Lateral Gene Transfer, Rearrangement and Reconciliation

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Abstract

Background. Models of ancestral gene order reconstruction have progressively integrated different evolutionary patterns and processes such as unequal gene content, gene duplications, and implicitly sequence evolution via reconciled gene trees. In unicellular organisms, these models have so far ignored lateral gene transfer, even though it can have an important confounding effect on such models, as well as a rich source of information on the function of genes through the detection of transfers of entire clusters of genes.

Result. We report an algorithm together with its implementation, DeCoLT, that reconstructs ancestral genome organization based on reconciled gene trees which summarize information on sequence evolution, gene origination, duplication, loss, and lateral transfer. DeCoLT finds in polynomial time the minimum number of rearrangements, computed as the number of gains and breakages of adjacencies between pairs of genes. We apply DeCoLT to 1099 gene families from 36 cyanobacteria genomes.

Conclusion. DeCoLT is able to reconstruct adjacencies in 35 ancestral bacterial genomes with a thousand genes families in a few hours, and detects clusters of co-transferred genes. As there is no constraint on genome organization, adjacencies can be generalized to any relationship between genes to reconstruct ancestral interactions, functions or complexes with the same framework.

Availability. [http://pbil.univ-lyon1.fr/software/DeCoLT/](http://pbil.univ-lyon1.fr/software/DeCoLT/)
**Introduction**

The evolution of genomes can be explored at two different scales. At the chromosome level, rearrangements have been studied from the 1930’s [1,2], and have progressively incorporated the possibility of unequal gene content, gene duplications and gene losses [3,4]. Later, but largely independently, in the 1960’s, the evolution of genomic sequences began to be modeled [5,6], and has more recently been extended to include the duplication, loss and the lateral transfer of genes via the reconciliation of gene trees with a species tree [7–11]. These two scales have only met on a few occasions through the integration of phylogenies and rearrangements [12,13], using reconciled phylogenies to account for the duplication and loss of genes. Here, we build on these ideas and reconstruct ancestral gene order based on reconciled phylogenies that account for gene origination, duplication, loss, and transfer.

We propose an algorithm to simultaneously reconstruct all gene organizations along a species phylogeny, minimizing the number of gains and breakages of *adjacencies* that link consecutive genes on chromosomes. We build upon the dynamic programming principle proposed by Bérard *et al.* [13] and extend it to consider reconciliations (containing lateral gene transfer) produced by Szöllősi *et al.* [14] as input.

We implement our algorithm naming the resulting software *DeCoLT*, in reference to DeCo [13] (Detection of Coevolution) with Lateral Transfers. We examine two datasets of gene trees from a single set of cyanobacteria species. The first set of gene trees is computed from sequence alignments only [15], and the second one is computed by a species tree aware method [16].

Our method and the efficiency of the computation is based on the hypothesis that adjacencies evolve independently from each other. While extant genomes consist either of a single or a relatively small number of linear or circular chromosomes, this hypothesis implies that reconstructed ancestral genomes may in theory exhibit more complex arrangements. For example an ancestral gene may be involved in more then two adjacencies, or a large number may have only a single adjacent gene. In the cyanobacteria dataset, extant genomes are all circular, and the ancestral genomes inferred by DeCoLT are also close to being circular with only a few deviations. Most deviations result from the absence of signal to reconstruct genomes in deep ancestors, but some are caused by errors in gene trees, leading to errors in ancestral gene contents. We observe that ancestral genome organizations computed from gene trees that are based on both the species tree and the sequence are closer to being circular than those computed from gene trees based on sequence alone. This validates the reconstruction principle we present here and confirms that species tree aware methods produce more accurate gene trees.
Context

A dated species tree is a rooted binary tree whose leaves are the extant genomes and internal nodes, the ancestral genomes, are totally ordered. The time interval between two consecutive internal nodes of a species tree defines a time slice (see Figure 1 for an example, where branches leading to A, B or the ancestor of C and D overlap two time slices while the others overlap one). Genomes contain a set of genes and a set of adjacencies, which are pairs of genes, the genes being the two extremities of the adjacency. In extant genomes, an adjacency means that two genes are immediately consecutive, with no other gene between them on the chromosome (regardless of their physical distance) so every gene belongs to exactly two adjacencies.

