Maximum Parsimony Inference of Phylogenetic Networks in the Presence of Polyploid Complexes

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Abstract.—Phylogenetic networks provide a powerful framework for modeling and analyzing reticulate evolutionary histories. While polyploidy has been shown to be prevalent not only in plants but also in other eukaryotic species, most work done thus far on phylogenetic network inference assumes diploid hybridization. These inference methods have been applied, with varying degrees of success, to data sets with polyploid species, even though polyploidy violates the mathematical assumptions underlying these methods. Statistical methods were developed recently for handling specific types of polyploids and so were parsimony methods that could handle polyploidy more generally yet while excluding processes such as incomplete lineage sorting. In this article, we introduce a new method for inferring most parsimonious phylogenetic networks on data that include polyploid species. Taking gene tree topologies as input, the method seeks a phylogenetic network that minimizes deep coalescences while accounting for polyploidy. We demonstrate the performance of the method on both simulated and biological data. The inference method as well as a method for evaluating evolutionary hypotheses in the form of phylogenetic networks are implemented and publicly available in the PhyloNet software package. [Incomplete lineage sorting; minimizing deep coalescences; multilabeled trees; multispecies network coalescent; phylogenetic networks; polyploidy.]

Hybridization and polyploidization have long been recognized as crucial factors in speciation and genomic and phenotypic novelties (Oxelman et al. 2017; Blischak et al. 2018). While in homoploid hybridization, the hybrid has the same number of chromosome sets as the two parental species, allopolyploid hybrids receive both chromosome sets from the parents, thus increasing the size of the chromosome set as compared to the two parents. Both types of hybridization result in reticulate evolutionary histories of the whole genomes that are best modeled by phylogenetic networks. Autopolyploidy, on the other hand, is whole-genome duplication (WGD) that involves a single lineage and does not violate a treelike evolutionary history at the level of the species phylogeny.

Polyploidy is prevalent across the eukaryotic branch of the Tree of Life. Many extant flowering plants are neopolyploids, and for the remaining diploid species, one or more rounds of ancient WGD events can be traced (Masterson 1994; Jiao et al. 2011), thus indicating that they are paleopolyploids. Although hybrid and polyploid species are less commonly observed in animals than plants, presumably owing to their potential reduced fitness, fish and amphibians are known to have high incidence of polyploidy (Glasauer and Neuhaus 2014; Berthelot et al. 2014; Woods et al. 2005). Furthermore, it is believed that at least two rounds of ancient WGD occurred in the vertebrate lineage (the 2R hypothesis) (Ohno 1970; Muffato and Crotta 2008). Fungi also include polyploids, with evidence supporting that the ancestor of the baker’s yeast Saccharomyces cerevisiae underwent WGD (Marcet-Houben and Gabaldón 2015).

Allopolyploidization has been attracting attention for decades as it reflects the joint effects of genome doubling and interspecific hybridization. Unlike autopolyploids, allopolyploids contain multiple divergent subgenomes, each derived from distinct parental species. The investigation of subgenome evolution has been propelled by recent advances in homoeology analyses (Clover et al. 2016; Sancho et al. 2021). From modeling and inference perspectives, allopolyploidy is of particular interest, as it results from hybridization of two species and gives rise to evolutionary histories in the form of phylogenetic networks. Although polyploidy could potentially be identified from the chromosome count, the task of determining its mode of origin is nontrivial, especially when the parental taxa are closely related. Moreover, other evolutionary processes, such as incomplete lineage sorting (ILS), complicate this task. Therefore, extending models such as the multispecies coalescent to account for polyploidy could provide a powerful approach inferring evolutionary histories of polyploids.

Yu et al. (2012, 2014) extended the multispecies coalescent to incorporate hybridization into the model, giving rise to the multispecies network coalescent (MSNC). Based on this generative process, PhyloNet (Than et al. 2008; Wen et al. 2018) implements a wide array of methods for inferring phylogenetic networks in the presence of incomplete lineage sorting and diploid hybrids, including parsimony methods (Yu et al. 2013), maximum likelihood and pseudolikelihood methods (Yu et al. 2014; Yu and Nakhleh 2015; Zhu and Nakhleh 2018), and Bayesian methods (Wen et al. 2016; Wen and Nakhleh 2018; Zhu et al. 2018). Since almost all these methods are computationally demanding (with the exception of Yu and Nakhleh (2015)), a divide-and-conquer approach was recently introduced to speed
them up (Zhu et al. 2019). Cao et al. (2019) illustrated the use of many of these inference methods, as well as network summarization methods, on data generated under the multispecies network coalescence, which assumes diploid hybridization.

