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Scaling up accurate phylogenetic reconstruction from gene-order data

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ABSTRACT

Motivation: Phylogenetic reconstruction from gene-order data has attracted increasing attention from both biologists and computer scientists over the last few years. Methods used in reconstruction include distance-based methods (such as neighbor-joining), parsimony methods using sequence-based encodings, Bayesian approaches, and direct optimization. The latter, pioneered by Sankoff and extended by us with the software suite GRAPPA, is the most accurate approach, but cannot handle more than about 15 genomes of limited size (e.g. organelles).

Results: We report here on our successful efforts to scale up direct optimization through a two-step approach: the first step decomposes the dataset into smaller pieces and runs the direct optimization (GRAPPA) on the smaller pieces, while the second step builds a tree from the results obtained on the smaller pieces. We used the sophisticated disk-covering method (DCM) pioneered by Warnow and her group, suitably modified to take into account the computational limitations of GRAPPA. We find that DCM-GRAPPA scales gracefully to at least 1000 genomes of a few hundred genes each and retains surprisingly high accuracy throughout the range: in our experiments, the topological error rate rarely exceeded a few percent. Thus, reconstruction based on gene-order data can now be accomplished with high accuracy on datasets of significant size.

Availability: All of our software is available in source form under GPL at http://www.compbio.unm.edu

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INTRODUCTION

Biologists can infer the ordering and strandedness of genes on a chromosome, and thus represent each chromosome by an ordering of signed genes (where the sign indicates the strand). These gene orders can be rearranged by evolutionary events such as inversions and transpositions and, because they evolve slowly, give biologists an important new source of data for phylogeny reconstruction—see, e.g. Downie and Palmer (1992), Olmstead and Palmer (1994), Palmer (1992), and Raubeson and Jansen (1992). Appropriate tools for analyzing such data may help resolve some difficult phylogenetic reconstruction problems. Developing such tools is thus an important area of research—indeed, the recent DCAF symposium organized by Sankoff and Nadeau (2000) was devoted to this topic.

A natural optimization problem for phylogeny reconstruction from gene-order data is to reconstruct an evolutionary scenario with a minimum number of the permitted evolutionary events on the tree. This problem is NP-hard for most criteria—even the very simple problem of computing the median of just *three* genomes (the median of k genomes is a genome that minimizes the sum of the pairwise distances between itself and each of the k given genomes) under such models was proved NP-hard by Pe'er and Shamir (1998) and Caprara (1999).

For some datasets (e.g. chloroplast genomes of land plants), biologists conjecture that rearrangement events are predominantly *inversions* (also called reversals). In other datasets, transpositions and inverted transpositions are viewed as possible, but their relative preponderance with respect to inversions is unknown. Sankoff proposed the *breakpoint* distance (the number of pairwise gene adjacencies present in one genome but absent in the other), a measure of distance between genomes that is independent of any particular mechanism of rearrangement; the *breakpoint phylogeny*, introduced by Blanchette *et al.* (1997), is the most parsimonious tree with respect to breakpoint distances.

The two software packages for reconstructing the breakpoint phylogeny, the original BPAnalysis of Sankoff and Blanchette (1998) and the more recent and much faster GRAPPA of Moret *et al.* (2001), both use as their basic optimization tool an algorithm for computing the breakpoint median of three genomes, although GRAPPA also supports inversion medians and inversion distance—see Moret *et al.* (2002b)—, the latter through the linear-time algorithm of Bader *et al.* (2001) and the former through the algorithms of Caprara (2001) and Siepel and Moret

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(2001). Extensive testing has shown that the trees returned by GRAPPA are superior to those returned by other methods used in phylogenetic reconstruction based on gene orders, such as distance-based methods and parsimony based on encodings—see Moret *et al.* (2002c) and Wang *et al.* (2002) for reviews of these other methods. The closely related software of Bourque and Pevzner (2002) is the only method that approaches its accuracy. (A recent Bayesian approach due to Larget *et al.* (2002) and an effort based on local perturbation of a minimum spanning tree from Wu and Gu (2003), while both showing promise, were tested on just one or two datasets and thus cannot as yet be properly evaluated.)