Genes of all genomes are partitioned into homologous families, and each family is organized in a gene tree, which is a rooted tree whose nodes are the genes, describing the pattern of descent within a family. Gene trees are reconciled with the species tree, which means that nodes and branches of gene trees are annotated to account for the particular history of the gene family. Possible events are origination (of the gene in the species tree), speciation (genes follow the species diversification), duplication, transfer, or loss. Transfers are the acquisition of a gene by a genome in the species tree from a genome outside the species tree. Indeed genes at the origin of transfers almost always belong to unsampled or extinct species [14]. That is why speciation does not only happen at the nodes of the species tree, but a gene can also leave the species tree by speciation, and be transferred back later (see Figure 1 for an example). Every event on a gene tree is associated to a branch and a time slice of the species tree.

The input to our method is a dated species tree, a set of reconciled gene trees and the set of extant adjacencies. Reconciled gene trees yield ancestral genes (dots inside green circles in Figure 1). The problem will be to construct the ancestral adjacencies, given this input. In practice the input is provided by methods and software described in Szöllősi et al.’s trilogy [11, 14, 16]. The first paper of this trilogy explains how to find the dated species tree, the second one how to reconcile gene trees taking extinct or unsampled species into account, and the third one how to reconstruct species aware gene trees.

We construct ancestral adjacencies in a manner that minimizes the number of rearrangements along the species phylogeny. This is computed as the smallest number of gains and breakages of adjacencies necessary to explain all extant adjacencies. For example, if we infer no ancestral adjacencies at all, then the value of this objective function is proportional to the number of extant adjacencies, because all of them are gained independently. If we propose an adjacency in an ancestral genome which is a common ancestor of a set of adjacencies, then the value decreases because all adjacencies in that set are explained by a unique gain (see Figure 1 where three extant adjacencies can be explained with two gains and a breakage).
Figure 1: The evolution of an adjacency within a dated species tree, along reconciled gene phylogenies. The gene trees are blue and purple, while red horizontal edges are adjacencies. The time slices $t_0, \ldots, t_3$ indicate in which order the speciation nodes (big green nodes) occur, and are used to localize genes in the species tree (a branch and a time slice give the coordinates of a gene or an event). Red crosses mean gene losses, for example in the branches leading to $A$ or $C$ (adjacencies are lost when one extremity is lost), or an adjacency breakage, for example in the branch leading to $D$ (gene loss and adjacency breakages are different events, since a gene loss is not a rearrangement while a breakage is, and only rearrangements are counted in the objective function). Here one adjacency is gained in the branch leading to species $C$, one is broken in the branch leading to species $D$, and one is transferred from the branch leading to $B$ to the branch leading to $C$. The transfer implies first a speciation outside the species phylogeny, and then a transfer which can be in another time slice. A tandem duplication in the branch leading to $A$ gives a new adjacency between the two copies.
We then have to describe how an ancestral adjacency propagates within reconciled gene trees so that it can be recognized as an ancestor of an extant one.

**Propagation rules**

There is an adjacency between two genes only if they are in the same species (extant or ancestral) at the same time slice. If there is an adjacency between two ancestral genes $a$ and $b$, it is propagated to the descendants, in the absence of rearrangements, following the rules described in Table 1 according to the events happening to genes $a$ and $b$.

| event for $a$ | event for $b$ | adjacencies in descendants | graphical depiction | recurrence rules |
|--------------|--------------|---------------------------|--------------------|-----------------|
| GeneLoss     | any event    | $\emptyset$               | a
\[a\]
\[b\]
|              |              |                           | 2                  |                 |
| no event     | no event     | $a_1b_1$                  | a
\[a_1\]
\[b_1\]
|              |              |                           | 3                  |                 |
| no event     | GeneDuplication | $ab_1$ or $ab_2$       | a
\[a\]
\[b\]
\[a_1b_1b_2\]
|              |              |                           | 4                  |                 |
| GeneDuplication | GeneDuplication | $a_1b_1$ and $a_2b_2$ or $a_1b_2$ and $a_2b_1$ | a
\[a_1\]
\[a_1\]
\[b_1\]
\[b_1\]
\[a_2\]
\[a_2\]
\[a_2\]
\[b_2\]
|              |              |                           | 5                  |                 |
| Speciation   | Speciation   | $a_1b_1$ and $a_2b_2$    | a
\[a_1\]
\[b_1\]
\[a_2\]
\[a_2\]
\[b_2\]
|              |              |                           | 6                  |                 |

