Paleontology, Genomics, and Combined-Data Phylogenetics: Can Molecular Data Improve Phylogeny Estimation for Fossil Taxa?

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Abstract.—The genomics revolution offers great promise for resolving the phylogeny of living taxa, but does it offer any benefits for reconstructing relationships among extinct (fossil) taxa? Superficially, the answer would seem to be “no,” given that molecular data cannot be obtained for most fossil taxa. However, because fossil taxa often interdigitate among living taxa on the Tree of Life, molecular data may indirectly enhance phylogenetic accuracy for fossil taxa in the context of a combined analysis of morphological and molecular data for living and fossil taxa. Here, I use simulations to assess accuracy for fossil taxa in a mixed analysis of living and fossil taxa, before and after addition of molecular data to the living taxa. The results show conditions where the accuracy for fossil taxa is greatly increased by adding molecular data, sometimes by as much as 100%. In other cases, the increase is negligible, such as when fossil taxa greatly outnumber living taxa in the analysis. However, there were few cases where accuracy was significantly decreased by the addition of the molecular data, suggesting that this practice may range from highly beneficial to mostly harmless. Overall, the results suggest that improvements in molecular phylogenetics can potentially benefit phylogeny reconstruction for fossil taxa.

[Accuracy; fossils; genomics; morphology; phylogeny.]

The genomics revolution is currently transforming the field of phylogenetics and efforts to reconstruct the Tree of Life. Using tools from genomics, it is now possible to address phylogenetic questions with a staggering number of informative characters from multiple, unlinked loci (e.g., Rokas et al. 2003; Takezaki et al. 2004; Philippe et al. 2005; Hallstrom et al. 2007). Although some phylogenetic problems may remain persistent due to very short times between splitting events (e.g., Rokas and Carroll 2006; Wiens et al. 2008), there seems to be great potential to resolve the Tree of Life with phylogenomic approaches.

But what about fossil taxa? Is there any way that the new wealth of molecular data can improve phylogeny estimation for extinct taxa? On the surface, the answer would seem to be “no.” Apart from very recent fossil taxa (from which DNA data can sometimes be obtained) or other exceptional cases (e.g., Organ et al. 2008), the phylogenetic placement of fossil taxa is based entirely on morphological data. This is unfortunate because morphological data sets typically suffer from a limited number of characters relative to molecular data sets, and the number of characters is a key factor in estimating the correct phylogeny (review in Hillis and Wiens 2000). Furthermore, there may be problems of nonindependence among characters (e.g., Emerson and Hastings 1998; O’Keefe and Wagner 2001), which can strongly mislead analyses based on morphology alone (e.g., due to developmental processes that affect entire suites of characters, such as paedomorphosis [Wiens, Bonett, et al. 2005] or peramorphosis [Smith et al. 2007]). Thus, relationships among most extinct taxa are based entirely on morphological data, and these hypotheses may sometimes be precarious due to limited numbers of characters and potential nonindependence among these characters. Yet, there is no obvious way that the new wealth of unlinked molecular characters can directly benefit phylogenetic analyses for most fossil taxa.

However, molecular data might potentially improve phylogenetic accuracy for fossil taxa in the context of combined analyses of morphological and molecular characters for living and fossil taxa. The common practice in paleontologically based phylogenetic studies is to analyze morphological data alone, even when the analysis includes extant taxa (for two recent, high-profile examples, see Wible et al. 2007; Friedman 2008; for a summary, see Cobbett et al. 2007). A combined analysis of morphological and molecular data for living taxa should generally be more accurate than an analysis of morphology alone, given the increased number and independence of characters. If fossil taxa are included, then the combined analysis might lead to higher accuracy for the fossil taxa as well. This may be especially likely if the morphological data are insufficient to fully resolve relationships of the living and fossil taxa. Also, when molecular data improve the placement of a living taxon, this may “drag” closely related fossil taxa into more accurate positions as well.

