An Empirical Comparison Between BPAnalysis and MPBE on the *Campanulaceae* Chloroplast Genome Dataset

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Abstract

The *breakpoint phylogeny* is an optimization problem proposed by Blanchette *et al.* for reconstructing evolutionary trees from gene order data. These same authors developed and implemented `BPAnalysis` [4], a heuristic method (based upon solving many instances of the Travelling Salesman Problem) for estimating the breakpoint phylogeny. We present a new heuristic for estimating the breakpoint phylogeny which, although not polynomial-time, is much faster in practice than `BPAnalysis`. We use this heuristic to conduct a phylogenetic analysis of the flowering plant family *Campanulaceae*. We also present and discuss the results of experimentation on this real dataset with three methods: our new method, `BPAnalysis`, and the neighbor-joining method [21], using breakpoint distances, inversion distances, and inversion plus transposition distances.

Introduction

Phylogenetic tree reconstruction is a major aspect of much biological research. This is a very difficult computational problem because most optimization tasks related to tree reconstruction are NP-hard and can require years to solve on real datasets. With the recent introduction of whole genomes for use in phylogenetic reconstruction, the problem has become even more complex. In this paper, we present a new technique for reconstructing trees from gene order data and we compare this new technique to other approaches using chloroplast genomes from the flowering plant family *Campanulaceae*.

The genomes of some organisms have a single chromosome or contain single-chromosome organelles (such as mitochondria or chloroplasts) whose evolution is largely independent of the evolution of the nuclear genome for these organisms. Many single-chromosome organisms and organelles have circular chromosomes. Given a particular strand from a single chromosome, whether linear or circular, we can infer the ordering of the genes, along with directionality of the genes, thus representing each chromosome by an ordering (linear or circular) of signed genes. Note that picking the complementary strand produces a different ordering, in which the genes appear in the reverse direction and reverse order. The evolutionary process that operates on the chromosome can thus be seen as a transformation of signed orderings of genes.

The first heuristic for reconstructing phylogenetic trees from gene order data was introduced by Blanchette *et al.* in [4]. It sought to reconstruct the *breakpoint phylogeny* and was applied to a variety of datasets [5, 24].

A different technique for reconstructing phylogenies from gene order data was introduced by Cosner in [8]. This method also attempts to find the breakpoint phylogeny, but has a very different approach. We call this technique *Maximum Parsimony on Binary Encodings (MPBE)*. The MPBE method first encodes a set of genomes as binary sequences and then constructs maximum-parsimony trees for these sequences. Cosner used this method to reconstruct a phylogeny for 18 chloroplast genomes from the *Campanulaceae*. This analysis revealed an incredible diversity of genome rearrangements, including inversions, insertions, deletions, gene duplications, and putative transpositions. Transpositions in particular have only rarely been hypothesized for chloroplast evolution, therefore the inference of these events for the *Campanulaceae* was surprising. Also interesting were the extensive contractions and expansions of the inverted repeats, and the disruption of highly conserved operons. The variety of rearrangements far exceeds that reported in any group of land plants, making it challenging to determine the exact numbers and the evolutionary sequence of rearrangement events. Several of these events are of particular interest because they have not been encountered elsewhere, or because they are common in the *Campanulaceae*.

In this paper, we report on an extensive phylogenetic analysis of a subset of the original dataset (12 of the original 18 genera) using a simple version of the MPBE technique. We also compare the performance of this method to two other methods for phylogeny reconstruction based upon gene order data: `BPAnalysis` (the heuristic designed and implemented by Blanchette *et al.* [4]) and the polynomial-time, distance-based method of neighbor-joining [21], using a variety of distance measures.

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Definitions

We assume a fixed set of genes $\{g_1, g_2, \dots, g_n\}$. Each genome is then an ordering of some multi-subset of these genes, each gene given with an orientation that is either positive (g_i) or negative ($-g_i$). The multi-subset formulation allows for deletions or duplications of a gene. A linear genome is then simply a permutation on this multi-subset, while a circular genome can be represented in the same way under the implicit assumption that the permutation closes back on itself. For example, the circular genome on gene set $\mathcal{G} = \{g_1, g_2, \dots, g_6\}$ given by $g_1, g_2, -g_3, g_4, g_6, g_2$ has one duplication of the gene g_2, has a deletion of the gene g_5, and has a reversal of the gene g_3. That same circular genome could be represented by several different linear orderings, each given by rotating the linear ordering above. Furthermore the ordering $g_1, g_2, \dots, g_n$, whether linear or circular, is considered equivalent to that obtained by considering the complementary strand, i.e., to the ordering $-g_n, -g_{n-1}, \dots, -g_1$.

In tracing the evolutionary history of a collection of single-chromosome genomes, we use inversions, transpositions and transversions (inverted transpositions), because these events only rearrange gene orders; a more complex set of structural changes has been considered in [8].

Let G be the genome with signed ordering $g_1, g_2, \dots, g_n$. An *inversion* between indices i and j, for $i < j$, produces the genome with linear ordering

$$g_1, g_2, \dots, g_{i-1}, -g_j, -g_{j-1}, \dots, -g_i, g_{j+1}, \dots, g_n$$

If we have $j < i$, we can still apply an inversion to a circular (but not linear) genome by simply rotating the circular ordering until the two indices are in the proper relationship—recall that we consider all rotations of the complete circular ordering of a circular genome as equivalent.