A *history* is a set of ancestral and extant adjacencies. In a history, any adjacency which does not have a parent identified by the propagation rules yields an adjacency *gain*. A *breakage* is inferred when an adjacency is present in the history but one of its descendants according to the propagation rules is not. The *cost* of a history is the number of gains and breakages it yields. We are computing a minimum cost history.
| event for $a$ | event for $b$ | adjacencies in graphical depiction | recurrence rules |
|--------------|-------------|----------------------------------|-----------------|
| no event     | SpeciationOut | $ab_1$ | 7 |
| SpeciationOut | SpeciationOut | $a_1b_1$ and $a_2b_2$ or $a_1b_2$ and $a_2b_1$ | 8 |
| no event     | SpeciationOutside | $ab_1$ or $ab_2$ | 9 |
| SpeciationOutside | SpeciationOutside | $a_1b_1$ and $a_2b_2$ | 9 |
| Transfer     | any event    | $ab$ | 10 |

Table 1: Propagation rules for the adjacencies: a function of the events happening to their extremities. We denote $a_1$ and $a_2$ (resp., $b_1$ and $b_2$) as the children of $a$ (resp., $b$). Recurrence rules then follow exactly the propagation rules, adding the possibility of rearrangements at each step. Speciation means speciation inside the species tree, SpeciationOut means the parent is inside and one child is inside and the other child is outside the species tree, while SpeciationOutside means that the parent (and possibly the children) are outside. Note that “no event” is any non-leaf event (leaves of gene trees are labeled with Extant, see the recurrence rules), while “any event” is any non-leaf or leaf event. Note that “no event” cannot occur with speciation, since both trees are reconciled (see Figure 1), while “no event” happens with “no event” when both genes change time slice without any event (for example, the change from $t_1$ to $t_2$ in the branch between the root and its right child in Figure 1).
Algorithm

We compute a minimum cost history by writing a dynamic programming algorithm following the propagation rules and adding adjacency gains and breakages with costs that are considered in the optimization. In order to solve a more general problem and to present the recurrence formulas more clearly, gains and breakages are assigned a cost, which could be different, and we minimize on the number of events weighted by their cost. In practice we always use the algorithm with equal costs, thus minimizing the sum of the number of gains and breakages.

Classes of adjacencies

Two adjacencies are homologous with respect to a particular history if they descend from a common ancestor following the propagation rules. Homology of adjacencies is an equivalence relation. We first state a necessary condition for a set of adjacencies to be homologous in order to restrict the search space for homology.

Two extant adjacencies \(a_1b_1\) and \(a_2b_2\) are possibly homologous if there are two ancestral genes \(a\) and \(b\) of an ancestral genome \(G\), such that \(a\) (resp., \(b\)) is an ancestor of \(a_1\) and \(a_2\) (resp., \(b_1\) and \(b_2\)). This simply tells us that in order to find a common ancestor of two adjacencies, there has to exist two genes being the extremities of this adjacency. So if two adjacencies are homologous with respect to a particular history then they are possibly homologous (the definition of possible homology is independent from any history). Possible homology, defined on two adjacencies, is obviously a symmetric and reflexive relation. It is also transitive, partitioning the set of extant adjacencies into equivalence classes.

Consequently, homology can be searched within a class. For each class \(\{a_1b_1, \ldots, a_kb_k\}\), there are two genes \(a\) and \(b\), such that \(a\) (resp. \(b\)) is an ancestor of all \(a_i\) (resp., \(b_i\)). Among the possible such genes \(a\) and \(b\) for a class, we call the highest distinct ones the roots of that class. We then work with the disjoint subtrees rooted by \(a\) and \(b\), and find a history following the propagation rules for all adjacencies whose extremities are descendants of \(a\) and \(b\). Hence, it is sufficient to search within pairs of trees to construct a history.