Many researchers might hesitate to add molecular data to improve phylogeny estimation for fossil taxa because the resulting data matrix would likely be dominated by missing data. For example, if the molecular data set contained 2000 characters (a relatively small number) and the morphological data set contained 100 (a relatively large number), then any fossil taxon in the combined data matrix would contain at least 95% missing data cells. Large numbers of missing data cells have traditionally been considered problematic for phylogenetic analysis (e.g., Rowe 1988; Donoghue et al. 1989; Hulsenbeck 1991; Novacek 1992; Wiens and Reeder 1995; Wilkinson 1995; Grande and Bemis 1998; Ebach and Ahyong 2001, but see Anderson 2001; Kearney 2002). However, recent simulation and empirical studies
suggest that highly incomplete taxa can be accurately placed in phylogenetic analyses, if the overall number of characters is large and the characters that are present are reasonably accurate, despite vast numbers and high proportions of missing data cells (e.g., Wiens 2003; Driskell et al. 2004; Philippe et al. 2004; Wiens, Fetzner, et al. 2005; Manos et al. 2007; Wiens and Moen 2008).

Even if one accepts that extensive missing data are not necessarily problematic, there may still only be a limited set of circumstances under which molecular data will improve phylogeny estimation for fossil taxa. For example, if most taxa in the combined data matrix are fossils, then it is hard to imagine that adding molecular data for a limited number of living taxa will dramatically increase phylogenetic accuracy for most species. Furthermore, the DNA data must estimate the correct tree or at least must not be entirely misleading. Similarly, the morphological data must not be entirely uninformative or strongly misleading either; even in the combined analysis, the accurate placement of the fossils is still ultimately dependent on the morphological data.

Several previous studies have combined fossil and molecular data in phylogenetic analyses (e.g., Eernisse and Kluge 1993; Shaffer et al. 1997; Jordan and Hill 1999; O’Leary 1999; Sun et al. 2002; Gatesy et al. 2003; Asher et al. 2005; Xiang et al. 2005; Hermens et al. 2006; Rothwell and Nixon 2006; Magallón 2007; Manos et al. 2007; O’Leary and Gatesy 2008). Among these studies, several found that the addition of molecular data changed the position of at least some fossil taxa (e.g., the extinct crocodilian genus *Borealosuchus* is monophyletic when analyzed using morphological data alone but becomes paraphyletic when molecular data are added; Gatesy et al. 2003). Unfortunately, even if the placement of fossil taxa differs after inclusion of the molecular data, there may be little basis for determining whether phylogeny estimation has moved closer to the true phylogeny for the organisms in question (i.e., without knowing what the true phylogeny is).

In this study, I use simulations to address whether phylogeny estimation for fossil taxa might potentially be improved by adding molecular data to a combined analysis of living and fossil taxa, and under what conditions this may (or may not) occur. Computer simulations provide a context where the true phylogeny is known. Therefore, they offer a means to compare the accuracy of different approaches with phylogeny reconstruction (i.e., how well each approach estimates the true phylogeny). Admittedly, computer simulations require many simplifying assumptions and may be inappropriate to address some types of questions (e.g., do morphological data yield accurate phylogenies for mammalian fossil taxa?). However, they may be useful for addressing more general questions, such as whether adding one set of characters can improve accuracy for taxa that entirely lack data from those characters.

Previous simulation and empirical studies have suggested that adding sets of characters with data for only some taxa can sometimes improve the overall accuracy of the entire tree (e.g., Wiens 1998a; Wiens, Fetzner, et al. 2005). However, these studies did not address whether relationships among the less complete taxa were actually improved and were not designed to mimic the combination of molecular and fossil data. Many previous studies have also discussed whether adding fossil taxa improves the estimated relationships among living taxa (e.g., Gauthier et al. 1988; Donoghue et al. 1989; Huelsenbeck 1991; Eernisse and Kluge 1993; Wiens 2005; Rothwell and Nixon 2006); here, I ask instead whether adding molecular data to living taxa can improve estimated relationships among fossil taxa.

**Materials and Methods**

The basic design of the simulations was as follows. DNA and morphological characters were simulated on the same 16-taxa phylogeny. Certain taxa were designated to be fossils (morphology only). A matrix of morphological data was generated for all the 16 taxa. A matrix of combined morphological and molecular data for all the 16 taxa was also generated, but this combined matrix contained only missing data cells for the molecular characters for the fossil taxa. These data matrices were then analyzed using parsimony and Bayesian methods. The morphological data were analyzed alone, and accuracy was estimated for relationships among the fossil taxa (i.e., the tree was pruned to include only the fossil taxa, and the similarity of the estimated tree to the known tree for the fossil taxa alone was assessed). The combined matrix was then analyzed, and again accuracy was assessed for the fossil taxa alone. This basic procedure was then repeated hundreds of times and for different simulated conditions, such as different numbers of characters, branch lengths, and tree shapes. The main question of the study is whether accuracy for the fossil taxa is higher before or after the addition of the molecular data to the living taxa.

