. As we shall see, Fitch relations are characterized by HLC and IC.

We start with proving the necessity of HLC.

**Lemma 1.** Let $X$ be a Fitch relation on $L$ and $(T, \lambda)$ be some edge-labeled tree that explains $X$. Then, we have $N[y] \in \mathcal{E}(T)$ for all $y \in L$ and $N[X] \subseteq \mathcal{E}(T)$.

**Proof.** Let $X$ be a Fitch relation and $(T = (V,E), \lambda)$ be an edge-labeled tree that explains $X$. Moreover, let $y \in L$ be chosen arbitrarily. By definition, $N[y]$ contains always the vertex $y \in L$ and therefore, $|N[y]| \geq 1$. 

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**Figure 2:** Shown is the graph representation for all possible relations $X \subseteq [L \times L]_{irr}$ with $|L| = 3$. The relations are grouped into allowed ($A_1 - A_8$) and forbidden ($F_1 - F_8$) triangles. The figure is adopted from [16].
If $|N[y]| = 1$, then $N[y] = \{y\} = C_T(y) \in \mathcal{C}(T)$. Now, assume that $|N[y]| \geq 2$. Clearly, it holds that $N[y] \subseteq L = C_T(\rho_T)$. Therefore, we can choose a vertex $v \in V$ with $N[y] \subseteq C_T(v)$ such that $|C_T(v)|$ is minimal. Moreover, $v$ must be an inner vertex, since $|C_T(v)| \geq |N[y]| \geq 2$. This and $y \in C_T(v)$ imply that there is a vertex $w \in V$ with $(v, w) \in E$ and $v \prec w \preceq y$. Since $T$ is phylogenetic and due to the minimality of $|C_T(v)|$, we can conclude that $N[y] \not\subseteq C_T(w)$. The latter implies that there exists a vertex $x \in N[y] \setminus C_T(w) \subseteq C_T(v)$. Since $x \notin C_T(w)$, the leaf $x$ and the vertex $w$ are incomparable in $T$. The latter, together with $(v, w) \in E$ and $v \prec x$, implies that $v = \lca(x, w)$. Moreover, $w \preceq y$ immediately implies that $v = \lca(x, y)$. Since $x \in N[y]$ implies $(x, y) \notin X$ and since $(T, \lambda)$ explains $X$, we can conclude that there is no 1-edge on the path from $v = \lca(x, y)$ to $y$.

Note, we have chosen $v$ such that $N[y] \subseteq C_T(v)$. Assume for contradiction that $N[y] \neq C_T(v)$. Hence, there is a vertex $x' \in C_T(v) \setminus N[y]$. Since $x', y \in C_T(v)$, we obtain $y \prec \lca(x', y)$. Since $x' \notin N[y]$, we have $(x', y) \in X$. This, together with the fact that $(T, \lambda)$ explains $X$, implies that there is a 1-edge on the path from $\lca(x', y)$ to $y$. However, since $v \preceq \lca(x', y) \preceq y$, this 1-edge is also contained on the path from $v$ to $y$; a contradiction. Hence, $N[y] = C_T(v) \in \mathcal{C}(T)$.

Finally, since for all $N[y] \in N[X]$ we have $N[y] \in \mathcal{C}(T)$, we can conclude that $N[X] \subseteq \mathcal{C}(T)$. \hfill \Box

Using Lemma 1 and the fact that (every subset of) the cluster-set $\mathcal{C}(T)$ of a phylogenetic tree $T$ is always hierarchy-like, we immediately obtain the following

**Corollary 1.** Every Fitch relation $X$ satisfies the hierarchy-like-condition (HLC).

To show the necessity of IC, we start with following lemma.

**Lemma 2.** Let $X$ be a Fitch relation on $L$ and $(T, \lambda)$ be some edge-labeled tree that explains $X$. Moreover, let $y \in L$ be a leaf and chose $v \in V(T)$ such that $N[y] = C_T(v)$. Then, the following two properties are satisfied:

(a) There is no 1-edge on the path from $v$ to $y$.

(b) If $N[y] \neq L$, then $(\text{par}(v), v)$ is a 1-edge.

