

Reconciled trees and incongruent gene and species trees

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ABSTRACT. We present a method for visualising and quantifying the relationship between a pair of gene and species trees that constructs a third tree termed the reconciled tree. Given a gene tree and a species tree the reconciled tree represents the history of the gene tree embedded within the species tree. The reconciled tree is constructed from one or more subtrees of the species tree, and contains the gene tree as a subtree. The relationship between the gene and species tree can be expressed in terms of the number of gene duplications and gene losses required to construct the reconciled tree. This number can be used as an optimality criterion for selecting the species tree that best accounts for the observed gene tree.

Introduction

The key assumption that motivates molecular systematics is that evolutionary trees for genes also contain information about the evolutionary relationships of organisms. Indeed, it is often assumed that gene trees and species trees are isomorphic; once the gene tree is obtained the species tree can be obtained simply by relabelling the leaves of the gene tree with the names of the corresponding species. However, two observations contradict this assumption: (1) species may contain more than one copy of the same gene, and (2) different gene trees may imply different species trees. If two or more copies of the gene are sequenced then relabelling the gene tree with the species names will result in some species occurring more than once. In this case there is no longer a one-to-one correspondence between the gene and species trees, raising the problem of how to extract the latter from the former. If different gene trees support different species trees (that is, the gene trees are incongruent) then this raises the question of how to choose among these alternative species trees.

Our goal in this paper is to outline an approach for visualising the relationship between gene and species trees. This method employs a third tree which we call the reconciled tree. The term comes from Goodman's goal [7] of reconciling incongruent gene and species trees. The reconciled tree corresponds to a map between the gene

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and species tree which associates each node in the gene tree with a node in the species tree. Unless the reconciled tree is identical to, or a subtree of, the species tree then the reconciled tree has associated with it a cost that is the sum of the number of gene duplication and gene loss events required to reconcile the gene and species trees. Given that we can compute the “cost” of reconciling a gene and species tree, this cost can be used as an optimality criterion for choosing the species tree that yields the least costly reconciled tree for a given gene tree. Because of the vast number of evolutionary trees for even a few species [6] we will typically need to rely on heuristics to search for optimal species trees. We outline the use of techniques for characterising the search landscape that allow insight into the performance of various search strategies for finding the optimal species tree. We use this technique to reanalyse the 53 gene trees studied by Guigó *et al.* [8].

To avoid potential confusion it is useful to clarify how this approach differs from consensus methods [1, 11] which it superficially resembles. Consensus methods operate on two or more trees with the same terminal labels and are used to display the extent to which two or more trees agree on relationships among the same set of objects. Reconciled trees, however, operate on trees for different entities (e.g., genes and organisms) which are in some sense associated (it is this association that allows us to compare the trees by establishing a relationship between the terminal labels in the two trees). Furthermore, a reconciled tree results from embedding one tree into another. In an important sense, which we elaborate on below, the reconciled tree combines information from both the trees being compared, unlike consensus methods which represent only shared information.

Maps between trees

Central to the concept of a reconciled tree is the notion of a map between two trees. This idea was first introduced by Goodman *et al.* [7] and has recently attracted renewed attention [4, 8, 13, 16]. For simplicity, let us initially assume that we have only a single gene in each of our study species. To distinguish between genes and species we will use the convention of labelling species by the letters a, b, c, \dots , and the genes from those species by $1, 2, 3, \dots$, where gene 1 is from species a , gene 2 from species b , and so on. Let G be a binary gene tree for n sequences obtained from n species, and S be the binary species tree (Figure 1).

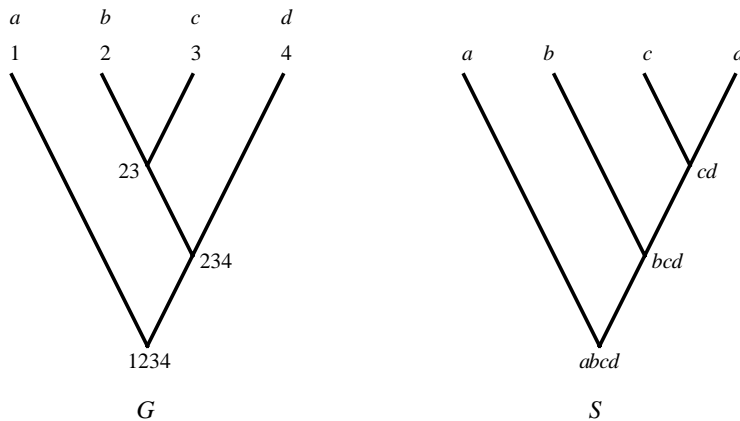


FIGURE 1. Example gene and species trees G and S

For any node $g \in G$, let $\eta(g)$ be the set of species in which occur the extant genes descendant from g . Also, for any $g \in G$, let $M(g) \in S$ be the smallest node in S that includes g , that is the smallest cluster satisfying $\eta(g) \subseteq M(g)$. The node $M(g) \in S$ corresponds to the most recent common ancestor of all the species in which either g (if g is a leaf) or all the genes descendant from g occur. The map between the internal nodes in the two trees in Figure 1 is shown in Figure 2.