Kamneva et al. (2017) evaluated various inference tools in PhyloNet (Than et al. 2008; Wen et al. 2018) for inferring phylogenetic networks of polyploid strawberries, Fragaria (Rosaceae), with species that ranged from triploid to decaploid. As Blischak et al. (2018) correctly pointed out, “Though not specifically built for polyploids, PhyloNet can model multiple haplotypes in each lineage. It can hence infer hybridization in these species when the assumptions of its model (the coalescent) are not violated, making it most appropriate for recent allopolyploids.”

While existing inference tools in PhyloNet were not designed for handling polyploidy, there are several existing methods that are designed specifically to model polyploidy events (Oxelman et al. 2017). Many of these methods have relied on multilabeled species trees, or MUL-trees, to model polyploids. As multiple copies of a locus could be present in the genome due to polyploidization, a MUL-tree extends standard phylogenetic trees by allowing multiple leaves to be labeled by the same taxon name (Fig. 1a–d). There is a straightforward connection between a phylogenetic network and a MUL-tree, as illustrated in Figure 1a,b. Indeed, in one of the earliest works in this area, Huber and Moulton (2006) provided an algorithm for converting a MUL-tree into a phylogenetic network, which was later implemented in the PADRE software (Lott et al. 2009). This connection between phylogenetic networks and MUL-trees was the basis for computations under the MSNC in Yu et al. (2012, 2014) before moving towards computations directly on the phylogenetic network in subsequent implements in PhyloNet. The software tool GRAMPA (Thomas et al. 2017) uses MUL-trees to reconcile a set of gene trees parsimoniously with a given species tree to postulate polyploid events.

To the best of our knowledge, the only statistical inference methods specifically designed for handling allopolyploids are the AlloppNET method of Jones et al. (2013) and its extension (Oxelman et al. 2017). AlloppNET uses Bayesian Markov chain Monte Carlo (MCMC) to sample, using multilocus DNA sequence data, the posterior distribution of phylogenetic networks that contain diploid and allotetraploid species. The method allows for multiple individuals per species and samples, in addition to the phylogenetic network topology, parameters including divergence and hybridization times as well as population sizes. Like other statistical inference methods in PhyloNet, AlloppNET is computationally intensive, in particular as it employs reversible-jump MCMC to sample the transdimensional space of phylogenetic networks. Furthermore, as mentioned above, AlloppNET allows for only diploid and allotetraploid species.

As described in Blischak et al. (2018), producing an all-encompassing stochastic model of polyploidization would be a massive undertaking due to the complexities of processes occurring during and after polyploidization events. In this article, we take a maximum parsimony approach to phylogenetic network inference in the presence of general allopolyploidy events. Recognizing that the parsimony method of Yu et al. (2013) as implemented in PhyloNet was not designed to handle allopolyploids, Oberprieler et al. 2017 coupled it with a permutation scheme where multiple analyses are conducted in each of which only two copies from the polyploid are mapped to one parent and the other copies are mapped to a second parent, and then reporting the optimal result over all these analyses. In this work, we extend the method of Yu et al. (2013) to properly handle polyploids without the need for a permutation approach. We implemented both an inference method (MPAllopp) and a scoring method in PhyloNet and assessed their performance on the simulated and biological data used in (Jones, 2017), (Marcussen et al., 2014), and (Joly et al., 2009). We report on the accuracy and running time of MPAllopp, and compare it to AlloppNET as well as existing methods in PhyloNet that were not designed to handle polyploid hybridization. We show that MPAllopp outperforms other methods in PhyloNet that were not designed to handle allopolyploidy and performs similarly to AlloppNET in terms of accuracy while being much faster.

FIGURE 1. Allopolyploidy, phylogenetic networks, and MUL-trees.

a) Phylogenetic network depicting allopolyploidization involving the ancestor of X, Y, and Z. b) MUL-tree representation of the network. c) Gene tree inside the branches of the phylogenetic network. d) Gene tree with two copies of the gene in each of the species X, Y, and Z. e) Gene tree where the signal for the allopolyploids is confounded by ILS and gene loss. f) Gene tree where the signal for the allopolyploids is confounded by ILS and gene loss.
METHODS

Minimizing Deep Coalescences in the Presence of Allopolyploidy

Maddison (1997) proposed minimizing deep coalescences (MDC) as a parsimony criterion for reconciling a gene tree with a given species tree, as well as for inferring a species tree from a collection of gene trees, both under the assumption that gene tree heterogeneity is caused by ILS. Maddison and Knowles (2006) later implemented and tested a heuristic for inferring a species tree under the MDC criterion. Than and Nakhleh (2009) provided a mathematical characterization of the number of deep coalescences given a clade in the species tree (without having the species tree itself), which allowed for developing exact algorithms for species tree inference under the MDC criterion. To account for hybridization and introgression simultaneously with ILS, Yu et al. (2013) introduced the MDC criterion for inferring phylogenetic networks from a collection of gene trees whose heterogeneity is assumed to be caused by ILS and introgression. All these works on the MDC criterion naturally allow for including multiple individuals per species.