Recurrence formulas within one class

For any two gene tree nodes \(a\) and \(b\), for which \(s(a) = s(b)\), let \(c_1(a, b)\) be the minimum cost of a history for the two gene subtrees rooted at \(a\) and \(b\), assuming there is an adjacency between \(a\) and \(b\), and let \(c_0(a, b)\) be the minimum cost of a history for two gene subtrees rooted at \(a\) and \(b\), assuming there is no adjacency between \(a\) and \(b\). The values of \(c_1(a, b)\) and \(c_0(a, b)\) are recursively computed, according to the events annotating \(a\) and \(b\). Thus we have to enumerate all cases.
Given a node \( u \), \( u_1 \) is the first (or only child of \( u \) in the case that \( u \) has only one child), while \( u_2 \) is the second child. We write \( E(u) \) to denote the event at node \( u \), where \( E(u) = \text{Extant} \) when \( u \) is a leaf of a gene tree corresponding to an extant gene.

**Case 1** \( E(a) = \text{Extant} \) and \( E(b) = \text{Extant} \) (both nodes are leaves).

In this case, if \( ab \) is an adjacency then \( c_1(a, b) = 0 \) and \( c_0(a, b) = \infty \), else \( c_1(a, b) = \infty \) and \( c_0(a, b) = 0 \).

**Case 2** \( E(a) = \text{GeneLoss} \) (one of the genes is lost, any event may happen to the other).

In this case \( c_1(a, b) = 0 \) and \( c_0(a, b) = 0 \).

**Case 3** \( E(a) = \text{NoEvent} \) and \( E(b) = \text{NoEvent} \) (both gene trees are changing time slice without any event).

In this case \( c_1(a, b) = \min\{c_1(a_1, b_1), c_0(a_1, b_1) + C(\text{Break})\} \) and \( c_0(a, b) = \min\{c_0(a_1, b_1), c_1(a_1, b_1) + C(\text{Gain})\} \).

**Case 4** \( E(a) \in \{\text{Extant}, \text{NoEvent}, \text{Speciation}, \text{SpeciationOut}\} \) and \( E(b) = \text{GeneDuplication} \)

In this case we suppose that the duplication of \( b \) happens before any event in the gene tree containing \( a \). Here, \( c_1(a, b) = D_1 \), and \( c_0(a, b) = D_0 \), where

\[
D_1 = \min\left\{ \begin{array}{l}
    c_1(a, b_1) + c_0(a, b_2), \\
    c_0(a, b_1) + c_1(a, b_2), \\
    c_1(a, b_1) + c_1(a, b_2) + C(\text{Gain}), \\
    c_0(a, b_1) + c_0(a, b_2) + C(\text{Break})
\end{array} \right. \\
D_0 = \min\left\{ \begin{array}{l}
    c_0(a, b_1) + c_0(a, b_2), \\
    c_0(a, b_1) + c_1(a, b_2) + C(\text{Gain}), \\
    c_1(a, b_1) + c_0(a, b_2) + C(\text{Gain}), \\
    c_1(a, b_1) + c_1(a, b_2) + 2 \times C(\text{Gain})
\end{array} \right.
\]

**Case 5** \( E(a) = \text{GeneDuplication} \) and \( E(b) = \text{GeneDuplication} \).

In this case \( c_1(a, b) = \min(D_1, D_2, D_2) \) where

\( D_1 \) (defined in Case 3) is the cost in the case where the \( a \) duplication comes first.