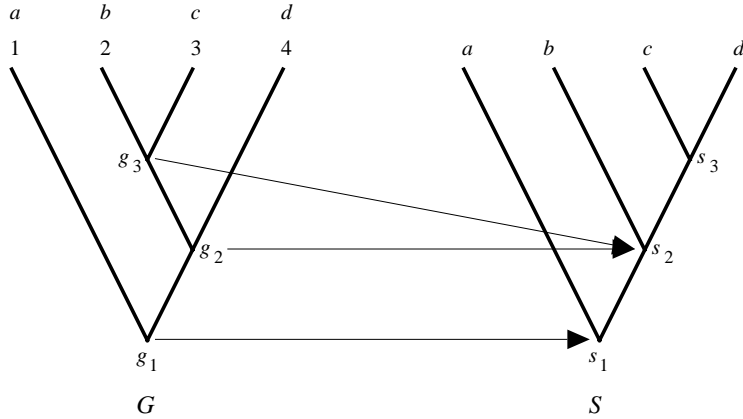


FIGURE 2. Mapping from gene tree G into species tree S .

Constructing a map amounts to finding for each node $g \in G$ the most recent common ancestor of all the species containing genes that descend from g . In the example in Figure 2, the descendants of g_3 are found in species b and c , hence g_3 corresponds to the most recent common ancestor of these species in S , namely s_2 .

Duplications. If each g has a unique image $M(g)$ then G and S are said to be *consistent*. If G and S are not consistent then there will be cases where more than one node in G maps onto the same node in S . These cases are termed duplications.

DEFINITION 1. A duplication is an internal node $g \in G$ for which $M(g) = M(g_l)$ or $M(g) = M(g_r)$, where g_l and g_r are the left and right children of g , respectively.

For the trees in Figure 2, $M(g_2) = M(g_3) = s_2$, hence there is a duplication at g_2 .

Reconciled trees

Reconciling two incongruent gene and species trees requires postulating a combination of gene duplications and losses [7]. A duplication results in two copies of the gene, hence we would expect all the descendants of the species lineage in which the duplication took place to possess those two copies. If they do not then we must postulate gene losses. Figure 3 shows the gene tree G from Figure 1 embedded in its species tree S , and the corresponding reconciled tree.

The duplication at g_2 results in two pairs of gene lineages. Three gene losses (one each in species b , c , and d) are required to account for the absence of one or other of the two gene lineages in those species. If there had been no gene losses then the gene tree would comprise seven leaves. This complete gene tree is the reconciled tree [14, 16].

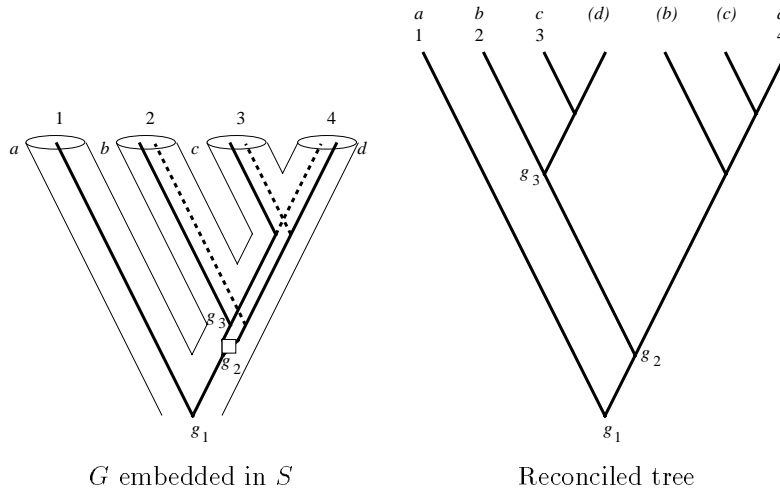


FIGURE 3. Embedding of a gene tree into a species tree, and the “unfolded” gene tree forming the reconciled tree.