While the method of Yu et al. (2013) could in practice be used on data with allopolyploid species while treating multiple gene copies as alleles from different individuals, the criterion is not mathematically designed for handling polyploids. Let us illustrate this issue with the scenario depicted in Figure 1c. The gene tree shown inside the branches of the phylogenetic network has two copies from each of the three taxa X, Y, and Z, due to the polyploid hybridization event involving taxa B and C. If each of the two copies are treated as alleles from two different individuals, then this gene history will be heavily penalized by the MDC criterion as lineages failed to coalesce on the four branches connected to X, to Y, to Z, and to the most recent common ancestor (MRCA) of X and Y. Indeed, the MDC score of this gene tree given the phylogenetic network is 4 in this case. However, when considering that this is a polyploid hybridization event and the two lineages correspond to two different copies of the gene, these lineages should not be expected to coalesce on these four branches, and the true score of this network is 0. This example illustrates the need for a new method to properly handle polyploid hybridization events.

To address this issue, we propose a method that extends the MDC criterion to accommodate polyploidy. Our method is based on the idea of assigning subgenomes to each gene copy, where each subgenome corresponds to a different copy of the gene. This allows us to properly handle polyploid hybridization events without heavily penalizing the network score.

**Given a collection G of gene trees, inferring a phylogenetic network Ψ under the criterion of minimizing the number of extra lineages amounts to computing**

Ψ* ← argminΨ \( \sum_{g \in G} X_L(T, g) \)  \( (2) \)

Based on this formulation, the inference is done by walking the space of phylogenetic networks using the implementation of Yu et al. (2013), while evaluating the number of extra lineages on the MUL-tree representation of each network, using Equation (1).
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FIGURE 2. Allele mappings from a gene tree to a MUL-tree and the number of extra lineages. a) The set of all eight possible allele mappings for the gene tree of Figure 1d and the MUL-tree of Figure 1b given that each copy of allopolyploids came from distinct subgenome, along with the number of extra lineages that results from each of the allele mappings. b–c) The reconciliations that correspond to allele mappings #1 and #7, respectively, of the gene tree within the branches of the MUL-tree. Shown next to each of the MUL-tree branches is the number of extra lineages on that branch, which is the number of lineages that persist without coalescing on that branch minus 1.

limitations of the method as formulated are that it cannot detect homoploid hybridization and assumes no recombination between parental subgenomes.

PhyloNet Implementation

We implemented the inference based on Equation (2) in PhyloNet (Than et al. 2008; Wen et al. 2018) as a new command called InferNetwork_MP_Allopp. This method takes as input a set of gene trees and reports the top-k species networks and their MUL-trees based on the MUL-tree score. If during the search there are multiple networks with the same scores encountered, those networks will be reported if their scores are within the top-k scores, although the total number of networks returned will be truncated at k, which by default is set to 5.

- The subgenome assignment. This method makes use of the known subgenome identity for each sampled allopolyploid allele to determine the (reduced) set of candidate allele mappings to use during the phylogenetic network inference. We refer to the new method that makes use of this information as MPAllopp (“MP” is for maximum parsimony). In the absence of information about the assignment of gene sequences to subgenomes, MPAllopp considers all possible subgenome assignment per-locus. However, this requires the addition of heuristics due to the exponential growth in the number of ways alleles may be mapped to subgenomes (i.e., we may have to consider an extraordinarily large number of sets of assignments) when computing the parsimony score.

- The maximum number of reticulations allowed during the search. If this number is set at 0, then the method searches the space of genome trees (Thomas et al. 2017; Oxelman et al. 2017) only.

- Whether the hybrid species are known. If the hybrid species are known and specified by the user, and the maximum number of reticulations allowed equals the number of specified hybrid species, the method searches only networks that have the specified hybrids; that is, it does not detect any other potential hybrids. If the maximum number of reticulations allowed is greater than the number of specified hybrid species, the method could detect additional hybrids. If the hybrids are not specified, then the method identifies hybrids.

