A Critique of Thompson and Ramírez-Barahona (2023) or: How I Learned to Stop Worrying and Love the Fossil Record

Eric R. Hagen[1,*]

[1]Department of Ecology & Evolutionary Biology, University of Toronto
[*]Address correspondence to: eric.hagen@utoronto.ca

Abstract

Last year, a study published in *Biology Letters* by Thompson and Ramírez-Barahona (2023) argued that, according to analyses of diversification on two massive molecular phylogenies comprising thousands of species, there is no evidence that angiosperms (i.e., flowering plants) were affected by the Cretaceous-Paleogene mass extinction. Here I show that these conclusions are flawed from both methodological and philosophical perspectives. I demonstrate that the methods used in their study possess statistical limitations that strongly reduce the power to detect a true mass extinction event using data similar to those analyzed by Thompson and Ramírez-Barahona (2023). Additionally, I use their study as a springboard to examine the relationship between phylogenetic and fossil evidence in diversification studies.

1 Background

Since the introduction of molecular phylogenies and the comparative methods that rely on them, practitioners have defied expectations of what was thought possible. For example, when Felsenstein [1] famously introduced phylogenetic independent contrasts, reviewers were skeptical that there would ever be sufficient robust phylogenies for the method to prove useful [2]. Another example is the development of methods to estimate extinction rates from phylogenies that are entirely composed of extant taxa; however, this issue is much stickier. Nee et al. [3] demonstrated that extinction rates can be estimated, and mass extinction events can be inferred, from lineage-through-time (LTT) plots that only contain information about lineage "births." Estimation of extinction rates from phylogenies of extant taxa proved controversial: Rabosky [4] went so far as to argue that extinction rates should not even be estimated from molecular phylogenies, while others, such as Beaulieu and O'Meara [5], have argued that reliable extinction estimates can be gleaned from phylogenies that are sufficiently large using sophisticated methods that incorporate things like rate heterogeneity. Most recently, Louca and Pennell [6] demonstrated that speciation and extinction rates cannot be reliably inferred from LTTs derived from time-calibrated trees, inspiring widespread concern about the degree to which inferences from phylogenies are stretched.

However, optimism does remain. Recently, Thompson and Ramírez-Barahona [7] attempted to detect a signature of the Cretaceous-Paleogene (KPg) mass extinction on two very large phylogenies of flowering plants. They failed to do so, arguing that this demonstrated the resilience of angiosperms, likely due to their rich adaptations. This finding was discussed in several popular science articles, including in the New York Times. However, their paper contains two issues. The first is that this finding disagrees with several robust analyses of the angiosperm fossil record [8], the fern fossil record (i.e., "fern spikes" [9]), and the fossil record of phytophagous insects [10], which display evidence of plants having undergone widespread extinction at the KPg boundary. While disagreeing with previous evidence is not necessarily an indication of an incorrect result, especially since the fossil record is imperfect, the fact that Thompson and Ramírez-Barahona [7] have offered an answer to a paleobotanical problem using extant-only data can reasonably raise suspicion.

This reply investigates the aspects underlying the second issue with their study, overextending the capabilities of analyses of molecular phylogenies. Despite numerous papers sounding alarms about limitations of phylogenetic comparative methods (both warranted and unwarranted), they continue to be applied to problems for which they can offer but limited information and ultimately produce suspect results. Here, I argue that the methods employed by Thompson and Ramírez-Barahona [7] cannot confidently support the conclusion that flowering plants were not subjected to the ravages of the end-Cretaceous extinction. Specifically, I discuss the lack of phylogenetic information deep in the tree as well as pitfalls of the TESS [11] and CoMET [12] models, and I conclude with a brief discussion of the relative merits of phylogenetic comparative methods (PCMs) and fossils for answering questions about extinction events in the distant geologic past.

2 Lack of Information

The supposed ability of Thompson and Ramírez-Barahona [7] to reconstruct the severity of the KPg extinction event on flowering plants from extant species alone relies on the fact that signatures of diversification rate heterogeneity are often retained by phylogenies. However, the evolutionary patterns gleaned from analyses of molecular phylogenies as opposed to those of the fossil record sometimes differ widely [13]. This should not be particularly surprising given the fact that a very small proportion of organisms throughout geologic time are successfully buried and fossilized, preserved to the present, and ultimately excavated by humans [14]. Moreover, many evolutionary avenues, even ones that produce large clades over time, may ultimately leave no survivors to the present that can be included in molecular phylogenies.