\( D_2 \) is the cost in the case where the \( a \) duplication comes first:

\[
D_2 = \min\left\{ \begin{array}{l}
    c_1(a_1, b) + c_0(a_2, b), \\
    c_0(a_1, b) + c_1(a_2, b), \\
    c_1(a_1, b) + c_1(a_2, b) + C(\text{Gain}), \\
    c_0(a_1, b) + c_0(a_2, b) + C(\text{Break})
\end{array} \right.
\]
\( D12 \) is the cost in the case where the \( a \) and \( b \) duplications are simultaneous: \( D12 = \min \) (over all 16 of the following cases):

\[
\begin{pmatrix}
(1) & c_1(a_1, b_1) + c_1(a_2, b_2) + c_0(a_1, b_2) + c_0(a_2, b_1), \\
(2) & c_1(a_1, b_1) + c_1(a_2, b_2) + c_0(a_1, b_2) + c_1(a_2, b_1) + C(Gain), \\
(3) & c_1(a_1, b_1) + c_1(a_2, b_2) + c_1(a_1, b_2) + c_0(a_2, b_1) + C(Gain), \\
(4) & c_1(a_1, b_1) + c_1(a_2, b_2) + c_1(a_1, b_2) + c_1(a_2, b_1) + 2 \cdot C(Gain), \\
(5) & c_1(a_1, b_1) + c_0(a_2, b_2) + c_0(a_1, b_2) + c_0(a_2, b_1) + C(Break), \\
(6) & c_1(a_1, b_1) + c_0(a_2, b_2) + c_0(a_1, b_2) + c_1(a_2, b_1) + C(Gain) + C(Break), \\
(7) & c_1(a_1, b_1) + c_0(a_2, b_2) + c_1(a_1, b_2) + c_0(a_2, b_1) + C(Gain) + C(Break), \\
(8) & c_1(a_1, b_1) + c_1(a_2, b_2) + c_0(a_1, b_2) + c_0(a_2, b_1) + C(Break), \\
(9) & c_0(a_1, b_1) + c_1(a_2, b_2) + c_0(a_1, b_2) + c_1(a_2, b_1) + C(Gain) + C(Break), \\
(10) & c_0(a_1, b_1) + c_1(a_2, b_2) + c_1(a_1, b_2) + c_0(a_2, b_1) + C(Gain) + C(Break), \\
(11) & c_0(a_1, b_1) + c_0(a_2, b_2) + c_1(a_1, b_2) + c_1(a_2, b_1), \\
(12) & c_0(a_1, b_1) + c_1(a_2, b_2) + c_1(a_1, b_2) + c_1(a_2, b_1) + C(Gain), \\
(13) & c_1(a_1, b_1) + c_0(a_2, b_2) + c_1(a_1, b_2) + c_1(a_2, b_1) + C(Gain), \\
(14) & c_0(a_1, b_1) + c_0(a_2, b_2) + c_1(a_1, b_2) + c_0(a_2, b_1) + C(Break), \\
(15) & c_0(a_1, b_1) + c_0(a_2, b_2) + c_0(a_1, b_2) + c_1(a_2, b_1) + C(Break), \\
(16) & c_0(a_1, b_1) + c_0(a_2, b_2) + c_0(a_1, b_2) + c_0(a_2, b_1) + 2 \cdot C(Break)
\end{pmatrix}
\]

And, \( c_0(a, b) = D00 \), where

\[
D00 = \min \left\{ D0, \begin{array}{l}
D0 = c_0(a_1, b) + c_0(a_2, b), \\
c_0(a_1, b) + c_1(a_2, b) + C(Gain), \\
c_0(a_1, b) + c_0(a_2, b) + C(Gain), \\
c_1(a_1, b) + c_1(a_2, b) + 2 \cdot C(Gain)
\end{array} \right\}
\]

**Case 6** \( E(a) = \text{Speciation} \) and \( E(b) = \text{Speciation} \).

We assume without loss of generality that \( s(a_1) = s(b_1) \) and \( s(a_2) = s(b_2) \). Here, \( c_1(a, b) = S1 \) and \( c_0(a, b) = S0 \), where

\[
S1 = \min \left\{ \begin{array}{l}
c_1(a_1, b_1) + c_1(a_2, b_2), \\
c_1(a_1, b_1) + c_0(a_2, b_2) + C(Break), \\
c_0(a_1, b_1) + c_1(a_2, b_2) + C(Break), \\
c_0(a_1, b_1) + c_0(a_2, b_2) + 2 \cdot C(Break)
\end{array} \right\}
\]

\[
S0 = \min \left\{ \begin{array}{l}
c_0(a_1, b_1) + c_0(a_2, b_2), \\
c_1(a_1, b_1) + c_0(a_2, b_2) + C(Gain), \\
c_0(a_1, b_1) + c_1(a_2, b_2) + C(Gain), \\
c_1(a_1, b_1) + c_1(a_2, b_2) + 2 \cdot C(Gain)
\end{array} \right\}
\]

**Case 7** \( E(a) \in \{\text{Extant}, \text{NoEvent}, \text{Speciation}\} \) and \( E(b) = \text{SpeciationOut} \).

In this case \( c_1(a, b) = c_1(a, b_1) \) and \( c_0(a, b) = c_0(a, b_1) \).

