Mean and Variance of Phylogenetic Trees

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Abstract.— We describe the use of the Fréchet mean and variance in the Billera-Holmes-Vogtmann (BHV) treespace to summarize and explore the diversity of a set of phylogenetic trees. We show that the Fréchet mean is comparable to other summary methods, despite its stickiness property, and that the Fréchet variance is faster and more precise than commonly used variance measures. These mean and variance measures are more theoretically justified, and more robust, than previous estimates of this type, and can be estimated reasonably efficiently. This leads to applications such as mean hypothesis testing. (Keywords: phylogenetics, treespace, Fréchet mean, Fréchet variance, two-sample test)

Sets of related phylogenetic trees are commonly encountered in evolutionary biology. For example, one might encounter such a set as the output of an inference program like MrBayes [Ronquist et al. (2012)], or as a set of gene trees on some given set of species. For this paper, we think of any such set as being a sample from an underlying distribution on the set of all phylogenetic trees with a fixed leaf set.

Here, we consider a mathematically-founded basis for describing the mean of such a distribution, using the representation of trees as elements of a geometric space and looking for the Fréchet mean: the tree that minimizes the sum of the squared distance between the mean and the elements of a sample from the distribution. This formulation also allows the identification of the Fréchet variance, which is the actual sum of the squared distances between the sample elements and the Fréchet mean.

We describe algorithms for computing the Fréchet mean and variance. They are fairly efficient to compute, and we show that the mean gives a good way of summarizing the uncertainty found in a set of trees. A particularly nice property of the mean is that it may result in a non-fully-articulated tree, but does so in a statistically appropriate way. We also describe computation of the variance. The Fréchet variance is a better measure of statistical uncertainty than simpler measures (like the number of topologies found in a set of trees), and is faster to compute than the sum of all pairwise distances between trees, for a large set of trees.
Our results show that the treespace-based measure of phylogenetic distance that originated with [Billera et al. (2001)] can in fact be used in practical applications.

**BACKGROUND**

We begin by describing the treespace in which we are working, and some properties of that space and its distance measure. We also consider other ways of summarizing a collection of trees besides the Fréchet mean and variance, and some of their properties.

**Treespace**

The *Billera-Holmes-Vogtmann (BHV) treespace*, $T_n$, [Billera et al. (2001)] contains all unrooted phylogenetic trees with edge lengths and a given set of $n + 1$ labelled leaves. For this paper, we fix the set of leaf labels to be $\{0, 1, ..., n\}$. Any of these trees can be thought of as rooted by fixing leaf 0 as the root. In this paper, we will defined the BHV treespace as a subspace of $\mathbb{R}^N$, where $N = 2^n - 1$ is the number of possible splits on $n + 1$ leaves, or equivalently, the number of possible partitions of the set of leaves into two sets, each of size at least 1. Each coordinate of $\mathbb{R}^N$ corresponds to a different split, where the order of the splits does not matter, but is fixed. Note that by allowing partitions of size 1, we include splits corresponding to edges ending in leaves. The original definition [Billera et al. (2001)] ignores these edges, but notes that they can be included, as we have done here.

Given a tree $T$ with $n + 1$ leaves and edge lengths, it corresponds to the following vector in $\mathbb{R}^N$: for every edge $e$ in $T$ with length $|e|_T$, let the coordinate corresponding to the split induced by $e$ be $|e|_T$. Let the coordinates corresponding to splits not induced by edges in $T$ be 0. Let $T_n$ be the set of vectors in $\mathbb{R}^N$ that correspond to trees, as just described. Not all non-negative vectors in $\mathbb{R}^N$ correspond to trees due to split incompatibility. Two splits are *incompatible* if they cannot be induced by edges existing in the same tree. For example, a *cherry* is a pair of adjacent leaves in a tree, and the corresponding split separates the two adjacent leaves from all others. No tree with $n \geq 4$ can have both $\{1, 2\}$ and $\{1, 3\}$ as cherries, so the corresponding splits $\{1, 2\}\{0, 3, 4, ..., n\}$ and $\{1, 3\}\{0, 2, 4, 5, ..., n\}$ are incompatible, and no vectors in $T_n$ have positive values in both these coordinates. The *topology* of a tree is the set of all splits induced by the edges of that tree. A binary tree, in which all interior nodes have degree 3, contains $2n - 1$ splits, while degenerate or non-binary trees will contain fewer than $2n - 1$ splits. See Figure 1. For more details, including a combinatorial description of the space, see [Billera et al. (2001)].