A *transposition* on the (linear or circular) ordering G acts on three indices, i, j, k, with $i < j$ and $k \notin [i, j]$, and operates by picking up the interval $g_i, g_{i+1}, \dots, g_j$ and inserting it immediately after g_k. Thus the genome G above (with the additional assumption of $k > j$) is replaced by

$$g_1, g_2, \dots, g_{i-1}, g_{j+1}, \dots, g_k, g_i, g_{i+1}, \dots, g_j, g_{k+1}, \dots, g_n$$

Once again, if we have $j > i$, we can still apply the transposition to a circular (but not linear) genome by first rotating it to establish the desired index relationship.

An *edit sequence* describes how one genome evolves into another through a sequence of these evolutionary events. For example, let G be a genome and let $p_1, p_2, \dots, p_k$ be a sequence of evolutionary events operating on G; then $p_1, p_2, \dots, p_k(G)$ defines a genome G'. When each operation is assigned a cost, then the *minimum edit distance* between two genomes G and G' is defined to be the minimum cost of any edit sequence transforming G into G'. When the cost of each operation is finite, any two genomes have a finite edit distance. (Similarly, if the probability of each edit operation is given, we can define the edit sequence of maximum probability.)

The *inversion distance* between two genomes is the minimum number of inversions needed to transform one genome into another. The inversion distance between two genomes is computable in polynomial time for signed genomes [14, 16] and is available in software as `signed_dist`, but is NP-hard [6]—indeed APX-hard [2]—in the unsigned case. The *transposition distance* between two genomes is the minimum number of transpositions needed to transform one genome into the other. Computing the transposition distance is of unknown computational complexity but, for the case of linear genomes, Bafna and Pevzner [1] have found a 1.5-approximation algorithm. When both transpositions and inversions are allowed, nothing is known about the computational complexity or approximability of computing edit distances, although heuristics have been developed for that purpose and are available in software. (In particular, `derange2` [3] is a fast heuristic for estimating these distances, and has been used both by Sankoff *et al.* [23] and by us.)

Another genome metric that is not a direct evolutionary metric is the *breakpoint distance*. Given two genomes G and G' on the same set of genes, a breakpoint in G is defined as an ordered pair of genes (g_i, g_j) such that g_i and g_j appear consecutively in that order in G, but neither (g_i, g_j) nor $(-g_j, -g_i)$ appear consecutively in that order in G'. For instance, if $G = g_1, g_2, -g_4, -g_3$ and $G' = g_1, g_2, g_3, g_4$, then there are exactly two breakpoints in G: $(g_2, -g_4)$, and $(-g_3, g_1)$; the pair $(-g_4, -g_3)$ is not a breakpoint in G' since (g_3, g_4) appear consecutively and in that order in G'. The *breakpoint distance* is the number of breakpoints in G relative to G' (or vice-versa, since the measure is symmetric).

An *evolutionary tree* (or *phylogeny*) for a set S of genomes is a binary tree with $|S|$ leaves, each leaf labeled by a distinct element of S. A putative evolutionary tree is "correct" as long as this leaf-labeled topology is identical to the true evolutionary tree (which we do not know for real data sets). The process of tree reconstruction generally involves the inference of additional aspects of the tree. For example, a given method may infer weights on the edges (also called "branch lengths"), genomes at internal nodes (i.e., "ancestral" genomes), or probabilities for each type of evolutionary event on each edge of the tree. These topologies and additional parameters are estimated in order to optimize some objective criterion; the three basic optimization criteria in use by biologists are *Maximum Parsimony*, *Distance-Based Methods*, and *Maximum Likelihood*. We briefly review the first two criteria.

Maximum parsimony: Assume that we are given a tree in which each node is labelled by a genome. We define the cost of the tree to be the sum of the costs of its edges, where the cost of an edge is one of the edit distances between the two genomes that label the endpoints of the edge. Finding the tree of minimum cost for a given set of genomes and a given definition of the edit distance is the problem of *Maximum Parsimony for Rearranged Genomes (MPRG)*; the optimal trees are called the maximum-parsimony trees. (The MPRG problem is related to the more usual maximum-parsimony problem for biomolecular sequences, where the edit distance between two sequences is just the number of positions in which they differ, or the Hamming distance.)

Distance-based methods: Distance-based methods for tree reconstruction operate by first computing all pairwise distances between the taxa in the dataset, thus computing a representation of the input data as a distance matrix d. In the context of genome evolution, this calculation of distances is done by computing minimum edit distances, based upon some cost function for each of the allowed operations (inversions, transpositions, etc.). Given the distance matrix d, the method computes an edge-weighted tree whose leaf-to-leaf distances closely fit the distance matrix. Since almost all optimization problems related to tree reconstruction are NP-hard, the most frequently used distance-based methods are polynomial-time methods such as neighbor-joining [21]; these do not explicitly seek to optimize any criterion, but can have good performance in empirical studies. In particular, neighbor-joining has had excellent performance in studies based upon simulating biomolecular sequence evolution and is probably the most popular distance-based method.