**Case 8** \( E(a) = \text{SpeciationOut} \) and \( E(b) = \text{SpeciationOut} \).
We assume without loss of generality that $a_1$ (resp., $b_1$) is the child that remains inside the species tree, while $a_2$ (resp., $b_2$) is the child that leaves the tree. In this case, $c_1(a, b) = c_1(a_1, b_1) + \min\{c_1(a_2, b_2), c_0(a_2, b_2) + C(Break)\}$ and $c_0(a, b) = c_0(a_1, b_1) + \min\{c_0(a_2, b_2), c_1(a_2, b_2) + C(Gain)\}$.

**Case 9** $E(b) = \text{SpeciationOutside}$ *(one of the genes is outside of the species tree, any event may happen to the other).*

Here, we compute values for $a$ and $b$ only if $\sigma(a)$ or $\sigma(b)$ are defined, and in this case compute values only if $\sigma(a) = \sigma(b)$. Then, if $E^*(a) = \text{NoEvent}$ and $E^*(b) = \text{Speciation}$, then $c_1(a, b) = D1$ and $E^*(b) = D0$ (both defined in Case 4). Else, $E^*(a) = E^*(b) = \text{Speciation}$ and then $c_1(a, b) = S1$ and $c_0(a, b) = S0$ (both defined in Case 6).

**Case 10** $E(a) = \text{Transfer}$ *(one of the genes is a transfer, any event may happen to the other).*

In this case, since $s(a) = s(b)$, the values $c_1(a, b)$ and $c_0(a, b)$ have already been computed recursively, and hence they are these values. Observe that if $E(b) \notin \{\text{Transfer, GeneLoss}\}$ then $(a, b)$ will form the root of an equivalence class.

**Backtracking Procedure**

First, the dynamic programming matrix $M[a, b]$ containing a cell for each pair $(a, b)$ of nodes in the respective gene trees is created by following the recurrence rules for each equivalence class. However, all of the nodes $u$ such that $E(u) = \text{SpeciationOutside}$ are outside of the species tree, and hence $s(u)$ is undefined. This means that $M[a', b']$ for such pairs $(a', b')$ where $E(a) = E(b) = \text{SpeciationOutside}$ have not computed in $M$, and so the recurrence rules cannot track the propagation of a possible adjacency in the species tree above such a pair $(a', b')$ to a pair below, that has been co-transferred back into the species tree. We now present a preprocessing step that artificially assigns an a species and an event to such nodes $(a', b')$ to allow the recurrence rules (i.e., Case 9) to be able to track such propagation. After this step, the recurrence rules can be applied again, with Case 9 in effect, to obtain the full $M$. Then, a classical backtracking procedure can be used to reconstruct ancestral adjacencies along a minimum path in $M$. This preprocessing step is done in a manner that guarantees that the tracking of such propagation will result in a minimum cost history. We now detail this preprocessing step.

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1 $\sigma$ (resp., $E^*$) is another type of species (resp., event) assignment function like $s$ (resp., $E$) that is defined during a preprocessing step of the backtracking procedure, which is described in the next section. This rule does not become effective until this step is executed.
For each cell $M[a, b]$ where $E(a) = E(b) = SpeciationOut$, we do a (depth-first) search to find all nodes $a'$ (resp., $b'$) below $a$ (resp., $b$) such that $E(a') = (resp., E(b')) = Transfer$. Since each $a'$ (resp., $b'$) is a point where the SpeciationOut event at $a$ (resp., $b$) returns to the species tree, let $M'$ be the set of all such pairs $(a', b')$ (i.e., cells in $M$) such that $s(a') = s(b')$. It is only these “frontier” cells $M'$ that have the possibility to connect $(a, b)$ with the lower parts of the respective trees (the lower parts rooted precisely at each $(a, b) \in M'$), through the propagation of an adjacency. Other such descendants of $(a, b)$ can never receive adjacencies from $(a, b)$, so we treat them as losses and consider them no further.