Although GRAPPA runs over one billion times faster than the initial BPAnalysis implementation, it remains an exponential-time algorithm. On a modern workstation, it typically takes one hour to finish a 13-taxon analysis, but nearly one month to finish a 15-taxon one. Bayesian methods rarely scale well even for sequence-based data: it may take months to run an analysis of a 1000-taxon dataset through Markov Chain Monte Carlo (MCMC) methods. Distance-based methods run quickly even on large datasets, but their accuracy decreases rapidly with increasing number of taxa, as shown by Moret *et al.* (2002a) and Nakhleh *et al.* (2002). Thus our best hope for accurate reconstruction is to design a way to scale the current GRAPPA software suite so as to tackle much larger problems; any such approach must reduce the size of the problem(s) that GRAPPA will be required to solve.

APPROACHES TO SCALING

A standard approach to scaling is to compute the smallest possible nontrivial trees: *quartet trees*, defined on just four taxa. Quartet methods rely on finding the optimal 4-leaf tree for each quartet and using this information to build the overall tree. Several theoretical methods (quartet cleaning and others) as well as one practical method (quartet puzzling) have been proposed to use quartet trees—see St. John *et al.* (2001) for a recent review and experimental comparison of these methods. In the case of gene orders, however, computing the best tree for a quartet is itself NP-hard (it includes finding the median of three genomes as a special case). Moreover, having to consider all quartets means that, on large datasets, many quartets will have very large pairwise distances, in which case determining the best quartet tree becomes chancy—each of the three possible trees will have poor scores. Finally, quartet-based methods, while running in polynomial time, tend to be slow: all of them must take $\Omega(n^4)$ time by definition. In previous work—see Tang *et al.* (2002)—, we conducted preliminary experiments with both quartet optimization methods and tree-building from quartets, the latter with the quartet-puzzling method of Strimmer and von Haeseler (1996) (the best quartet-based method in the experiments of St. John *et al.* (2001) and the one used by biologists); we found that quartet-puzzling, even with optimal quartet trees, lagged far behind our new DCM-GRAPPA (discussed below) in both speed and accuracy, although it did construct more accurate trees than pure neighbor-joining.

A more sophisticated approach to the decomposition problem should use a type of divide-and-conquer approach, in which the set of taxa is decomposed into a collection of subsets, each of which optimizes some criterion designed to make reconstruction on the subset as accurate and efficient as possible. The best such approach to date is the family of disk-covering methods (DCM), introduced by Warnow and her group—see Huson *et al.* (1999a), Huson *et al.* (1999b), and Huson *et al.* (1999c)—and since shown to produce better results on sequence-based data than any other distance- or parsimony-based method through experimental studies of Moret *et al.* (2002a), and Nakhleh *et al.* (2001a,b). We combined the DCM2 approach of Huson *et al.* (1999c) with GRAPPA, limiting the size of the subsets (disks in the DCM terminology) to at most 13 taxa through a combination of threshold choices and recursive calls to the DCM decomposition itself, yielding a DCM-GRAPPA software for tree reconstruction from gene-order data. We then tested the performance of DCM-GRAPPA through extensive simulations.

OUR EXPERIMENTAL APPROACH

We ran simulation studies of DCM-GRAPPA, using neighbor-joining (NJ)—see Saitou and Nei (1987)—and DCM-NJ—see Huson *et al.* (1999c)—as controls. We generated both uniformly distributed trees and random birth-death trees, the latter with the program r8s of Sanderson (2002). We generated trees with 20, 40, 80, 160, 320, 640, and 1280 taxa (the last to test scalability); on each tree, we evolved signed permutations of 50, 100, and 200 genes (a range that covers organellar genomes), using evolutionary rates (r, the expected number of evolutionary events along a tree edge) of 2, 4, and 8.

For each combination of parameter settings, we generated 10 datasets and examined the mean and variance of the outcomes. All our experiments were run on Athlon 1900XP machines with 2GB of main memory running Linux.

Given an inferred tree (reconstructed phylogeny), we can assess the topological accuracy by computing the *Robinson-Foulds* (RF) distance due to Robinson and Foulds (1981) with respect to the true tree. (Note that the true tree may not be the model tree itself, because the evolutionary process may cause no changes on some edges of the model tree—the true tree is defined to be the result

of contracting such edges in the model tree.) For every tree there is a natural association between every edge and the bipartition on the leaf set induced by deleting the edge from the tree. An edge is said to be *missing* in a tree if there is no edge defining the same bipartition in the tree. If an edge in the true tree is missing in the inferred tree, this edge is then called a *false negative* (FN). Similarly, a *false positive* edge (FP) is an edge of the inferred tree that is missing in the true tree. The RF distance is the total number of false negative and positive negative edges. (These measures can also be normalized by dividing them by the number of internal edges in the true tree.)