The reconciled tree R has two important properties which allow it to depict the relationship between the gene and species tree. The first property is that the observed gene tree is a subtree of the reconciled tree (Figure 3). The second property is that if we label each leaf of the reconciled tree with the corresponding species label then the clusters of the reconciled tree are all clusters of the species tree (Figure 4).

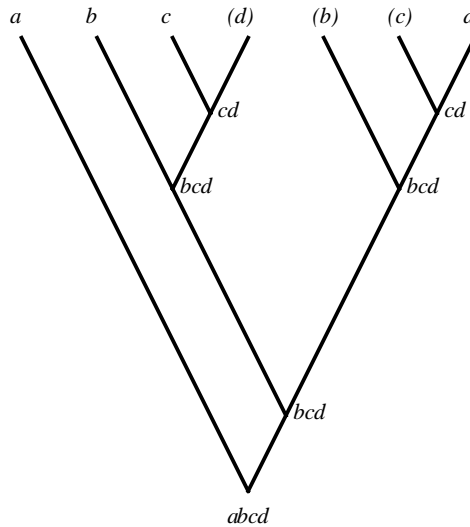


FIGURE 4. Relabelled reconciled tree.

Each cluster in the reconciled tree is also a cluster in the species tree.

However, whereas in S the clusters of any two children of a node $s \in S$ are disjoint, in the reconciled tree any two child clusters of an internal node $r \in R$ are either disjoint or identical. The later case corresponds to a duplication.

Computing numbers of losses. If the gene tree is a strict subtree of the reconciled tree, then there must have been gene losses. To compute the number of losses we can colour the leaves of the reconciled tree with either $\{1\}$ (presence) or \emptyset (absence) of the gene. Each internal node g is assigned the colour $c_g = c_{g_l} \cup c_{g_r}$. If $c_{g_l} \cap c_{g_r} = \emptyset$ then one of the node's children has lost a gene. Hence we can compute the number of losses in a single post-order traversal (i.e., from leaf vertices to root) of the reconciled tree. In examples we have tried this procedure finds the same number of losses as the formulae in [8] and [13] which compute losses as a function of the number of nodes between $M(g)$ and $M(g_l)$ and $M(g_r)$ for each internal node in the gene tree. For formal proof of the equivalence of these measures see Eulenstein (in this volume).

Constructing the reconciled tree. In a reconciled tree a duplication is indicated by a node whose two children have the same cluster (Figure 4). Hence the reconciled tree is assembled from subtrees of S . The following is a sketch of the algorithm in [16] for constructing a reconciled tree R for gene tree G and species tree S :

- : Step 1. Let $R = S$. Colour each leaf in R with $\{1\}$.

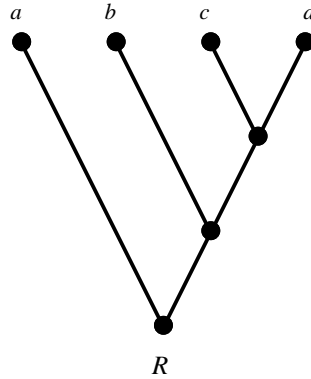


FIGURE 5. Step 1: Each leaf is coloured with $\{1\}$.

- : Step 2. Traverse G in preorder (i.e., from root to leaf vertices). For each node g that is a duplication go to step 3. If the tree has been completely traversed go to step 5.
- : Step 3. Find the node $r \in R$ that corresponds to $M(g)$. Copy the subtree in S rooted at $M(g)$ and add this to R below r . Inserting this subtree creates an additional node x which corresponds to the gene duplication at g . Figure 6 shows this step for the trees in Figure 1.
- : Step 4. Colour the descendants of the two subtrees rooted at x to reflect the presence of the gene. Let x_l and x_r be the left and right children of x , respectively. For each terminal descendant i of $x_l \in R$, if $i \in \eta(g_l)$ then colour it $\{1\}$; otherwise colour it \emptyset . We reverse the colours for x_r , the right child of x .

The internal nodes are coloured using the rule $c_g = c_{g_l} \cup c_{g_r}$. The result of this step for the trees in Figure 1 is shown in Figure 7, where $\bullet = \{1\}$ and $\circ = \emptyset$. Return to step 2.