There is a distance metric associated with the BHV treespace called the *BHV distance* or the *geodesic distance*, which was also defined in [Billera et al. (2001)]. Consider all trees in $T_n$ with the same topology. Because these trees all correspond to the same set of splits, their vectors have exactly the same set of non-zero coordinates, which can take on any positive values. Thus, if the number of non-zero coordinates is $d$, this set of trees corresponds to a $d$-dimensional Euclidean orthant, which is the non-negative part of $\mathbb{R}^d$. 
Figure 1: (a) Five and (b) three of the fifteen orthants in the Billera-Holmes-Vogtmann (BHV) treespace $\mathcal{T}_4$. For ease of visualization, the five dimensions corresponding to the leaf edges are not included. Thus, each binary tree topology is represented by a quadrant (2-dimensional orthant), with the two quadrant axes corresponding to the lengths of the interior edge splits. The axis between two quadrants corresponds to a tree topology that is not fully resolved. The geodesic (shortest) path between two trees is shown by a dashed line and may pass through different orthants depending on the branch lengths of the endpoint trees, as shown by the geodesics between $T_1$ and $T_2$ and between $T'_1$ and $T'_2$.

When the tree topology is binary, $d = 2n - 1$. For any two trees with the same topology, the BHV distance is the Euclidean distance between their corresponding vectors in that orthant. For two trees with different topologies, the BHV distance is the length of the shortest path between them that remains in the treespace. The length of any path can be computed by calculating the Euclidean distance of the path restricted to each orthant that it passes though, and summing these lengths. The shortest path is called a geodesic, and will pass from one orthant to the next orthant through lower-dimensional boundaries corresponding to trees with fewer splits. See Figure 1 for an example of two geodesics in $\mathcal{T}_4$.

The BHV treespace is connected, since for any two trees are connected by a path through the origin. This may or may not be the geodesic. Billera et al. (2001) showed that the BHV treespace is globally non-positively curved (Bridson and Haefliger 1999), which implies that geodesics are unique. Owen and Provan (2011) gave a polynomial time algorithm for computing the geodesic distance between two trees that runs in $O(n^4)$ time, but which is faster for trees with common splits.

Note that if the $L_1$ metric, which is also known as the Manhattan or taxicab metric,
is used in each orthant instead of the $L_2$ or Euclidean metric, then the length of a (no longer unique) geodesic between two trees is the same as the weighted Robinson-Foulds distance (Robinson and Foulds 1979) between them (see St. John (2017) for a good summary).

Mean and variance

In Euclidean space, the Fréchet mean, or centre of mass, is the point minimizing the sum of the squared distances to the sample points, and is equivalent to the coordinate-wise average of the sample points. The Fréchet mean was similarly defined for treespace by Miller et al. (2015) and Báčák (2014), independently. For a set of sample trees $\{T_1, T_2, ..., T_r\}$, the Fréchet mean, or simply, mean, is the tree $t$ which minimizes $\sum_{i=1}^{r} d(t, T_i)^2$, where $d(\cdot, \cdot)$ is the BHV distance between the two trees. The Fréchet variance, or simply variance, is that minimum sum of squared distances. This mean is unique because treespace is non-positively curved. Both Miller et al. (2015) and Báčák (2014) gave an algorithm for approximating the mean and variance based on the Law of Large Numbers for non-positively curved space derived by Sturm (2003).

We briefly mention some interesting properties of the mean. See Miller et al. (2015, Section 5) for details and proofs. The mean tree is not necessarily a refinement of the majority-rule consensus tree (which contains all splits found in a majority of the trees $T_i$). Any split that appears in the mean tree appears in at least one of the sample trees, and any split that appears in all the sample trees appears in the mean tree. Finally, the mean tree is “sticky” (Hotz et al. 2013), in that perturbing one of the sample trees does not always change the mean tree. This “stickiness” only happens when the mean is on a lower-dimensional orthant in treespace, which corresponds to a degenerate tree topology. This implies that the mean will be unresolved more often than one might expect or wish, similar to the majority-rule consensus tree. This is not a coincidence, as the majority-rule consensus tree is the median tree under the Robinson-Foulds distance (Barthélemy and McMorris 1986), and Pattengale (2005) further showed that the weighted majority-rule consensus tree is the median under the weighted Robinson-Foulds distance. Recall that using an $L_1$ metric on each orthant in this space instead of the Euclidean metric gives the weighted Robinson-Foulds distance instead of the BHV distance.