Overall, we found that the reconstructions produced by DCM-GRAPPA demonstrated excellent topological accuracy (within a few percent of optimal) throughout the range of parameters tested.

BACKGROUND

We briefly review the DCM approaches, focusing on the DCM2 method that we used, and the basic ideas behind GRAPPA.

The disk-covering methods

The disk-covering methods are a class of phylogenetic reconstruction 'meta-methods' that operate in conjunction with a given 'base method,' such as maximum parsimony or maximum likelihood. These methods operate by dividing a set of taxa into overlapping subsets (the 'disks'), constructing trees on the subsets using the base method, and then merging the subtrees into a supertree.

Warnow and her group devised two types of DCM. The first method, DCM1, produces many decompositions for the dataset; for each such decomposition, it computes a (possibly different) supertree; finally, it chooses one of these supertrees according to some criterion such as maximum parsimony or maximum likelihood. DCM1 was designed to be used with fast base methods, such as neighbor-joining, because it involves up to $O(n^3)$ phylogenetic reconstructions of subsets of taxa. In contrast, DCM2 produces fewer decompositions (potentially only one, although not in the way we used it) and thus can use computationally expensive methods such as maximum parsimony and maximum likelihood. Since our base method is the very expensive GRAPPA, we use DCM2.

Both methods operate by creating a graph from the distance matrix: each taxon becomes a vertex and an edge is placed between two taxa whenever their pairwise distance falls below a given threshold. (DCM2 typically uses the smallest threshold that results in a connected graph.) Edges are then added (greedily, since a minimum addition is NP-hard) to the graph to make it chordal (i.e. the graph does not contain simple cycles with more than 3 vertices). A chordal graph has a linear number of maximal cliques and these cliques can be found in polynomial time; moreover, minimal vertex separators in chordal graphs are maximal cliques.

DCM2 uses a vertex separator technique for its decomposition: if $G = (V, E)$ is the chordal graph it has obtained, it computes (in quadratic time) a separator $X \subseteq V$ such that X is a maximal clique and $G' = (V - X, E')$ has components $A_1, A_2, \ldots, A_r$ where $\max_i |X \cup A_i|$ is minimized. The overlapping subproblems are then $X \cup A_i$ for $i = 1, 2, \ldots, r$. These subproblems overlap in a single 'spine,' the separator X, a property exploited in the supertree merging phase, which uses a strict consensus merger specialized for DCM2 subtrees.

GRAPPA

GRAPPA is our re-implementation and elaboration on the original BPAnalysis of Sankoff and Blanchette (1998). In order to identify the best reconstructed tree, the program examines every possible tree topology on the given taxa, scoring each (using a sum of tree edge lengths) and retaining the tree(s) of lowest score. Scoring each tree is itself an NP-hard problem, since it requires reconstructing internal genomes. In the code, it is carried out heuristically through local iterative improvement: initial internal genomes are assigned in some way, then the tree is repeatedly traversed, replacing each internal genome by the median of its three neighbors if such a replacement reduces the sum of tree edge lengths, and continuing until no change takes place. Finally, computing the median is itself NP-hard, but fast solutions have been provided by Moret *et al.* (2001) for breakpoint medians and by Caprara (2001) and Siepel and Moret (2001) for inversion medians—although it should be noted that all of these methods will display exponential behavior for large pairwise distances. The study of Moret *et al.* (2002b) showed unequivocally that inversion medians are preferable to breakpoint medians (even though exact breakpoint medians can be found faster than exact inversion medians), so inversion medians are used throughout this study.