Now, let $T_a$ and $T_b$ be the subtrees of the trees containing $a$ (resp., $b$), rooted at $a$ (resp., $b$) with the leaves $a'$ (resp., $b'$), such that $(a', b') \in M'$. It is the propagation of adjacencies within this tree we need to analyze in order to most parsimoniously (in terms of gains and breakages of adjacencies) connect $(a, b)$ with its frontier cells $M'$. Since all internal nodes $u \in T_a$ (resp., $v \in T_b$), i.e., nodes of degree larger than one have $E(u) = (resp., E(v) =) SpeciationOutside$, it follows that $s(u)$ and $s(v)$ are undefined, and so there is no restriction on possible adjacencies between any such pair $(u, v)$ in the propagation from $(a, b)$ down to the frontier $M'$. In constructing a minimum cost history that explains all extant adjacencies, we may hence assume the most parsimonious of such scenarios. One way to do this is to impose an artificial species subtree topology $T_S$ that both $T_a$ and $T_b$ must follow in this propagation, in such a way that recurrence rules (i.e., Case 9) will allow the computation of a minimum cost history. We hence take this approach in the following.

If the topology of $T_a$ was identical to $T_b$, it is easy to see that we could choose $T_S$ to be $T_a$. The topology of $T_b$ is different from $T_a$ in general, however we can still choose $T_S$ to be $T_a$, and then we can force $T_b$ to follow (i.e., we reconcile $T_b$ in) $T_S$ in a manner that minimizes the number of duplications of nodes in $T_b$. This is because such a duplication is the only situation that would invoke line 3 of D1 in Case 9 during the computation of the history—no other lines of Case 9 have a Gain. Such a reconciliation that achieves this is the LCA reconciliation [17], so we use this.

We use this topology $T_S$ to assign an artificial species and event to each internal node of $T_a$ and $T_b$ so that the recurrence (Case 9) may follow this pair of trees. Since the topology of $T_a$ is that of $T_S$, we simply set $\sigma(u)$ to some unique integer, and $E^*(u) = Speciation$ for each $u \in T_a$. We then set $\sigma(v)$ for $v \in T_b$ to $\sigma(u)$ for the $u$ that $v$ maps to in the LCA reconciliation. The reconciliation of $T_b$ also introduces duplication and loss events to $T_b$. For either such event, we set $E^*(v) = NoEvent$ for the corresponding nodes, and then for the remainder of the (Speciation) nodes $v'$, we set $E^*(v') = Speciation$. Now that we have assigned these artificial species and events to the these SpeciationOutside nodes, we can simply apply the recurrence rules again (this time making Case 9 effective) on the trees to compute the full $M$. Then, after applying the
classical backtracking procedure on $M$, the optimal (minimum cost) history is then obtained by choosing the minimum among $c_1 + C(Gain)$ and $c_0$ on the roots of all classes.

**Complexity**

Let $m$ be the number of gene trees, and $n$ be the maximum number of genes in a gene tree. Since there are as many as $n$ time slices $t_0, \ldots, t_n$ (see Figure 1) for any tree, there can be as many as $n^2$ events (most of them are NoEvent events) in a given tree. The number of equivalence classes is $O(m^2)$, and hence there are $O(m^2n^4)$ comparisons computed during the initial construction of dynamic programming matrix $M$ before the preprocessing step. The preprocessing step, involving (linear-time computable) LCA reconciliation [17], for a pair of trees takes time $O(n)$, for an overall time of $O(m^2n)$. The second run of the recurrence rules after the preprocessing step is again $O(m^2n^4)$, for an overall (polynomial) running time of $O(m^2n^4)$.