Evaluating Phylogenies: The False Positive and False Negative Rate

Let T be a tree leaf-labelled by the set S. Given an edge e in T, the deletion of the edge from T produces a bipartition π_e of G into two sets. The set $C(T) = \{\pi_e : e \in E(T)\}$ uniquely defines the tree T; this characterization is called the *character encoding* of T. Given a collection of trees $T_1, T_2, \ldots, T_k$, each leaf-labelled by S, we define the *strict consensus* of the trees to be that unique tree T_{sc} defined by $C(T_{sc}) = \cap_i C(T_i)$. This is the maximally resolved tree which is a common contraction of each tree T_i. Character encodings are used to compare trees and to evaluate the performance of a phylogenetic reconstruction method. Let T be the "true" tree and let T' be the estimate of T. Then the *false negatives* of T' with respect to T are those edges e that obey $\pi_e \in C(T) - C(T')$, i.e., edges in the true tree that the method fails to infer. The *false positives* of T' with respect to T are those edges e that obey $\pi_e \in C(T') - C(T)$, i.e., edges in the inferred tree that do not exist in the true tree and should not have been inferred. Note that every trivial bipartition (induced by the edge incident to a leaf) exists in every tree. Consequently, false positives and false negatives are calculated only with respect to the internal edges of the tree and expressed as a percentage of the number of internal edges.

Previous Work in Reconstructing Trees from Gene Order Data

Most of the work done by computer scientists related to genome rearrangements has focused on the problem of computing minimum edit distances, i.e., inversion distances or inversion plus transposition distances between two linear (or circular) orderings on (signed or unsigned) genes. Most of these problems are not yet known to be solvable in polynomial time, so that heuristics of unknown accuracy are used. We focus instead on the problem of inferring phylogenetic trees from the gene order data, a problem that has seen comparatively little work to date.

Distance-Based Methods for Reconstructing Trees

There has been little use of distance-based methods for reconstructing phylogenies from gene order data. However, in a recent publication, Blanchette *et al.* [5] evaluated two of the most popular polynomial-time distance-based methods for phylogenetic reconstruction, neighbor-joining and Fitch-Margoliash [11], for the problem of reconstructing the phylogeny of metazoans. They calculated a breakpoint distance matrix for inferring the metazoan phylogeny from mitochondrial gene order data. The trees obtained by these methods were unacceptable because they violated assumptions about metazoan evolutionary history. Later, they examined a different dataset and found the result to be acceptable with respect to evolutionary assumptions about that dataset [22].

Computational Complexity of MPRG

MPRG seems to be the optimization criterion of choice. Indeed, most approaches to reconstructing phylogenetic trees from gene order data have explicitly sought to find the maximum-parsimony tree with respect to some definition of genomic distances (inversion distances or a weighted sum of inversions, transpositions, and transversions). All these problems are NP-hard however, or of unknown computational complexity. Even the fundamental problem of computing optimal labels (genomes) for the internal nodes is very difficult. When only inversions are allowed, it is NP-hard, even for the case where there are only three leaves [7].

Breakpoint Phylogeny

Recently, Blanchette *et al.* [5] proposed a new optimization problem for phylogeny reconstruction on gene order data. In this problem, the tree sought is that with the minimum number of breakpoints, rather than that with the minimum number of evolutionary events (e.g., inversions, transpositions, transversions, etc.). It has long been known that the breakpoint distance is at most twice the inversion distance for any two genomes. For some datasets, however, there can be a close-to-linear relationship between the breakpoint distance and either the inversion distance or the weighted sum of inversions and transpositions. When a linear relationship exists, the tree with the minimum number of breakpoints is also the tree with the minimum number of evolutionary events. Consequently, when a close-to-linear relationship exists, the tree with the minimum number of breakpoints may be close to optimal with respect to the number of evolutionary events. Blanchette *et al.* [5] observed such a close-to-linear relationship in a group of metazoan genomes (the correlation coefficient between the two measures for their set was 0.9815) and went on to develop a heuristic for finding the breakpoint phylogeny.

Computing the breakpoint phylogeny is NP-hard for the case of just three genomes [20], a special case known as the *Median Problem for Breakpoints (MPB)*. Blanchette *et al.* however, showed that the MPB reduces to the Travelling Salesman Problem (TSP) and designed special heuristics for the resulting instances of TSP. Their heuristic approach to solving the breakpoint phylogeny exactly solves numerous instances of the TSP. Specifically, their algorithm considers each tree topology in turn; for each tree, it fills in internal nodes by computing medians of triplets of genomes iteratively (until no change occurs) and scores the resulting tree. The best tree is returned at the end of the procedure. This heuristic is computationally intensive on several levels. First, the number of unrooted binary trees on n leaves is $(2n-5)\cdot(2n-7)\cdot\ldots\cdot 3)$, so that the outer loop is exponential in the number of genomes. Secondly, the inner loop itself is computationally intensive, since computing the median of three genomes is NP-hard [20] and since the technique used by Blanchette *et al.* involves solving many instances of TSP in a reduction where the number of cities equals the number of genes in the input. Finally, the number of instances of TSP can be quite large, since the procedure iterates until no further change of labelling occurs within the tree. Thus the computational complexity of the entire algorithm is exponential in *each* number of genomes and the number of genes.