The Fréchet variance of a set of trees quantifies how spread out a set of trees is from their mean. We will show that in our experiments, as sequence length increases and there is more information about the tree to be reconstructed, the variance of samples of trees from the bootstrap and posterior distributions decreases.

Sturm (2003) showed that a Law of Large Numbers holds, meaning that as the sample size increases, the sample means of a distribution over $T_n$ converge to the true mean. Barden and Le (2017) proved a Central Limit Theorem on the BHV treespace, showing that the distribution of the sample means converges to a certain Gaussian distribution.

Other measures of centre among a set of trees
We compare the Fréchet mean to three other commonly used measures of centre or consensus in phylogenetics. The first is the majority-rule tree, which is the tree containing exactly those splits appearing in a majority of the input trees (Margush and McMorris 1981). The majority-rule consensus tree and the Fréchet mean are not, in general, refinements of each other (Miller et al. 2015).

The other two measures of centre come from tree search algorithms. In these cases, the tree search procedures produce a distribution of trees along with a most probable tree, which acts like a centre. Specifically, we look at maximum likelihood search using RAxML (Stamatakis 2014), which produces a distribution of bootstrap trees (Felsenstein 1985) where the maximum likelihood (ML) tree can be considered as a centre. Alternatively, using a Bayesian approach to phylogenetic inference, the implementation MrBayes (Ronquist et al. 2012) returns a posterior distribution representing the most probable tree, with the maximum a posteriori tree (MAP) tree often being used as its summary tree.

Other measures of variance

The variance or amount of variability in a set of trees is less established as a measure than the summary tree. Often, a visual representation, such as SplitsTree (Huson and Bryant 2006) or DensiTree (Bouckaert 2010), is used to represent the diversity of a set of trees. Unfortunately, these methods cannot be assessed or compared quantitatively. Instead, we will compare the Fréchet variance with several quantitative measures of variance, namely the number of different tree topologies in the set, the number of different splits in the set, and the sum of the squared geodesic distances between each pair of trees in the set. The later measure was proposed in Chakerian and Holmes (2012) as a method for approximating the variance, and used by Ponciano et al. (2012) to measure the variance of posterior distributions. It requires \(O(m^2 n^4)\) time to estimate the variance of \(m\) trees on \(n\) leaves.

One can also explore the variability of a set of trees using pairwise comparison methods applied to all pairs of trees in the set. One can compare trees using both the BHV distance and the more commonly-used Robinson-Foulds (RF) distance (Robinson and Foulds 1981). The RF distance between trees \(T_1\) and \(T_2\) is defined as the number of splits in \(T_1\) but not in \(T_2\), plus the number of splits in \(T_2\) but not in \(T_1\). That is, it is the size of the symmetric difference between the split sets of \(T_1\) and \(T_2\). Note that this definition only uses the tree topologies, and not the edge lengths of the trees.

Related work

The most closely related work to ours is that of Benner et al. (2014). They investigated the behaviour of both the mean and the median (the tree minimizing the sum of distances, instead of the sum of squared distances, to the sample trees). Bačák (2014) summarize posterior distributions returned by Bayesian tree reconstruction methods. The authors simulated sequences of lengths 50, 100, 250, and 500 using a 14-taxon tree of plants and the
F81 evolutionary model (Felsenstein 1981). They show that the mean and median estimates are comparable to the majority-rule consensus estimate, and in some instances perform better, and also investigate how the Fréchet variance changes with sequence length.

Our work was conceived independently, and while the overall aim of the work is the same, the scope of our experiments are much broader, considering tree distributions generated from both maximum likelihood and Bayesian methods; the general GTR evolutionary model; trees with more taxa; and a more comprehensive look at the Fréchet variance.

