Keywords: Bioinformatics, Phylogenetics, Simulation, Tree Generator, Supertrees.

Abstract: More and more evolutionary and molecular biologists are interested in building alternative supertrees. Often, developing new approaches or testing new metrics requires relevant datasets that are not always easy to obtain. In order to solve this problem of lack of data, we propose a new approach and developed a program in Python to generate overlapping phylogenetic trees with biological events to simplify the process of obtaining this type of data. The new tool takes the number of phylogenetic trees the user wants to generate, the maximum number of leaves per tree to generate, and the average level of leaf overlap between phylogenetic trees as input parameters. The program returns to the user a set of phylogenetic trees in Newick format, respecting the parameters given as input, in order to use them to infer a supertree (or supertrees). This data can be an important resource for research; the user can download the generated data and use it later in their relevant application tasks.

Availability and implementation: The generator is freely and publicly available to the entire scientific community on the GitHub platform, without any registration, https://github.com/tahiri-lab/gptree under the MIT licence. The pipeline is written in Python 3.7.

1 INTRODUCTION

More and more phylogenetic researchers are dealing with phylogenetic supertrees in order to improve our understanding of species evolution. A supertree is assembled from individual gene trees, but these trees can be defined in different, but mutually overlapping, sets of taxa. Studies on supertrees are essential to reconstructing the phylogenetic trees of all living species (i.e., the OpenTree Online project (Hinchliff et al., 2015)). Among the unsolved problems, classification problems play an important role and have several practical applications, i.e., the creation of the tree of life in order to know and understand the biodiversity of species.

The reliability of species phylogeny can be validated through the merging of related gene trees, considering the present topological conflicts (Maddison et al., 2007). As part of this aspect, it is possible to examine the consensus tree issue, where trees for merging are constructed for the same set of taxa, or the supertree issue, where trees for merging are constructed for different but overlapping sets of taxa. There are a number of methods for inferring a consensus tree, examples of which are the majority-rule consensus trees, the extended majority-rule consensus tree, and the strict consensus tree (Bryant, 2003). It is worth noting that, in practice, researchers do not often work with phylogenies on the same set of taxa, and, therefore, the issue of inferring a supertree becomes important (Bininda-Emonds, 2004). One approach is to combine a collection of small phylogenetic trees with a partial level of overlap between them into complex supertrees (Wilkinson et al., 2007).

We should also highlight the aspects of tree clustering in situations, where combining the overlapping trees into a supertree can be performed for the identified clusters. There are a number of approaches to tree clustering that include k-means (Stockham et al., 2002), k-medoids (Tahiri et al., 2018), MultiPolar Consensus (MPC) method (Bonnard et al., 2006), and Multiple Consensus Trees (MCT) method (Guénoche, 2013). Recently, (Tahiri et al., 2022a) have introduced a method for building alternative consensus trees and supertrees using k-means and the Robinson and Foulds (RF) distance alongside with adaptation of
several popular cluster validity indices.

Researchers who work with phylogenetic trees and supertrees may experience a lack of availability of datasets that they can use to test their solutions, for example, classification, clustering, and testing new metrics for comparing trees ((Swenson et al., 2010); (Tahiri et al., 2022b)). In this context, the creation of software for generating overlapping phylogenetic trees to infer supertrees that can be used later by scientists, i.e., in biology, medicine, ecology, bioinformatics, may become a relevant tool for phylogenetic studies.

Generation of a supertree through its inference from a set of trees as the core of the solution should have the functionality to generate phylogenetic trees with a certain set of features, including the different number of leaves in trees, the presence of overlapping between trees, and the incorporation of biological events, which provide the generated set with the property of approximation to the processes of evolution rather than randomness.

2 RELATED WORK

There are various software solutions for the generation of individual phylogenetic trees based on different approaches and with additional features incorporated. An overview of some of these implementations is shown in Table 1.