The accuracy of `BPAnalysis` for the breakpoint phylogeny problem depends upon the accuracy of its component heuristics. While it evaluates every tree, the labelling given to each tree is only locally optimal: although it solves TSP exactly at each node, it labels nodes with an iterative method that can easily be trapped at a local optimum. In our experiments, we have found that `BPAnalysis` often needed to be run on several different random starting points in order to score a given tree accurately. This is typical of hill-climbing heuristics [18], but will affect the running time proportionally.

Our New Method: Maximum Parsimony on Binary Encodings of Genomes (MPBE)

Our new technique has two phases. In the first phase, we encode the input genomes as a collection of binary sequences and analyze these sequences under maximum parsimony. In the second phase, we screen the set of maximum-parsimony trees found, and select those that minimize the number of breakpoints, number of inversions, or number of inversions/transpositions/transversions.

Phase I: Solving Maximum Parsimony on Binary Encodings of Genomes

We now show how we define the binary sequences. We note all ordered pairs of signed genes (g_i, g_j) that appear consecutively in at least one of the genomes. Each such pair defines a position in the sequences (the choice of index is arbitrary). If (g_i, g_j) *or* $(-g_j, -g_i)$ appear consecutively in a genome, then that genome has a 1 in the position for this ordered pair, and otherwise it has a 0. These "characters" can also be weighted. (In this study, we did not weight any characters; however, in the study reported in [8], character weighting was used, along with other characters such as gene segment insertions and deletions, duplications of inverted repeats, etc. Thus, the method can be extended to allow for evolutionary events more complex than gene order changes.)

Now let $H(e)$ be the Hamming distance between the sequences labelling the endpoints of the edge e—the Hamming distance between two sequences is the number of positions in which they differ. We define the *Binary Sequence Maximum Parsimony (BSMP)* problem as follows: the input consists of a set S of binary sequences, each of length k; the output is a tree T with leaves labelled by S and internal nodes labelled by additional binary sequences of length k in such a way as to minimize $\sum H(e)$ as e ranges over the edges of the tree. The trees with the minimum score are called maximum-parsimony trees.

Our first phase then operates as follows. First, each genome is replaced by a binary sequence. The BSMP problem is then solved exactly or approximately, depending upon the dataset size; BSMP is NP-hard [12], but fast heuristics exist that are widely available in standard phylogeny software packages, such as `PAUP` [25]. Although no study has been published on the accuracy of these heuristics on large datasets, it is generally believed that these heuristics usually work well on datasets of size up to about 40 genomes. Moreover, exact solutions on datasets of up to about 20 genomes can be obtained through branch-and-bound techniques in reasonable amounts of time; consequently, BSMP has been solved exactly in some cases.

Phase II: Screening the Maximum-Parsimony Trees

Once the maximum-parsimony trees are obtained, the internal nodes are labelled by circular signed gene orders by giving the topology of the maximum-parsimony tree as a constraint to `BPAnalysis`, thus producing a labelling which (hopefully) minimizes the breakpoint distance of the tree. The labelling also allows us to score each tree for the minimum number of inversions (by scoring each edge using `signed_dist` [13]), or for the minimum number of inversions/transpositions/transversions events (by scoring each edge using `derange2` [3]), depending upon the relative costs of these events. Depending upon which objective criterion is desired, the tree that minimizes the total cost is then returned.

Running Time of MPBE

The computational complexity of MPBE, while less than that of `BPAnalysis`, remains high. Evaluating a single tree topology in the search space takes polynomial time—more precisely, takes $\Theta(nk)$ time, where n is the number of genomes and k is the number of genes in each genome, but the search for the maximum-parsimony trees is based upon hill-climbing through the space of tree topologies. Thus finding the maximum parsimony trees is exponential in the number of genomes but only polynomial in the number of genes. Labelling the internal nodes of each maximum-parsimony tree by using constraint trees for `BPAnalysis` is expensive, but we generally only examine a small percentage of the space of trees and thus reduce the computational cost significantly by comparison to the exhaustive search strategy of `BPAnalysis`. Evaluating the cost of each tree with respect to inversion or inversion/transposition/transversion distances is quite fast.

Accuracy of MPBE for the Breakpoint Phylogeny

We now show that MPBE should be seen as a heuristic for the breakpoint phylogeny problem. Suppose T is the breakpoint phylogeny for the set $G_1, G_2, \ldots, G_n$ of genomes. Each node in T is labelled by a circular ordering of signed genes and the number of breakpoints in the tree is minimized. If each node in the tree is then replaced by the binary encoding, using the technique described earlier, the parsimony length of the tree (given these sequences at each node) is exactly twice the number of breakpoints in the tree. Thus, seeking a tree with the minimum number of breakpoints is exactly the same as seeking a tree (based upon binary encodings) with the minimum parsimony length, *provided that* each binary sequence can be realized by a circular ordering of signed genes. (Not all binary sequences are derivable from signed circular orderings on genomes!) This is why we have included Phase II in our method.

The accuracy of the MPBE method with respect to the breakpoint phylogeny problem depends upon several issues. The major issue is whether the topology of the breakpoint phylogeny will be one of the maximum-parsimony trees obtained in the first phase (in our experiments it has been, but this may not hold true throughout the parameter space, and further experiments will be needed to establish this). The other issues are simpler: since each of the problems we solve (maximum parsimony on binary sequences, the median problem for breakpoints, and the inversion/transposition/transversion distance) is either known or conjectured to be NP-hard, the accuracy of the heuristics will determine whether we find globally optimal or only locally optimal solutions.