OUR NEW METHOD: DCM-GRAPPA

In combining the DCM approach with GRAPPA, we have to face two issues. DCM2 uses for its threshold the smallest value that will produce a connected graph, but the sizes of the resulting disks are unpredictable (although larger thresholds tend to produce larger disks). Since we cannot realistically run GRAPPA on more than 15 taxa, we must either limit the choice of thresholds to those that produce sufficiently small disks (but may fail to produce connected graphs) or use a recursive decomposition of the larger disks. However, the proofs of convergence and guarantees offered for DCM2 hold only when the graph produced is connected and only for a one-level decomposi-

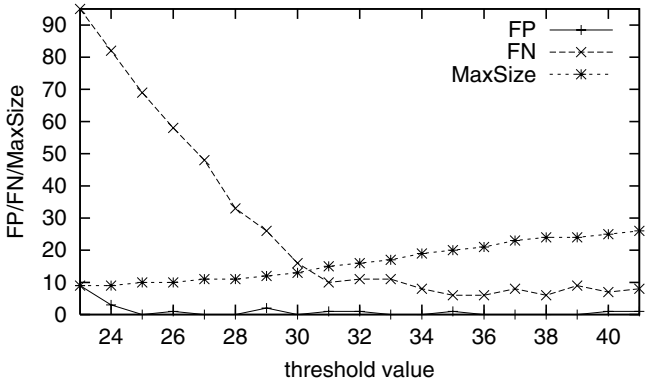


Fig. 1. Number of false positives, number of false negatives, and maximum disk size as a function of threshold values for 320 genomes, 100 genes, and evolutionary rate $r = 4$

tion. In other words, we must address, at least experimentally, the question of whether our adaptations damage the quality of results returned by a DCM2 approach. To examine how large the disks should be when DCM-GRAPPA converges, we ran experiments to examine how the error rates and disk sizes vary with different threshold values. Figure 1 shows a typical example (using uniform random trees). The figure indicates that, as the threshold value increases, the maximum disk size increases and the error rate decreases. Error rates hit their lowest level for a maximum disk size of 20—an encouraging result, since it suggests that DCM-GRAPPA will not need to handle unrealistically large disks to obtain good accuracy on datasets of medium size (several hundred taxa). For larger datasets, the 'good' threshold value tends to produce several disks larger than our limit of 13; on these datasets we used the recursive approach with very good results, as documented below.

Our second problem has to do with resolving ties. Since DCM2 creates subsets that have closely related taxa (as confirmed in our experiments), GRAPPA will often return a number of trees with the same 'best' score. DCM2 does not have a specific tiebreaker nor any way to take into account all equally 'good' trees. Past experience with DCM2 used on DNA sequence data shows that the best results are obtained when an optimal tree refinement (OTR) phase is added as a last step, because the tree returned by the strict consensus merging step of DCM2 is likely to have unresolved edges; thus using a consensus step on the subtrees, which would further reduce resolution before merging, would be counterproductive. We investigated the impact of choosing different 'best' trees by introducing a random selection and running the same dataset through DCM-GRAPPA many times. Pleasingly, and perhaps somewhat surprisingly, we found that the results showed very little variance within the range of datasets we explored; thus our results below are obtained with an arbitrary selection among competing 'best' subtrees.

EXPERIMENTAL RESULTS FOR TREE RECONSTRUCTION

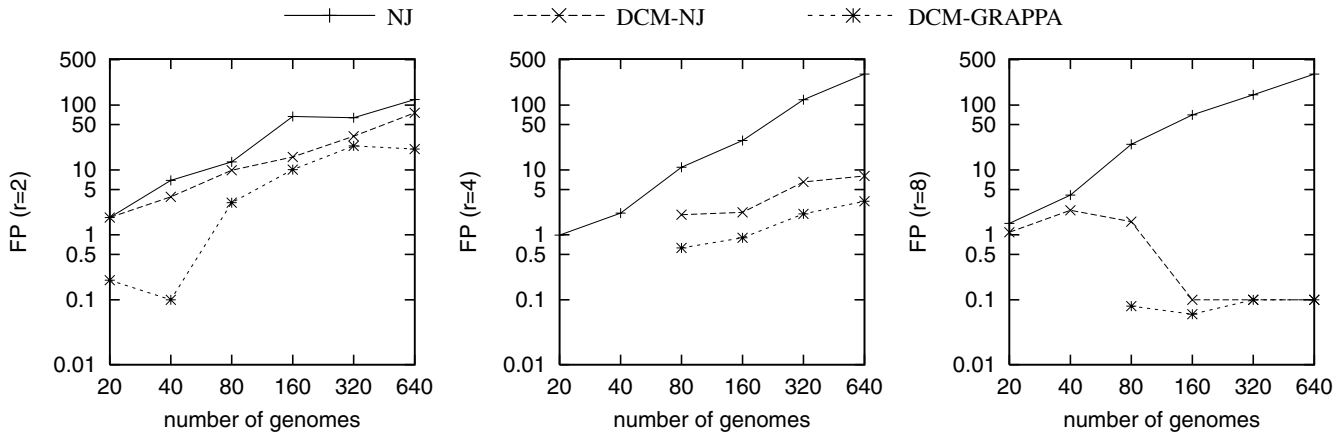
Our experiments had two main goals: to assess the accuracy of our new methods under a variety of parameters and to evaluate the scalability (the time/accuracy tradeoff) of these approaches as the number of taxa increases. We ran tests for up to 640 taxa under various rates of evolution—the r parameter, which denotes the expected number of evolutionary events along a tree edge—and on three types of tree distributions—uniform, birth-death, and a family proposed by Aldous (2001) as more closely modelling published phylogenies in the biological literature. We also ran a few tests for 1280 taxa, but only under a limited range of parameter settings.