In addition to phylogenetic network inference, we provide the user with the option to evaluate, rather than infer, competing hypotheses. Given a phylogenetic network \( \Psi \) and a set of gene trees \( G \), the parsimony score of the \( \Psi \) is given by

\[
\sum_{g \in G} XL(U(\Psi), g)
\]

This analysis is enabled by the new PhyloNet command DeepCoalCount_AlloppNet. We envision this command used in at least two contexts. First, if the user has two or more evolutionary hypotheses (in the form of phylogenetic networks), this command can be used to assess which of these hypotheses has the best score. Second, if the inference method above returns a phylogenetic network that does not match some biological knowledge, the user can manipulate...
the inferred network to obtain one that matches the biological knowledge and compare the two. In other words, this command can be used in an exploratory mode.

Last but not least, a brief explanation of the nature of heuristic searches is in order. Inferring the optimal phylogenetic network according to Equation 2 is computationally very demanding. Therefore, the algorithm implemented by the command InferNetwork_MP_Allopp performs a random walk in the space of phylogenetic networks, evaluates phylogenetic networks encountered during the walk, and returns the best (i.e., the one with the lowest parsimony score) network among all those encountered. For each run, the search starts from either a random tree or a starting tree quickly estimated from the data using MDC (Than and Nakhleh 2009) or NJ, (Liu and Yu 2011). Then the search explores the network space by modifying the current topology. Six moves were employed to alter the network topology: i) reticulation edge addition, which creates a new hybridization event; ii) reticulation edge deletion, which removes one hybridization event; iii) reticulation edge tail relocation, which modifies a parent lineage of a hybridization event; iv) reticulation edge direction flip, which reverses the direction of the "gene flow" underlying the hybridization event; v) reticulation edge replacement, which replaces an existing hybridization event by a completely different one; and vi) reticulation edge head relocation, which modifies the hybrid lineage resulting from the hybridization event. A new network is proposed by applying a randomly chosen operation from the six, proportional to their associate weights, to the current network. The score of the resulting network is calculated, and it is accepted or rejected based on this score. If the score of the proposed topology is worse, we reject the proposal, and the search continues from the current network. If it is accepted, the resulting network replaces the current one and the search proceeds from it. This type of heuristics is not guaranteed to find the optimal network, which is why it is recommended to run the command multiple times and return the optimal solution among all the runs (in the simulation experiments below, we ran the method 30 times on each data set). The search heuristic computes the parsimony score of each network candidate encountered during the search by considering the set of all allele mappings that satisfy the known subgenome assignment.

If the subgenome assignment is not known, we found that it was only possible to estimate this assignment and the genome tree with the addition of further heuristics in more complex scenarios, due to the previously mentioned combinatorial explosion. We implemented a similar heuristic for reducing the number of mappings as in Thomas et al. (2017) to efficiently evaluate allele mappings without exhaustively considering all of them as their number could be prohibitive even for a small number of species. This heuristic works as follows for each gene tree:

1. Determine the set of clades $C_1, \ldots, C_n$ that include all leaf species with polyploid alleles and exclude all species with diploid alleles.

2. For each clade $C_i$:
   - If the sister node $D_i$ to the root node of $C_i$ is diploid, then the leaves of $C_i$ will be mapped to the subgenome most related to the genome of $D_i$.
   - If the leaves of $C_i$ come from different polyploid species, then they will be mapped to the same subgenome.

Simulations

We used the AlloppDT simulator (Jones 2012) and parameter settings of Jones et al. (2013) and Jones (2017) to simulate a collection of data sets. The data were generated under four different evolutionary scenarios with 1, 2, 3 and 3 reticulations (Fig. 3), where for each scenario the mutation rate, the number of individuals per species, and the number of genes were varied. In addition, we increased the number of genes to 100 to emulate the kinds of data sets enabled by next-generation sequencing. In total, there were 116 model conditions, each with 10 replicate data sets (Table 1). Since the gene tree-based inference methods considered in this study require rooted gene trees, an outgroup species was added for the purpose of rooting the estimated gene trees Table 1 and was excluded in the species phylogeny reconstruction. It would be possible for molecular clock methods to root the gene trees by finding the tree with the best ultrametric fit, but this may be unreliable when the strict molecular clock assumption is violated.