This paper focuses on the Fréchet mean and variance of a set of trees, which is a point summary of the data. Other work has look at 1-dimensional summaries, or best fit lines, in BHV treespace (Nye 2011, 2014; Feragen et al. 2013), and extensions to multi-dimensional summaries or a generalization of Principal Components Analysis (PCA) (Nye et al. 2016). Confidence sets on the BHV treespace are constructed in Willis (2016), and a full Central Limit Theorem is given in Barden and Le (2017).

**Methods**

**Computation of the mean and variance**

The mean tree is the tree that minimizes the sum of the squared BHV distances from itself tree to the sample trees. This is equivalent to the centre of mass in Euclidean space. We compute an approximation of the mean tree using the iterative implementation described in Miller et al. (2015). In this implementation, a new approximation of the mean tree is returned each iteration, and these approximations converge to the true mean tree as the number of iterations grows. To decide when to stop the iterative algorithm, we use a program option to check for convergence using a Cauchy sequence of length 10 with an epsilon of $10^{-6}$. This means that we stop the iterative algorithm when the pairwise BHV distances between the last 10 mean approximations were all less than or equal to $10^{-6}$. In all of our experiments, the means converged within 285 000 iterations. We also use the random permutation heuristic. We validated this choice of epsilon by choosing one repetition for each sequence length, and computing the (approximate) mean of the corresponding posterior distribution sample 10 times using the chosen parameters. For an epsilon of $10^{-6}$, the average BHV distance between pairs of approximate means of the same sample was on the order of $10^{-5}$, which we considered acceptable. Computing the mean and variance took 10-35 minutes on a 3.5 GHz 6-Core Intel Xeon E5 Processor. The program and source code are available at http://comet.lehman.cuny.edu/owen/code/SturmMean.tar.gz.

**Simulated Data**
We simulated 10 sets of DNA sequences for a variety of lengths (500, 1000, 1500, 2000, 2500, 3000, 3500, 4000 base pairs), using the tree of 44 mammals from Murphy et al. (2001), which is shown in Figure 2 as the reference tree. The sequences were simulated with Seq-Gen version 1.3.3 (Rambaut and Grass 1997) using the GTR model with the following parameter settings: the proportion of invariable sites is 0.18; the equilibrium frequencies for A, C, G, T are 0.21, 0.31, 0.3, and 0.18, respectively; and the GTR rate matrix is (1.5, 4.91, 1.34, 0.83, 5.8, 1), where the entries are the transition rate from A to C, A to G, A to T, C to G, C to T, and G to T. These parameter values were estimated in Hillis et al. (2005) for this reference tree.

For each set of sequences for each length, we ran RAxML Version 8 (Stamatakis 2014) to compute the Maximum Likelihood (ML) tree and 1000 bootstrap sample trees. For the RAxML settings, we used the GTR + I + Γ evolutionary model. We conducted a full analysis (option -f a) using rapid bootstrapping (option -x), which was recommended in the RAxML user manual when computing a large number of bootstrap replicates, since every 5th bootstrap tree is used as a starting point for the ML tree search. For each set of sequences, at each length, we also ran MrBayes 3.2 (Ronquist et al. 2012) to compute the MAP tree and sample 1000 trees from the posterior distribution. For the MrBayes settings, we used the GTR+I+Γ evolutionary model, and ran for 5,000,000 iterations, sampling every 1000 generations. We used the last 1000 sampled trees as the posterior distribution.

For each sample distribution of 1000 trees, we estimated the Fréchet mean and variance, using SturmMean (Miller et al. 2015) with the settings explained in the previous
We also computed the majority-rule consensus tree, and three other measures of variation for each sample: the number of different tree topologies in the sample, the number of different splits in the sample, and the sum of the squared BHV distance between each pair of trees in the sample. We compared the mean tree to the majority-rule consensus, ML, and MAP trees as follows. For each sequence length, repetition, and distribution type (bootstrap or posterior) there was one mean tree, one majority-rule consensus tree, and either an ML or MAP tree, depending on whether the sample was from the bootstrap or posterior distribution. Adding the reference tree to this set, we computed the RF distance between each pair of trees. We also computed the BHV distance if both trees in the pair had meaningful branch lengths. The mean trees, ML trees, and reference tree all had meaningful branch lengths, while the majority-rule consensus tree and MAP trees did not.