Chloroplast DNA Structure

Chloroplast DNA is generally highly conserved in nucleotide sequence, gene order and content, and genome size [19]. The genomes contain approximately 120 genes which are involved in photosynthesis, transcription, translation, and replication. Major changes in gene order, such as inversions, gene or intron losses, and loss of one copy of the inverted repeat, are usually rare. Therefore, they are extremely useful as phylogenetic markers because they are easily polarized and exhibit very little homoplasy when properly characterized [9]. In groups in which more than one gene order change has been detected, the order of events is usually readily determined (e.g., [17, 15]). Chloroplast DNA gene order changes have been useful in phylogenetic reconstruction in many plant groups (see [9]). These changes have considerable potential to resolve phylogenetic relationships and they provide valuable insights into the mechanisms of cpDNA evolution.

The *Campanulaceae* cpDNA Dataset

We have used the chloroplast genomes of the flowering plant family *Campanulaceae* for a test case of our technique. In earlier work [8], Cosner obtained detailed restriction site and gene maps for 18 genera of the *Campanulaceae* and used a variant of the MPBE analysis described above to obtain a phylogenetic analysis of these genera. When restricted to gene order rearrangements, 6 of the genera are shown to be duplicates of other genera (they differ only in terms of insertions and deletions of gene segments, or expansions and contractions of the inverted repeat). In order to compare our technique with `BPAnalysis` and other reconstruction techniques based upon gene orders, we reduced the dataset to 12 genera and 105 genes after eliminating repeated genes. We included the tobacco genome as an outgroup for this analysis, thus producing a dataset of 13 genomes.

This chloroplast dataset is interesting to us for several reasons. The phylogeny reconstructed by our analysis is extremely close to that obtained by other analyses, including neighbor-joining analyses based upon either breakpoint distances or inversion and transposition distances. The genome phylogeny is also very similar to the gene phylogenies that have been reconstructed. Thus, it represents an *easy* test case for the topology estimation problem and it is reasonable to assume that the topology is reliable. However, because of the diversity of types of evolutionary events, it suggests an interesting model of genome evolution and also makes the inference of the gene orderings for the internal nodes quite complicated.

Chloroplast Data Analysis

We used gene maps to encode each of the 13 genera as a circular ordering of signed gene segments. We represent each circular ordering as a linear ordering, beginning at gene segment 1. In order to conserve space (and make the rearrangements easier to observe), we have represented each ordering compactly by noting the maximal intervals of consecutive gene segments with the same orientation. Thus the sequence 1, 2, -4, -3, 5, 6, 7, 10, 8, 9 would be represented as (1–2)(4–3)(5–7)(10)(8–9). Tobacco has the "unrearranged" ordering 1, 2, ..., 105, which we represent as (1–105). Figure 1 gives the compact representations of the genomes for the 13 genera.

We used these 13 circular orderings as input to `BPAnalysis`. The program spent over 43 hours of computation time without completing. We also encoded these orderings with our binary encoding technique and conducted an analysis of the resulting binary sequences under maximum parsimony using the branch-and-bound procedure of `PAUP`. We obtained four maximum parsimony trees from this dataset. (The sequences, as well as the four MP trees, are available on our web page[26]. They can also be calculated directly from the gene order data. Note that the ordering of the sequence of breakpoints does not affect the results.) We then inferred circular orderings of signed gene segments for each internal node by giving each of the four binary MP trees as a constraint tree to `BPAnalysis`. This produces a tree in which each node (internal and leaf) is represented by circular signed orderings on genes, potentially minimizing the number of breakpoints in the tree. (An actual minimization is not guaranteed, because `BPAnalysis` uses hill-climbing on each fixed-tree and thus may find only a local minimum.) We then scored each tree for the number of breakpoints. Interestingly, the labelling of internal nodes obtained by `BPAnalysis` produced the same number of breakpoints on all four trees, .

We note that the best breakpoint score obtained in 43 hours of computation by `BPAnalysis` from the original orderings was 96—substantially larger than the