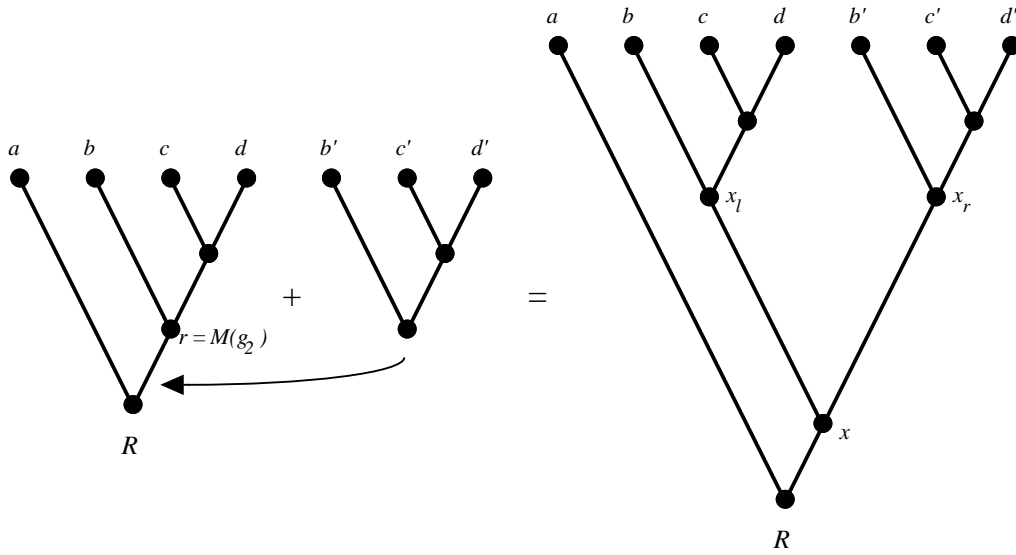
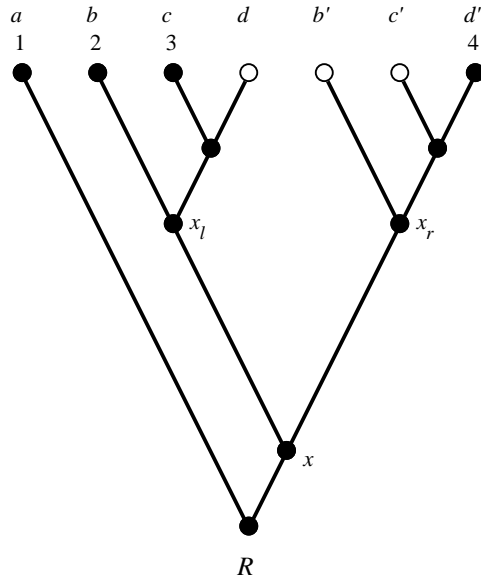
FIGURE 6. Step 3: Adding the subtree rooted at $M(g_2)$.

FIGURE 7. Step 4: Colouring the reconciled tree.

The reconciled tree with nodes coloured to indicate the presence (\bullet) or absence (\circ) of a gene lineage.

: Step 5. Compute the number of losses as described above.

Properties of reconciled trees. The formal properties of reconciled trees have been little explored. One possibility for their exploration is modelling them using multisets [12] as sketched by Page [16]. Clearly the reconciled tree for a given G and S is unique, but this is not sufficient to guarantee the following conjectures:

1. The reconciled tree $R(G, S)$ minimises the sum d of duplications.
2. The reconciled tree $R(G, S)$ minimises the sum $(d + l)$ of duplications and losses.
3. For a given G , all S that minimise $(d + l)$ also minimise d .

These questions are believed to be open at the time of writing, and invite investigation.

Note that the mathematical results presented here are not restricted to binary gene and species trees. The mapping and construction of reconciled trees is well-defined and consistent for polytomous trees. However, interpretation of such trees must be cautious. There are two possible interpretations of polytomies: *hard* and *soft* [10]. Soft polytomies may be resolved in different ways, which may give rise to reconciled trees with different costs.

Embedding subtrees. If we are comparing more than one gene tree with a species tree then it will often be the case that not all the genes are known in all the species of interest. While the algorithms for mapping two trees and for constructing the reconciled tree are still applicable in this case, the number of losses computed needs to be interpreted carefully. For example, in the algorithm given above a gene tree for four genes may be perfectly consistent with a larger species tree (on $n > 4$ species say), but the lack of genes in the remaining $(n - 4)$ of those species will be counted as losses. Given the uneven taxonomic sampling in the sequence data bases (e.g., the predominance of mammals among the 101 genes in the SWISPROT data base listed by Guigó *et al.* [8]) a more reasonable interpretation may be that these species simply have not been sequenced for that gene.

