

## Predicting the Ancestral Character Changes in a Tree is Typically Easier than Predicting the Root State

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**Abstract.**—Predicting the ancestral sequences of a group of homologous sequences related by a phylogenetic tree has been the subject of many studies, and numerous methods have been proposed for this purpose. Theoretical results are available that show that when the substitution rates become too large, reconstructing the ancestral state at the tree root is no longer feasible. Here, we also study the reconstruction of the ancestral changes that occurred along the tree edges. We show that, depending on the tree and branch length distribution, reconstructing these changes (i.e., reconstructing the ancestral state of all internal nodes in the tree) may be easier or harder than reconstructing the ancestral root state. However, results from information theory indicate that for the standard Yule tree, the task of reconstructing internal node states remains feasible, even for very high substitution rates. Moreover, computer simulations demonstrate that for more complex trees and scenarios, this result still holds. For a large variety of counting, parsimony- and likelihood-based methods, the predictive accuracy of a randomly selected internal node in the tree is indeed much higher than the accuracy of the same method when applied to the tree root. Moreover, parsimony- and likelihood-based methods appear to be remarkably robust to sampling bias and model mis-specification. [Ancestral state prediction; character evolution; majority rule; Markov model; maximum likelihood; parsimony; phylogenetic tree.]

A fundamental challenge in evolutionary biology is to understand how the traits we observe today in different species evolved from some common ancestral state. A phylogenetic tree linking the species in question provides the usual way to study this question (Liberles 2007). With a tree, one can attempt to reconstruct the evolution of the traits that we observe at the leaves of the tree by estimating the ancestral state at the root of the tree and at the other interior nodes. Typical questions of interest include: what the likely ancestral state was, whether a particular trait evolved just once in the tree or several times, and how reliable our estimates of ancestral states at internal nodes of the tree are. It is this last question that we are concerned with in this article. Using both mathematical and simulation-based analyses, we provide new results concerning the performance of various methods for predicting the ancestral states in a tree. Our work complements and builds on earlier work in this area (Maddison 1995; Zhang and Nei 1997; Mossel 2003; Li et al. 2008, 2010; Fischer and Thatté 2009; Gascuel and Steel 2010; Zhang et al. 2010) much of which has focused on the mathematical performance of maximum parsimony, with an emphasis on tree root prediction rather than on the global scenario of all changes along the tree. Two recent papers have further investigated the relative merits and limitations of various ancestral state reconstruction methods; Royer-Carenzi et al. (2013) show that the performance ranking of likelihood-based methods heavily depends on the tree topology, while Susko and Roger (2013) deals with reconstructing ancestral state frequencies, rather than the precise state that occurred on every sequence site. The performance of maximum parsimony under symmetric  $r$ -state substitution models on caterpillar, completely balanced and Yule trees was also investigated

in Yang et al. (2011), which, in addition, considered whether using all the leaves, or a just carefully selected subset of them, improves predictive accuracy (cf. Fischer and Thatté 2009).

Some of our results apply to all possible methods in placing upper bounds on the reliability of any estimates. However, we are also interested in comparing the performance of particular methods, such as *Majority Rule* (MR), *Maximum Parsimony* (MP) and *Maximum Likelihood* (ML). These methods require increasing levels of knowledge concerning the tree (for MR the tree is irrelevant, for MP we require the tree topology but not the branch lengths, and for ML we require the tree, the branch lengths and a substitution model). Moreover, there are efficient techniques for predicting the scenario of ancestral changes (e.g., for ML, there is the algorithm by Pupko et al. [2000]). We formally demonstrate that these methods can perform very differently on the same tree, and that while ML is “best” if the information required is available, MR can either be much better or much worse than MP, depending on the tree structure and the branch lengths.

A further focus of this article is the question of whether the root can be predicted with more or less accuracy than the other internal nodes. The question is of interest since although there are more leaves below the root than below an internal node, the root is also the most ancient node in the tree, and thus is the “most distant” from the data we observe today. The impact of this distancing effect is quantified explicitly in the next section (Equations 3–5).

We first show that there are situations where the root can be estimated either much more accurately or much less accurately than the internal nodes. Turning to Yule pure-birth trees, we establish a result that seems

slightly surprising at first: in certain regimes, where it is impossible to predict the state at the root of the tree accurately, it is still possible to estimate the ancestral state at a randomly chosen internal node with some accuracy.

The structure of this article is as follows: we first present basic definitions and an information-theoretic lemma, before mathematically investigating the relative performance of different methods for predicting both the root state and the states at internal nodes. We then analyze the expected performance of methods on Yule trees mathematically. Simulations confirm this analysis and show that the results still apply with more realistic, nonmolecular clock trees and complex models of character evolution. We end with a brief discussion.

## MATHEMATICAL CONCEPTS AND TOOLS

### Definitions and Models

Consider any method  $M$  for predicting ancestral states at the internal nodes of a rooted tree  $T$  based on data that consist of the observed state at each leaf  $l$  in the leaf set  $\mathcal{L}$  of  $T$ . Given any particular assignment of states  $x_{\mathcal{L}}$  to the leaves of  $T$ , we will let  $M(x_{\mathcal{L}}, T, v)$  denote the state that method  $M$  estimates to be the ancestral state for internal node  $v$ . A standard assumption in statistical phylogenetics is that the states have evolved on the tree according to some stochastic process (model). In this case, the assignment of states to the set of leaves (denoted here as  $X_{\mathcal{L}}$ ) and to any node  $v$  of the tree (denoted here as  $X(v)$ ) are random variables.

We are interested in the probability that any given method  $M$  is able to predict the state at  $v$  correctly given the states at the leaves, that is  $\mathbb{P}(M(X_{\mathcal{L}}, T, v) = X(v))$ . We call this probability the *predictive accuracy* of method  $M$  at a node  $v$  of  $T$ , and denote it as  $PA_M(v, T)$ . Thus:

$$PA_M(v, T) := \mathbb{P}(M(X_{\mathcal{L}}, T, v) = X(v)).$$

We are also interested in the average of this probability over all the internal nodes of the tree (including the root), and so we let  $\overline{PA}_M(T)$  denote the average value of  $PA_M(v, T)$ , where this average is taken over all internal nodes of  $T$ . Equivalently,  $\overline{PA}_M(T)$  is the predictive accuracy of  $M$  at an internal node selected at random.

There is a trivial “lower” bound for these predictive accuracy measures over all methods  $M$ , namely the one we obtain by the rather naive “method” in which the leaf data are ignored altogether and the state at  $v$  is estimated to be the most probable *a priori* state under the model (in this case  $M(X_{\mathcal{L}}, T, v)$  is independent of  $X_{\mathcal{L}}$ ).

In this article, we deal with time-reversible continuous Markovian processes. Therefore if  $(\pi_1, \dots, \pi_r)$  denotes the (unique) stationary distribution on the  $r$  states then this trivial lower bound on  $PA_M(v, T)$ , for any node  $v$  of  $T$ , is just:

$$\pi := \max_i \{\pi_i\}. \quad (1)$$

For instance, for the Jukes–Cantor model or the Kimura 2ST model, we have  $\pi = 1/4$ . Both  $PA_M(v, T)$  and  $\overline{PA}_M(T)$

lie between  $\pi$  and 1, with 1 corresponding to perfect prediction, and  $\pi$  corresponding to a prediction that is no better than the naive method that ignores the data. We refer to  $\pi$  as the *trivial bound* for  $PA_M(v, T)$  and  $\overline{PA}_M(T)$ .

Note, however, that  $\pi$  is not a universal lower bound for  $PA_M(v, T)$  and  $\overline{PA}_M(T)$ ; for example, an even worse “method” is to systematically predict the state having the lowest *a priori* probability.

In (Gascuel and Steel 2010) we defined a general time-reversible (GTR) process on  $r$  states to be a *conservative model* if the original state is always more probable than any of the alternative states; formally:

$$\pi_{ii}(t) > \pi_{ij}(t), \text{ for all } t \geq 0 \text{ and all } i \neq j, \quad (2)$$

where  $\pi_{ij}(t)$  denotes the transition probability of ending in state  $j$  after time  $t$  given that  $i$  was the initial state. Equation (2) is referred to as the “forward inequality” in Sober and Steel (2011).

Notice that if  $\pi_j$  denotes the equilibrium distribution for any conservative model, then  $\pi_{ij}(t)$  converges to  $\pi_j$  as  $t$  increases and for all initial states  $i$ . Furthermore, under the conservative assumption, by taking the limit of Equation (2) as  $t \rightarrow \infty$ , we have  $\pi_i \geq \pi_j$  for all  $i, j$ . This implies that any conservative model on  $r$  states has a uniform equilibrium distribution  $(1/r, 1/r, \dots, 1/r)$ , and so (from Equation (1)), we have  $\pi = 1/r$ .