**Proof.** Let $X$ be a Fitch relation on $L$, $(T = (V, E), \lambda)$ be an edge-labeled tree that explains $X$ and $y \in L$ be an arbitrary leaf. Lemma 1 implies that we can choose a vertex $v \in V$ with $N[y] = C_T(v)$. Clearly, if $y = v$, then the lemma is trivially satisfied. Hence, assume that $v \neq y$.

Obviously, $v$ is an ancestor of $\lca(x, y)$ for every $x \in C_T(v) = N[y]$. Moreover, since $T$ is phylogenetic, there is a vertex $z \in C_T(v) = N[y]$ with $\lca(z, y) = v$. Since $z \in N[y]$ we have $(z, y) \notin X$. This and the fact that $(T, \lambda)$ explains $X$ imply that the path from $\lca(z, y) = v$ to $y$ does not contain a 1-edge, which proves Property (a).

We continue with proving Property (b). Since $N[y] = C_T(v)$ and $N[y] \neq L$, we can conclude that $v \neq \rho_T$. Since $v \neq \rho_T$ and $T$ is phylogenetic, there must be a parent $\text{par}(v)$ of $v$ and a vertex $x' \in C_T(\text{par}(v)) \setminus C_T(v)$. Hence, $x' \notin C_T(v) = N[y]$ and thus, $(x', y) \in X$. By the choice of $x'$, we have $\lca(x', y) = \text{par}(v)$. Since $(T, \lambda)$ explains $X$, we can conclude that there is a 1-edge along the path from $\lca(x', y) = \text{par}(v)$ to $y$. Moreover, Property (a) implies that there is no 1-edge along the path from $v$ to $y$. Taken the latter arguments together, $(\text{par}(v), v)$ must be a 1-edge. \hfill \Box

**Lemma 3.** Every Fitch relation $X$ satisfies the inequality-condition (IC).

**Proof.** Let $X$ (on $L$) be a Fitch relation and $(T = (V, E), \lambda)$ be an edge-labeled tree that explains $X$. In order to prove the statement we need to show that $|N[y]| \leq |N|$ is satisfied for every $y \in L$ and every $y \in N := N[y]$.

Let $y' \in L$ and let $y \in N := N[y']$. Clearly, if $y = y'$, then $|N[y]| = |N|$. Thus, assume that $y \neq y'$. Lemma 1 implies that $N \in \mathcal{C}(T)$. Hence, there is a vertex $v \in V$ such that $C_T(v) = N$. Since $y \in N = C_T(v)$, we can conclude that $v$ is an ancestor of $y$ in $T$.

In what follows, we distinguish two mutually exclusive cases, either $v = \rho_T$ or $v \neq \rho_T$. If $v = \rho_T$, then $N = L$. Hence, $N[y] \subseteq L = N$ and therefore $|N[y]| \leq |N|$. Now, assume that $v \neq \rho_T$ and therefore, $N = C_T(v) \neq L$. Again, Lemma 1 implies that $N[y] \in \mathcal{C}(T)$ is a cluster. Hence, there is a vertex
\[ w \in \mathcal{C}(T) \text{ such that } N[y] = C_T(w). \] Note that we have by definition \( y \in N[y] = C_T(w). \) This, together with \( y \in N = C_T(v) \), implies that \( y \in C_T(v) \cap C_T(w). \) Since \( C_T(v) \cap C_T(w) \neq \emptyset \) and \( \mathcal{C}(T) \) is a hierarchy, we can conclude that \( C_T(v) \cap C_T(w) \in \{ C_T(v), C_T(w) \}. \) The latter implies either \( v \preceq w \) or \( w \prec v. \)

First, assume that \( w \prec v. \) Since we have \( C_T(v) = N \neq L \), we can apply Lemma 2 (b) to conclude that \( \lambda(\text{par}(v), v) = 1. \) This, together with \( w \prec v \), implies that there is a 1-edge on the path from \( w \) to \( y \); a contradiction to Lemma 2 (a) applied on \( N[y] = C_T(w). \) Hence, \( w \prec v \) is not possible, and therefore, it must hold that \( v \preceq w. \) Thus, we have \( N[y] = C_T(v) \subseteq C_T(v) = N \), and therefore, we obtain \( |N[y]| \leq |N| \).