For each gene tree, DNA sequences of length 500 were generated using Seq-Gen Version 1.3.2 (Rambaut and Grass 1997) under the HKY model. Then gene trees were reconstructed from the simulated sequence alignments using IQ-TREE Version 2.1.2 (Minh et al. 2020), and rooted by the outgroup.

| Table 1. AlloppDT parameters for data sets with allopolyploidy. Based on the species phylogenies in Fig. 3, we varied the parameters and obtained 116 conditions. For each condition, 10 replicates were simulated. |
|---|
| **Scenario** | **Parameters** |
| **DEF** | G (Number of genes) ∈ {1, 3, 10, 100} |
| | N (Individuals per species) ∈ {1, 3, 9} |
| | T (Mutation rates) ∈ {4e−9, 2e−8, 1e−7} |
| | substitutions per site per generation |
| | H (Root height) = 0.02 substitutions per site |
| | G (Number of genes) ∈ {1, 3, 10, 100} |
| | N (Individuals per species) ∈ {1, 3} |
| | T (Mutation rates) = 2e−8 substitutions per site per generation |
| | H (Root height) = 0.045 substitutions per site |
FIGURE 3. Multilabeled species trees used in allopolyploid simulations (taken from Jones (2017)). a) Scenario D with 5 species has one hybridization event that gave rise to the allopolyploid clade \{x,y,z\}. b) Scenario E with five species has two hybridization events that led to the allopolyploid clades \{x, y\} and \{x,z\}. c) Scenario F with five species has three hybridization events that resulted in the allopolyploid clades \{x\}, \{y,z\}, and \{x\}. Scenario J with 13 species has three hybridization events that produced the allopolyploid clades \{v, t, u\}, and \{x, y, z, w\}.

Comparison with other methods—The AlloppNET input XML files were generated by AlloppDT with the same model parameters and MCMC settings as used in Jones (2017). This includes coestimating the assignment of alleles to subgenomes.

We tested MPAllopp (with known subgenome assignment) and compared it to three methods:

- InferNetwork_MPL in PhyloNet, which implements the maximum pseudolikelihood method of Yu and Nakhleh (2015) (this is labeled MPL below).
RESULTS AND DISCUSSION

Results on Simulated Data

Treating Gene Copies as Alleles From Different Individuals: Performance of MPL and MP—MPL and MP are not intended to model the evolutionary relationship between subgenomes, and hence, unlike our new methods do not include any mechanism for assigning alleles to subgenomes. Despite this, we wished to analyze whether MPL and MP could accurately model the relationship between species, by treating alleles from different subgenomes as being randomly sampled from a species without subgenome structure.

The accuracy results of the methods on data sets with low, moderate, and high levels of ILS are shown...
As the results show, both MPL and MP infer very accurate networks when using the true gene trees of scenario D with low levels of ILS, which is the simplest of all four scenarios considered, as it contains a single hybridization event. However, this observation changes significantly for the same scenario when the level of ILS increases and/or when gene tree estimates are used. In particular, while still using the true gene trees, as the level of ILS increases, the error rates of both methods increase, with MP performing slightly better. We hypothesize that MP performs slightly better because it is a simple summary method that does not rely on the mathematical assumptions of the coalescent model, which are violated here and are used explicitly by MPL. Specifically, coalescent methods assume that alleles can be inherited at random from any individual chromosome in the previous generation, but when subgenomes are nonrecombining, alleles can only be inherited from chromosomes belonging to the same subgenome. For low levels of ILS, gene tree estimation error significantly impacts the performance of the methods; however, that gene tree estimation error does not make the performance much worse in the presence of higher levels of ILS. We determined that the difference in error rates is somewhat artifactual, as it is being caused by the inability to resolve relationships between individuals at each locus within each extant species, due to very short coalescent times when ILS is low (Supplementary Fig. S1 available on Dryad at http://dx.doi.org/10.5061/dryad.4xgx2580).

Scenario E has two hybridization events, Scenario F has three hybridization events, and Scenario J has three hybridization events and is much larger. Therefore, these scenarios are more challenging for the methods than Scenario D, and they increase in complexity from
FIGURE 6. Boxplots of inference accuracy on simulated data with high ILS (mutation rate of $1 \times 10^{-7}$) over 10 replicates. Data sets with 100 genes were too large for AlloppNET to converge within a reasonable amount of time, so they are omitted from the 100-gene results shown here. Furthermore, scenario J is not shown since only one mutation rate ($2 \times 10^{-8}$) was used.

Scenario E to F to J. We observe the impact of the increase in complexity on the performance of MPL and MP, as both infer less accurate networks on these scenarios than on the data of Scenario D.