### Ancestral State Prediction Methods

We consider three main classes of methods for predicting ancestral states (majority rule, maximum parsimony, and maximum likelihood), each of which requires a different degree of knowledge concerning the tree. The simplest method is MR, which does not use the tree topology to make predictions, relying just on the states of the leaves. This method estimates the state at a node  $v$  as the state that occurs most frequently among the leaves that lie in the clade that has  $v$  as its root (any tie is broken uniformly at random). A simple method that takes the tree topology (but not its branch lengths) into account is MP which estimates the state at a node to be the one that minimizes the number of substitutions required to explain the evolution of the states observed at the leaves on the tree. Finally, if one knows the tree topology, the branch lengths and a substitution model for describing the evolution of the states in the tree, then ML provides a further approach to estimating ancestral states (Pagel 1999).

For both MP and ML estimation, it is clear how to estimate the state at the root of the tree. For other internal nodes, there are various options, which we discuss later. For example, it is always possible (and straightforward) to use a root prediction method based on the subtree rooted with  $v$ . This approach is expected to be less accurate than if we were to consider all tree leaves when predicting any given node  $v$ , and our simulations indicate that the difference is substantial. But mathematical proofs are easier and give a lower

bound for the predictive accuracy of more sophisticated approaches (to be described later). Note, moreover, that for MR, accounting for all tree leaves is possible but without any practical interest as the (majority) prediction is the same for all internal nodes. We thus consider here that MR is used only with a node’s descendants.

For the mathematical analysis, we mostly deal with MR and MP. In the results that follow, we also describe some other simple methods for predicting ancestral states in a tree. Note, however, that some of the likelihood approaches (namely the method that involves predicting the maximum posterior probability ancestral state) can be shown to confer the largest predictive accuracy amongst all methods (cf. theorem 3.1 of Steel and Székely [1999]). This means that our positive results (convergence of the predictive accuracy to 1) obtained with MR or MP in models that have equal *a priori* probabilities of states, also apply to ML methods.

*Predicting the Ancestral States Simultaneously at all Nodes*

One can also try to predict the exact history of character evolution on a tree—in other words, the ancestral state at every internal node. In general, this last task is difficult to guarantee with any accuracy, particularly when the tree is large. It might seem that this problem is hopeless; however, the states at the internal nodes are highly correlated and so the probability of an accurate complete reconstruction for large trees may not be small.

Although few results are available to guarantee the accuracy of reconstructing a complete scenario of changes, there exists a rigorous and explicit lower bound on the accuracy of reconstruction, under very strong assumptions of very low substitution rates, even for trees with many taxa. More precisely, suppose the state changes are rare enough that any two edges with changes are separated by at least three edges with no changes. Then the MP reconstruction of the character state changes in the tree is not only unique but it is guaranteed to coincide exactly with evolution of the character within the tree. This is a combinatorial result, but it translates to a stochastic bound—if the probability of transitions are low enough then we are almost sure to be able to reconstruct the transitions within the tree accurately. For details, the reader is referred to proposition 9.5.1 of Steel and Penny (2005), which provides an example: if  $n = 10,000$ , and the probability of a substitution under (say) a Jukes–Cantor model on each edge is  $2 \times 10^{-4}$ , we obtain an accuracy for complete reconstruction of 0.99. This result is a “worst-case” analysis, and in practice, accurate reconstruction may be possible at a higher substitution rate.

*Information Loss*

Information theory provides a useful way to obtain a bound on predictive accuracy that applies across all methods. To describe this, first recall that the

*mutual information* of two random variables  $X$  and  $Y$  is defined by:

$$I(X; Y) = \sum_{x,y} \mathbb{P}(X=x \ \& \ Y=y) \ln \left( \frac{\mathbb{P}(X=x \ \& \ Y=y)}{\mathbb{P}(X=x)\mathbb{P}(Y=y)} \right).$$

$I(X; Y)$  is a nonnegative symmetric measure which vanishes precisely when  $X$  and  $Y$  are independent, and which has a number of attractive properties (Cover and Thomas 1991). Moreover, if the mutual information between the states at the leaves and the state at an internal node is small, then no method can accurately predict the ancestral state at that node from the observed states at the leaves. This is sometimes formalized in the form of Fano’s Lemma (Cover and Thomas 1991); however, we describe a bound that is more explicit for our purpose, the proof of which is provided in the Appendix.

**Lemma 1.** *For any ancestral state estimation method  $M$  (deterministic or randomized), and any internal node  $v$  in a tree  $T$ , we have:*

$$PA_M(v, T) \leq \pi + \sqrt{\frac{1}{2} I(X(v); X_{\mathcal{L}})}.$$

As an application of this lemma, suppose we have a two-state symmetric process, and we are interested in predicting the state at the root  $\rho$  from the leaf states by some method. For a single leaf  $l \in \mathcal{L}$ , if we let  $x = \exp(-2qt)$ , where  $q$  is the substitution rate, and  $t$  is the temporal distance from the root to the leaf, then  $I(X(\rho); X_l)$  is equal to  $\frac{1}{2}(1+x)\log(1+x) + \frac{1}{2}(1-x)\log(1-x)$  and this is never more than  $x^2$  (Evans et al. 2000). Thus,

$$I(X(\rho); X_l) \leq \exp(-4qt), \tag{3}$$

Moreover, for the two-state symmetric model, Evans et al. (2000) established the inequality:

$$I(X(\rho); X_{\mathcal{L}}) \leq \sum_{l \in \mathcal{L}} I(X(\rho); X_l). \tag{4}$$

Thus, for this model, the information that the leaves provide about the ancestral root state is never more than the cumulative information of each leaf taken independently (as would apply if the tree was a star tree). In particular, for a given tree  $T$  with  $n$  leaves and the two-state symmetric model (3) and (4) give:

$$I(X(\rho); X_{\mathcal{L}}) \leq n \exp(-4qt), \tag{5}$$

for an ultrametric tree of height  $t$ , with  $q$  the substitution rate. Lemma 1 now tells us that the predictive accuracy of any method to estimate the root state will decay towards the trivial bound unless the number  $n$  of leaves grows exponentially with the expected number of substitutions between the root and any given tip ( $qt$ ); indeed, the number of taxa required for accurate reconstruction of the root state cannot be much less than some constant times  $e^{4qt}$ .

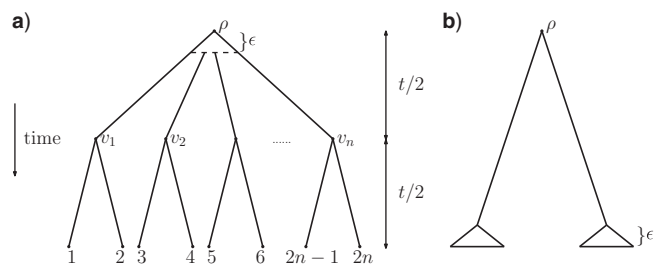


FIGURE 1. a) A tree for which the state at the root of the tree can be estimated with much higher accuracy than any of the more recent nodes. The top portion of the tree can be any tree (including a polytomy or a fully resolved tree). b) A tree for which the root state of the tree is difficult to estimate accurately, but the states at the other nodes can be predicted accurately.

#### MATHEMATICAL RESULTS I: THE RANGE OF POSSIBILITIES

We have described earlier an application of Lemma 1 that shows that, in a certain regime, all methods must have low predictive accuracy in estimating the root state. However it oversimplifies matters to say that this is because the root is simply “too ancient”; there are some trees for which the root can be estimated with higher accuracy than a more recent node (an example is provided in figure 5b of Sober and Steel [2011]).

Here we carry this a step further, and show that there are trees for which the state at the ancient root node can be predicted with arbitrarily high accuracy, and yet none of the other internal nodes can have their state predicted with an accuracy much higher than the trivial bound. We also demonstrate a less surprising converse relationship. This is summarized in the following result.

**Theorem 2** For any conservative GTR process, and for  $\delta > 0$ , the following hold:

- (1) There are trees and branch lengths for which the root state can be predicted with an accuracy of at least  $1 - \delta$  but no method can infer the states at any nonroot node with an accuracy that is better than the trivial bound plus  $\delta$ .
- (2) There are trees and branch lengths for which the states at all the nonroot internal nodes can be predicted with an accuracy of at least  $1 - \delta$  but no method can infer the states at the root of the tree with an accuracy that is better than the trivial bound plus  $\delta$ .

*Proof.* A formal proof of Theorem 2 is provided in the Online Appendix 1 (<http://dx.doi.org/10.5061/dryad.sc724>); here, we describe the intuition behind the proof informally. Part (1) considers the tree shown in Fig. 1(a), in which many lineages radiate within a short period of time ( $\epsilon$ ) near the root (the topology of the tree in this portion is not important) and all the other branches shown are of the same (large) length. Here, the root state can be accurately predicted as  $n$  becomes large because, although each leaf provides little information about the root state when  $t$  is large, collectively, these leaves (for

large enough values of  $n$ ) allow us to infer the root state with an accuracy as close to 1 as we wish. By contrast, any other internal node has just three adjacent nodes, each of which will be far from that node for large values of  $t$ , and in this case, there is no advantage obtained by increasing  $n$ . Notice that the estimation of the root state is consistent with Lemma 1 (and consequential bounds such as (5)), since we are first selecting a large value of  $t$  and with this fixed, we let  $n$  tend to infinity.