One solution to this problem is to construct the reconciled tree from the subtree that results from pruning the species for which the gene locus is unknown. An alternative is to introduce a third colour, “?”, for those leaves in the reconciled tree that correspond to species that lack any representatives of the gene, that is $s \in (S \setminus G)$. If neither child of g has colour “?” then the rule presented in section 3.1 still applies. However, if either one of the other child, but not both, is “?” then g takes the colour of the other child. If both children are “?” then $g = “?”$ The advantage of constructing the reconciled tree for the complete set of species is that the reconciled tree again highlights those species which we might expect to harbour undiscovered sequences related to the subset of known sequences. For a different treatment of the same problem see Mirkin [13].

Kinds of duplications. For ease of presentation so far we have considered only the case where each species has a single gene, which is the only case considered by Guigó *et al.* [8] and Mirkin [13]. However, we may have gene trees in which more than one sequence is available from the same species. In this instance there will be one or more $g \in G$ where $\eta(g_l) \cap \eta(g_r) \neq \emptyset$. However, we can show that for any such node g , either one or both of its children will map onto the same node in the species tree and hence be correctly interpreted as a gene duplication:

LEMMA 1. *For $g \in V(G)$ with children g_l and g_r such that $\eta(g_l) \cap \eta(g_r) \neq \emptyset$, the embedding mapping M described above is such that either $M(g) = M(g_l)$ or $M(g) = M(g_r)$ or both.*

PROOF. Let $M(g_l) = x$, $M(g_r) = y$, and $M(g) = z$. Then x is the smallest superset in S of $\eta(g_l)$, y is the smallest superset in S of $\eta(g_r)$, and z the smallest superset in S of $\eta(g)$. Choose $a \in \eta(g_l) \cap \eta(g_r)$. Suppose that $z \notin \{x, y\}$. Then

we must have $z \supset x$ and $z \supset y$ since S is a tree. Since $a \in g_i$ then $x \cap y = \emptyset$, a contradiction. \square

Heuristic searches for optimal species trees

The cost of mapping a gene tree into a species tree can be used as an optimality criterion for choosing among alternative species trees. If the species tree is unknown then a natural candidate for it is the tree that yields the least costly reconciled tree [8, 16, 19]. Given the large numbers of possible trees for even moderate numbers of species [6] we will usually have to rely on heuristics which do not guarantee to find the globally optimal solution.

One approach is to search tree space using tree perturbations such as the well-known Nearest Neighbour Interchange (NNI) [23]. An initial starting species tree is chosen and its cost is computed by reconciling it with the gene tree. The start tree is then perturbed in search of a better tree. If one is found, the search continues from the better tree, repeating until no perturbation produces an improvement. This strategy of hill climbing is sensitive to the initial starting tree, and to the conformation of the landscape for the problem instance [3]. In particular, if the search landscape has several locally optimal peaks the heuristic search may find a species tree which is locally optimal but far removed from the global optimum. This problem can be clearly illustrated using the recent study by Guigó *et al.* of 53 genes from a range of eukaryotes [8].

Eukaryote example. Guigó *et al.* took 53 gene trees and searched for the least-cost species tree with which to reconcile them.

Tree perturbations and the search landscape. Taking a landscape approach to the investigation of tree space is a fruitful method of determining the nature of phylogenetic signal in a data set. In this section we describe how landscape-based methods were used to assess the ruggedness of the landscape of the solution space to this problem instance.

We performed 50 simple hill-climbing heuristic searches, with randomly chosen starting trees, using each of two sets of tree perturbations to move between estimated species trees. At each step in a search, the current tree would be perturbed until either a better tree (with lower total cost) was found, or all instances of the perturbation were tried without success, at which point the search would be halted. The initial trees, chosen at random, were identical for the two search strategies.

The first tree perturbation used was Nearest Neighbour Interchange (NNI, [23]) by itself; the second set of perturbations alternated between NNI and Cut and Paste (C&P, also known as Subtree Pruning and Regrafting or SPR [22]). Thus the adjacencies in the second landscape included those in the first.

The NNI search was found to be markedly poorer in recovering least-cost solutions (trees) to this problem instance: in all 50 NNI runs the best tree found, of cost 171, was obtained just once. The best tree found using the ALT method had a cost of 159 (36 duplications and 123 losses), which was obtained 6 times. Note also that all but one of the NNI searches was less successful than all the ALT searches.

Figure 8 shows the costs of the best trees found in each of the searches, for NNI and ALT methods, plotted against maximal steepest climb length [3]. From this figure we can deduce that the landscape induced by the NNI adjacencies is more “rugged” than that induced by (NNI + C&P) as with the ALT search.

Since our best tree found was still obtained only 6 times in the 50 runs, we must stress the importance of performing multiple searches from random starting points [9]. We cannot begin to have confidence in the global optimality of our best solutions found, until we have encountered them many times.