Trachelium
(1–15)(76–56)(53–49)(37–40)(35–26)(44–41)(45–48)
(−36)(25–16)(90–84)(77–83)(91–96)(55–54)(105–97)
Campanula
(1–15)(76–49)(39–37)(40)(35–26)(44–41)(45–48)
(−36)(25–16)(90–84)(77–83)(91–96)(55–54)(105–97)
Adenophora
(1–15)(76–49)(39–37)(29–35)(40)(26–27)(44–41)(45–48)
(−36)(25–16)(90–84)(77–83)(91–96)(55–54)(105–97)
Symphyandra
(1–15)(76–56)(39–37)(49–53)(40)(35–26)(44–41)(45–48)
(−36)(25–16)(90–84)(77–83)(91–96)(55–54)(105–97)
Legousia
(1–15)(76–56)(27–26)(44–41)(45–48)(36–35)(25–16)
(90–84)(77–83)(91–96)(5–8)(55–53)(105–98)(28–34)
(40–37)(49–52)(−97)
Asyneuma
(1–15)(76–57)(27–26)(44–41)(45–48)(36–35)(25–16)(89–84)
(77–83)(90–96)(105–98)(28–34)(40–37)(49–52)(−97)
Triodanus
(1–15)(76–56)(27–26)(44–41)(45–48)(36–35)(25–16)
(89–84)(77–83)(90–96)(55–53)(105–98)(28–34)(40–37)
(49–52)(−97)
Wahlenbergia
(1–11)(60–49)(37–40)(35–28)(12–15)(76–61)(27–26)
(44–41)(45–48)(−36)(54)(25–16)(90–84)(77–83)(91–96)
(−55)(105–97)
Merciera
(1–10)(49–53)(28–35)(40–37)(60–56)(11–15)(76–61)
(27–26)(44–41)(45–48)(−36)(54)(25–16)(90–85)(77–84)
(91–96)(−55)(105–97)
Codonopsis
(1–8)(36–18)(15–9)(40)(56–60)(37–39)(44–41)(45–53)
(16–17)(54–55) (61–76)(96–77)(105–97)
Cyananthus
(1–8)(29)(36–26)(40)(56–60)(37–39)(25–9)(44–48)
(55–49)(61–96)(105–97)
Platycodon
(1)(8)(2–5)(29–36)(56–50)(28–26)(9)(49–45)(41–44)
(37–40)(16–25)(10–15)(57–59)(6–7)(60–96)(105–97)
Tobacco
(1–105)

Figure 1: 12 genera of *Campanulaceae* and the outgroup Tobacco, as circular orderings of signed gene segments

breakpoint score obtained by our parsimony analysis of binary sequences.

We then scored each tree (using the labels assigned by `BPAnalysis`) for the inversion distance and for the inversion/transposition/transversion distance. We used weights of 2.1 for transpositions and transversions and 1 for inversions. Using this weighting scheme, the first tree has a total of 40 inversions and 12 transpositions/transversions; the second has 48 inversions and 18 transpositions/transversions; the third has 40 inversions and 12 transpositions/transversions; and the fourth has 67 inversions and 32 transpositions. See our web page for figures with edge weights labelled [26]. Thus, the first and third trees are superior (under this analysis) to the second and fourth. We then evaluated the first and third trees with respect to the inversion distance, given the labelling on internal nodes obtained by `BPAnalysis`:

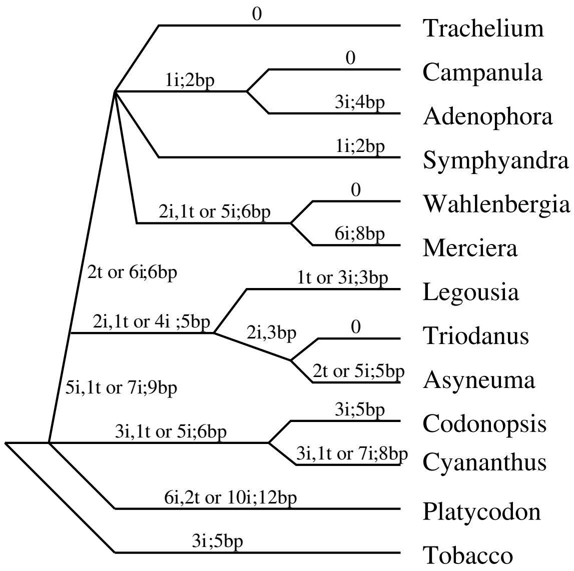


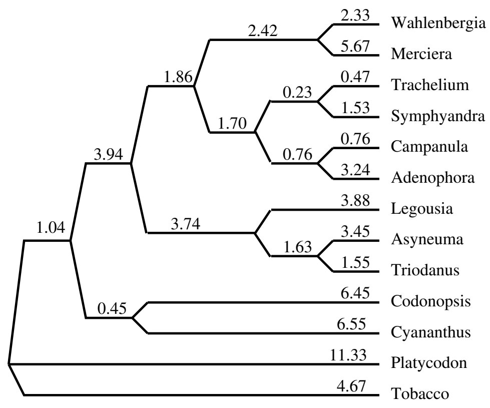
Figure 2: The reconstructed phylogeny of 12 genera of *Campanulaceae* and the outgroup tobacco based upon an MPBE analysis of 185 binary characters. The number of inversions and transpositions is given above each edge followed by the number of inversions in an inversion-only scenario; the number of breakpoints is given last.

the first tree has a total number of 68 inversions, while the third has 67. Both trees have zero-length edges (i.e., the endpoints of some edges have the same gene orderings); when these edges are contracted, the two trees are identical. The contracted tree is shown in Figure 2.

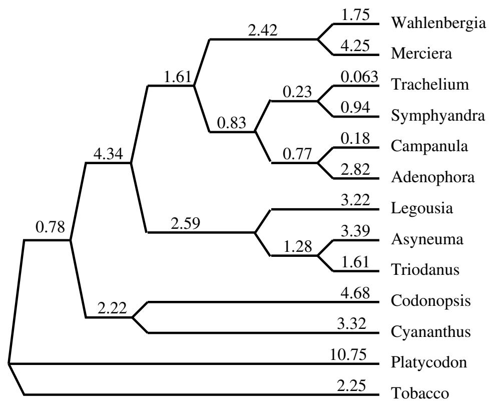
Interestingly, that tree is also a contraction of each of the trees obtained by the MPBE analysis [8] on the original 18 genera encoded using a larger set of characters (in which insertions, deletions, duplications, contractions/expansions of the inverted repeat, etc., were also used).