A 16-taxa phylogeny was simulated using programs written by the author in C. In many of the simulations, the tree was unrooted and was either entirely asymmetric (Fig. 1a) or symmetric (Fig. 1b), to test the robustness of the results to different tree shapes using the most extreme shapes possible. The same set of branch lengths was assumed for both DNA and morphological characters (assuming that lengths in both data sets are primarily influenced by the amount of time between splitting events). The first set of simulations assumed equal lengths for all branches throughout the tree, but with different lengths used in different simulations.

The morphological data sets consisted of either 20 or 100 characters, representing relatively low and high numbers for a morphological data set for 16 species. In 7 plant and vertebrate studies reviewed in Table 2, the number of morphological characters per taxon ranges from 1.750 to 8.944 (below 5.5 in 6 of the 7 studies), with a mean of 3.991, which is similar to the range and midpoint used in the simulations (1.25–6.25, midpoint = 3.75). The simulated morphological characters were all binary, given that most morphological characters in most morphological data sets appear to be binary.
The molecular data sets consisted of 2000 DNA sequence characters. Sequences were evolved assuming a 3:1 transition:transversion ratio and initial base frequencies of $A = 37\%$, $G = 12\%$, $C = 24\%$, and $T = 27\%$ (parameter values based on mammalian sequences as reported by Zwickl and Hillis 2002). Although a more complex and realistic model could have been used, this added complexity would be irrelevant to this study (the accuracy of parsimony or Bayesian analysis with DNA sequence data is not the issue here). The most important property of the DNA sequence data is that it can accurately resolve relationships among the living taxa with a large number of characters. Many molecular data sets have >2000 characters, but previous studies suggest that the DNA data consistently resolve the entire tree correctly under the conditions examined here using parsimony, likelihood, and Bayesian methods (Wiens 2003; Wiens and Moen 2008). Furthermore, under conditions where the combined analysis had the lowest accuracy in this study, doubling the number of characters had little discernible impact (results not shown).

Branch lengths used were 0.01, 0.05, 0.10, and 0.20, where a branch length is defined here as the probability of a character-state change occurring along that branch. Although analysis of the DNA data is potentially accurate for all these lengths under the conditions analyzed here, these different lengths strongly affected the accuracy of the morphological data. For binary data, a length of 0.20 represents high levels of homoplasy (the consistency index is roughly 0.26, meaning that each character changes an average of 4 times across the tree). This branch length is less problematic for the DNA sequence data because, given multiple changes in the same character, DNA characters can often evolve to a different state (no homoplasy), whereas for binary characters, multiple changes must involve homoplasy. Conversely, short branch lengths are also potentially problematic for the morphological data because the combination of the limited rate of change and limited number of characters leads to a paucity of informative character changes (i.e., for 16 taxa and a length of 0.01 for each branch, only about 15% of the characters are parsimony informative).

For a given matrix, 4, 8, or 12 taxa were chosen to be fossils. In each simulation, these fossil taxa were evenly dispersed among the living taxa. Thus, when there were 4 fossil taxa, these species were A, F, K, and P (Fig. 1); for the 8 fossil taxa, these taxa were B, D, F, H, J, L, N, and O; and for 12 fossil taxa they included all taxa but A, E, L, and P. Alternately, these taxa could have either been placed randomly or clustered together. If the fossil taxa were all clustered into one clade and the living taxa in another, one would not expect the molecular data to be able to improve phylogeny estimation for them; this seems so obvious as to not be worth testing quantitatively. Alternately, if taxa were placed randomly, we would expect the results to be generally similar to those from even placement (on average). I focused exclusively on even spacing to represent the situation where the addition of molecular data is at least potentially useful for the fossil taxa.

Phylogenetic analyses were initially conducted using parsimony, as this is the method that is most widely used for reconstructing relationships among fossil taxa. However, some analyses were also conducted using Bayesian analysis, given that recent versions of MrBayes (Huelsenbeck and Ronquist 2001) allow a likelihood
model for morphological data (Lewis 2001) to be imple-
mented and combined with analyses of DNA sequence
data. Parsimony analyses were conducted using PAUP*
version 4.0b10 (Swofford 2002).