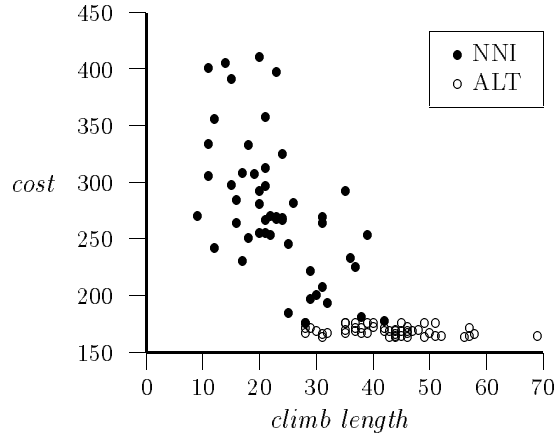


FIGURE 8. The maximal steepest climb lengths for 50 NNI and 50 ALT searches.

In the above figure we see the markedly poorer performance of simple NNI searching compared with the ALT search (q.v.). In 50 NNI searches none found the best found with the ALT search.

Guigó *et al.*'s preferred species tree is shown in Figure 9(a), for which they found 46 duplications and 101 losses. Using the algorithm for constructing reconciled trees we also found 46 duplications but an additional 44 losses, for a total cost of $46 + 145 = 191$. The same cost was found using Mirkin's formula [13], hence we suspect that Guigó *et al.*'s value for the losses is an error. Guigó *et al.* also report that their best species tree is wholly consistent with 18 of the gene trees, however we find it is consistent with only 17.

Our best tree found using the ALT searches has a cost of 159 (36 duplications + 123 losses) and is consistent with 25 of the gene trees (Figure 9(b)).

Using Page's program COMPONENT [15] we found that the Guigó *et al.* tree is 7 NNI steps from their seed tree (Figure 9(c)). This distance is substantially shorter than most of our NNI climbs, but comparison of the searches on this basis is confounded by the difference in counting methods of the authors and of Guigó *et al.*. It is interesting to note that the best tree found (Figure 9(b)) in the ALT searches is 13 NNI steps from their seed tree, and 15 NNI steps from their best tree found, so in a very loose sense the heuristic search strategy adopted by Guigó *et al.* went in the wrong "direction" from the seed tree.

The best trees found. A more thorough search led to the discovery of 11 more trees, each requiring 36 duplications and 123 losses, and which differed only slightly from each other. The most biologically reasonable one is that shown in Figure 9(b). The Adams consensus tree [2] of these 12 trees is shown in Figure 10(a), and the strict consensus is in Figure 10(b).