Topological accuracy

Figures 2 and 3 show the average numbers[†] of false positive and false negative edges for DCM-GRAPPA, DCM-NJ, and plain NJ (which is always dominated by the other two) for datasets of 100 genes and varying numbers of taxa, on uniform trees. (Because NJ always produces binary trees, its FN and FP values will be equal whenever the true tree is itself binary.) Two main observations should be made. First, DCM-GRAPPA is remarkably accurate within the range of our tests: for $r = 4$ and $r = 8$ and for up to 640 taxa, the normalized RF error rate stayed below 0.02—i.e. we never saw more than 10 edges in error, out of 637 internal edges. When the evolutionary rate is low, the accuracy decreases because many ties arise in the optimization process; moreover the disk size increases, since more organisms are closely related, thereby increasing the running time of DCM-GRAPPA (although this does not substantially affect the running time of DCM-NJ). Figure 4 shows the relationship between rates of evolution and false negatives and false positives. For our limited results on 1280 taxa, the RF rates stayed below 0.02, for fewer than 20 edges in error out of 1277 internal edges.

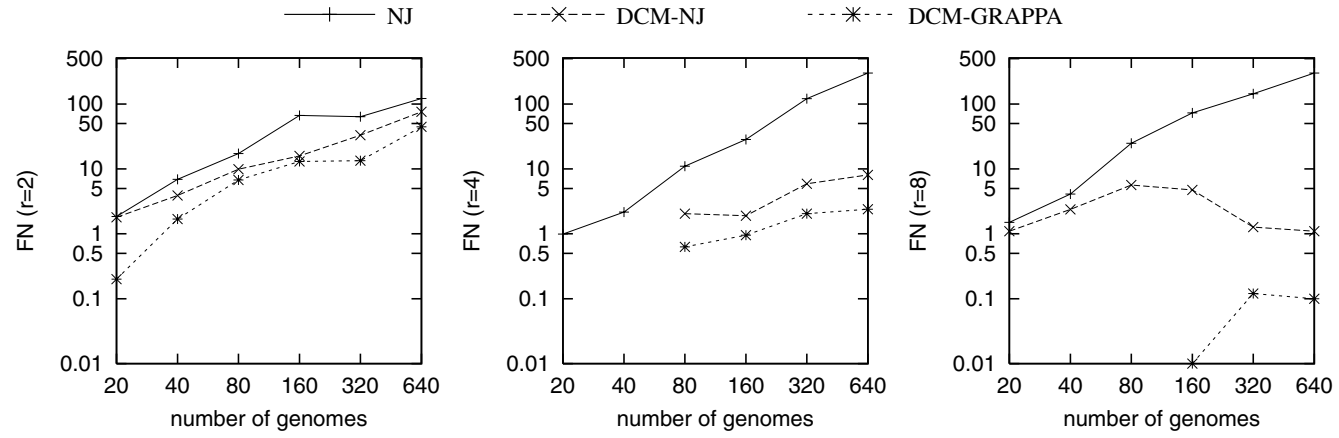
Secondly, DCM-NJ, which consistently outperforms most methods when used with sequence data, also does well with gene-order data, but it is consistently outperformed by DCM-GRAPPA, in spite of the fact that it explores all possible thresholds—a distinct advantage at low evolutionary rates, where the good thresholds are significantly larger than for more realistic evolutionary rates.