The justification of Part (2) is much simpler when we consider the tree in Fig. 1b. If the height ( $\epsilon$ ) of the two subtrees is sufficiently small and the length of the two edges that are incident with the root node are sufficiently long, then every internal node can be predicted with high accuracy, while the root node cannot. In this case, referring again to Lemma 1, for estimating the root state we fix  $n$  and  $\epsilon$  but let  $t$  tend to infinity. ■

The accuracy of methods for estimating an internal node (including the root node) can vary considerably between methods—for some tree shapes, one method can have high predictive accuracy while another may have a low one; for a different tree shape, the relative predictive accuracy of these two methods can be reversed.

**Theorem 3** For any  $0 < \delta < 1$  and the two-state symmetric substitution process:

- (1) there are trees and branch lengths for which the predictive accuracy of MP for root state estimation is at least  $1 - \delta$ , while that of MR is less than the trivial bound plus  $\delta$ ;
- (2) there are trees and branch lengths for which the predictive accuracy of MR for root state estimation is at least  $1 - \delta$ , while that of MP is less than the trivial bound plus  $\delta$ ;
- (3) assuming the branch lengths of the trees in Part (1) and Part (2) are known, the predictive accuracy of ML is at least  $1 - \delta$  in both cases.

*Proof.* A formal proof of Theorem 3 is provided in the Online Appendix 2; but again we describe the intuition behind the proof informally.

For Part (1), consider a tree of the shape shown in Figure 2a, with ultrametric branch lengths, where  $L$  is large and  $\epsilon$  is small. Then regardless of the tree topology of  $T_0, T_1$  and  $T_2$ , when  $n$  is large MR is essentially determined by the distribution of states in the leaves of  $T_0$ , since this subtree will contain nearly all the leaves. However, when  $L$  is large, the root of this subtree ( $v$ ) is distant from the root node ( $\rho$ ), so the majority estimate from  $T_0$  will be a poor indicator of the state at this ancestral node  $\rho$ . By contrast, MP performs well if the edges in the two right-hand subtrees  $T_1$  and  $T_2$  are both sufficiently short and they attach close to the root of the tree—for any given large value of  $L$  (which, in turn, fixes the height of the tree), this can always be achieved by making  $n$  a sufficiently large power of 2,

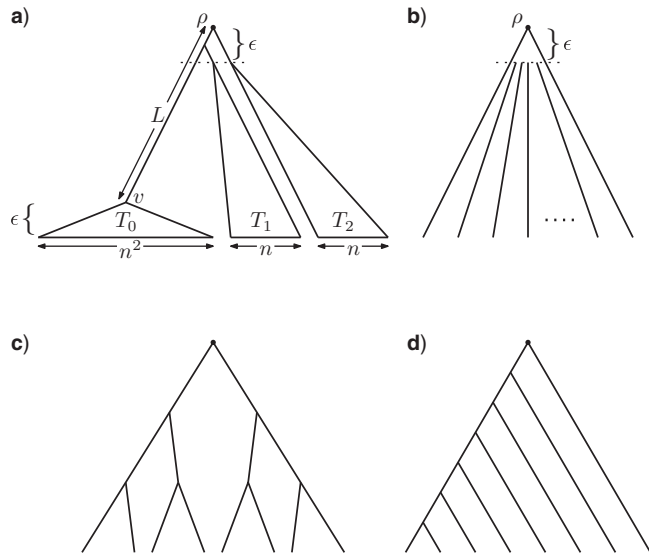


FIGURE 2. The accuracy of root state estimation methods as  $n$  grows with the depth of the tree fixed. Portions of the tree not shown in detail are described in the text. a) A tree for which the accuracy of MP converges to 1, but that of MR declines toward the trivial bound; b) a “rapid radiation” scenario where the accuracy of MR converges to 1, but the accuracy of MP converges to a lower value as  $n$  grows. c) An example of a completely balanced tree on eight leaves, and d) a caterpillar tree on eight leaves.

and selecting a completely balanced binary tree for  $T_1$  and  $T_2$  (a completely balanced tree on eight leaves is shown in Fig. 2c). In that case, proposition 2.1 of Gascuel and Steel (2010) assures us that the first pass of the Fitch–Hartigan algorithm will be an accurate predictor of the root of  $T_1$  and  $T_2$ , and these accurate estimates effectively determine the predicted state at the root  $\rho$  regardless of the possibly unreliable estimate of the root state in the more leaf-rich left-hand tree  $T_0$ .

For Part (2), consider the tree in Figure 2b which has  $n$  leaves and all its pendant edges are of the same long length, but all its interior edges are very short (this might arise, for example, in an early “rapid radiation” scenario). If this initial radiation is sufficiently short, then each node in the top part of the tree will be in the same state as the root of the tree, and so the states at the leaves represent independent and identically perturbed samples of this state. MR has a predictive accuracy converging to 1 as  $n$  grows (the height of the tree is assumed to be fixed) since each leaf provides a (nearly) independent estimate of the root state. However, for MP the topology of the top part of the tree plays a crucial role—for instance, if one leaf is incident with the root, then this leaf (which is a poor indicator of the root state by itself) can have a large influence on predictions of the root state under MP. More generally, we show that if the topology of the top part of the tree is a “caterpillar tree” (an example of such a tree is shown in Fig. 2d) then the predictive accuracy of MP will converge to a value that is no more than the trivial bound plus  $\delta$  as  $n$  tends to infinity, provided that (i) the height of the tree is sufficiently large (relative

to  $\delta$ , not  $n$ ) and that the height of the top part of the tree ( $\epsilon$ ) is sufficiently small. For full details of these arguments, the reader is referred to the Online Appendix 2 (<http://dx.doi.org/10.5061/dryad.sc724>).

For Part (3), the two-state symmetric model has a uniform distribution of states at the root so ML estimation maximizes the predictive accuracy and so is more accurate than both MP and MR, as noted earlier (from theorem 3.1 of Steel and Székely [1999]). Incidentally, if the branch lengths are not known, this is no longer the case even for the two-state model, since if the branch lengths are regarded as parameters in the ML estimation of the root state, and one is inferring these branch lengths from the single character under study, then this method becomes identical to MP (Tuffley and Steel 1997). ■

Theorem 3(2) shows that the predictive accuracy of MP in estimating the root state can be close to the trivial bound if the top part of the tree has the shape of a caterpillar tree. However, such trees are highly unbalanced, and so it is pertinent to ask whether the predictive accuracy would improve if the tree was more balanced. We thus consider the extreme case of a completely balanced tree (i.e., there are  $2^h$  leaves all at depth  $h$  from the root, as in Fig. 2c with  $h=3$ ), and then make some remarks concerning the case of random trees. For the case of a completely balanced tree we have a quite different predictive accuracy result to the caterpillar in the following result (the proof of which is in Online Appendix 3, (<http://dx.doi.org/10.5061/dryad.sc724>)).

**Proposition 4.** Consider the tree shown in Figure 2b. If the top part of the tree has a completely balanced topology then as  $\epsilon$  converges to zero, and  $n$  grows (the height of the tree is fixed), the predictive accuracy of MP in estimating the root state in a symmetric two-state model converges to 1.

Replacing the completely balanced tree with a Yule–Harding topology appears to lead to similar limiting behavior as for the completely balanced tree, though we do not have a rigorous proof of this claim. In any case, the convergence in the Yule–Harding setting is quite slow. For example, with  $n=1,000$ , and with each of the long pendant edges in Figure 2b having a substitution probability of 0.45, the predictive accuracy of MP for estimating the root state for a Yule–Harding topology can be calculated exactly (by the recursion described in the Online Appendix 3, (<http://dx.doi.org/10.5061/dryad.sc724>)) and it turns out to be around 0.69; by contrast, for MR, the predictive accuracy is more than 0.99.

Alternatively, if we were to replace the completely balanced tree with a tree topology selected from the uniform distribution on rooted trees (the so-called “PDA” distribution) then the resulting predictive accuracy of MP has a limit that is strictly less than 1 (since a PDA tree has a positive probability ( $\approx 0.5$ ) of having a leaf adjacent to the root).

MATHEMATICAL RESULTS II: EXPECTED ACCURACY UNDER  
YULE TREES

The examples described so far that exhibit the limits of predictability and unpredictability involve trees that are in some sense “extreme” cases. Thus it is pertinent to ask what we should expect for “typical” phylogenetic trees. This requires specifying some model for generating a tree and branch lengths, and in evolutionary biology the simplest such model is the Yule pure-birth model. Despite the simplicity of this model, it nevertheless provides a reasonable approximation to the shape of empirical evolutionary trees (McPeck 2008). In this section, we study the predictive accuracy of ancestral state reconstruction in a Yule tree that is grown either for a fixed time  $t$  (in which case the number of leaves is a random variable) or is sampled when it has  $n$  leaves (in which case the tree height is a random variable). We are interested in limiting results (i.e., what happens as  $t$  or  $n$  becomes large?). The predictive accuracy for the state at the root node, or at a randomly selected internal node is dependent on the ratio of two parameters, the speciation rate (in the Yule model) and the substitution rate.