We analyzed and tested these generators and evaluated the possibility of using them for the specified task. Some applications are aimed at random tree generation, and such solutions are usually part of a larger software product with broader functionality (e.g., a web service (T-Rex) or a library for working with phylogenetic trees in Python (ete3)). Most of these simulators are capable of introducing additional features into the generated trees, which include birth and death events (e.g., based on the Gillespie algorithm (Gillespie, 1977)) and horizontal gene transfer (HGT). The most popular languages for the generators considered are Python, Java, and C.

None of the existing tree simulators provides the functionality to generate phylogenetic tree sets with partially overlapping sets of taxa, so there is a need to propose an approach to get this kind of data (with the possibility to use the existing simulator as a base generator). We chose the AsympTree library (Schaller et al., 2022) version 2.2 as the basic simulator for generating a single tree (or a pair of species trees and gene trees), since it is the closest option according to the requirements we set for the tree: the availability of evolutionary scenarios (duplication, loss, and horizontal gene transfer events), the possibility of setting additional parameters (such as loss, duplication, and HGT rates), and the Python programming language (this is the programming language that commonly used to prototype solutions using a wide range of data and algorithm libraries for bioinformatics and machine learning). We use ete3 library as an additional tool for working with generated trees (using the features available in Python). It provides a possibility to visualize the data, calculate comparison parameters of two trees (including RF distances), and save the generated trees in Newick format. More information about this is in the following sections.

Special attention should be given to horizontal gene transfer, which plays an essential role in evolution ((Davin et al., 2018); (Wolfe and Fournier, 2018); (Bapteste et al., 2004)) and its incorporation into the dataset makes the data closer to realistic. Since each gene has its own evolutionary history, which can be represented by its own phylogenetic tree, it (the gene) can demonstrate evolutionary patterns based, in particular, on horizontal gene transfer events (Tahiri et al., 2022a). HGT in AsympTree is implemented as follows (Schaller et al., 2022):

1. A species tree is generated.
2. A copy of a gene present at time $t$ in any branch of the species tree is moved to another branch of the species tree. This branch, where the copy of the gene is placed, is chosen uniformly among the branches available at time $t$, but without considering the branch with the parent gene.

Visualization of the presence of HGT in the generated dataset will be shown in the Results and Discussion section. The selected generator of a single phylogenetic tree with the presence of duplication, loss, and horizontal gene transfer events was used as the basis for the pipeline, which is described in the next section.

3 METHODS

Several basic conditions have to be fulfilled to generate a phylogenetic tree dataset in order to obtain a phylogenetic supertree:

1. Generation of trees with different numbers of leaves in each tree;
2. Presence of simulated biological events in trees (in particular, horizontal gene transfer);
3. Presence of overlaps between trees.

The level of overlap of the two trees we determine
<table>
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<tr>
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<tr>
<td>T-Rex (Random tree generator module)</td>
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<td>Zombi</td>
<td>Birth-death model</td>
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by the following formula 1:

\[
OL(T_1, T_2) = \frac{n(T_1, T_2)}{n(T_1) + n(T_2) - n(T_1, T_2)},
\]

where, \(OL(T_1, T_2)\) is the overlap level between tree 1 (denoted \(T_1\)) and tree 2 (denoted \(T_2\)), \(n(T_1, T_2)\) is the number of common leaves between \(T_1\) and \(T_2\), \(n(T_1)\) is the number of leaves in \(T_1\), \(n(T_2)\) is the number of leaves in \(T_2\).

As mentioned above, an analysis of current solutions in phylogeny and bioinformatics did not detect the availability of dataset “generators” with the above conditions. In this work, we propose a dataset generation pipeline of phylogenetic trees for use in generating supertrees. A scheme of the pipeline is shown in Figure 1.

The main steps in the proposed workflow are as follows:

1. Input step. The user enters the initial values:
   - \(L_{\text{min}}\) is the minimum possible number of leaves for each tree;
   - \(L_{\text{max}}\) is the maximum possible number of leaves for each tree;
   - \(N_{\text{gen}}\) is the number of trees to be generated;
   - \(p_{\text{level}}\) is the average level of overlap (common leaves) between the trees in the set.