To show that HLC and IC are also sufficient for Fitch relations, we first define an edge-labeled tree \( \mathcal{T}(X) = (T, \lambda) \) based on \( N[X] \) and prove that \( \mathcal{T}(X) \) explains \( X. \)

**Definition 6.** Let \( X \) be a relation on \( L \) that satisfies HLC. The edge-labeled tree \( \mathcal{T}(X) = (T, \lambda) \) has the following cluster set

\[ \mathcal{C}(T) = N[X] \cup \{ L \} \cup \{ \{ x \} : x \in L \} \]

and each edge \((\text{par}(v), v)\) of \( T \) obtains the label

\[ \lambda(\text{par}(v), v) = 1 \iff C_T(v) \in N[X], \text{i.e., there is some } y \in L \text{ with } N[y] = C_T(v). \]

We emphasize that all non-trivial clusters of \( \mathcal{T}(X) \) are provided by the elements in \( N[X] \). Now, we are in the position to show that HLC and IC are sufficient for Fitch relations.

**Lemma 4.** Let \( X \) be a relation on \( L \) that satisfies HLC and IC. Then, \( \mathcal{T}(X) \) is well-defined. Moreover, \( \mathcal{T}(X) \) explains \( X \), and thus, \( X \) is a Fitch relation.

**Proof.** Since \( X \) satisfies HLC, the set \( N[X] \) is hierarchy-like. Hence, \( \mathcal{C}(T) \) as in Def. 6 is indeed a hierarchy. This, together with Theorem 1, implies that \( T \) is well-defined. In particular, \( T \) is a phylogenetic tree on \( L \). The edge-labeling \( \lambda : E(T) \to \{ 0, 1 \} \) in Def. 6 is only based on the existence of some \( y \in L \) with \( N[y] = C_T(v) \), and thus, is well-defined as well. In summary, the edge-labeled tree \( \mathcal{T}(X) = (T = (V, E), \lambda) \) is well-defined.

Now, we prove that \( \mathcal{T}(X) \) explains \( X. \) To this end, we will show that \( (x, y) \in X \) if and only if there is a 1-edge on the path from \( \text{lca}(x, y) \) to \( y \) in \( \mathcal{T}(X). \)

First, suppose that \((x, y) \in X\). By definition \( N[y] \in N[X] \) and, by construction of \( T \), we have \( N[y] \in N[X] \subseteq \mathcal{C}(T). \) Thus, there is some vertex \( v \in V \) with \( N[y] = C_T(v). \) Since \((x, y) \in X\), we have \( x \notin N[y] \) and therefore \( N[y] \neq L. \) The latter implies that \( v \neq \text{par}(v) \), and hence, \( \text{par}(v) \) exists. By construction of \( \lambda \), we have \( \lambda(\text{par}(v), v) = 1. \) Now, \( x \notin N[y] = C_T(v) \) and \( y \in N[y] = C_T(v) \) imply that \( \text{lca}(x, y) \preceq \text{par}(v). \) Hence, the 1-edge \((\text{par}(v), v)\) is located on the path from \( \text{lca}(x, y) \) to \( y. \)

Conversely, assume that \( x, y \in L \) are distinct vertices such that the path from \( \text{lca}(x, y) \) to \( y \) contains a 1-edge \((\text{par}(v), v)\). Hence, by construction of \( \lambda \), we have \( C_T(v) \in N[X] \).

We continue to show that \( N[y] \subseteq C_T(v). \) Since \( v \) is located on the path from \( \text{lca}(x, y) \) to \( y \), we have \( v \preceq y \) and thus, \( y \in C_T(v). \) Since \( X \) satisfies IC and \( y \in N := C_T(v) \in N[X] \) it must hold that \( |N[y]| \leq |N| = |C_T(v)|. \) Moreover, since \( X \) satisfies HLC, the set \( N[X] \) is hierarchy-like. Since \( C_T(v), N[y] \in N[X] \) and \( y \in C_T(v) \cap N[y], \) we have either \( C_T(v) \subseteq N[y] \) or \( N[y] \subseteq C_T(v). \) However, \( |N[y]| \leq |C_T(v)| \) immediately implies that \( N[y] \subseteq C_T(v) \) must hold.