Our main result seems at first somewhat surprising—in some regions of parameter space the root state cannot be inferred accurately, yet the state of a randomly selected internal node can be. Before stating this result more formally, we first need to define precisely what we mean for predictive accuracy when the tree  $T$  is randomly generated. Let  $\mathcal{T}$  denote a random rooted phylogenetic tree generated by the Yule model. Consider any ancestral state reconstruction method  $M$ . Then conditional on  $\mathcal{T}=T$  the predictive accuracy for estimating the state at the root of the tree is  $PA_M(T, \rho)$ . Thus the predictive accuracy of  $M$  for root state inference of a Yule tree is:

$$PA_{M;Y}(\rho) := \mathbb{E}[PA_M(\mathcal{T}, \rho)]$$

where  $\mathbb{E}$  refers to expectation with respect to the randomly generated Yule tree  $\mathcal{T}$ .

Similarly, the predictive accuracy of  $M$  for inferring the state at an internal node selected uniformly at random in a Yule tree is denoted  $\overline{PA}_M(T)$ , and so:

$$\overline{PA}_{M;Y} := \mathbb{E}[\overline{PA}_M(\mathcal{T})].$$

We begin by summarizing known results for the special case of the symmetric two-state model.

**Proposition 5.** Consider a Yule tree grown for time  $t$  (from a single lineage), and a two-state symmetric stationary substitution process on the tree. Then, as  $t$  grows:

- (1) the predictive accuracy of MP for estimating the root state of the tree converges to the trivial bound ( $\frac{1}{2}$ ) if and only if the speciation rate is less than six times the substitution rate;
- (2) the predictive accuracy of any method for estimating the root state converges to the trivial bound if the

speciation rate is less than **four** times the substitution rate.

The proof of part (1) is from Gascuel and Steel (2010). For Part (2), Inequality (5) applies once we condition on the number of leaves of the Yule tree. Consequently, an upper bound on the mutual information between the root state and the leaf states is the expected value of  $N_t \exp(-4qt)$  where  $N_t$  is the number of leaves in a Yule tree grown for time  $t$ . Moreover, the expected value of  $N_t$  is  $\exp(\lambda t)$ , where  $\lambda$  is the speciation rate. Thus the predictive accuracy decays exponentially fast to zero if  $\lambda < 4q$  (this result was described further in Li [2011]).

We now turn to the main result in this section, which formalizes the notion that it is easier to predict the state at a randomly selected node in a Yule tree than the root state.

**Theorem 6**

- (1) With a Yule tree  $\mathcal{T}$ , and any GTR process, the accuracy of any method in predicting the state at the root (i.e.,  $PA_{M;Y}(\rho)$ ) converges to the trivial bound as  $n$  grows (or as  $t$  grows), when the substitution rate passes a particular threshold dependent on the speciation rate.
- (2) With a Yule tree  $\mathcal{T}$ , and any conservative GTR substitution process there exists a (very simple) method  $M$  for which the accuracy of predicting the state at a randomly selected node (i.e.,  $\overline{PA}_{M;Y}$ ) does not converge to the trivial bound as  $n$  (or  $t$ ) grows, for any fixed substitution rate.

The formal proof of this theorem is provided in the Appendix, but here we offer some informal comments as to the underlying intuition behind the claims. Part (1) is similar to the statement of part (1) of the Proposition 5 but it differs from it in an important way: the previous theorem was restricted to the two-state symmetric model, while here we are dealing with more general processes (the price we pay for the extra generality is that the bounds obtained are weaker).

Turning to Part (2), a key observation is that in any rooted binary tree at least half of the internal nodes are adjacent to at least one leaf, and the lengths of the pendant edges they are incident with have expected length of  $1/2\lambda$  on average (Mooers et al. 2012). Thus, for at least half the internal nodes that are adjacent to a leaf, selecting the state of that leaf as an estimate leads to nontrivial predictive accuracy, regardless of the speciation and substitution rates, and  $n$ . Note that the assumption that the model is conservative is required only for the proof of Part (2); also the assumption in Part (2) of a Yule (pure-birth) process could be weakened to allow extinction also, since the expected average of the branch lengths across the extant pendant edges

is still bounded, even for trees produced by a critical birth-death process (Stadler and Steel 2012). ■

The simplicity of this method makes the proof easy; more sophisticated methods, such as ML or maximum posterior probability estimation will be more accurate. In other words, the (positive) result in Part (2) stands for a large variety of methods, while the (negative) result in Part (1) applies for all possible methods.

#### SIMULATION RESULTS

Theorem 6 states that for any method, the predictive accuracy of reconstructing the tree root vanishes as  $n$  (or  $t$ ) grows, when the speciation/substitution rate ratio passes below a particular threshold. With the two-state symmetric model, this threshold is equal to 6 for parsimony and is at least 4 for any other reconstruction method (e.g., ML). Moreover, Theorem 6 shows that the accuracy of a very simple method in predicting a randomly selected internal node does not vanish as  $n$  (or  $t$ ) grows, when this rate ratio is fixed. Due to the simplicity of the reconstruction method used in this proof, this result still holds for more sophisticated approaches, such as those based on parsimony or likelihood. However, Theorem 6 does not provide any quantification. We do not know how quickly the ability to reconstruct the tree root vanishes or the extent to which the internal nodes can be reconstructed. This section uses computer simulations to answer these questions. We first simulate Yule trees with variable numbers of tips and speciation/substitution rate ratios, and assess the accuracy of a number of reconstruction methods based on majority, parsimony and likelihood. The results show that reconstructing internal nodes is indeed much easier than reconstructing the tree root for Yule trees. In a second series of simulations, we show that these results still holds when using more realistic, nonmolecular clock trees and a standard substitution model for DNA sequences. All procedures used in these simulations are quite standard (Yule tree, MP, ML, etc.). To simplify the analyses and comparisons performed in this study, all these procedures have been implemented in a unique program written in Common Lisp and available in the Online Supplementary Material (<http://dx.doi.org/10.5061/dryad.sc724>).

#### *Yule Trees and the Two-State Symmetric Model*

We generated Yule trees with  $n=10$ ,  $n=100$ , and  $n=1,000$  tips. Next, binary (0/1) sequences of length 50 were randomly generated and evolved along the tree using the symmetric two-state model. The speciation/substitution rate ratio was equal to 1, 2, 3, 4, 5, 6, 8, 12, and 20, thus having a focus on the 4–6 region where the accuracy of the various methods is expected to drop and be clearly different from one method to another.

We compared a number of reconstruction methods:

- **Majority:** For any given node  $v$ , we select the majority state among  $v$ 's descendants; in the case

of a tie, we randomly select 0 or 1, with equal probability. This method uses partial information when predicting a nonroot node  $v$ , as only the descendants of  $v$  are accounted for. Moreover, the predictions are made independently for each of the tree nodes.

- **Parsimony:** We have the choice among several options:

- **Parsimony-Down:** just as with Majority, we only look into the subtree rooted with  $v$ , using the standard Fitch–Hartigan algorithm (Fitch 1971; Hartigan 1973). Due to partial information we do not expect high accuracy using Parsimony-Down.

- **Parsimony-Acctran and Parsimony-Deltran:** we use now all tips to predict the ancestral state of any given internal node, and thus expect better results than with Parsimony-Down (and Majority). Acctran and Deltran are two heuristic procedures (Swofford and Maddison 1987; Maddison and Maddison 2003). Both select one most parsimonious global change scenario (among many, for most data-sets). Acctran means “accelerated transformations” and favors substitutions close to the tree root, thus tending to avoid convergent substitutions, but accepting reverse substitutions. Deltran means “delayed transformations” and favors substitutions close to the tips, thus preferring convergent substitutions rather than reverse substitutions. Acctran is typically used with morphological characters (convergent evolution is then unlikely), while Deltran is often used with geographic annotations (convergent evolution then means multiple introductions into some country or region and is quite possible; see e.g., Wallace et al. [2007]).

These two procedures aim at solving ambiguous nodes. With two states, when the tree root is ambiguous, we make a random decision with equal probabilities (0.5), and then all other node ambiguities are solved automatically thanks to the properties of these procedures. With more than two states (e.g., DNA), some ambiguities may remain and the ambiguous nodes are randomly assigned on the fly during tree traversal, with equal probabilities among remaining states. By the end we obtain a global, optimal scenario without any ambiguity.