We also compared the lengths of the sampled trees and their centres. Here we define the length of a tree $T$ with edge set $E$ to be $\sqrt{\sum_{e \in E} |e|^2}$. This is the BHV distance of tree $T$ to the BHV treespace origin. To investigate stickiness of the mean, we compare the length of the mean tree of a distribution with the average length of trees in that distribution, to see if the mean is much shorter than its component trees. We also compare the lengths of the mean and the average lengths of trees between the posterior and bootstrap distributions generated from the same sequence set. In all cases, we use the Wilcoxon ranked signed test (Wilcoxon 1945) to test the hypothesis that the two distribution of lengths are the same.

Finally, we conducted a mean hypothesis test on the bootstrap and posterior samples for the first repetition of 4000 base pairs. Recall that a mean hypothesis test is a type of two-sample test that tests if the means of two samples are the same. Rejecting this hypothesis implies that the samples are from different distributions. We computed the BHV distance between the means of these bootstrap and posterior samples, and compared it to the BHV distance between the means of a random partition of the two samples. Since a full permutation test is not feasible (since each sample contains 1000 trees), we estimated it using 500 randomly chosen permutations. Note we are following the method for performing two-sample hypothesis testing on trees that was suggested in Feragen et al. (2013).

**Results**

To visualize what the trees from one repetition of the experiment look like, for the first repetition of the 4000 base pair sequence length experiment, we computed the BHV distance between 100 trees from each of the bootstrap and posterior samples, the reference tree, the ML tree and the two mean trees. We use classic Multi-dimensional Scaling (MDS) (Kruskal 1964) to reduce this to two dimensions (Fig. 3). The two means and the ML tree are in the middle of their respective samples. The two samples are separated in space and the reference tree is closer to the posterior sample, but not near its centre. The reference tree, two means, ML tree, and 62 of the trees from the two samples have the same topology. The other common topologies appear in both the bootstrap and posterior
samples. This suggests that the difference between the two clusters in Figure 3 is primarily due to branch lengths, which the BHV distance takes into account, instead of topology.
Figure 3: A sample of trees from the first repetition of the 4000 base pair sequence length embedded in 2 dimensions using classic Multi-dimensional Scaling. The trees from the bootstrap and posterior samples form two clusters, most likely due to differences in branch lengths instead of topology.
Comparison of the mean tree with other measures of centre

First we compare the mean trees to the reference tree and the other summary trees, showing that they are close. The four plots in Figure 4 use the RF and BHV distances to compare the Fréchet mean of each bootstrap and posterior sample to its corresponding majority-rule consensus tree, the reference tree, and either ML or MAP tree, as appropriate. In all cases, the distance between the Fréchet mean and the other trees decreases as the sequence length increases. The mean tree is closer to the ML or MAP and majority-rule consensus tree than to the reference tree, with those three trees almost always having the same topology for sequences of length 3000 base pairs and longer.

Next we consider the relation of the reference tree to the reconstructed trees. The four plots in Figure 5 use the RF and BHV distance to compare the reference tree to the mean tree, majority-rule consensus tree, and ML or MAP tree of each sample. These plots combined with the previous Figure 4 show that the reconstructed trees are closer to each other than to the reference tree. As expected, all trees become closer to the reference tree as the sequence length increases, since this gives more information about the reference tree, improving its reconstruction. Interestingly, under the BHV distance, the mean tree of the bootstrap distribution is on average closer to the reference tree than the ML tree.

Variance

We compare the different measures of variance in a set a trees, namely the number of different topologies, the number of different splits, the Fréchet variance, and the sum of the squared BHV distance between all pairs of input trees, in Figure 6. As the sequence length increases, there is more information about the underlying tree, and so we expect RAxML and MrBayes to both do a better job at inferring this tree and be more certain about it. This should be reflected by a decrease in variance in the bootstrap and posterior distributions, and thus samples, as the sequence length increases, which is what we see in Figure 6. For all measures, the posterior samples have a lower variance than the bootstrap samples. This matches previous observations that the posterior probabilities are higher than bootstrap frequencies for well-supported clades (Erixon et al. 2003; Douady et al. 2003; Huelsenbeck and Rannala 2004), since trees in a lower variance sample are not as spread out, and thus have fewer different splits, than trees in a higher variance sample. In general, we find that there is more uncertainty in the variance estimate for the other metrics than the Fréchet variance.