Finally, it worth noting that increasing the numbers of genes does not seem to affect the performance of these two methods, with the only exception observed when using 100 gene trees on the low-ILS conditions of Scenarios D and E, where the performance improved significantly over smaller numbers of genes when using gene tree estimates.

While inference under the MSNC using MPL could result in the correct phylogenetic network topology, several of the branch lengths could be inferred incorrectly. Consider the scenario of Figure 1c. Since inference under the MSNC assumes that $x_1$ and $x_2$ are two alleles of species X, and the same for the pair ($y_1,y_2$), and the pair ($z_1,z_2$), the lengths of all four branches in the subtree $(X,Y,Z)$ would be underestimated to account for the absence of coalescence events on these branches. In other words, while MPL happened to provide good results in some of these simulations in terms of the phylogenetic network topology, it did so at the expense of the branch lengths. For example, for scenario F (see Fig. 3c), the coalescent times of the homeologous alleles from the tetraploid z have to be more ancient than the divergence time between species a and b. That is to say, no homoeologs could coalesce along the two horizontal edges or the branch connected to z. So, even when the tetraploid z was correctly inferred as the hybrid species, MPL worked by forcing the age of the hybridization to be zero.

Accounting for Allopolyploidy Explicitly: Performance of MPAllopp and AlloppNET—For low levels of ILS, MPAllopp outperforms both MPL and MP in particular when using gene tree estimates. Furthermore, increasing the number of genes has a noticeable positive impact on the performance of MPAllopp. For moderate and high levels of ILS, MPAllopp outperforms both methods significantly when using both the true or estimated gene trees, across all scenarios. For low levels of ILS,
AlloppNET and MPAllopp performed almost identically on Scenario D. For Scenarios E and F, AlloppNET outperformed MPAllopp slightly when using 1 or 3 genes, but they both had almost identical performance when using 10 genes. For higher levels of ILS, AlloppNET could outperform MPAllopp, especially when using a larger number of genes. However, increasing the number of genes also increases the computational requirements of AlloppNET. For 100-gene data sets, 29/30 AlloppNET chains converged (effective sample size for log-posterior of genes also increases the computational requirements of AlloppNET). For 100-gene data sets, 29/30 AlloppNET chains converged (effective sample size for log-posterior density samples ≥ 200) for scenarios D, E, and F given a single individual and high ILS. But for other model conditions only 26/240 chains converged after at least 46 h of running time, so we excluded AlloppNET from our analysis of 100-gene data sets.

Overall, counting the number of times AlloppNET and MPAllopp inferred the correct network, MPAllopp successfully recovered the true network in 44% of replicates across all combinations that are being compared of scenario, mutation rate, and number of individuals (56% of the replicates when using the true gene trees). AlloppNET, on the other hand, performed slightly better, inferring the true network in 48% of the cases. We also noted that MPAllopp had an advantage over AlloppNET when the number of individuals per species increased to 9 under high ILS, with a mean error of 1.57 as compared to 1.71.

As mentioned above, AlloppNET is more powerful than the other methods studied here in that it estimates other evolutionary parameters (beside the network topology) including sampling gene trees from the posterior distribution. Normally AlloppNET is initialized with user-supplied assignments of alleles to subgenomes, but will coestimate these assignments along with the gene and species phylogenies. To assess the quality of gene trees estimated by AlloppNET, we quantified their accuracy and compared it to that of gene trees estimated from the sequence alignments using IQ-TREE. As AlloppNET returns a sample of gene trees per locus, we computed for each locus the maximum clade credibility (MCC) tree. As Supplementary Figure S1 available on Dryad shows, AlloppNET infers more accurate gene trees, owing mainly to the fact that AlloppNET samples the gene trees and phylogenetic networks simultaneously. The average gene tree error of data sets with low, moderate, and high ILS were 0.39, 0.27, and 0.23, respectively for IQ-TREE gene trees, whereas they were 0.27, 0.21, and 0.15, respectively for AlloppNET.

We also evaluated the performance of MPAllopp when the subgenome assignment is unknown, a method we call MPAllopp*. Based on this evaluation, estimates of the species network are much higher in error when the subgenome assignment must be estimated using the heuristic we describe in Methods (Supplementary Fig. S2 available on Dryad). Running time was generally unaffected, except for scenario J where it increased by up to 20 minutes (Supplementary Fig. S3 available on Dryad).