- **Parsimony-Local:** we first compute the most parsimonious state assignments for each node, and then select one of these remaining states (independently and with equal probabilities) for every ambiguous

node. Computations are performed using the DownPass algorithm that is described in the MacClade user guide (Maddison and Maddison 2003). DownPass is faster but equivalent to re-rooting the tree on every node  $v$  and running the Fitch–Hartigan procedure, with a slightly modified last step because  $v$  has now three root descendants. DownPass provides for every node the list of ancestral states corresponding to at least one optimal scenario, from which we randomly select one state in case of ambiguity. This method achieves local decisions, as opposed to Acctran and Deltran which search for a global, optimal scenario. Thus, the selected scenario may not be one of the most parsimonious scenarios, and it is easy to see with examples that it may be not parsimonious at all. This actually occurs for most of the data sets generated in this study. We tested this (nonstandard) procedure to assess the importance of having a global consistence among predictions, and for the sake of comparisons with ML approaches, where we have a similar choice between using the most likely joint scenario (the global view) or the marginal likelihood of each of the nodes (the local view).

- **Maximum likelihood:** We have similar options as we have with parsimony:
  - Likelihood-Down: just as with Majority and Parsimony-Down we only look into the subtree rooted with  $v$  using the standard pruning algorithm (Felsenstein 1981), and then select the most likely state.
  - Likelihood-Best: we use the dynamic programming algorithm proposed by Pupko et al. (2000) to infer the most likely joint (global) change scenario.
  - Likelihood-Marginal: to locally select the most likely state for every node  $v$ , we use the marginal probabilities obtained using the pruning algorithm after re-rooting the tree on  $v$  (with an easy adaptation for the last three-descendant step).

We measured the accuracy of all these methods in reconstructing the ancestral state at: (1) the tree root and (2) any randomly selected internal node (including the tree root). These accuracy measures are simply the proportions of correct root and node predictions in the simulated data. We also measured (3) the method accuracy in reconstructing the changes that occurred along the tree branches. Let  $e$  be a randomly selected branch, and let  $i$  and  $j$  be the states observed during simulations at both ends of  $e$  for a given site; the change between  $i$  and  $j$  is correctly reconstructed when the studied method correctly reconstructs both  $i$  and  $j$  (note

that  $i$  may be equal to  $j$ ). With pendant branches, the leaf state is known, and thus only interior branches are accounted for in this measure. This “branch accuracy” measure is used to assess and compare the performance of local/global methods. Let us assume that node prediction has a success probability of  $p$  and that it is independent from one node to another (as we basically expect with Parsimony-Local and Likelihood-Marginal), then the success of reconstructing the changes along the internal branches should be equal to  $p^2$ . If we now assume that the prediction successes are fully correlated at both ends of  $e$ , that is, both predictions are simultaneously correct or wrong, then the expected success of predicting the change along  $e$  is equal to  $p$ ; for example, with  $p=0.7$ , we expect values of 0.49 and 0.7, respectively with independent (local) and perfectly concerted predictions, that is, very different accuracies.

The three accuracy measures (root, node, and branch) are averaged over the 500 trees, 50 sites, and  $(n-1)$  internal nodes or  $(n-2)$  internal branches, for each of the speciation/substitution rate ratios and  $n$  values. The results are displayed in Figure 3 and in the Online Appendix 4 (<http://dx.doi.org/10.5061/dryad.sc724>). The main findings are as follows:

1. Regarding root prediction, the results are congruent with Gascuel and Steel (2010). Likelihood is best, as expected. Majority is better than Parsimony and is surprisingly accurate, despite its simplicity and the fact that it does not use the tree topology. However, these results should not be overemphasized. In many cases (e.g., with morphological or geographical characters), we may have some sampling bias in the number of occurrences of some of the character states, in which case Majority is expected to perform poorly. For example, when half of the tips with a given character state (say 0) are not sampled, while all other tips (those having 1) are sampled, the accuracy of all methods drops but Parsimony becomes better than Majority; with 100 tips and a speciation/substitution rate ratio of 6, the accuracy of reconstructing the tree root is 0.70, 0.74, and 0.79 for Majority, Parsimony and Likelihood, respectively, against 0.80, 0.77, and 0.83 with no sampling bias. With this (strong) sampling bias, Majority is thus more affected than Parsimony and Likelihood, which both appear remarkably robust. Moreover, the ability of MP and ML to account for all tips information to predict internal nodes is one more important advantage of these methods over MR.
2. As expected from Theorem 6(1), the performance of all methods in predicting the tree root drops down when the number of tips increases. For example, with a rate ratio of 4, the accuracy is  $\sim 0.8$ ,  $\sim 0.7$ , and  $\sim 0.65$  with  $n=10$ ,  $n=100$ , and  $n=1,000$ , respectively. Moreover, with  $n=1,000$  and a rate



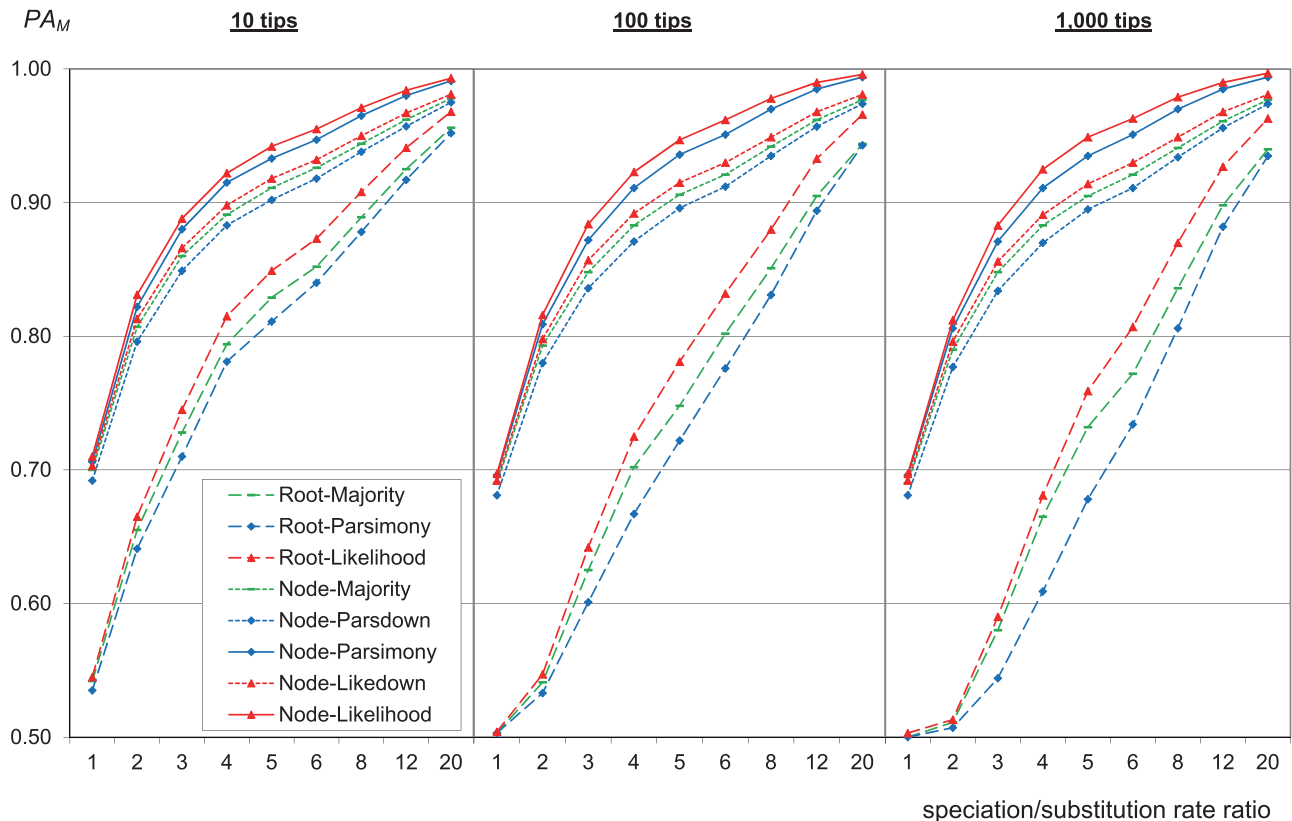


FIGURE 3. A comparison of the accuracy of various methods for reconstructing the root state (denoted Root in the legend) and any randomly selected interior node (denoted Node). Majority, Parsdown and Likedown only use the descendants of the node to be predicted, while Likelihood and Parsimony use all tips in their predictions (this distinction does not hold when predicting the tree root). All parsimony methods (except Parsimony-Down, referred to as Parsdown for short) have very similar results, so here we use Parsimony-Acctran in Root- and Node-Parsimony. Similarly, we use Likelihood-Best (Pupko et al. 2000) in Root- and Node-Likelihood. The X-axis is the speciation/substitution rate ratio;  $PA_M$  (Y-axis) is the predictive accuracy (proportion of correct predictions) of method  $M$ ; 500 trees with sequences of length 50 were simulated for each of the conditions (number of tips and rate ratio).

ratio of 1 and 2, the accuracy of all methods is nearly the same as that of random predictions, while with a rate ratio of 5, where Parsimony is expected to be poor (see above), we see a clear gap between this method and the two others. Actually, based on these simulations, it seems that the transition value for Majority should be less than 6 (as it is for Parsimony) and may possibly be 4 (the best possible value).