Furthermore, since \((\text{par}(v), v)\) is located on the path from \( \text{lca}(x, y) \) to \( y \), we can conclude that \( x \notin C_T(v). \) This and \( N[y] \subseteq C_T(v) \) imply that \( x \notin N[y]. \) Hence, the definition of \( N[y] \) implies that \( (x, y) \in X. \)

To summarize, for any two distinct vertices \( x, y \in L \) we have \((x, y) \in X\) if and only if there is a 1-edge on the path from \( \text{lca}(x, y) \) to \( y \) in \( \mathcal{T}(X). \) Therefore, \( \mathcal{T}(X) \) explains \( X. \) Hence, \( X \) is a Fitch relation.

As a consequence of the results above, we obtain the following new characterization.
Theorem 4. A relation $\mathcal{X}$ is a Fitch relation if and only if $\mathcal{X}$ satisfies HLC and IC.

For the sake of completeness we show that $\mathcal{F}(\mathcal{X})$ is least-resolved w.r.t. a Fitch relation $\mathcal{X}$.

Proposition 1. The tree $\mathcal{F}(\mathcal{X})$ is the unique least-resolved tree that explains the Fitch relation $\mathcal{X}$.

Proof. By construction of $\mathcal{F}(T)$ all non-trivial clusters of $T$ are provided by $\mathcal{N}[\mathcal{X}]$. Hence, for each non-trivial cluster $C_\lambda(v)$ of $T$ there is a vertex $y \in L$ with $N[y] = C_\lambda(v)$. The latter and the construction of $\lambda$ imply that all inner edges of $\mathcal{F}(\mathcal{X})$ are 1-edges. Moreover, since $\mathcal{F}(\mathcal{X})$ explains $\mathcal{X}$, we can apply Property (a) of Lemma 2 and conclude that there is no 1-edge on the path from $v$ to $y$. However, since all inner edges of $\mathcal{F}(\mathcal{X})$ are 1-edges, the path from $v$ to $y$ is simply the edge $(v, y)$. Hence, $(v, y)$ is an outer 0-edge. Therefore, each inner vertex $v \neq \rho_0$ is incident to an outer 0-edge. Now, we can apply Theorem 3 to conclude that $\mathcal{F}(\mathcal{X})$ is the unique least-resolved tree that explains $\mathcal{X}$. □

3.2 Characterization via Three-Vertex Subrelations

Theorem 2 provides a characterization in terms of forbidden and allowed triangles. All allowed triangles $\Delta \in \{A_1, A_2, \ldots, A_8\}$ share a common property, namely if $\Delta$ contains an edge $(c, b)$ but no edge $(a, b)$, then $(c, a)$ must be an edge in $\Delta$ and either both $(a, c), (b, c)$ are an edge in $\Delta$ or neither of them is an edge in $\Delta$. This, in fact, characterizes allowed triangles as shown in the next lemma.

Lemma 5. Let $\mathcal{X}$ be a relation on $L$. Then, the following two conditions are equivalent:

1. For every $\{x, y, z\} \in \binom{\mathcal{X}}{3}$ the subgraph of $\mathcal{X}$ that is induced by $\{x, y, z\}$ is isomorphic to one of the allowed triangles $A_1, A_2, \ldots, A_8$.

2. for every $\{a, b, c\} \in \binom{\mathcal{X}}{3}$ with $(c, b) \in \mathcal{X}$ and $(a, b) \notin \mathcal{X}$, we have $(c, a) \in \mathcal{X}$ and either $(a, c), (b, c) \in \mathcal{X}$ or $(a, c), (b, c) \notin \mathcal{X}$.