Let's assume, for the sake of argument, that the tree is roughly accurate in its representation of evolutionary patterns taken by the clade(s) represented, and that it represents all or nearly all the extant species recognized by taxonomists. If this is the case for the phylogenies used by Thompson and Ramírez-Barahona [7], one might assume they should be able to accurately infer signatures of past mass extinctions. However, this is not necessarily the case. As one travels from the tips toward the root of a phylogeny, whether one is performing an ancestral state reconstruction or a diversification study or any other type of phylogenetic comparative analysis, the amount of information available to an investigator deteriorates. O'Meara and Beaulieu [15] demonstrate this on a simulated tree with one million tips, arguing that analyses and inferences of evolutionary events deep on phylogenetic trees rely on the few lineages that survived to the present and were thus able to be sequenced and included in a molecular phylogeny. These lineages may comprise a completely unrepresentative sample with little information about the evolutionary events that shaped their clade and the more inclusive clades in which they are nested.

In the case of Thompson and Ramírez-Barahona [7], in one of the phylogenies they analyzed, produced by Smith and Brown [16], only about 0.7% of all speciation events occur at or before 65 mya. Examined in another way, there are only 548 lineages present at the 65 mya time slice, compared to about 77,000 tips in the Smith and Brown tree. The imbalance of information in many large phylogenies has spurred other scientists to develop and increasingly employ tip rate analyses (e.g., [17]; [18]) that take advantage of the glut of information available near the present, but which unfortunately are unable, at least at this point in time, to provide information about past mass extinction events in the distant past, and thus do not form a viable alternative for analyses like that of Thompson and Ramírez-Barahona [7].

3 Issues with TESS and CoMET

Thompson and Ramírez-Barahona [7] fit diversification models to log-transformed LTTs of two flowering plant phylogenies using TESS [11] and estimate diversification dynamics on each using the CoMET model [12], which is implemented in the TESS R package. The decision to use these methods to answer their question of whether angiosperms were affected by the KPg mass extinction is a curious one. While the CoMET model is interesting in that it uses compound Poisson process models to distinguish between several different types of diversification rate shifts across phylogenies, the authors clearly state in their discussion that CoMET is not an ideal model for a study like the one conducted by Thompson and Ramírez-Barahona [7]. Specifically, May et al. [12] claim that, in their simulation study of their own method, CoMET almost never showed strong support for the true mass extinction model when the mass extinction was located in the more ancient (i.e., rootward) half of the tree. In these scenarios, the mass extinction model was favored only 8.4% of the time with diversification rate shifts, and only 6% of the time without such shifts. Since the KPg mass extinction occurs close to the halfway point in time along the Smith and Brown phylogeny, one could reasonably expect Thompson and Ramírez-Barahona [7] to mention this issue among the other caveats and potential pitfalls they discuss. However, they do not. Additionally, May et al. [12] point out that CoMET inferences of mass extinction event times often do not coincide with the times inferred from fossil record evidence of mass extinctions. This is also goes unmentioned in Thompson and Ramírez-Barahona [7].

Comprehensive tests of the performance of CoMET have previously been published (see Culshaw et al. [19], which found high rates of Type II error when mass extinction survival rates were not very low and when trees were not very large). Therefore, I performed a limited number of tests involving the Smith and Brown tree [16] and simulated trees of similar sizes. To test the degree to which TESS and CoMET can accurately identify the generating models of simulated trees, I performed a similar analysis to that of Thompson and Ramírez-Barahona [7] using the phylogeny published by Smith and Brown [16]. I assumed a sampling fraction of about 22.5%, calculated by dividing the number of tips in the Smith and Brown tree (about 77,000) by the estimate of about 350,000 total angiosperm species published by Paton et al. [20]. After extracting branching times from the tree, I created three simulated trees in TESS to which I could fit the data using the following branching-process models: constant birth-death, episodic birth-death, and a birth-death model including a mass extinction. The marginal likelihoods of each model were estimated using stepping-stone sampling in TESS, and the relative support of the models were compared using Bayes factors (see [21]). Although the mass extinction model was decisively favored over episodic birth-death, this analysis yielded extremely strong support for the constant birth-death model over both the episodic and mass extinction models according to Bayes factors exceeding 1.9 million, astronomically higher than the “decisive” threshold of 100 advocated by Jeffreys [21].