3. However, this decrease in the accuracy of predicting the tree root is rather slow; for example, with a rate ratio of 3, where all methods should become analogous to random guessing (accuracy = 0.5) with large  $n$ , we still see a clear signal with  $n = 1,000$ . Moreover, with large rate ratios, the decrease is even slower; for example, with a rate ratio of 20, we see very little difference between  $n = 10$  and 1,000.
4. If we now compare the accuracy of reconstructing interior nodes, the performance of methods that use all the tips is nearly the same, disregarding

whether the predictions are done globally or locally. Specifically, Parsimony-Acctran, Parsimony-Deltran, and Parsimony-Local have nearly identical accuracy, and the same holds for Likelihood-Best and Likelihood-Marginal (see Online Appendix 4, (<http://dx.doi.org/10.5061/dryad.sc724>)). This somewhat surprising finding does not come from the simulation protocol. For example, with Parsimony, there is a large number of nodes (up to 30% with high substitution rates) with an ambiguous ancestral annotation, meaning that Parsimony-Local produces highly suboptimal scenarios (but relatively accurate node predictions!). Moreover, when examining the accuracy in reconstructing the changes along the tree branches, we see (Online Appendix 4, (<http://dx.doi.org/10.5061/dryad.sc724>))) that local and global methods perform nearly identically. Actually, we see a slight advantage (of at most 2%) for Parsimony-Acctran and Parsimony-Deltran over Parsimony-Local, while Likelihood-Best and Likelihood-Marginal are almost undistinguishable (the former is possibly

a bit better than the latter with 10 tips and the opposite may hold with 1000 tips). Moreover, the accuracy value of both local and global approaches is just slightly above  $p^2$ , where  $p$  is the node accuracy, meaning that all of these methods perform nearly the same as if they were achieving independent (local) predictions from one node to another. This is obviously not true for global methods like the approach of Pupko et al. (2000), but their accuracy has not been improved so far (their interpretability likely is). Thus, in the following and in Figure 3, we present and discuss the results of Parsimony-Acctran (the most standard parsimony option) and Likelihood-Best (the most rational option with maximum likelihood in this context). For the sake of conciseness, these methods are simply referred to as Parsimony and Likelihood.

5. The two best methods for predicting interior nodes are Likelihood and Parsimony, in this order, and the difference between both is surprisingly small ( $\sim 1\%$  or less in all conditions). This contrasts with root prediction, where the gap is much higher (up to  $\sim 8\%$ ). Methods using descendant tips only are clearly behind, with Likelihood-Down being the best of these, Parsimony-Down the worst and Majority in between, as expected from the results on root prediction.
6. The accuracy of all methods in predicting interior nodes is remarkably similar regardless of the number of tips. It is difficult to see any difference between  $n = 100$  and 1000, and the results with  $n = 10$  are neither worse nor better than with  $n = 100$  and  $n = 1000$ . This finding is most likely to be explained by the fact that in a Yule tree, the subtrees are also Yule trees, meaning that in large Yule trees, most of the nodes are contained in small Yule trees with only a few tips. We thus observe very fast convergence of the node prediction accuracy for all methods and conditions, which contrasts with the slow degradation of performance when reconstructing the tree root, which we discussed above (especially for high rate ratios).
7. The main result from these simulations is that there is a large gap in accuracy when predicting the tree root and interior nodes, especially with low rate ratios where root prediction accuracy vanishes for all methods. For example, continuing the above example with a rate ratio of 4, the accuracy in reconstructing interior nodes is  $\sim 0.9$  for all methods and values of  $n$ . In other words, reconstructing the interior nodes is (relatively) easy, while reconstructing the tree root is difficult most of the time and is just impossible for large values of  $n$  and small rate ratios. We will show in the next section that this statement still holds when using more realistic, nonmolecular trees and a standard DNA substitution model.

#### *Nonmolecular-Clock Trees and the HKY+ $\Gamma$ Substitution Model*

We reuse here the simulation protocol of Gascuel and Steel (2010), where we compared the accuracy of various methods in predicting the root state. Similar simulations were used to benchmark the topological accuracy of a large variety of tree-building programs (Guindon and Gascuel 2003; Desper and Gascuel 2004), and their features and parameterizations may be seen as biologically realistic. Here we summarize the main components of this protocol; additional explanations and justifications can be found in the previously mentioned references.

We first generated a Yule tree with  $n = 25, n = 50, n = 100, n = 200,$  and  $n = 400$  leaves. This molecular-clock tree was then perturbed by multiplying every branch length (independently) by  $(1 + X)$ , where  $X$  was an exponential variable with parameter 0.5. The factor  $(1 + X)$  was used (as opposed to, say,  $X$ ) to avoid an excessive number of very small branches. The observed departure from the molecular clock, as measured by the ratio between the longest and shortest root-to-leaf path lengths, was equal to  $\sim 3.5$  on average, a value that is typical in published phylogenies. Finally, the whole tree was rescaled so that the average root-to-leaf distance was uniformly distributed between 0.1 (relatively low divergence) and 1.0 (high divergence). In the previous set of simulations, the height of the tree was increasing with  $n$ , but here we assume that the average height is kept constant, while we increase the taxon sampling density. We thus expect that the larger the  $n$ , the more accurate the various methods will be in reconstructing the ancestral root and interior node states. We generated 500 trees using this procedure for each tree size  $n$ .

DNA-like sequences of 100 sites were evolved along these trees using the HKY model (Hasegawa et al. 1985) with a transition/transversion rate ratio ( $\kappa$ ) of 4.0 (the default value in most software) and the equilibrium frequencies of A, C, G, and T being equal to 0.15, 0.35, 0.35, and 0.15. This HKY model was combined with a discrete gamma distribution of parameter 1.0 with six rate categories. We thus obtained 500 data sets of 100 sites for each tree size  $n$ .

Tested methods were essentially the same as in previous simulation study. However, the Pupko et al. (2000) algorithm (Likelihood-Best) is not able to cope with the gamma model of site rates and was not used here. Moreover, the Maximum-Likelihood approach uses a number of parameters (branch lengths, nucleotide frequencies, transition/transversion rate ratio, gamma distribution) which are not used by other approaches (Majority and Parsimony) and are generally not (or approximately) known in practical cases. Thus, we used the Maximum Likelihood approach under three settings:

1. Likelihood-Down: just as in previous simulations, this method uses only the descendants of the node to be predicted, combined with HKY+ $\Gamma_6$  and a complete knowledge of the model parameters.

2. Likelihood-Marginal: again, we use the same approach as in previous simulations, based on the marginal distribution of state posterior probabilities for every tree node, combined with HKY+ $\Gamma$ 6 and a complete knowledge of the model parameters. This approach somehow provides the “best possible” result that can be obtained with our data sets.
3. Likelihood-Marginal-JC: we used again the same marginal posterior probabilities, but assumed the simple Jukes–Cantor (JC) model which ignores the differences in nucleotide frequencies and transition/transversion rates which were employed to generate the data. Moreover, as this JC model was used without a gamma distribution of site rates, the branch lengths were only approximate. This approach is thus expected to provide a more realistic view of maximum likelihood performance, compared to the performance of parsimony that is based on similar simplifying assumptions, but does not use (even only approximate) branch lengths.

The same accuracy criteria were used as in previous experiments. All results are displayed in Figure 4

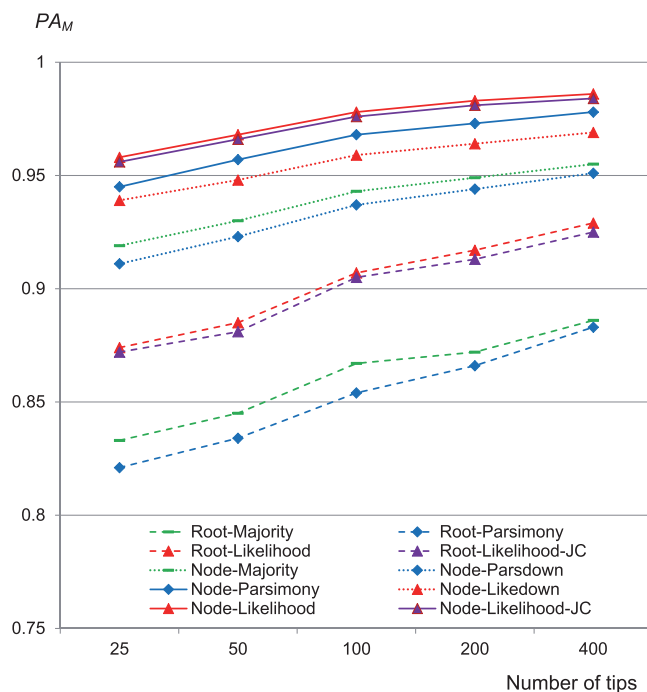


FIGURE 4. Accuracy of the methods with nonmolecular-clock trees and a HKY+ $\Gamma$  substitution model. Root- and Node-Likelihood results are obtained using Likelihood-Marginal with HKY+ $\Gamma$ 6. Root- and Node-Likelihood-JC are based on the same method but use JC model instead. Node- and Root-Parsimony use the Acctran algorithm. Majority, Parsdown and Likedown only use the descendants of the node to be predicted; the latter is launched with HKY+ $\Gamma$ 6. The X-axis is the number of tips ( $n$ );  $PA_M$  (Y-axis) is the predictive accuracy (proportion of correct predictions) of method  $M$ ; 500 trees with sequences of length 100 were simulated for each value of  $n$ .