Proof. Let $\mathcal{X}$ be a relation on $L$. Recall that $\mathcal{X}[\{u, v, w\}]$ denotes the subgraph of $\mathcal{X}$ that is induced by the vertices $u, v, w \in L$. In this proof, we use the labels of the vertices for the allowed and forbidden triangles as shown in Fig. 1.

Assume for contraposition that Condition (2) is not satisfied. Hence, there is a subset $\{a, b, c\} \in \binom{\mathcal{X}}{3}$ such that $(c, b) \in \mathcal{X}$ and $(a, b) \notin \mathcal{X}$, but

(i) $(c, a) \notin \mathcal{X}$ or

(ii) either $(a, c) \in \mathcal{X}$ and $(b, c) \notin \mathcal{X}$ or $(a, c) \notin \mathcal{X}$ and $(b, c) \in \mathcal{X}$.

Put $\Delta := \mathcal{X}[\{a, b, c\}]$. Since $(c, b) \in \mathcal{X}$ and $(a, b) \notin \mathcal{X}$, the in-degree of $b$ in $\Delta$ must be one. Since none of the graphs $A_1, A_2, A_3$ and $A_4$ contains a vertex with in-degree one, $\Delta$ cannot be isomorphic to $A_1, A_2, A_3, A_4$.

Assume for contradiction that $\Delta$ is isomorphic to $A_5$. Since $A_5$ contains only one vertex with in-degree one namely $y$, we obtain $b = y$. Moreover, $(c, b) \in \mathcal{X}$ and $(a, b) \notin \mathcal{X}$ imply $x = a$ and $z = c$. Since $(z, x) \in E(A_5)$, we have $(c, a) \in E(\Delta) \subseteq \mathcal{X}$. Hence, Case (i) cannot be satisfied. Now, $(y, z), (x, z) \notin E(A_5)$ implies that $(b, c), (a, c) \notin E(\Delta) \subseteq \mathcal{X}$, and therefore, Case (ii) cannot be satisfied. The latter two arguments lead to a contradiction, since at least one of the Cases (i) or (ii) has to be satisfied. Thus, $\Delta$ cannot be isomorphic to $A_5$.

Assume for contradiction that $\Delta$ is isomorphic to $A_6$. Analogously as in the case for $A_5$, we have $b = y, x = a$ and $z = c$ and, by similar arguments, we obtain that $\Delta$ cannot be isomorphic to $A_6$.

Assume for contradiction that $\Delta$ is isomorphic to $A_7$ or $A_8$. For both graphs, $(c, b) \in \mathcal{X}$ and $(a, b) \notin \mathcal{X}$ imply $z = c$. Since $b$ has in-degree one in $\Delta$, we observe that $b \in \{x, y\}$ for both graphs. Due to symmetry, we can w.l.o.g. choose $a = x$ and $b = y$. Since $(z, x) \in E(A_7) \cap E(A_8)$, we have $(c, a) \in E(\Delta) \subseteq \mathcal{X}$. Hence, Case (i) $(c, a) \notin \mathcal{X}$ is not possible. If $\Delta$ is isomorphic to $A_7$, then we have $(a, c), (b, c) \in E(\Delta) \subseteq \mathcal{X}$, since $(x, z), (y, z) \in E(A_7)$. Now, if $\Delta$ is isomorphic to $A_8$, then we have $(a, c), (b, c) \notin E(\Delta) \subseteq \mathcal{X}$, since $(x, z), (y, z) \notin E(A_8)$. Again, the latter arguments lead to a contradiction, since at least one of the Cases (i) or (ii) has to be satisfied. Thus, $\Delta$ cannot be isomorphic to $A_7$ or $A_8$. 8
Thus, if Condition (2) is not satisfied, then $\Delta$ cannot be isomorphic to one of $A_1, \ldots, A_8$, and therefore, Condition (1) is not satisfied.