Running Times of the Methods—We recorded the computational time in CPU minutes for each method on each data set; the results for low, moderate, and high levels of ILS are shown in Figures 7, 8, and 9, respectively. As expected, AlloppNET was in general the slowest method (71 min on average), and MP was the second slowest (25 min on average). The parsimony methods typically took 1–2 min to complete; however, for MP, the variation in running time was noticeable when gene tree estimation error was involved, especially when more individuals were included. In fact, MP failed to complete on 2 and 28 data sets of gene tree estimates with 3 and 9 individuals per species, respectively.

As the results show, Scenario D in general takes the least amount of time for the methods to analyze since it is the simplest in terms of the number of hybridization and the location of the hybridization events. Scenarios E and F are more complex and take more time, and Scenario J, which is the largest, takes the most time. Furthermore, in many cases of 3 or more genes, AlloppNET takes time that is two order of magnitude larger than that of MPAllopp. For 100 gene trees, and as mentioned above, AlloppNET did not converge within 46 h. Finally, MP had the largest variability in running time over data sets with the same parameter settings, a trend that is clearest on data sets with high ILS levels.

In summary, the simulation results show that AlloppNET and MPAllopp have comparable performance in terms of accuracy of the phylogenetic network topology, yet MPAllopp is much faster than AlloppNET.

Analysis of Biological Data Sets

A Triticum (Poaceae) data set—We used the bread wheat data set with 275 gene trees from Marcussen et al. (2014). The data set includes the allohexaploid Triticum aestivum (Ta), its five diploid relatives Triticum monococcum (Tm), Triticum urartu (Tu), Aegilops tauschii (At), and Aegilops speltoides (Asp), and Aegilops tauschatii (At), together with three outgroup species Hordeum vulgare (Hv), Brachypodium distachyon (Bd), and Oryza sativa (Os). AlloppNET was not run on this data set since it only deals with diploids and tetraploids. Given that the bread wheat contains six sets of chromosomes, we set the maximum number of allowable reticulations to 2 and 3. Using our method with the genome identities being assigned to the alleles of the allopolyploid bread wheat, we obtained the optimal results after 30 runs of search, as shown in Figure 10.

Although there are three equally parsimonious networks inferred, they share the same underlying MUL-tree. As described above, MPAllopp traverses the network space while evaluating the network based on its MUL-tree representation. In this case, three networks correspond to the same MUL-tree. This nonuniqueness of the network notwithstanding, the three networks point to an evolutionary hypothesis with
two hybridization events leading to the formation of *T. aestivum*. Furthermore, the identified putative diploid progenitors, namely *Ae. speltoides, Ae. tauschii* and *T. urartu*, were consistent with previous studies (Marcussen et al. 2014). The three networks differ in the order in which these three species hybridized to give rise to the two hybridization events shown.

*A Pachycladon (Brassicaceae) data set—* We reanalyzed the *Pachycladon* data set with five nuclear single-copy genes from Joly et al. (2009). We included eight tetraploid species of the *Pachycladon* genus, coupled with two diploids *Arabidopsis thaliana* and *Lepidium apelatum* as in Jones et al. (2013). For each gene, BEAST Version 2.6.4 was employed to infer a sample of trees, based on which we computed the maximum clade credibility tree by the utility program TreeAnnotator in BEAST. This produced five gene tree estimates in total (Supplementary Fig. S4 available on Dryad). We ran MPAllopp supplied with the genome identities of the allele copies from the *Pachycladon* species, varying from one to three reticulations. Results are shown in Figure 11 and Supplementary Figure S5 available on Dryad.

The main difference between these two phylogenetic networks is the placement of *P. wallii*: In Figure 11a, *P. wallii* is placed as basal to the clade formed by *P. latisiliqua, P. enysii, P. fastigiata*, and *P. stellata*, whereas in Figure 11b it is grouped with *P. latisiliqua* as sisters. The latter is in full agreement with the result reported in Jones et al. (2013) using the same data. Interestingly, *P. wallii* is traditionally regarded as a sister species to *P. novaezelandiae* considering that they both live in the southern South Island of New Zealand. Such conflicts were probably caused by missing data as *P. novaezelandiae* only has sequences for the CHS gene in this data set, and one of the gene copy was grouped within the *P. latisiliqua et al. clade while the other was a sister to *P. novaezelandiae*. It is also worth mentioning that though six networks with equal score were returned, there are only four unique underlying
MUL-trees: Figure 11a and Supplementary Figure S5a available on Dryad are associated with the same MUL-tree despite different number of reticulations, so are Figure 11b and Supplementary Figure S5b available on Dryad. This again illustrates the identifiability issue for the conversion between MUL-trees and networks.