and in the Online Appendix 5 (<http://dx.doi.org/10.5061/dryad.sc724>). The main findings are as follows (some are similar to what we already observed in previous simulations and are merely summarized):

1. Majority performs better than Parsimony-Down, both in reconstructing the root state and the interior nodes.
2. The three all-tips parsimony methods (Acctran, Deltran, and Local) perform nearly identically, with a slight (probably nonsignificant) advantage for Acctran.
3. To predict the state of interior nodes, methods using all tips are clearly better than methods using node descendants only; for example, Parsimony-Acctran is better than Likelihood-Down, despite the fact that the latter uses a complete knowledge of the substitution model (branch lengths, nucleotide frequencies, etc.).
4. Again, we do not see any significant difference between methods performing local and global predictions (see the results of Acctran, Deltran, and Parsimony-Local in the Online Appendix 5, (<http://dx.doi.org/10.5061/dryad.sc724>)). Moreover, for all methods, the accuracy in reconstructing the changes along the tree branches is roughly equal to the square of the node accuracy, just as if predictions were made independently at both branch extremities (Online Appendix 5, (<http://dx.doi.org/10.5061/dryad.sc724>)).
5. Again, we see a large gap between predicting the root and predicting the interior node states. Notably, the gain with Parsimony is 10% or more. Despite its simplicity, Parsimony appears to be quite accurate at predicting the interior node states and the changes along the tree branches.
6. As expected, all method accuracies increase with  $n$  (representing taxon sampling density) but the amelioration is relatively slow ( $\sim 5\%$  for all methods when predicting the root state) compared to augmentation of the taxon number (from 25 to 400).
7. Lastly, the most surprising finding is the remarkable performance of maximum likelihood when used with the over-simplistic JC model. The results are nearly the same as with HKY+ $\Gamma$ 6 for both the tree root and interior nodes. Compared to Parsimony, this Maximum Likelihood approach is clearly better at predicting the root state, thanks to the use of (approximate) branch lengths, while Parsimony uses the tree topology only. This robustness is quite encouraging regarding the use of Maximum Likelihood with real data, and can be related to the robustness we have already observed with biased sampling (see above), and the apparent robustness of Maximum Likelihood methods regarding topological errors reported by [Hanson-Smith et al. \(2010\)](#).

## CONCLUDING COMMENTS

Ancestral state reconstruction based on a phylogenetic tree allows biologists to estimate where and when important innovations (the gain, loss or change of some character state) may have occurred in the evolutionary history of a set of taxa. Such approaches are also useful in phylogeography (e.g., Slatkin and Maddison 1989; Wallace et al. 2007) to study the evolution of epidemics and their movement and exchanges moving from one country to another.

In this article, we have applied mathematical methods and simulations to quantify how reliable such predictions are likely to be under a variety of models of sequence evolution. We showed that predicting the state at a given node that is deep in the tree can be provably arbitrarily close to the trivial bound, even though for a randomly selected node in a Yule tree the accuracy always lies distinctly above the trivial bound.

Yet, this story also has some twists—there are trees for which the root can be predicted more accurately than any other node. And Majority Rule, which ignores the tree structure in estimating the root state, has an accuracy that is not much worse than other methods. This may go some way toward explaining the apparent robustness of ancestral state prediction to the choice of tree, by Hanson-Smith et al. (2010) who stated that “incorporating phylogenetic uncertainty very rarely changes the inferred ancestral state and does not improve the accuracy of the reconstructed ancestral sequence.” Moreover the prediction methods are relatively robust against other factors such as model mis-specification, sampling bias, and approximate parameter and branch length values.

Among the three main methods we consider, ML, MP, and MR, the first method (ML) is the most accurate, but it also requires knowing the most about the tree and model. Regarding MP and MR we showed that the relative performance of each depends very much on the tree and its branch lengths, and that neither is universally better than the other. However on Yule trees MR tends to be slightly more accurate than MP for estimating the root state, but less accurate at estimating randomly selected nodes in the tree than certain versions of parsimony which account for all tree tips.

For future works, a fundamental mathematical question is to determine, for the two-state symmetric model, whether the critical speciation-to-substitution ratio at which the accuracy of MR (and ML) decays asymptotically to the trivial bound occurs at the lowest possible value 4 or some value higher than 4 (as is the case for MP, which has its transition at the ratio 6). Results from Bartoszek and Sagitov (2013), which deals with a symmetric model (on a continuous rather than discrete state space) on Yule trees may provide one way to show that the critical speciation-to-substitution ratio for MR and ML coincide. However, for more general discrete state models (beyond the two-state symmetric case) one cannot expect the two ratios (for MR and ML) to coincide, due to results in Mossel (2001) and Sly (2011).

It would also be interesting to further explore the prediction of the edges on which particular state changes occurred, and the accuracy of complete ancestral reconstruction in the tree. The only theoretical result known (described earlier) requires very strong assumptions, and is far from optimal. Moreover, our simulation results indicate that the dependence between predictions made at two edge extremities is not well accounted for by current approaches, which could be a route to design more accurate methods aimed at predicting the changes that occurred along the tree.

## SUPPLEMENTARY MATERIAL

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.sc724>.

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## APPENDIX: MATHEMATICAL PROOFS

*Proof of Lemma 1*

*Proof.* Firstly observe that for any two discrete random variables  $X$  and  $Y$ , the mutual information  $I(X; Y)$  equals the Kullback–Leibler divergence  $D(P_{X,Y} || P_X P_Y)$  between  $P_{X,Y}$  (the joint probability distribution of  $X$  and  $Y$ ) and  $P_X P_Y$  (the corresponding product of marginal probability distribution, and which therefore treats  $X$  and  $Y$  as independent). Now, Pinsker’s inequality (cf. Cover and Thomas [1991]) states that for any two probability distributions  $P$  and  $Q$  we have:

$$\sqrt{\frac{1}{2}D(P||Q)} \geq \sup_A \{|P(A) - Q(A)|\},$$

where the supremum is over all events  $A$ . Combing this with the previous observation gives:

$$\sqrt{\frac{1}{2}I(X; Y)} \geq \sup_A \{|P_{X,Y}(A) - P_X P_Y(A)|\}, \quad (6)$$

Now, suppose that  $M$  is any (deterministic or randomized) method for predicting  $X$  from  $Y$ , and let  $E$  be the event that  $M(Y) = X$  (note, we allow  $M$  to be

“random” as some methods result in ties that then are generally broken randomly).

Then, from (6), we have:

$$\sqrt{\frac{1}{2}I(X; Y)} \geq P_{X,Y}(E) - P_X P_Y(E),$$

and so, equivalently:

$$P_{X,Y}(M(Y)=X) \leq \sqrt{\frac{1}{2}I(X; Y)} + P_X P_Y(E). \quad (7)$$

Now,

$$\begin{aligned} P_X P_Y(E) &= \sum_{x,y} P_X P_Y(X=x, Y=y) P(M(y)=x) \\ &= \sum_{x,y} P(X=x) P(Y=y) P(M(y)=x). \end{aligned}$$

Thus if we let  $a_x = P(X=x)$ ,  $b_y = P(Y=y)$  and  $c_{xy} = P(M(y)=x)$ , then

$$P_X P_Y(E) = \sum_{x,y} a_x b_y c_{xy}. \quad (8)$$

Setting  $A = \max_x \{a_x\}$ , Equation (8) now gives:

$$P_X P_Y(E) \leq A \sum_{x,y} b_y c_{xy} = A \sum_y b_y \sum_x c_{xy} = A \sum_y b_y = A,$$

where the second equality relies on the fact that for each  $y$  we have  $\sum_x c_{xy} = 1$ .

Thus, since  $A = \max_x P(X=x) = \pi$  we obtain  $P_X P_Y(E) \leq \pi$ , and applying this to (7) in the case where  $X = X(v)$ ,  $Y = X_{\mathcal{L}}$  we obtain the claimed inequality. ■

*Proof of Theorem 6*

*Proof of Part (1):* We exploit a result from Mossel and Steel (2005) (Equation (14.8)) which says that for any method  $M$  if we take  $v = \rho$  (the root of a fixed tree  $T$ ) then

$$PA_M(\rho, T) \leq \pi + \sum_{v \in \mathcal{L}} \exp(-qt(v)), \quad (9)$$

where  $t(v)$  is the sum of the branch lengths from the root to leaf  $v$  and  $q = \sum_j \min_{i \neq j} q_{ij}$ , where  $q_{ij}$  is the transition rate from state  $i$  to state  $j$ .