The morphological data were first analyzed alone, and
then, accuracy was assessed after pruning the tree to in-
clude only the fossil taxa. The combined molecular and
morphological data were then analyzed and again accu-
tracy was assessed for the pruned tree, including only the
fossil taxa. Parsimony analyses consisted of a heuristic
search with tree–reconstruction branch swapping
and 20 random-taxon-addition sequence replicates.
Accuracy was assessed based on the number of nodes
shared between the estimated and the true phylogenies,
using a single shortest tree from each parsimony search.
When averaged across replicates, a single tree from each
replicate should approximate the average accuracy from
comparing each shortest tree with the true phylogeny.
Analyses using parsimony used 200 replicates for a given
set of simulated conditions.

Bayesian analyses were conducted using MrBayes ver-

tion 3.04 (Huelsenbeck and Ronquist 2001). In general,
default options for Bayesian analysis were used. The
DNA sequence data were analyzed using the Hasegawa–
Kishino–Yano model (Hasegawa et al. 1985; accommo-
dating unequal base frequencies and unequal transition
and transversion rates), and morphological data were
analyzed using the model of Lewis (2001). One hundred
replicates were analyzed for each set of conditions. Each
Bayesian analysis used 40 000 generations, and the first
10 000 generations were discarded as burn-in. Although
this may seem like an unusually small number of gen-

erations relative to most empirical studies (which typi-
cally use several million), similar analyses using a larger
number of generations show that this number is ade-
quate (Wiens and Moen 2008). Furthermore, the results
show near-perfect accuracy for many of the Bayesian
analyses, indicating that there are generally few random
errors (if any) generated by a failure to reach stationar-
ity. Following standard practice, the estimated Bayesian
phylogeny was based on a majority-rule consensus of
the post-burn-in trees. PAUP* was used to generate these
consensus trees and to compare the estimated Bayesian
phylogenies with the true phylogeny.

The initial set of analyses used equal branch lengths,
which is not necessarily realistic. Two additional sets
of analyses were therefore conducted. First, I used ran-
domly generated branch lengths on the fully asymmetric
and symmetric unrooted topologies, with mean branch
lengths of 0.01 (range 0–0.02, with lengths drawn from
a uniform distribution), 0.05 (0–0.10), 0.10 (0–0.20), and
0.20 (0–0.40), that could be easily compared with the
other results. A different length was selected for each
branch in each replicate, but again the same length was
used for both the molecular and the morphological
characters.

Second, I simulated the data on two rooted topologies
(Fig. 1c,d) with ultrametric branch lengths (i.e., the sum
of the branch lengths from the root to the terminals is
the same for all taxa). These topologies were randomly
generated using a Yule (pure birth) model of speciation
with Mesquite, version 1.05 (Maddison and Maddison
2004). This model generated both a topology and rela-
tive branch lengths. The length of each branch in each
topology was then rescaled, so that a given set of sim-
ulations was conducted on each topology using mean
branch lengths of 0.01, 0.05, 0.10, or 0.20. In this case, the
different branch lengths are equivalent to different over-
temoral scales for the phylogeny, from relatively re-
cent (0.01) to more ancient (0.20). In theory, the analyses
could have been conducted on hundreds of randomly
simulated topologies rather than just 2, but such ran-
domization would have made it very difficult to test the
effects of combining data from living and fossil taxa (i.e.,
the major focus of the study), and the effects of differ-
tree shapes and branch lengths are addressed in the
other simulations.

An important property of fossil taxa is that they may retain
more ancestral states than living taxa (e.g.,
Gauthier et al. 1988; Donoghue et al. 1989; Huelsenbeck
1991). Most simulations in this study treated the fos-

til taxa as equivalent to living, morphology-only taxa.
However, a set of analyses were conducted on the rooted
topologies in which the fossil taxa retained all the char-
acter states of their immediate ancestral node, to assess
whether this impacted the results. Of course, in the real
world, fossil taxa would presumably retain only some
fraction of these ancestral states, but this extreme sce-
nario was intended to offer the strongest contrast with
the other simulations.