Table 1: The false negative rate matrix of trees from various reconstruction methods on the *C*ampanulaceae data in Figure 1. MPBE1 through MPBE4 are the four most parsimonious trees by the MPBE method. NJ(BP), NJ(I), and NJ(IT) are the neighbor joining trees from the distance matrix using MPBE estimation, `derange2` with inversion only, and `derange2` with cost ratio $inversion : transposition = 1 : 2.1$.

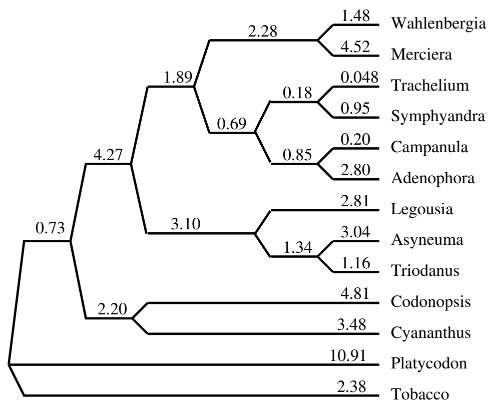
	NJ(BP)	NJ(INV)	NJ(IT)	MPBE1	MPBE2	MPBE3	MPBE4
NJ(BP)	0	0	0	1	2	1	2
NJ(INV)	0	0	0	1	2	1	2
NJ(IT)	0	0	0	1	2	1	2
MPBE1	1	1	1	0	1	1	2
MPBE2	2	2	2	1	0	2	1
MPBE3	1	1	1	1	2	0	1
MPBE4	2	2	2	2	1	1	0



(a) Neighbor joining tree on the distance matrix of binary encodings.



(b) Neighbor joining tree on the distance matrix from `signed_dist`, *inversion only.*



(c) Neighbor joining tree on the distance matrix from `derange2`, *cost ratio is* $inversion : trans-position = 1 : 2.1$.

Figure 3: The reconstructed phylogeny of 12 genera of *Campanulaceae* and the outgroup tobacco using neighbor joining on various distance estimation methods.

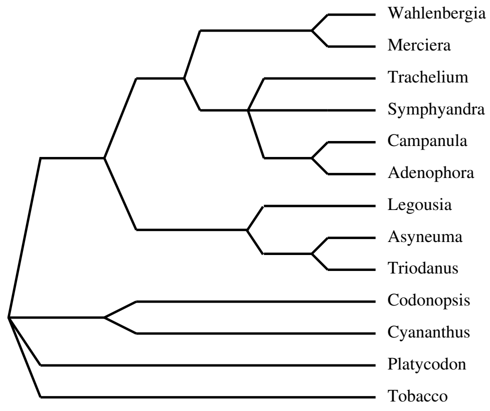


Figure 4: The consensus tree of the four MPBE trees and three neighbor joining trees from the 12 genera dataset of *Campanulaceae* and the outgroup tobacco. The tree has 8 internal edges, out of at most 10 internal edges allowed in a 13-taxa phylogeny.

We also computed neighbor-joining trees (using `Phylip` [10]) on two different distance matrices: the inversion/transposition/transversion distance matrix (computed with `derange2` with relative weights of 1, 2.1, and 2.1) and the breakpoint matrix (computed using `BPAnalysis`). We show the `derange2` distance matrix in Table 2; the other distance matrices are available on our web page [26].

All of the trees we reconstructed (whether by neighbor-joining or maximum parsimony) are fairly similar, differing pairwise in at most two edges. Table 1 shows the false negative rate for the 7 trees: 3 neighbor-joining trees and 4 MP trees. The similarity between all the trees reconstructed indicates a high level of confidence in the the accuracy of the common features of the phylogenetic reconstructions. The conditions under which these genomes evolved (low

Table 2: The distance matrix computed with `derange2` and a 2.1 weight ratio

	Tra	Cam	Ade	Sym	Leg	Asy	Tri	Wah	Mer	Cod	Cya	Pla	Tob
Tra	0.0	1.0	4.0	1.0	8.3	10.4	8.3	4.1	8.1	15.2	14.1	19.2	10.0
Cam	1.0	0.0	3.0	2.0	9.3	11.4	9.3	5.1	9.2	15.1	15.2	20.2	11.2
Ade	4.0	3.0	0.0	5.1	12.1	14.3	12.1	8.1	11.2	16.2	15.2	20.2	13.1
Sym	1.0	2.0	5.1	0.0	9.2	11.4	9.3	5.1	9.1	14.2	13.3	20.2	11.1
Leg	8.3	9.3	12.1	9.2	0.0	8.4	4.1	12.2	14.3	18.1	16.1	23.2	14.2
Asy	10.4	11.4	14.3	11.4	8.4	0.0	4.2	12.4	16.2	18.2	16.2	21.1	12.2
Tri	8.3	9.3	12.1	9.3	4.1	4.2	0.0	12.2	14.4	18.2	15.2	21.2	12.2
Wah	4.1	5.1	8.1	5.1	12.2	12.4	12.2	0.0	6.0	18.1	16.2	23.1	14.2
Mer	8.1	9.2	11.2	9.1	14.3	16.2	14.4	6.0	0.0	17.2	16.3	24.1	16.1
Cod	15.2	15.1	16.2	14.2	18.1	18.2	18.2	18.1	17.2	0.0	8.3	18.2	10.2
Cya	14.1	15.2	15.2	13.3	16.1	16.2	15.2	16.2	16.3	8.3	0.0	16.3	10.2
Pla	19.2	20.2	20.2	20.2	23.2	21.1	21.2	23.1	24.1	18.2	16.3	0.0	13.3
Tob	10.0	11.2	13.1	11.1	14.2	12.2	12.2	14.2	16.1	10.2	10.2	13.3	0.0