[†] Missing data are values of 0, which cannot be shown on a logarithmic scale.



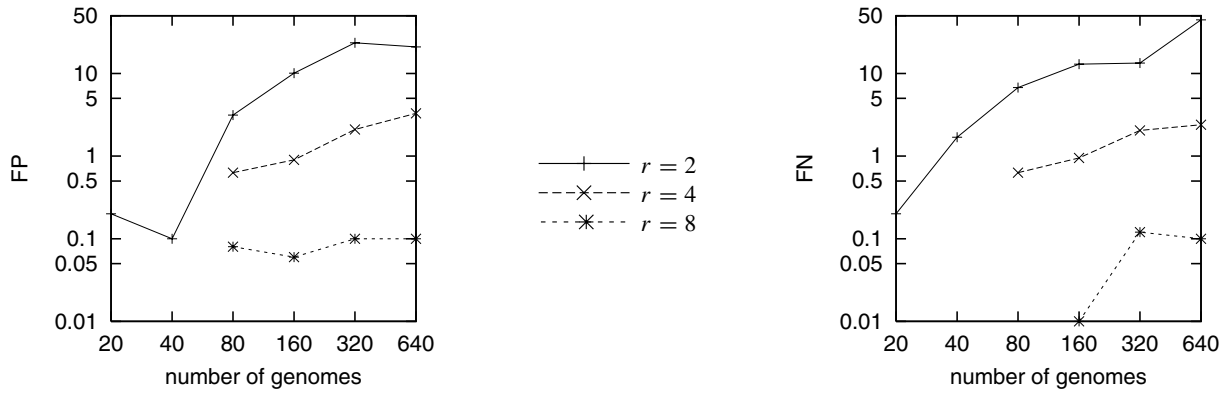
(a) evolutionary rate $r = 2$ *(b) evolutionary rate $r = 4$* *(c) evolutionary rate $r = 8$*

Fig. 2. Average numbers of false positives for the three algorithms as a function of the number of taxa, for 100 genes and three evolutionary rates. (Missing values equal 0.)



(a) evolutionary rate $r = 2$ *(b) evolutionary rate $r = 4$* *(c) evolutionary rate $r = 8$*

Fig. 3. Average numbers of false negatives for the three algorithms as a function of the number of taxa, for 100 genes and three evolutionary rates. (Missing values equal 0.)



(a) false positives *(b) false negatives*

Fig. 4. Average numbers of false positives and false negatives for DCM-GRAPPA as a function of the number of taxa, for 100 genes and three evolutionary rates. (Missing values equal 0.)

Tree topologies

A few words should be said about the topology of model trees. The data shown in Figures 2 and 3 are for uniform random trees, not for birth-death trees; in terms of relative accuracy, it more closely matches what we have found on biological datasets. For instance, on the Campanulaceae dataset of Cosner *et al.* (2000), which has 13 genomes of 105 genes, NJ obtains trees with score 70, DCM-NJ trees with score 71, and GRAPPA trees with score 64. On the more challenging dataset of Raubeson *et al.* (2001), which has 11 genomes of 38 genes, NJ obtains very poor trees with score 113, DCM-NJ obtains trees with score 101, and GRAPPA obtains good trees with score 82. In contrast, birth-death trees (regardless of their deviation from ultrametricity and regardless of the choice of evolutionary events) produced curious results: for the most part, they do not lead to good DCM decompositions, so that DCM-GRAPPA runs very slowly; and all three methods, NJ, DCM-NJ, and DCM-GRAPPA return equally good (very good!) topologies, except at very high rates of evolution, when NJ starts faltering. These findings are at odds with our limited experience on real datasets, where GRAPPA invariably outperforms NJ by a substantial margin. Uniform trees are not a perfect match either: in our simulations, DCM-NJ often performs almost as well as DCM-GRAPPA, which is not true on real datasets. Mooers and Heard (1997) observed long ago that neither the uniform nor the birth-death trees are a good match for the tree shapes seen in most published phylogenies; Aldous (2001) proposed a single-parameter family which can generate, for suitable settings of the single parameter β, both birth-death trees ($\beta = 0$) and uniform random trees ($\beta = -1.5$) and suggested that setting β to -1 gave a better fit. We also used this type of trees in our experiments and noted that they produced results falling somewhere between uniform trees and real datasets—and thus not quite the desired solution. Unfortunately, large (or even medium-sized) biological datasets of gene-order data are still under construction, so our tests were limited to simulations. Obviously, further research is needed in the production of realistic model topologies for simulations.