Another important property of fossil taxa is that they
may be missing many characters due to preservational
artifacts. In the previous analyses, I assumed that the
fossil taxa were complete for all morphological charac-
ters. But in reality, fossil taxa may lack data for certain
types of morphological characters that can be scored only
in living taxa (e.g., soft anatomy). Furthermore, a given
fossil taxon may be known from only a few incompletely
preserved specimens, and so each taxon may be miss-
ing a more or less random subset of the morphologi-

cal characters that could have been preserved. A limited
set of simulations was conducted to assess the effects of
randomly placed missing data in the fossil taxa, using
the baseline simulation conditions (asymmetric and
symmetric topologies with fixed branch lengths, 100
morphological characters, 8 fossil taxa). The morphologi-
cal data for the fossil taxa were arbitrarily made 50%
incomplete. Thus, for each taxon in each replicate, 50%
of the morphological characters were randomly selected
and replaced with missing data cells. Although preser-
vation of characters in real fossil taxa presumably is not
completely random, this simulation should represent a
“worst-case scenario” for the distribution of missing data
among characters, given that random missing data cells

These topologies were randomly

Second, I simulated the data on two rooted topologies

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shown. Similarly, even relatively small differences in
mean accuracy between approaches appeared to be sta-
tistically significant, and such tests are not explicitly
presented.

RESULTS

Overall, the results show that adding molecular data
increases accuracy for the fossil (morphology only) taxa
under a wide variety of conditions for both parsimony
and Bayesian analysis. Results for different levels of
completeness (see Fig. 2 for one set of results; complete
results are given in Supplementary Appendix 1, http://
www.sysbio.oxfordjournals.org/) show that the benefits
for adding molecular data to the fossil taxa are
strongest when 50% or 75% of the taxa in the matrix are
living. If only 25% of the taxa have molecular data, the
overall effects on accuracy for the fossil taxa are gener-
ally negligible. All further simulations were conducted
for the case where 50% of the taxa are living and 50% are
fossils.

The baseline results (equal branch lengths) for parsi-
mony (Fig. 3) show substantial increases in accuracy for
adding the molecular data when the number of char-
acters is low (for intermediate to high rates and both
symmetric and asymmetric trees), when there are
many characters but branch lengths are long, and more
generally for the symmetric tree topology (which shows
lower accuracy for the morphological data than the
asymmetric tree, given the same number of characters
and same branch lengths). Results are very similar for
Bayesian analysis under the same conditions (Fig. 4).
The results are also similar when the branch lengths are
allowed to vary randomly (within a set range) for both
parsimony and Bayesian analysis (Supplementary
Appendix 2).

For the first simulated topology under the Yule model
(Fig. 5), the results are again similar, showing some ben-
efit to adding the molecular characters under compara-
bled conditions (e.g., few characters, long branch lengths).
The results are generally similar for the second simu-
lated topology (Fig. 6), but there is a cost in accuracy
for adding the molecular data in the Bayesian analysis
when branch lengths are very long, 100 morphological
characters are sampled, and fossil taxa are equivalent to
living taxa (in terms of retaining ancestral states). For the
first topology, there is very little difference in the results
when the fossil taxa retain all the character states of
their immediate ancestors (Fig. 5). However, for the sec-
ond simulated topology, the accuracy is higher at higher
rates of change when the fossils retain their ancestral
states, and adding the molecular data improves accu-

![Figure 2](https://academic.oup.com/sysbio/article-abstract/58/1/87/1676618)

Figure 2. Results from simulations showing the accuracy of phylogeny estimation for fossil taxa in a combined analysis of living and
extinct taxa, both with (filled circles) and without (open circles) the addition of molecular data to the living taxa. These results show the effects
of varying the number of extant taxa (with molecular data) relative to the fossil taxa (morphology only), with 4 (25%), 8 (50%), or 12 (75%) of the
16 taxa extant. In these simulations, the branch length is 0.05 (intermediate low) for both the molecular and the morphological data sets. Results
are based on parsimony. There are 2000 DNA sequence characters in the combined data set. Each data point represents the average accuracy
from 200 replicates.
FIGURE 3. Results from simulations showing the accuracy of phylogeny estimation for fossil taxa in a combined analysis of living and extinct taxa, both with (filled circles) and without (open circles) the addition of molecular data to the living taxa. These results show the effects of different branch lengths on parsimony analysis when 50% of the taxa are extant and 50% fossils. Each data point represents the average accuracy from 200 replicates.

The presence of random missing data in the morphological characters for the fossil taxa reduced accuracy relative to analyses when the fossil taxa were more complete, as might be expected (Table 1). However, the presence of random missing data in the fossil taxa did not prevent the combined analysis from increasing accuracy for the fossil taxa (Table 1).