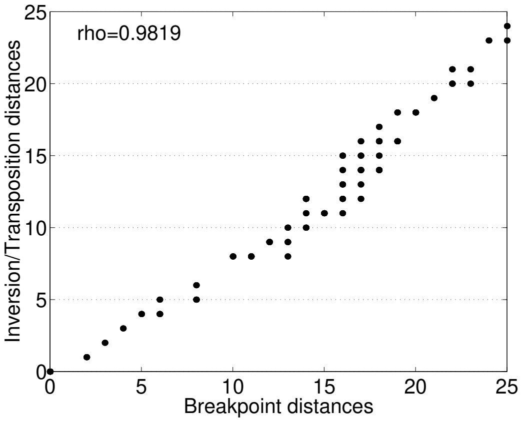


Figure 5: Comparison of distance calculations on the *Campanulaceae* Chloroplast dataset with a correlation coefficient of $rho = 0.9819$.

rates of evolution and a large number of gene segments) are probably responsible for this high level of similarity, which is observable at various levels. For instance, the breakpoint distance and the inversion/transposition/transversion distance (using relative costs of 1, 2.1, and 2.1) are very closely related, as illustrated in Figure 5. (The high correlation coefficient indicates that the two distances stand in a nearly linear relationship to each other.) These observations suggest that this dataset forms an easy case for phylogeny reconstruction; other evolutionary conditions may prove more difficult for phylogenetic reconstruction and may distinguish between different methods.

Software Issues

Running time may be a real issue with respect to reconstructing phylogenetic trees. While neighbor-joining is fast (technically $O(n^3)$, but very fast in practice), none of the other methods are. Maximum parsimony is NP-hard (and some searches do take a long time), but by comparison `BPAnalysis` is significantly slower. On our dataset (13 genomes, 105 gene segments), maximum parsimony took 0.15 seconds to find the four best trees using the branch-and-bound option in `PAUP`. However, for the same dataset, `BPAnalysis`, after running for 43 hours, had still not searched more than a small fraction of the tree space.

Even calculating the distance matrix between every pair of signed circular genomes in a large data set is computationally challenging. `derange2` is fast, but inexact: because it heuristically computes the distance between two genomes by using inversions, transpositions, and inverted transpositions (transversions) using a greedy strategy, it only allows an operation if that operation decreases the breakpoint distance between the two genomes. Consequently, it can miss minimal edit sequences, as we observed in our tests. Hannenhalli's software `signed_dist` for pairwise distances runs in slow polynomial-time ($\Theta(k^4)$ to compute distances between a pair of genomes on k genes); in order to compute all pairwise distances, it requires $\Theta(n^2k^4)$ time. For our dataset, k was 105 and n was 13.

We timed each method on the chloroplast dataset. Finding the four maximum-parsimony trees with `PAUP` took 0.15 seconds on a Macintosh G4. Labelling the internal nodes with `BPAnalysis` took 0.38 seconds for each tree. Computing inversion distances using `signed_dist` took 45.65 seconds per edge and computing inversion/transposition/transversion distances using `derange2` took 0.01 seconds per edge. Our experiments thus suggest that `BPAnalysis` evaluates approximately 120 trees a minute; at this rate, since the number of trees on 13 leaves is 13,749,310,575, `BPAnalysis` would take well over 200 years to complete its search of tree space for our problem. Blanchette *et al.* did complete their analysis of the metazoan dataset, which has 11 genomes on a set of 37 genes. This is a much easier problem, as there are far fewer trees to examine (only 2,027,025) and as scoring each tree involves solving a smaller number of TSP instances on a much smaller number of cities (37 rather than 105). Overall, it is clear that datasets of sizes such as ours are currently too large to be fully analyzed by `BPAnalysis`. More effective implementations of the basic concept, such as hill-climbing or branch-and-bound through the tree space and abandoning strict optimality in solving the TSP instances in favor of a fast and reliable heuristic (such heuristics abound in the TSP literature), could make the method run fast enough to be applicable to datasets comparable to ours.

Conclusions

In view of these results, our new method stands as a good compromise between speed and accuracy. Neighbor-joining is faster (guaranteed polynomial-time), but returns only one tree and thus tells us little about the space of near-optimal trees, while `BPAnalysis` is much slower. Furthermore, our preliminary results confirm that our new method returns results as good as any of the other methods, and does so within very reasonable times, even on sizes at which `BPAnalysis` cannot run to completion in a reasonable amount of time.

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