Recursive DCM-GRAPPA

For datasets with more than a few hundred taxa, the disk size frequently exceeds 15; for these big disks, we must recursively call DCM to decompose each big disk into smaller ones. For DNA sequence data, Warnow and her group found that the accuracy of the reconstructed tree was poor for small and large thresholds and good for a substantial range of values in the middle—their plots show broad U-shaped curves. We observed the same behavior for small to medium threshold values, leading us to conjecture that leaving larger thresholds unexplored is not a major problem. Since we cannot predict which threshold value is the best, we use the maximum disk size as a stopping criterion. For example, for 320 taxa, the maximum disk size is set to 30 and DCM-GRAPPA starts with the minimum threshold value, increasing the threshold as it goes; as soon as a threshold value produces a disk larger than 30, the computation stops. For any disk larger than 12, we use DCM-GRAPPA instead of GRAPPA to reconstruct the disk, thereby introducing a possible recursion; the maximum allowed disk size for the recursive DCM-GRAPPA is set to $\frac{2}{3}$ of the global maximum disk size. If the recursive instance itself produces a disk larger than 12, it invokes another instance of DCM-GRAPPA and further scales the allowed disk size by $\frac{2}{3}$.

Running time

Detailed running times were not our objective here, since the various methods differ sharply: on a typical dataset of 640 taxa, NJ takes a minute, DCM-NJ takes 2–3 hours, and DCM-GRAPPA takes 10–12 hours for $r = 4$ and $r = 8$, but about two days for $r = 2$ (which gives rise to much larger disks). What interested us was the total running time of DCM-GRAPPA and the time used in each call to GRAPPA. Our test cases ran in a matter of hours (up to 12 hours for the most time-consuming ones, except for $r = 2$ where it could take 4 times longer). Disks were normally dispatched quickly: a typical 13-taxon disk took on the order of 40 minutes. Exceptions obviously must arise: some of the optimization subproblems solved by GRAPPA can take a long time—for instance, we encountered one disk of 5 taxa that required 10 hours. Yet such cases are obviously very rare—since the code ran in a matter of hours in spite of the thousands of disks that GRAPPA processes for each dataset. (Our implementation caches all processed disks: avoiding the recomputation of just a few expensive disks may cut the running time by a factor of 10.) A few hours to a day of computation on a workstation is common in biological practice—researchers have been known to run analyses for months on clusters of workstations (see Rice *et al.* (1997) for a well known example).

The other contributing factor to large running times is the number of genes in each genome. For our test range (which corresponds to typical organellar genomes), median computation is not a problem; for nuclear genomes with a thousand or more genes, condensation (replacing ordered subsequences of genes by a single entity) works well in the current GRAPPA implementation, but the presence of large pairwise distances prevents condensation from reducing the genome to a workable size. Thus datasets of nuclear genomes with large pairwise distances remain computationally intractable to date, although that

problem can be remedied, at least in part, by better taxon sampling.

CONCLUSIONS

We have shown that the time-consuming, but accurate approach to phylogeny reconstruction from gene-order data first proposed by Sankoff, then refined by our group, can be placed within a divide-and-conquer framework to scale it up to larger problems. Our DCM-GRAPPA scales gracefully to a thousand genomes, returning remarkably accurate results in our simulations within a few hours to a day of computation. As larger datasets of gene-order data are produced by the many existing projects dealing with organellar evolution, we will have an accurate tool available for their phylogenetic analysis.

The principal remaining challenge is to handle unequal gene contents; using our GRAPPA framework, we have made significant strides in this direction in terms of handling gene duplication events—for which see Marron *et al.* (2003) and Tang and Moret (2003). The other major challenge is to extend our results from genomes of organellar size (no more than a few hundred genes) to the much larger nuclear genomes (thousands of genes) by devising fast new algorithms for computing inversion medians.

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