By contraposition, assume that Condition (1) is not satisfied. Hence, there is a subset $\{a, b, c\} \in \binom{N}{3}$, such that the induced subgraph $\mathcal{X}[\{a, b, c\}]$ is not isomorphic to $A_1, \ldots, A_7$ or $A_8$; and therefore, $\mathcal{X}[\{a, b, c\}]$ is isomorphic to one of $F_1, \ldots, F_8$. First, we observe that for every forbidden triangle $F_1$ to $F_8$ the vertices are labeled such that $(c, b) \in \mathcal{X}$ and $(a, b) \notin \mathcal{X}$, see Figure 1. We also observe that $(c, a) \notin \mathcal{X}$ for $F_1, F_2, \ldots, F_8$ and $F_7$, and that $(a, c) \notin \mathcal{X}$ and $(b, c) \in \mathcal{X}$ for $F_8$.

Either way, we have found a subset $\{a, b, c\} \in \binom{N}{3}$ such that $(c, b) \in \mathcal{X}$ and $(a, b) \notin \mathcal{X}$, but $(c, a) \notin \mathcal{X}$ or $(a, c) \notin \mathcal{X}$ and $(b, c) \in \mathcal{X}$. Thus, Condition (2) is not satisfied.

Based on Theorem 2 and Lemma 5 we obtain the following new characterization of Fitch relations.

**Theorem 5.** A relation $\mathcal{X}$ on $L$ is a Fitch relation if and only if for every subset $\{a, b, c\} \in \binom{N}{3}$ with $(c, b) \in \mathcal{X}$ and $(a, b) \notin \mathcal{X}$, we have $(c, a) \in \mathcal{X}$ and either $(a, c), (b, c) \in \mathcal{X}$ or $(a, c), (b, c) \notin \mathcal{X}$.

### 4 An Alternative Proof of Theorem 2

The key idea of the proof of Theorem 2 in [16, Section 5], which proceeds by induction on the number of leaves, is to consider the superposition of trees explaining two induced subrelations $\mathcal{X}_1, \mathcal{X}_2$, each of which is obtained from $\mathcal{X}$ by removing a single vertex from $L$. This proof, however, is quite involved and very technical, and includes plenty of case studies. The characterization of Fitch relations in terms of HLC and IC allows us to establish a significantly shorter and simpler proof of Theorem 2, which we present here. We emphasize that this new proof is solely based on Theorem 4 and Lemma 5.

**Alternative proof of Theorem 2.** We omit the “if-direction” of the proof of Theorem 2, since the “if-direction” in [16] is fairly simple and straightforward to obtain. It is based on full enumeration of all 16 edge-labeled trees on three vertices, which eventually shows that none of the forbidden triangles can be explained by a tree.

For the “only-if-direction”, let $\mathcal{X}$ be a relation on $L$ and assume, for contraposition, that $\mathcal{X}$ is not a Fitch relation. Thus, Theorem 4 implies that $\mathcal{X}$ does not satisfy HLC or IC.

Assume first that $\mathcal{X}$ does not satisfy IC. Hence, there is a neighborhood $N \in N[\mathcal{X}]$ and a vertex $a \in N$ such that $|N[a]| > |N|$. Since $N \in N[\mathcal{X}]$, there is a vertex $b \in L$ such that $N[b] = N$. Note that $a, b \in N[b]$. The latter, together with $|N[a]| > |N| = |N[b]|$, implies that $a \neq b$ and the existence of a vertex $c \in N[a] \setminus N[b]$. In particular, we have $c \neq a$ and $c \neq b$ and hence $\{a, b, c\} \in \binom{N}{3}$. Since $c \notin N[b]$, it must hold that $(c, b) \in \mathcal{X}$. Since $a \in N[b]$, it must hold that $(a, b) \notin \mathcal{X}$. Since $c \in N[a]$, it must hold that $(c, a) \notin \mathcal{X}$. Therefore, Lemma 5(2) is not satisfied, which implies that Lemma 5(1) cannot be satisfied. Therefore, $\mathcal{X}$ must contain one of the forbidden triangles $F_1, \ldots, F_8$.