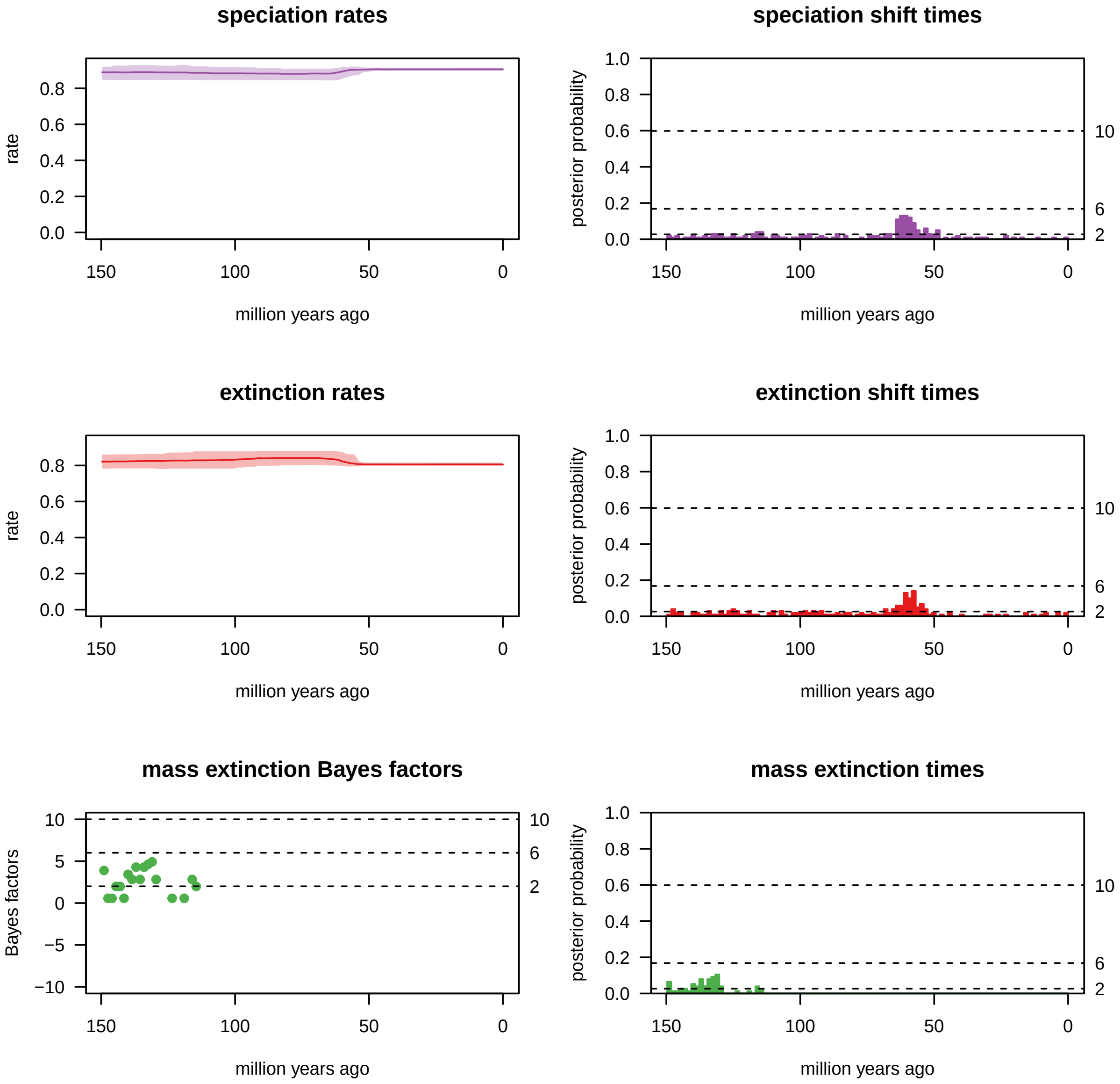

Figure 1: CoMET fails to detect a mass extinction at 66 mya on a simulated phylogeny of similar size and age to the Smith and Brown tree used in the analysis by Thompson and Ramírez-Barahona [7]. In this simulation, 30% of lineages survive the mass extinction; CoMET successfully detected mass extinction events on similar simulated trees with lower survival rates.