In practice, the number of equivalence classes is much smaller–closer to $m$ than $O(m^2)$, and the majority of the $O(n^2)$ events each tree are NoEvent events. On the cyanobacteria dataset of ($m = 1099$ families from $n = 36$ genomes, our implementation, DeCoLT, of this algorithm constructed the adjacencies in under 3 hours on a standard desktop computer.

**Cyanobacteria ancestral genomes**

The algorithm has been implemented and run on two datasets. They both have the same species tree (depicted in Figure 2), on the same set of 36 extant genomes from cyanobacteria and the same extant adjacencies.

They differ by their set of gene trees. One of them is the sequence trees, which are maximum likelihood trees constructed from a model of sequence evolution using multiple alignments of protein sequences of extant genes from each family, taken from [11]. The other is the ALE trees, which are maximum likelihood trees constructed from a model of sequence evolution in conjunction with a birth and death branching model to account for origination, duplication, transfer and loss, taken from [16]. As transfers are very likely to involve lineages outside any given phylogeny [14], reconciled trees have nodes leaving the species tree (SpeciationOut) and nodes transferring to the species tree (Transfer).

For both datasets, ancestral adjacencies were computed using DeCoLT. The degree of each ancestral gene (the number of adjacencies it belongs to) was computed, and we then plotted the proportion of ancestral genes having degree $k$ for $k$ between 0 and 6 (Figure 3). There are almost no genes with degree larger than 2 in either dataset. The proportion of genes with
Figure 2: Cyanobacteria dated species tree. The size (area) and colours of internal nodes is the ratio of the number of adjacencies over the number of genes in every ancestral species.

Figure 3: On the x axis is the degree of a gene, that is, the number of adjacencies it belongs to, and on the y axis there is the proportion of genes with this degree. In black, there are the values for the sequence trees and in red for ALE trees.
degree 2 increases from 17% for sequence trees to 31% for ALE trees. This means that we: (i) accurately reconstruct ancestral adjacencies because they all have a circular structure; and (ii) the quality of gene trees nearly doubles the resolution of ancestral genomes. Finally, having only 31% of ancestral genes with degree two means that a large part of the gene order signal is lost in this very deep branch. However, this is not the case for ancestral genomes. The size of the nodes on Figure 2 indicates the ratio between the number of adjacencies and the number of genes in each genome (the ideal ratio is 1). In the Prochlorococcus clade, over 80% of the genomes are reconstructed whereas it drops to nearly 0% in deeper nodes.

We found that 64 clusters of genes were co-transferred: transferred adjacencies were detected, as well as 28 clusters of co-duplicated genes during the evolution of cyanobacteria. Most are simply pairs of genes, but there is a cluster of 4 co-transferred genes, four clusters of 3 co-transferred genes, and two clusters of 3 co-duplicated genes.

**Discussion**

The optimizing property of the algorithm follows from the exact translation of the propagation rules into the recurrence formulas, adding all possibilities of rearrangements every time. It is a generalization of the reconstruction of discrete ancestral characters solved by Sankoff-Rousseau type algorithms [18]. To see this, one can observe that our framework is strictly equivalent to the Sankoff-Rousseau algorithm [18] in the case where there are no events in the trees.

Further improvements in the method would consist in adding the possibility of homolog replacement when a gene is transferred: for the moment any transfer yields rearrangements whereas some genes might replace an homologous one, keeping the gene order unchanged. We could also think of avoiding rearrangements caused by origination and losses of genes, which, for the moment, necessarily yield several adjacency gains and losses.

Future work will also consist in deriving function information from co-transfers, and trying the same principles on other kinds of relations than adjacencies, starting for example from the relation between domains forming the same gene.

**Author’s contributions**

MP, GS, VD and ET devised the algorithm. MP programmed the software. ET wrote the paper.
Acknowledgements

Thanks to Sèverine Bérard and Thomas Bigot. MP is funded by a Marie Curie Fellowship from the Alain Bensoussan program of ERCIM and and GS is funded by the Marie Curie Fellowship 253642 ”Geneforest”, and the Albert Szent-Györgyi Scholarship A1-SZGYA-FOK-13-0005. This work is funded by the Agence Nationale pour la Recherche, Ancestrome project ANR-10-BINF-01-01.
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