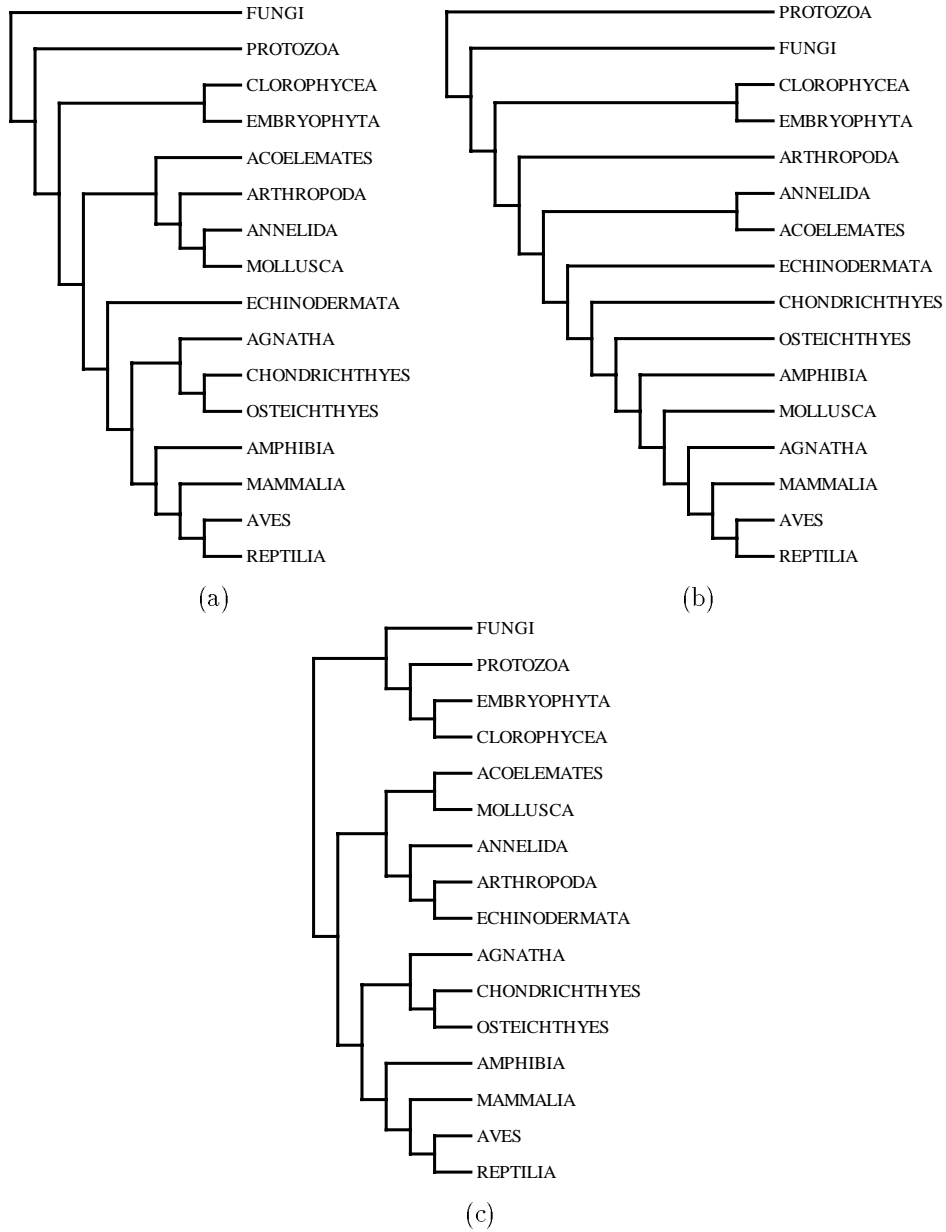


FIGURE 9. Three species trees for the 53 gene trees: (a) Guigó *et al.*'s tree; (b) our tree; (c) Guigó *et al.*'s seed tree.

Note that of the original 53 gene trees, only 4 had a mollusc sequence, and only one had an Agnathan, so it is perhaps not surprising that it is these two taxa whose position is so odd in the optimal trees we found.

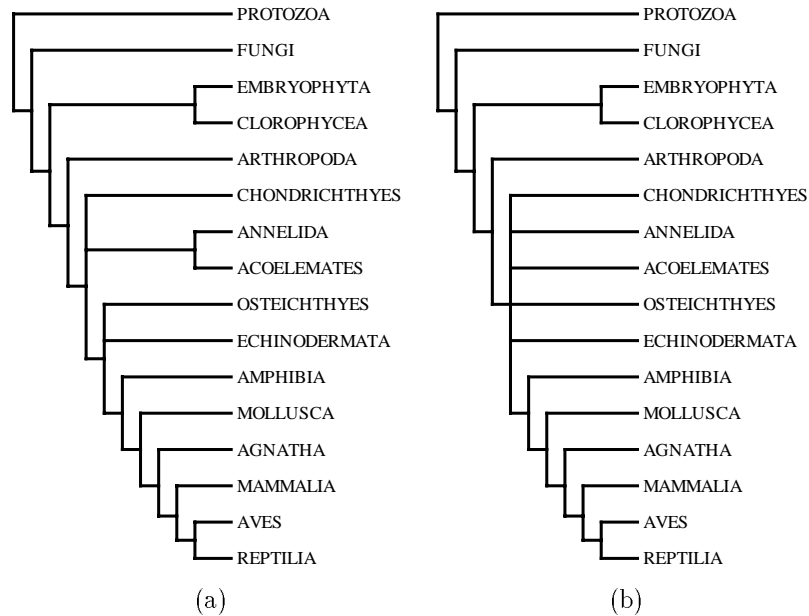


FIGURE 10. Adams and Strict consensus trees of the 12 least-cost species trees found from 53 gene trees.

Limitations

Allele phylogenies and coalescence. In this paper we have focussed on interpreting reconciled trees in terms of gene duplications and losses. Descendants of one or more gene duplications are paralogous [5]. However, orthologous sequences (which by definition have not undergone gene duplication) may also be present in multiple copies (alleles) and may yield gene trees which are discordant with the species tree. In this context “duplications” inferred by the reconciled tree are not literally gene duplications; rather they represent coalescences (instances of common ancestry) of intraspecific allele lineages. Rather than numbers of duplications and losses it may be more biologically meaningful to count other aspects of the reconciled tree, such as the number of times a pair of alleles from two different species fail to coalesce in the immediate ancestor of those species. Failures of the alleles into coalesce, depth to coalescence, and numbers of gene lineages present on each edge in the species tree are among the parameters that could be readily measured.