To test the degree to which TESS and CoMET can accurately identify mass extinction events in an analysis like that of Thompson and Ramírez-Barahona [7], I used *TreeSim* [22] to simulate phylogenies of similar size and age to the Smith and Brown phylogeny (each possessing a speciation rate (λ) of 0.2 and extinction rate (μ) of 0.1 prior to the mass extinction event, and λ of 0.3 and μ of 0.2 post-extinction event). Once again assuming a sampling fraction of 0.22 as in the Smith and Brown tree [16], I estimated diversification rates using the "tess.analysis" function (see Fig. 1) to test whether a mass extinction would be detected by the CoMET model. I opted for the function to run 10,000 iterations and to estimate hyperpriors empirically rather than setting them manually.

My analyses were successfully able to detect mass extinction on a phylogeny possessing 70,000 extant tips (originally with 73,062 additional extinct taxa) and a mass extinction at 66 mya through which only 10% of lineages survived. After increasing μ from 0.2 to 0.25, TESS also succeeded on a phylogeny of 70,000 extant tips (originally with 71,807 additional extinct taxa) with a root age of 143.2 mya and a mass extinction which 20% of lineages survived (the estimate calculated by Johnson [23]). However, as the percentage of lineages that survive the extinction event increases, the signal disappears. No mass extinction was detected on a phylogeny of 142,833 extant/extinct tips with a root age of 149.68 mya in which 30% of species survive the KPg, nor on a 141,651-tip tree with a root age of 152.76 mya in which 40% of species survive the KPg (see Fig. 1).

4 How I Learned to Stop Worrying and Love the Fossil Record

I have demonstrated that Thompson and Ramírez-Barahona [7] erred in their methodological choices and interpretations. However, I also believe that the paper suffers from a more philosophical issue. Thompson and Ramírez-Barahona [7] explain their findings by striking a middle ground between phylogenetic evidence and fossil evidence, concluding their paper by arguing that the contradiction they observe between their results and fossil evidence can be partially explained by the fossil record recording species-level extinction while the phylogenies they analyzed record the survival of higher taxa. I disagree with this interpretation: notwithstanding the study's methodological issues, I believe that the proper conclusion would have been deferring to the fossil evidence and pleading for further research.

Thompson and Ramírez-Barahona [7] are clearly aware of the respective strengths and limitations of phylogenetic and fossil evidence as they relate to their question of interest. Their conceptual error is treating these two types of evidence as having equal weight for determining whether the KPg significantly affected plants. While I am a comparative biologist who strongly believes in the power of molecular phylogenies as tools to learn about evolution, I believe that many comparative biologists do not, to quote O'Meara and Beaulieu [15], "recognize that *our methods are better suited for using the past to learn about the present survivors, not using the present survivors to learn about the past*" (p. 21; emphasis in the original). Between the end of the Cretaceous and the present, we can reasonably assume that many flowering plant clades originated, diversified, declined, and disappeared from the Earth, failing to earn themselves spots in a molecular phylogeny by surviving to the present. Only the fossil record can tell us about the lineages that did not live to be sequenced (i.e., the vast majority of those alive 66 million years ago).

This is not to say that the fossil record is perfect. Fossil data *can* be biased, and inferences about the past from phylogenies of extant taxa *can* be accurate. But, when examining events in deep time, fossils often contain much more information about evolutionary patterns than the deep internal branches of molecular phylogenies, and they should be treated as such. This relative weighing of evidence can be found in the literature surrounding estimates of the time at which angiosperms originated, where molecular clock analyses based on extant taxa frequently estimate much earlier origination times than fossil evidence would suggest (see [24]). On this front, the trustworthiness of the fossil record has been aggressively defended (see [25]), whereas molecular clock research is, by the admission of its architects, still evolving (e.g., [26]; [27]; [28]).

Instead of attempting to "corroborate" fossil evidence with phylogenetic analyses, it may be time for comparative biologists to stop worrying and love the fossil record.

5 Acknowledgments

I thank Jeremy M. Beaulieu, James D. Boyko, and C. Tomomi Parins-Fukuchi for stimulating conversations and helpful comments about this manuscript.

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