We first consider what happens if we allow  $t$  to grow (in which case the number of leaves at time  $t$ ,  $N_t$ , is a random variable) as this is simpler than allowing  $n$  to grow. For a Yule tree of depth  $t$  we have  $t(v) = t$  for each leaf  $v$ . Thus, conditional on  $N_t = n$ , Equation (9) gives

$$\mathbb{P}(M(X_{\mathcal{L}}, \rho) = X(\rho) | N_t = n) - \pi \leq n \exp(-qt),$$

and since  $PA_{M;Y}(\rho)$  is the expected value of this quantity (with respect to the Yule model) we have:

$$PA_{M;Y}(\rho, T) - \pi \leq \mathbb{E}[N_t] \exp(-qt).$$

Now  $\mathbb{E}[N_t] = \exp(\lambda t)$  and so  $PA_M(\rho, T) \leq \exp((\lambda - q)t)$ , which converges to zero, exponentially fast, if  $\lambda < q$ .

We now consider what happens if we allow  $n$  to grow, in which case the time  $\tau_n$  until the Yule tree has  $n$  leaves is a random variable. Firstly, observe that, for such a tree,  $t(v)$  takes the same value for all leaves  $v$  (since Yule trees are ultrametric). Conditional on  $\tau_n = t$  we have, from (Equation (9)):

$$\sum_{v \in \mathcal{L}} \exp(-qt(v)) = n \exp(-qt).$$

Thus,

$$PA_{M;Y}(\rho) - \pi \leq n \mathbb{E}[\exp(-q\tau_n)]. \quad (10)$$

Now it is a classic result (Kendall 1949) that the number  $N_t$  of leaves in a Yule tree generated for time  $t$  and speciation rate  $\lambda$  (starting with a single lineage) has a geometric distribution with parameter  $1 - \exp(-\lambda t)$ . In other words, we have:

$$\mathbb{P}(N_t \geq n) = (1 - \exp(-\lambda t))^{n-1}.$$

Moreover, by definition we have:

$$\mathbb{P}(\tau_n \leq t) = \mathbb{P}(N_t \geq n), \quad n \geq 1$$

and so, taking  $t = t_n = (1 - \delta) \frac{\ln(n)}{\lambda}$  where  $\delta > 0$  is chosen so that  $q > \lambda / (1 - \delta)$  we have:

$$\mathbb{P}(\tau_n \leq t_n) \sim \exp(-n^\delta) \quad (11)$$

and so the right-hand side of Equation (10) (namely  $n \mathbb{E}[\exp(-q\tau_n)]$ ) is equal to:

$$\mathbb{E}[n e^{-q\tau_n} | \tau_n \leq t_n] \cdot \mathbb{P}(\tau_n \leq t_n) + \mathbb{E}[n e^{-q\tau_n} | \tau_n > t_n] \cdot \mathbb{P}(\tau_n > t_n),$$

which, using Equation (11) we can further bound as follows:

$$n \cdot \exp(-n^\delta) + n \exp(-qt_n) \cdot (1 - \exp(-n^\delta)).$$

The first term in this last expression converges to zero as  $n$  tends to infinity, while the second term is bounded above by

$$n \exp(-qt_n) = n \exp(-q(1 - \delta) \ln(n) / \lambda) = n^{1 - q(1 - \delta) / \lambda},$$

which also converges to zero as  $n \rightarrow \infty$  since  $q > \lambda$  and  $\delta > 0$  has been chosen so that  $q > \lambda / (1 - \delta)$ .

Thus provided that  $q > \lambda$  we may select  $\delta$  sufficiently small (but positive) to ensure that the predictive accuracy of any method  $M$  on a Yule tree converges to the trivial bound as  $n$  tends to infinity.

*Proof of (2):* For any time-reversible continuous-time Markov process on  $r$  discrete states, the transition probability of being in the starting state after any given time  $t$  can be written as:

$$p_{ii}(t) = \pi_i + \sum_{j=2}^r a_j \exp(-b_j t), \quad (12)$$

where  $-b_2, \dots, -b_r$  are all strictly negative, and comprise the non-zero eigenvectors of the rate matrix, and where  $a_j > 0$  for all  $j$  (Aldous and Fill 2010). We will assume the  $b_i$  are ordered in increasing order of absolute value. Note that the constants  $b_i$  are proportional to the rate of the

process (in our setting the substitution rate), but the  $a_j$  are independent of this rate (and also of  $t$ ). Equation (12), and the condition  $p_{ii}(0) = 1$  implies that:

$$p_{ii}(t) \geq \pi_i + (1 - \pi_i)\exp(-b_r t).$$

For a conservative process we have  $\pi_i = \pi (= \frac{1}{r})$  and so

$$p_{ii}(t) \geq \pi + (1 - \pi)\exp(-b_r t) \quad (13)$$

Now let  $T$  be a binary tree, rooted on an ancestral node of degree 1 (we assume this rather than a root of degree 2 since we wish to model a Yule process grown from a single initial lineage). Thus if  $T$  has  $n$  extant leaves,  $T$  has  $n$  pendant edges and  $n$  ancestral nodes (including the root node). So, if  $v_0, v_1, v_2$  denote the number of ancestral nodes of  $T$  that are adjacent to 0, 1, and 2 extant leaves respectively, then  $v_0 + v_1 + v_2 = n$  and  $v_1 + 2v_2 = n$ , from which it follows that

$$v_0 = v_2. \quad (14)$$

This allows for the following randomised scheme that selects an ancestral nodes of the tree  $T$  uniformly at random. First select uniformly at random one of the pendant edges of this tree – call this randomly sampled edge  $e$ . If the ancestral node  $v$  of  $T$  that is incident with  $e$  is not adjacent with another extant leaf then select  $v$ . Otherwise (i.e., if  $v$  is adjacent to two extant leaves) toss a fair coin, and if the outcome is “heads” select  $v$ , while if the outcome is “tails” select uniformly at random any ancestral node of the tree that is not adjacent to any extant leaf.

This randomization scheme selects each of the  $n$  ancestral nodes of  $T$  with the same probability, since the probability that the selected node has 1, 2, or 0 adjacent extant leaves occurs with corresponding probabilities  $v_1/n$ ,  $2v_2/n \times \frac{1}{2} = v_2/n$  and  $2v_0/n \times \frac{1}{2} = v_0/n$  (by (14)), respectively (thereby in direct proportion to the numbers of such nodes) and each node within each of these three classes has equal probability of being selected.

Consider now the following method  $M$  for predicting the state at an ancestral node  $v$  of  $T$ . Given  $v$  select any leaf that is in the clade below  $v$ , and estimate the state at  $v$  by the state at this leaf. Then, conditional on the evolutionary time from  $v$  to the present being  $t$ , inequality (13) gives:

$$PA_M(v, T) \geq \pi + (1 - \pi)\exp(-b_r t).$$

Now if  $v$  is selected uniformly at random according to the above randomization scheme, then the probability that this process selects an ancestral node that is adjacent to a leaf is equal to the proportion  $((v_1 + v_2)/n)$  of ancestral nodes that are incident with at least one extant leaf, and this proportion (for any binary tree) is at least  $\frac{1}{2}$  by (14).

Thus, with probability at least  $\frac{1}{2}$  the node  $v$  selected by this process will have:

$$PA_M(v, T) \geq \pi + (1 - \pi)\exp(-b_r \theta), \quad (15)$$

where  $\theta$  is the length of the pendant edge  $e$  that was selected uniformly at random. On the other hand, if the node selected is not incident with an extant leaf then  $PA_M(v, T) \geq \pi$ , by the assumption that the model is conservative. So combining this with (15) we have, conditional on the length  $\theta$  of the randomly selected pendant edge  $e$ ,

$$\overline{PA}_M(T) \geq \pi + \frac{1}{2}(1 - \pi)\exp(-b_r \theta).$$

Let us now sample a Yule tree with  $n$  leaves that has grown from a single lineage (this is equivalent, under a uniform improper prior, to growing the tree until just before it has  $n + 1$  leaves). Then the length of a randomly selected pendant edge in a Yule tree (call it  $L$ ) has expected value  $\frac{1}{2\lambda}$  (Mooers et al. 2012). Thus, by Jensen’s inequality (applied to the convex function  $y = \exp(-x)$ ) the expected value of  $\exp(-b_r L)$  is at least  $\exp(-\frac{b_r}{2\lambda})$  and so

$$\begin{aligned} \overline{PA}_{M;Y} = \mathbb{E}[\overline{PA}_M(T)] &\geq \pi + \frac{1}{2}(1 - \pi)\exp\left(-\frac{b_r}{2\lambda}\right) \geq \pi \\ &+ \frac{1}{2}(1 - \pi)\left(1 - \frac{b_r}{2\lambda}\right). \end{aligned}$$

Alternatively, we can use the fact that  $L$  has an exponential distribution (Stadler and Steel 2012) to obtain a similar bound, noting that the expected value of  $\exp(-b_r L)$  is exactly  $1/(1 + \frac{b_r}{2\lambda})$ . This shows that  $\overline{PA}_{M;Y}$  is bounded away from the trivial bound, as claimed.

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