A potentially more interesting hypothesis based on the networks in Figure 11 is that this evolutionary history includes an autopolyploidization event at the most recent common ancestor of the in-group after the split from *A. thaliana*. That is, an alternative evolutionary scenario here is a species tree with no interspecific hybridization. These two alternative hypotheses have an equal score under the criterion optimized by MPAllopp and, thus, cannot be distinguished based on the data provided.

CONCLUSIONS

In this article, we introduced a new maximum parsimony method, MPAllopp, for inferring phylogenetic networks from gene tree topologies while accounting for polyploidy and incomplete lineage sorting simultaneously. The method employs a heuristic search for walking the network space while evaluating the parsimony score on the MUL-tree representation of the network.

The lack of a one-to-one mapping between MUL-trees and phylogenetic networks notwithstanding (Huber et al. 2016; Zhu et al. 2016), “seeing” the polyploid hybridization events in a MUL-tree is possible only for simplistic scenarios: a small number of taxa, a small number of hybridization events, a small number of gene extinctions, and, most importantly, the absence of confounding factors such as ILS (Fig. 1). Indeed, the parsimony algorithms and methods of Huber and Moulton (2006) and Thomas et al. (2017) do not account for ILS. Identifying the hybridization events computationally is the task of turning the MUL-tree into a phylogenetic network after a MUL-tree is inferred from the gene trees. Therefore, our method searches the phylogenetic network space directly by applying
Figure 9. Boxplots of running time (in CPU minutes) on simulated data with high ILS (mutation rate of $1 \times 10^{-7}$) over 10 replicates. Data sets with 100 genes were too large for AlloppNET to converge within a reasonable amount of time so they are omitted from the 100-gene results shown here. Furthermore, scenario J is not shown since only one mutation rate ($2 \times 10^{-8}$) was used.

| Runtime (min) | 1 gene | 3 genes | 10 genes | 100 genes |
|--------------|--------|---------|----------|-----------|
| 1 individual | MPL    | MP      | MPAllopp | AlloppNET |
| 3 individuals| MPL    | MP      | MPAllopp | AlloppNET |
| 9 individuals| MPL    | MP      | MPAllopp | AlloppNET |

Figure 10. Results on bread wheat data. Species networks inferred by MPAllopp from the 9-taxon wheat data set with 275 reconstructed gene trees from (Marcussen et al., 2014). The three networks have the same score under the criterion optimized by MPAllopp.

subnetwork transfer operations on networks, so that the inference result is a network, rather than a MUL-tree.

In addition, the method proposed here assumes allopolyploidization. However, in nature, homoploid hybrids, allopolyploids, and autopolyploids could
coexist, which would necessitate the development of methods incorporating various types of hybridization and polyploidization events. However, as we illustrated in the case of the Pachycladon data set above, some patterns in the phylogenetic network could potentially signal an autopolyploidization event. Currently, our method cannot infer autopolyploidy and requires the diploid progenitors to be sampled, as we have not implemented support for a data structure that can represent WGD in the absence of diploid progenitor species, or if those species never existed. We plan on implementing it in a future version.

As discussed in Blischak et al. (2018), statistical modeling of phylogenetic networks with polyploid hybridization is very complex. We believe that devising stochastic models and inference methods for restricted classes of polyploids, as in Jones et al. (2013); Oxelman et al. (2017), is most likely the way to make progress in this area.

In the last several years, there has been work on combining the multispecies coalescent model with a birth–death model of gene duplication and loss (Wu et al. 2014; Rasmussen and Kellis 2012; Du and Nakhleh 2018; Li et al. 2020). While these works do not consider hybridization or allelic introgression, Du et al. (2019) introduced a model that unifies the multispecies network coalescent and a birth–death model thus allowing for simultaneous modeling of incomplete lineage sorting, gene duplication and loss, and diploid hybridization. These works could be relevant for further advances in modeling polyploid hybridization in phylogenomic inference.

Statistical inference of phylogenetic networks is computationally much more demanding than inference of trees, severely limiting the sizes of data sets that can be analyzed with phylogenetic network methods. One approach to handling larger data sets is to analyze smaller subsets of the data (subsets in terms of taxa). This approach could be automated, as in Zhu et al. (2019) for example, but this requires developing methods for accurately estimating small networks with their evolutionary parameters and for merging these small networks into a network on the full data set. We view this as an essential direction for future research for phylogenetic network inference.

**Supplementary Material**

Data available from the Dryad Digital Repository: [http://dx.doi.org/10.5061/dryad.4xgx25b](http://dx.doi.org/10.5061/dryad.4xgx25b).

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