What time is it? Interactions between trees and fossils

Tom Carruthers

Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, USA.

email: tomcarruthers94@gmail.com

Molecular sequence data is not in itself informative about absolute evolutionary timescales. Fossils are therefore often analysed alongside molecular data in order to generate time-scaled reconstructions of the tree-of-life. Here, I analyse *interactions* between fossils and molecular based reconstructions of the tree-of-life, and explore the implications of these interactions for time-scaling the tree of life. I use simulations to explore two types of interaction: the *extinct lineage effect* and the *character evolution effect*. The extinct lineage effect describes the effect of extinct lineages on the expected delay between the origin of a clade and the age of its oldest known fossil. Meanwhile, the character evolution effect explores the impact of limited morphological character data on the lengths of this delay. Both of these effects can significantly distort the relationship between the age of clades and the origin of their oldest fossils, especially in datasets with many species. These impacts are entirely distinct from taphonomic biases often discussed in the context of the fossil record. I discuss the implications of these effects for divergence time estimation, highlighting that they are likely to impact methods that rely on both node and tip-calibration. This further suggests that both a fundamental re-appraisal of the purpose of divergence time estimation, alongside the development of new methods, is required.

Our understanding of evolutionary history is increasingly dependent on *time-calibrated phylogenetic trees* (estimates of the tree of life with branching events on an absolute timescale). Time-calibrated phylogenetic trees provide a context to investigate when, how quickly, and in what environments different lineages diversified, and the morphological traits and molecular processes underlying these patterns (e.g. Baldwin and Sanderson 1998; Lagomarsino et al. 2015; Berv and Field 2018; Muñoz-Rodríguez et al. 2019; Title et al. 2024). Recognising limitations in the informativeness of the different types data that underpin time-calibrated phylogenetic trees, including how these limitations interact with each other, is important for how we interpret time-calibrated phylogenetic trees, and consequently, our understanding of evolution (Britton 2005; Warnock et al 2012; Magallón et al. 2013; Brown and Smith 2018; Muñoz-Rodríguez et al. 2018; Carruthers et al. 2020a,b; Carruthers and Scotland 2021a,b; Budd and Mann 2024).

Molecular sequences (primarily DNA, and sometimes amino acids) are the primary source of data for estimating phylogenetic trees comprising extant organisms. Advances in sequencing technology and analytical methods have markedly improved understanding of the order of branching events in the tree of life. Broadly speaking, molecular data has enabled such advances because of the quantity of it that can be assembled (often 10s or 100s of thousands of bases per organism), and the relatively unambiguous way that it evolves (i.e. a single site on a strand of DNA can only be in state A, C, T or G) (Scotland et al. 2003; Wortley and Scotland 2006; Weitemier et al. 2014; Muñoz-Rodríguez et al. 2019). This contrasts to morphology, where datasets rarely comprise more than a few 10s of characters, and ambiguity often surrounds how characters are coded.

Nonetheless, molecular data provides at best partial information about *when* branching events in the tree of life happened. This is because the likelihood (in the context of phylogenetic inference) is, among other parameters, dependent on the total amount of molecular change along each branch in a phylogenetic tree. This in turn is a product of the *rate* of evolution along the branch, and the *time* duration of the branch. An infinite combination of rate and time durations on any given branch can have an equal likelihood, meaning that estimating time-calibrated phylogenetic trees is impossible without additional information (Britton 2005).

Often, *fossil calibration* provides this additional information. Broadly speaking, fossil calibration involves using fossils to generate an expectation about the age of some part(s) (clades) of a phylogenetic tree. This includes *node calibration*, where the oldest fossil for a clade is used as a minimum constraint for the age of a clade, and an assumption is made about how much older than the fossil the clade is expected to be. The assumption about how much older the clade is can be direct, for example, in Bayesian analyses prior probability distributions can be specified which are designed to describe expectations about the age of the clade in relation to the fossil (Ho and Phillps 2009). They can also be indirect, for example, a maximum constraint might be applied at another (deeper) node, which, given all the nodes are connected within a single tree, will influence expectations about the age of the focal node (e.g. Zuntini et al. 2024). Alternatively, *tip-calibration* (or derivations thereof such as the fossilised birth-death process) involves the explicit estimation of relationships of fossils relative to extant species in the phylogenetic tree. Combined with the known ages of the fossils, this enables time-calibration of the phylogenetic tree (Ronquist et al. 2012; Heath et al. 2014).