DISCUSSION

In this paper, I ask whether the addition of molecular data can potentially improve phylogeny estimation for fossil taxa. In theory, it seems that if fossil taxa interdigitate among living taxa on a phylogeny, then adding molecular data to the living taxa might improve phylogeny estimation for the fossil taxa, given that morphological data for both living and fossil taxa are included. To test this hypothesis, I simulated molecular and morphological data for both living (DNA + morphology) and fossil (morphology only) taxa. The results support the idea that molecular data can potentially improve phylogenetic accuracy for fossil taxa, with at least some increase under a variety of conditions for both parsimony and Bayesian analysis. In some cases, these increases can be quite dramatic (e.g., a roughly 100% increase; Fig. 3 and Supplementary Appendix 2). Perhaps just as importantly, I found few conditions where this practice led to a substantive decrease in accuracy (with one exception, discussed below). Based on these simulations, there is potentially much to gain but generally little to lose from combining data from molecules and fossils to improve our understanding of the phylogeny of extinct taxa.

These simulations also suggest the specific conditions where increases in accuracy seem most likely. First, the fossil taxa should interdigitate among the living taxa and should not be too numerous. As an extreme example, if one combines the fossil and living taxa and each are in separate clades, the molecular data will have little or no opportunity to improve estimation for the fossil taxa. Similarly, if the living and fossil taxa interdigitate but the fossil taxa are far more numerous, the overall accuracy of the tree (and the accuracy for the fossil taxa) may not be heavily influenced by the living taxa. Indeed, in simulations where only 25% of the taxa were living, the gains in accuracy for the fossil taxa were negligible (Fig. 2). However, there were often substantial improvements when 50% of the taxa were fossils (Figs 2–6 and Supplementary Appendix 2).

Second, the morphological data must be informative, but not too informative. The benefits of the combined analysis depend on there being enough variation in the morphology to help place the fossil taxa. When there were few informative characters (e.g., when there were
FIGURE 4. Results from simulations showing the accuracy of phylogeny estimation for fossil taxa in a combined analysis of living and extinct taxa, both with (filled circles) and without (open circles) the addition of molecular data to the living taxa. These results show the effects of different branch lengths on Bayesian analysis when 50% of the taxa are extant and 50% fossils. Each data point represents the average accuracy from 100 replicates.

only 20 characters and a branch length of 0.01), there was typically little improvement from adding the molecular data. Conversely, when the accuracy for the morphological data is very high, there is little that the molecular data can improve upon. In the real world, it seems likely that most morphological data sets for fossil taxa are neither completely uninformative (i.e., many nodes are resolved rather than being polytomies) nor are they likely to be entirely accurate (e.g., typically only some nodes are strongly supported by bootstrapping).

One set of results (Fig. 6) showed that the addition of molecular data led to significantly lower accuracy for the fossil taxa (accuracy for combined analysis = 57%) than analysis of the morphology alone (accuracy = 72%; based on a $t$-test, this difference is highly significant with $P < 0.0001$). This occurred for only one topology, and then only when the branches were very long (0.20), 100 characters were sampled, and the fossil taxa retained no more of their ancestral states than living taxa, and then only for Bayesian analysis (Fig. 6). Although it is reassuring that this occurs under such a restricted set of conditions, it is disconcerting that it occurs at all and that the ultimate cause is not obvious. The proximate cause of this pattern seems to be that fossil taxon L, with the longest terminal branch of any fossil taxon, is almost always misplaced toward the base of the tree (below clade F + G) in combined Bayesian analyses but not as frequently in analyses of the morphological data alone.

However, it is unclear why this should occur more often when molecular data are added, or why the problem is worse for Bayesian analyses than for parsimony. Interestingly, even though one might expect Bayesian analysis to be less sensitive to long-branch attraction than parsimony, previous results show that this is not necessarily true when analyzing binary data with very long branches, as analyzed here (e.g., Fig. 2c,d of Wiens 2005). Overall, this incongruous result shows that some caution is warranted when adding molecular data to Bayesian analyses of fossil taxa, even though the other results of this study (including Bayesian analyses with long, unequal branch lengths; Fig. 6) suggest that this practice is generally helpful or at least innocuous.