The measurements of the sum of the squared BHV distance between all pairs of sample trees and the Fréchet variance are almost identical. This is expected as these two measures are identical in Euclidean space, which is why the sum of squared BHV distance between all pairs was first suggested as a measure of variance. For large input sets, it is faster to compute the Fréchet variance than the BHV distance between all pairs. See the Discussion, below, for more details.
Figure 4: For each sample, the RF and BHV distances were calculated from its mean tree to its majority-rule tree, ML or MAP tree, and the reference tree. The distribution of these distances is shown using box plots. The mean tree approaches the ML or MAP, and consensus trees as the sequence length increases, with all three trees usually sharing the same topology by sequence lengths of 3000 base pairs.
Figure 5: For each sample, the RF and BHV distances were calculated from the reference tree to the sample’s mean tree, majority-rule tree, and ML or MAP tree. The distribution of these distances is shown using box plots. All three reconstructed trees approach the reference tree as the sequence length increases, with the mean tree being slightly closer on average than the ML tree under the BHV distance measure. The BHV distance between the reference and mean trees for the posterior distribution is shown in the lower-right graph in Figure 4.
Figure 6: The variance of the samples of the bootstrap and posterior distributions are measured using the number of different topologies, the number of different splits, the sum of the squared BHV distance between all pairs, and the Fréchet variance. For all measures, the posterior samples have lower variance on average than the bootstrap samples. The sum of the squared BHV distance between all pairs and the Fréchet variance are very similar. Note that the y axis for the number of different splits, sum of squares, and Fréchet variance are in log scale.
Tree length comparison

We also looked at the average tree lengths to determine the effect of mean stickiness. Some of the mean trees for shorter sequence lengths were not fully resolved due to the stickiness phenomenon, but all mean trees for the 4000 base pair sequence length were fully resolved or binary. However, even when mean trees are fully resolved, the stickiness property could still cause them to be closer to the origin of the sample than expected. In terms of measurable effects, this would translate into the mean trees being shorter (in terms of total edge length) on average than the trees from which they were computed. This matches what we found. For each 80 bootstrap and posterior samples, the length of the mean tree was strictly less than the average length of the corresponding sample trees. The $p$-value for each of these cases was less than $2.2 \times 10^{-16}$. While the mean trees had a shorter length than might be expected, this does noticeably affect the quality of the summary, as on average, the bootstrap and posterior mean trees were only 99.48% and 99.50%, respectively, of the average lengths of their respective samples.

We also compared the lengths of the ML trees of the bootstrap samples with their corresponding mean trees. In all 80 datasets the length of the ML tree was greater than the length of the corresponding mean tree. With $p$-value 0.005861, the length of the ML tree is greater than the average length of the trees in the corresponding bootstrap sample.

Interestingly, the average length of the trees in each posterior sample was always less than the average length of the trees in the corresponding bootstrap sample generated from the same set of sequences. This suggests the bootstrap samples are more spread out, which could also reflect posterior probabilities being higher for well-support clades than bootstrap probabilities. Unsurprisingly, the mean tree of the posterior distribution sample always had length less than the mean tree of the corresponding bootstrap sample.

Mean hypothesis test

For the first repetition of the 4000 base pair sequences, we performed a two-sample hypothesis test to compare the samples of the bootstrap and posterior distributions using the means. The null hypothesis stated that the two means of bootstrap and posterior distributions were the same. We tested this hypothesis using an approximate permutation test. In all cases, the distance between the means of a random partition of the two samples was strictly less than the distance between the means of the two samples themselves. Thus we reject the null hypothesis with an estimated $p$-value of 0.002, with a 95% confidence interval of $[0, 0.006]$. This means that we can assume the two distributions do not have the same mean, and thus are not the same. This was expected due to previous work showing the bootstrap and posterior probabilities are different (Erixon et al. 2003; Douady et al. 2003; Huelsenbeck and Rannala 2004).
Our experiments demonstrate that both the Fréchet mean and variance behave in the expected way on biological data. We have shown that the mean of samples of bootstrap and posterior distributions is comparable in accuracy to the ML and MAP trees, respectively, as well as the majority-rule consensus tree. Indeed the results seem to suggest that the original reference tree is slightly closer to the mean than to the other summary trees. While this possible gain may not be enough to warrant the cost of computing the mean, these results conclusively demonstrate that the mean is a valid summary method for a sample of trees. We believe the value of the mean comes from its sound mathematical backing, which enables more sophisticated statistical tests, like mean hypothesis testing.