2. Validation step. The input values should satisfy the following constraints: \(L_{\text{min}}\) is an integer and \(5 \leq L_{\text{min}} < 500\); \(L_{\text{max}}\) is an integer and \(L_{\text{min}} < L_{\text{max}} \leq 500\); \(N_{\text{gen}}\) is an integer and \(N_{\text{gen}} \leq 500\); \(p_{\text{level}}\) is a floating-point number in the range from 0.2 to 0.7 in increments of 0.05 (which corresponds to the range from 20% to 70%).

3. Original tree step. Generate the first tree with \(L_{\text{max}}\) leaves. Each tree is generated in 2 steps: first, a species tree is generated, and then a gene tree (with HGT) is generated on its basis. We distinguish separately the generation of the first tree as...
the base tree, which is used to calculate the level of overlap with the subsequent tree. The $L_{\text{max}}$ value is used to provide a large spread in the comparison of leaves in trees during calculations.

4. Pairwise tree step. Generate the next tree with a random number of leaves from the range ($L_{\text{min}}$, $L_{\text{max}}$).

5. Overlap level evaluation step. Calculate the average pairwise (without repetitions) level of overlap between trees and check how the new tree affects the average level of overlap. If they overlap in the specified range ($p_{\text{level}} - 0.01$; $p_{\text{level}} + 0.01$), this new tree is saved to the dataset (the user can adjust the size of this range). If not, return to step 4.

6. Repeat steps 4 and 5 until the dataset includes the required number of trees ($N_{\text{gen}}$).

7. Output step. Save the generated data (trees in Newick format) to a text file.

The user can save two datasets as a result of this workflow: the first dataset (main dataset) is a set of gene trees, the second dataset is a set of species trees (for individual purposes). In both datasets, the tree positions in the files correspond to each other. We will use individual pairs of trees to analyze the presence of horizontal gene transfer events.

This workflow can be extended for the case of inferring a single or multiple supertrees based on the generated data using additional packages. One of the most popular solutions for supertree inference is the CLANN tool (Creevey and McInerney, 2005), which is freely available on GitHub and widely used by a large number of researchers. In order to perform it, the user needs to follow several steps:

1. Dataset preparation (input): Generating phylogenetic trees defined on different, but partially overlapping sets of species, based on GPTree.

2. Prepare the input data: it can be data generated from the GPTree generator of phylogenetic trees with a different number of overlapping leaves.

3. Prepare a text file with commands: basic recommendations are provided in the tool documentation.

4. Run CLANN.

5. Save the output data.

A sample command file with recommended parameters is available on the project’s GitHub repository.
when the supertree inference is performed on the basis of a cluster of trees. The first option is to

Figure 2: Visualization of 6 trees from the generated dataset with the following parameters: $L_{\text{min}}=7$, $L_{\text{max}}=12$, $p_{\text{level}}=0.5$. Basic visual analysis shows that these trees have different numbers of leaves (one tree has 8 leaves, three trees have 9 leaves, one tree has 10 leaves, and one tree has 12 leaves), and they are within the given parameters. In addition, these trees have a certain proportion of common leaves, and, in fact, the average level of overlap is 0.5 with a small margin of error.

4 RESULTS AND DISCUSSION

This solution is implemented in Python and structured as an interactive Jupiter Notebook (in .ipynb format). The generator was tested in Google Colaboratory (Google Colab with a basic free version).

First, we generated and visualized a small number of trees in order to show their structure (Figure 2). The resulting image reflects the basic parameters set by the user.

Second, we tested the functionality of the phylogenetic tree generator with relation to three basic parameters: the number of generated trees with different set of leaves from the defined range, evaluation of the level of overlap between trees, and analysis of the presence of HGTs. The identified parameters and the basic results are shown in Table 2.