Major Assumptions

The results of this study generally seem promising for combining molecular and fossil data, but they are based on many simplifying assumptions. First, I treated the fossil taxa as evenly dispersed across the phylogeny of living taxa. If the fossil taxa were randomly distributed, then the improvements documented here would presumably be lessened somewhat in cases where the fossil taxa were more clumped than evenly dispersed among the living taxa. However, clumping of the fossil or living taxa should have no adverse effects on accuracy when the data are combined.
FIGURE 5. Results from simulations showing the accuracy of phylogeny estimation for fossil taxa in a combined analysis of living and extinct taxa, both with (filled circles) and without (open circles) the addition of molecular data to the living taxa. These results show the effects of different branch lengths on parsimony and Bayesian analyses when 50% of the taxa are extant and 50% fossils. One of the simulated topologies (Tree 1; Fig. 1c) was used, and the different branch lengths on the x-axis represent the mean of all internal and terminal branch lengths for the tree. A temporal position of 1 indicates that the fossil taxa were equivalent to living taxa, whereas a temporal position of 0 indicates that the fossil taxa retain all the character states of their immediate ancestors.

Second, I assumed a simple model of evolution for both the molecular and the morphological data, and that estimation of the molecular tree was straightforward. The purpose of this study was to test if an accurate molecular tree can help improve accuracy for fossil taxa. But the accuracy of many molecular trees is still in doubt (at least in part), even for trees based on large, multilocus data sets. For example, for relatively short but deep branches, there may be problems of long-branch attraction that extend across many genes (e.g., Rokas et al. 2005; Rokas and Carroll 2006). There may also be extensive discordance among gene trees due to incomplete lineage sorting on short branches throughout the tree, leading to weak support in the combined analysis of the genes (e.g., Rokas and Carroll 2006; Wiens et al. 2008).
FIGURE 6. Results from simulations showing the accuracy of phylogeny estimation for fossil taxa in a combined analysis of living and extinct taxa, both with (filled circles) and without (open circles) the addition of molecular data to the living taxa. These results show the effects of different branch lengths on parsimony and Bayesian analyses when 50% of the taxa are extant and 50% fossils. One of the simulated topologies (Tree 2; Fig. 1d) was used, and the different branch lengths on the x-axis represent the mean of all internal and terminal branch lengths for the tree. A temporal position of 1 indicates that the fossil taxa were equivalent to living taxa, whereas a temporal position of 0 indicates that the fossil taxa retain all the character states of their immediate ancestors.

For the morphological data, there may be many factors that may influence accuracy beyond the conditions simulated here (i.e., limited number of characters, high rates of homoplasy, and random missing data), such as correlated character evolution. An important caveat that should be made about these results is that if there is a strongly misleading signal in the morphological data that affects 1 or more of the fossil taxa, the molecular data may do little to directly improve the situation.

Comparisons with Empirical Studies

How do the simulation results compare with those from empirical studies? Here, I review results from 7 empirical studies that have combined molecular and morphological data to address the placement of fossil taxa (Table 2). These studies were chosen because they include trees from the morphological data alone and from the combined molecular and morphological data (including fossils). Many potential studies had to be
Table 1. Accuracy of parsimony analysis for fossil taxa in an analysis of living and extinct taxa, when analyzed using morphological data alone (morphology only) or with molecular data added to the living taxa (combined data), contrasting analyses in which the fossil taxa are complete for the morphological characters or have 50% of their characters randomly replaced with missing data cells

| Study                        | Taxa                          | Characters (morphology/DNA) | Resolved nodes (morphology/combined) |
|------------------------------|-------------------------------|-----------------------------|-------------------------------------|
| Shaffer et al. (1997)        | Turtles                       | 7/23                        | 115/1300                            |
| Gatesy et al. (2003)         | Crocodilians                  | 54/14                       | 164/2940                            |
| Asher et al. (2005)          | Mammals (rodents, lagomorphs) | 39/29                       | 228/5701                            |
| Asher and Hofreiter (2006)   | Mammals (tenreces)            | 3/20                        | 120/855                            |
| Rothwell and Nixon (2006)    | Plants (higher level)         | 26/30                       | 136/5072                            |
| Manos et al. (2007)          | Plants (juglandaceae)         | 5/27                        | 56/2006                            |
| O’Leary and Gatesy (2008)    | Mammals (Cetartiodactyla)     | 43/28                       | 635/40928                           |

Note: All results are from parsimony analysis (only 1 study included a Bayesian analysis of morphology). “Resolved nodes” refers to the number of dichotomous nodes in a strict consensus of the shortest trees from a given analysis.