From the results of the variance experiments, it is clear that the Fréchet variance is a faster and more precise measure of variance than existing alternatives. The variability in the number of topologies in the tree sets with the same sequence length is very high, in comparison to the other variance measures. Although the variability decreases when measuring the number of splits in the tree set, it is still higher than that of the sum of squared pairwise distances and the Fréchet variance for comparable sequence lengths.

The sum of squared pairwise distances and the Fréchet variance have very similar profiles, justifying the use of the sum of squared pairwise distances as a measure of variance in the literature. However for large sample sizes, the Fréchet variance is faster to compute. To compute the sum of squared geodesic distances for \( r \) trees, one must compute \( r(r - 1)/2 \) geodesic distances. In contrast, computing the Fréchet variance involves calculating the geodesic distance once per iteration of the algorithm. The number of iterations required depends on the desired precision of the mean. However, we have often obtained good results with 10,000-15,000 iterations, suggesting that when we are computing variances of more than approximately 200 trees, the Fréchet variance calculation will be faster than the sum-of-squared-distance calculation.

Our experiments on the tree lengths show that while the mean trees are shorter than the average length of the sample trees, it is by less than 1% on average. This demonstrates that stickiness leads to shorter mean trees, but that the difference in lengths is not likely to be significant and can be ignored.

One might think that we could use the mean to compute the species tree for a set of gene trees \( \text{[Miller et al. 2015, Example 5.5]} \). Even if we just restrict ourselves to the coalescent model for explaining gene tree diversity, this is still problematic in two regards. Under the coalescent model, the pendant edges of the gene trees must be at least as long as the pendant edges of the species tree, but will usually be longer. Since each pendant edge of the mean tree is computed by averaging the length of that edge in all input trees, the pendant edges of the mean of the gene trees will be at least as long, and usually longer, than the pendant edges of the true species tree. While it is possible that the topology of the mean tree might still match that of the species tree, as conjectured in \( \text{[Miller et al. 2015, Example 5.5]} \), our preliminary experiments suggest that stickiness greatly limits the amount of information in the mean tree, compared to other methods. In preliminary experiments, the mean tree of a set of simulated gene trees became essentially degenerate.
while the gene trees were still far away from the anomalous zone (Degnan and Rosenberg 2006). That is, the most common gene tree topology was still the same as the species tree topology. By essentially degenerate, we mean that the approximated mean tree was within a small distance of some degenerate tree. It is possible that an exact algorithm for computing the mean would give a non-degenerate mean tree topology even in these cases, but with an iterative algorithm, we can not be certain the mean is not degenerate, nor even that it would be informative.

**CONCLUSION**

We have shown that the Fréchet mean and variance behave in an expected way on biological data. We have further shown that the mean can be used in mean hypothesis testing to compare two samples of trees, and that the variance is a stable and reliable measure of the amount of variability in a sample of trees. This validation opens the door to new applications of these quantities.

One possible application of the variance is to determine when MrBayes or other Markov Chain Monte Carlo (MCMC) algorithms converge. Preliminary experiments on the MrBayes runs for the data in this paper show that comparing the variance of sliding windows of the sampled trees can identify the burn-in, but that the variance of the trees in the sliding window remains roughly the same after this period. However, there may be other datasets where the variance will continue to decrease after the burn-in, indicating a lack of convergence. The iterative algorithm for computing the mean is easily adapted into an online algorithm: at each iteration, instead of choosing a tree at random from the input set of trees, use the next tree generated by the MCMC chain. Unfortunately, it is not clear how to take advantage of this in computing the variance, which requires computing the BHV distance from the current mean approximation to all sample trees seen so far. However, algorithms for computing geodesics dynamically (Skwerer and Provan 2015) might help.

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