Horizontal Transfer. From a biological perspective perhaps the greatest limitation of this approach is that it excludes any possibility of horizontal transfer of genes between different species lineages [21]. Reconciled trees require that a species always acquires its genes from its immediate ancestors, whereas horizontal transfer implies that a species may have acquired a gene from another, contemporaneous lineage. Horizontal transfer introduces new complications because we are no longer simply interested in embedding one tree inside another. In particular, horizontal transfer establishes links between edges of the species tree. Given that horizontal transfer must take place between contemporaneous lineages not all the possible

pairs of edges will be valid horizontal transfers. Page [17] pointed out that transfers cannot take place between a lineage and either its descendants or its ancestors. However, by itself this rule is inadequate to ensure that only logically valid horizontal transfers are postulated [20]. Consider the example shown in Figure 11 of a species tree with two possible horizontal transfers indicated.

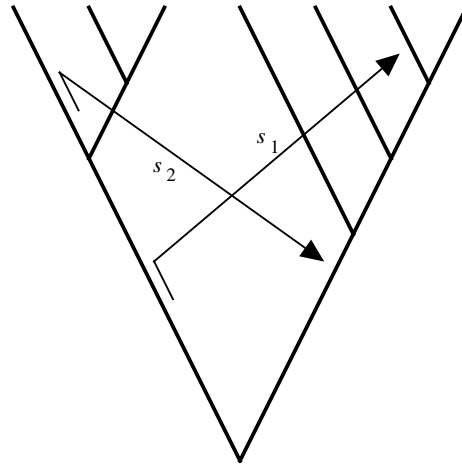


FIGURE 11. A pair of incompatible horizontal transfer events

In each case the transfer is between a pair of edges where neither edge is ancestral to the other, satisfying the rule in [17]. However, considered together these two horizontal transfers are mutually incompatible, as they stand. There is no ordering of the internal nodes of the species tree that will allow both switches to take place without one transfer going forward or back in time (Figure 12).

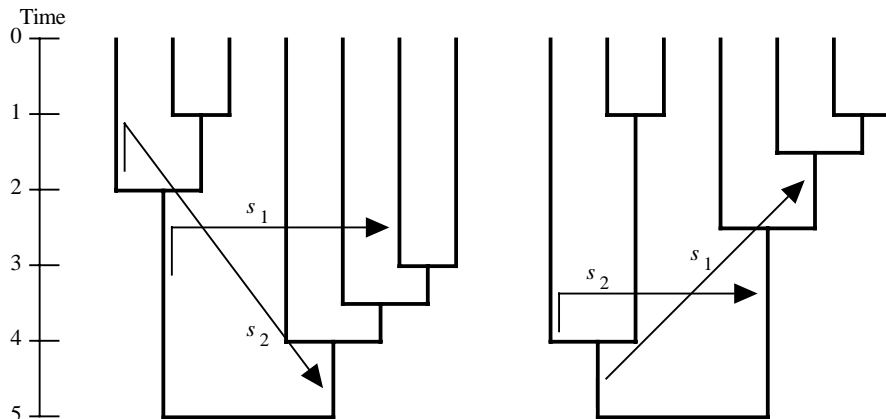


FIGURE 12. Two temporal orderings of the internal nodes of the tree in Figure 11

The horizontal transfers s_1 and s_2 are incompatible because there is no ordering of the internal nodes of the tree in Figure 11 that will ensure that both s_1 and s_2 take place between contemporaneous taxa.

Horizontal transfer introduces additional complexity because we have to consider the relative (temporal) order of internal nodes in the evolutionary tree. The

challenge is to develop methodologies which are capable of dealing with all the complexities introduced by horizontal transfers. This is being undertaken by the authors and is intended to be included in the next release of TREEMAP [18].

Conclusions

Reconciled trees are a simple way to visualise the relationship between a gene and a species tree. By displaying the complete history of the gene they allow us to see where gene duplications (both directly observed and inferred) occurred, and which species might yield further sequences of the same gene family. For these reasons we find them more intuitive than the labelling scheme adopted by Mirkin *et al.* [13]. The reconciled tree suggests a straightforward measure of the degree of fit between a gene tree and a species tree, namely the number of gene duplications and gene losses required to reconcile the two trees. This measure can be used as an optimality criterion for selecting among competing species trees. However, searches using this criterion must be conducted with care in order to avoid suboptimal species trees.

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