In summary, the 7 studies demonstrate that in empirical studies, molecular data can both improve resolution for fossil taxa and substantially change their phylogenetic placement. They also seem to support the idea that adding molecular data will improve phylogeny estimation for fossil taxa when the number of fossil taxa is limited relative to the number of living taxa and when the relationships of the fossil taxa are initially unresolved (increasing either resolution in empirical studies or accuracy in simulation studies). These studies also support the prediction that if there are extensive, strongly supported conflicts between the molecular and the
morphological data (and the fossil taxa outnumber the living taxa), then it may be more difficult for the molecular data to improve estimation for the fossil taxa.

**Implications for Molecular Dating Analyses**

Combined analyses of molecular and morphological data for living and fossil taxa may also be useful for researchers who use molecular data to determine ages of clades (i.e., fossil-calibrated molecular clock analyses). Typically, researchers determine the ages of extant clades by incorporating the estimated age for the oldest fossil taxon that is assumed to belong to each clade (e.g., Won and Renner 2006; Hugall et al. 2007; Roelants et al. 2007), where this assumption is often based on a previous phylogenetic analysis of morphological data that places the fossil taxon in that extant clade. An alternate approach is to undertake combined analyses, such that the molecular data can help inform the position of the relevant fossils (e.g., Manos et al. 2007). The results presented here suggest that such combined analyses may lead to improved phylogenetic placement for the fossil taxa, which may then in turn improve estimation of the divergence dates.

**Other Applications of Molecular Data to Phylogenetic Analysis of Fossils**

I began this paper by asking how phylogenomic data might improve phylogenetic analyses of fossil taxa. Although I focused on the efficacy of combined analyses of molecular and morphological data for living and fossil taxa, there are other ways that molecular data could directly or indirectly improve phylogeny estimation for fossil taxa. First, many of the same benefits noted here for combining molecular and fossil data might potentially be obtained by simply enforcing topological constraints in the analysis of fossil taxa, such that relationships among living taxa that are well established by molecular data are fixed in the analysis, without actually including the molecular data in the same matrix as the morphology (e.g., Doyle 2006; for comparison with related approaches, see Manos et al. 2007). Many of the same advantages and disadvantages may pertain to both this approach and the combined approach addressed here (e.g., both will depend on the fossil taxa interdigitating among living taxa). A major advantage of the constraint approach is that it might require less effort than assembling the molecular data and integrating them into a combined analysis. However, if relationships among the living taxa are not fully established by the molecular data, the constraints may lead to overestimating confidence in the relationships among both living and fossil taxa. Similarly, combining living and fossil taxa in the same matrix might actually improve relationships among living taxa as well, despite the disparity in the relative numbers of characters. This idea is supported by simulations (Wiens 2005) and addressed (if indirectly) in many empirical studies (e.g., Rothwell and Nixon 2006; Manos et al. 2007).

Integrated analyses of molecular and morphological data can also be used to improve the methodology of morphology-based phylogenetics, and these improvements can then be incorporated into paleontological studies. For example, if molecular data strongly establish relationships among a set of living taxa, then one can compare how well different methods for analyzing the morphological data perform at reconstructing these quasi-known relationships (e.g., different methods for coding polymorphic morphological characters; Wiens 1998b). Although such analyses are impossible for fossil taxa, methods that perform well in morphological analyses of extant taxa should also perform well for fossil taxa. Similarly, well-supported molecular phylogenies can reveal cases where morphology-based phylogenetics gives strongly misleading results, and critical analysis of the morphology can then offer insights into the processes that cause this to occur and how they might be ameliorated (e.g., Wiens, Bonett, et al. 2005).

**Conclusions and Prospects**

The results of this study suggest that the new flood of phylogenomic data has the potential to improve accuracy for fossil taxa, in the context of combined analyses of molecular and morphological data for living and fossil taxa. Of course, such combined analyses will not be a panacea for all problems in the phylogenetic analysis of fossil taxa, and even in these simulations, major increases in accuracy occur only under a finite set of conditions. Furthermore, there is no guarantee that this approach will always be effective in the real world, especially when there are strong conflicts between the molecular and the morphological data. However, the simulations do establish that such increases are theoretically possible, and empirical studies suggest that conditions where this seems likely to occur are common (i.e., when morphology alone does not resolve relationships among fossil taxa, but combined analysis does). Furthermore, and perhaps just as importantly, there were no simulated conditions where this approach consistently led to a significant decrease in accuracy for both parsimony and Bayesian analyses.

**Supplementary Material**

Supplementary material can be found at http://www.oxfordjournals.org/our_journals/sysbio/.

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