Figure 3 shows a visualization of the results by the level of overlap between trees. It should be noted that the distribution resembles a normal distribution. The bell-shaped curve can show the degree of diversity in the number of common leaves belonging to pairs of trees. In parts (a) and (b), we may see a small number of rare values (left and right), which actually are outliers. Part (b) may also help to evaluate the standard deviation, which can be relatively large for a flat bell-shaped distribution. The boundaries in the left and right tails region are also estimable using formula 1 (for the cases of $L_{\text{min}}$ and $L_{\text{max}}$ number of leaves) and considering the initial parameters set by the user.

We analyzed for the presence of horizontal gene transfer in the generated dataset. For this purpose, we used a random pair of species and gene trees and checked for the presence of HGT using the T-Rex web service (Boc et al., 2012). The results are shown in Figure 4. This service utilizes a polynomial-time algorithm with the possibility to choose between several optimization criteria (in particular, bipartition dissimilarity, Robinson and Foulds distance, and least-squares) and a bootstrap HGT detection (Boc et al., 2010). To infer a horizontal gene transfer scenario, the user needs to provide a pair of species and gene trees.

We observe that the generated dataset corresponds to our criteria. It should be noted that the generator works slower for overlap levels less than 0.2 and greater than 0.7, and therefore in the current version of the generator the recommended value of the overlap level is in the range (0.2, 0.7). In future releases of this generator, we plan to expand this range.

A version of Jupiter Notebook with code to analyze the generated dataset and examples can be found in the project repository on GitHub (https://github.com/tahiri-lab/GPTree).

The inference of supertrees based on the generated dataset is also an important subject. Depending on the objectives of the study, we can refer to the clustering approach, when the supertree inference is performed on the basis of a cluster of trees. The first option is to

<table>
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generate a large dataset, then cluster this dataset using existing algorithms (e.g., $k$-means or $k$-medoids, see (Tahiri et al., 2022a)) to build a supertree for each cluster that is found. The second option is to use the generator in a loop and generate several datasets of clusters for each set of parameters to infer a supertree.

One of the use cases of the developed tool is the generation of data to test new distance measures between phylogenetic trees defined on different, but mutually overlapping, sets of taxa, or supertrees. Although metrics between trees have been studied for more than 40 years, the story is different for supertrees since most distances were introduced in the last decade or so. The Robinson and Foulds distance is the most popular phylogenetic tree distance, but no such standard is known for supertrees. On the one side, it is possible to adapt existing metrics to compare phylogenetic trees (with overlapping leaves) in the context of clustering. This can help to effectively use phylogenetic tree clusters to infer supertrees. On
the other side, this foundation can help to evaluate, adapt and improve metrics for comparing phylogenetic supertrees relying on distance properties. Researchers can use simulated data with incorporated biological events in such tasks.

5 CONCLUSION

The analysis and comparison of several phylogenetic tree simulators did not reveal phylogenetic tree generators with partially overlapping taxon sets, which can be used in problems using phylogenetic supertrees. This paper proposes a new approach for generating a dataset of phylogenetic trees for further use in the building of phylogenetic supertrees. The generator takes the minimum possible number of leaves for each tree, the maximum possible number of leaves for each tree, the number of trees to be generated, and the average level of overlap (common leaves) between the trees in the set as input parameters. The output is a dataset consisting of gene trees with incorporated biological events, including duplication, loss, and horizontal gene transfer events. The trees in the generated dataset are in Newick format.

The generator presented in this paper can be useful for biologists, bioinformaticians, ecologists, and computer scientists to conduct experiments with phylogenetic trees and supertrees, for example, in the problems of developing new metrics of distance between supertrees, clustering, and classification problems. This solution is freely available in the repository on GitHub, where there are also additional scripts for testing the generated dataset with detailed comments, and sample datasets. The possibility to run the generator in Google Colaboratory makes it easier and more accessible for the scientific community.

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Conflict of Interest: none declared.

REFERENCES