Now assume that $\mathcal{X}$ does not satisfy HLC and thus, $N[\mathcal{X}]$ is not hierarchy-like. Hence, there are two neighborhoods $N, N' \in N[\mathcal{X}]$, such that $N \cap N' \notin \{\emptyset, N, N'\}$. The latter implies, in particular, $N \neq N'$. This and $N, N' \in N[\mathcal{X}]$ imply that there are two distinct vertices $y, y' \in L$ such that $N[y] = N$ and $N[y'] = N'$. Moreover, $N \cap N' \notin \{\emptyset, N, N'\}$ implies that $N$ and $N'$ are not disjoint. Since $y \in N[y]$ and $y' \in N[y']$, there are two mutually exclusive cases that need to be examined:

- (a) none of $y$ and $y'$ is contained in $N[y'] \cap N[y]$, and
- (b) at least one of $y$ and $y'$ is contained in $N[y'] \cap N[y]$.

**Case (a):** This case is equivalent to $y \in N[y] \setminus N[y']$ and $y' \in N[y'] \setminus N[y]$. Since $N[y'] \cap N[y] \neq \emptyset$, there is a vertex $x \in N[y] \cap N[y']$ with $x \neq y, y'$. Thus, $x, y$ and $y'$ are pairwise distinct. Since $y \notin N[y']$, we have $(y, y') \in \mathcal{X}$. Since $y' \notin N[y]$, we have $(y', y) \in \mathcal{X}$. Since $x \in N[y']$, we have $(x, y') \notin \mathcal{X}$, and since $x \in N[y]$, we have $(x, y) \notin \mathcal{X}$. Now, put $a := x, b := y'$ and $c := y$. Hence, we have found a subset $\{a = x, b = y', c = y\} \in \binom{N}{3}$ such that $(c, b) \in \mathcal{X}$, $(a, b) \notin \mathcal{X}$ and $(a, c) \notin \mathcal{X}$ and
(b, c) ∈ X. Thus, Condition (2) of Lemma 5 is not satisfied; and therefore, Condition (1) of Lemma 5 is not satisfied. Hence, X contains a forbidden triangle.

**Case (b):** This case is equivalent to y ∈ N[y] ∩ N[y'] or y' ∈ N[y] ∩ N[y']. We can assume w.l.o.g. that y ∈ N[y] ∩ N[y']. Since N[y] ∩ N[y'] ≠ {∅, N[y], N[y']}, there is an x ∈ N[y] \ N[y']. The latter, together with y, y' ∈ N[y'] and y ≠ y', implies that x, y and y' are pairwise distinct. Since x /∈ N[y'], we have (x, y') ∈ X. Since y ∈ N[y'], we have (y, y') /∈ X. Since x ∈ N[y'], we have (x, y) /∈ X. Now, put a := y, b := y' and c := x. Hence, we have found a subset {a = y, b = y', c = x} ∈ \( \binom{I}{2} \) such that (c, b) ∈ X and (a, b) /∈ X, but (c, a) /∈ X. Thus, Condition (2) of Lemma 5 is not satisfied; and therefore, Condition (1) of Lemma 5 is not satisfied. Hence, X contains a forbidden triangle.

In both Cases (a) and (b), the relation X contains a forbidden triangle, which proves the “only-if-direction”.

5 Summary

In this contribution, we gave two novel characterizations of Fitch relations X. One characterization is based on the neighborhoods N[y] of vertices y ∈ L that comprises vertex y and all vertices x ∈ L with (x, y) /∈ X. We have shown, that “well-behaved” collections N[X] of such neighborhoods (i.e., they satisfy HLC and IC) characterize Fitch relations. Furthermore, the tree \( \mathcal{T}(X) = (T, \lambda) \) with hierarchy \( \mathcal{C}(T) \) consisting of all members of N[X] together with the (possibly additional) sets L and \( \{x\} \) with \( x \in L \) and a well-defined labeling \( \lambda \) explains the Fitch relation X. In particular, \( \mathcal{T}(X) \) is the unique least-resolved tree for X. The second characterization is based on three-vertex induced subrelations and the observation that allowed triangles share a common simple property, namely, if X contains (c, b) but not (a, b), then (c, a) ∈ X and either both (a, c), (b, c) or none of them are contained in X. These results are used to establish a simpler and significantly shorter proof of the characterization theorem provided by Geiß et al. [16, Thm. 2].

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