It is uncontroversial to point out that information from the fossil record can be very incomplete. Taphonomic processes (processes that occur during the formation of fossils) inevitably lead to incomplete preservation, and variation in such processes e.g. between harder and softer organisms, creates further complexity. Geological processes can cause further loss and distortion of information by moving fossils and making some easier to sample than others (Darwin 1859 – opening of chapter 5 of The Origin; Behrensmeyer et al. 2000).

In terms of using fossils when estimating time-calibrated phylogenetic trees, limitations in the information contained within the fossil record are important in two interlinked ways. First, they can cause large and unpredictable delays between the origin time of a clade and the formation of the earliest fossils for that clade. Second, even if fossils exist for a clade, characters enabling fossils to be assigned to clades may be incompletely preserved making it difficult or impossible to use the fossils when estimating time-calibrated phylogenetic trees. These two issues make it hard to generate reasonable expectations about the relationship between the age of known fossils for a clade and the actual age of the clade, which can lead to major inaccuracies in time-calibrated phylogenetic trees (Wilf and Escapa 2015; Wilf et al. 2017; Carruthers and Scotland 2020). Regardless, fossil calibration generally remains the only form of evidence that is directly informative about time when estimating time-calibrated phylogenetic trees.

Alongside inherent limitations in the information contained within the fossil record, specific characteristics of phylogenetic trees may cause an additional degradation of information that interacts with the fossil record. In the following I explore two potentially interlinked examples of such phenomena.

The first example (hereafter the *extinct lineage effect*) rests on the fact that a molecular phylogenetic tree only comprises extant lineages. Assuming extinction rate (μ) > 0, there may be many (necessarily unsampled) extinct lineages such that for much of the history of the clade represented by the phylogenetic tree, the number of extinct lineages vastly outnumbers extant lineages (Fig.1). Assuming the diversity of a clade at a point in time (i.e. the sum of all the species/branches that existed at that time) bears some relation to the probability that a fossil for that clade is sampled at that time (an inherent assumption of the fossilised-birth-death process (Heath et al. 2014)), large numbers of extinct lineages will distort when a fossil is sampled. Importantly, if there are a large number of extinct lineages for a clade that are not part of a more nested extant clade, this may increase the probability that a fossil for the clade is sampled that is both significantly younger than the clade and does not belong to any more nested extant clades (Fig.1).

The second example (hereafter the *character evolution effect*) relates to the nested structure of a phylogenetic tree (with younger nested clades being more specific with respect to the relationships they describe than older and more general clades) and the limited morphological character data that is inevitable in any dataset. Specifically, limited morphological character data may cause fossils that actually belong to younger nested clades to be assigned to older and more general clades (Fig.1).

It is reasonable to hypothesise that the strength of the character evolution effect will increase in large phylogenetic trees for very diverse clades. In very diverse clades, there is, by definition, a larger increase in diversity from the origin of the clade to the present. Assuming the diversity of a clade bears some relation to the probability that a fossil for that clade is sampled, in very large clades there will be a proportionally greater probability of sampling fossils closer to the present (that actually belong to nested clades). This provides more opportunities for the character evolution effect to occur (i.e. for young fossils to be incorrectly assigned to more general clades). Further, in large phylogenetic trees and with limited morphological data, the proportion of clades for which morphological synapomorphies (derived similarities shared by the members of a clade) can be identified will tend to decrease. Fossils that are sampled from clades without synapomorphies must be assigned to more general clades, thus presumably strengthening the character evolution effect. Finally, in large phylogenetic trees, there is simply more potential for fossils to be assigned to much deeper nodes than to which they actually belong (owing to the fact that large phylogenetic trees contain longer sequences of serially nested clades).

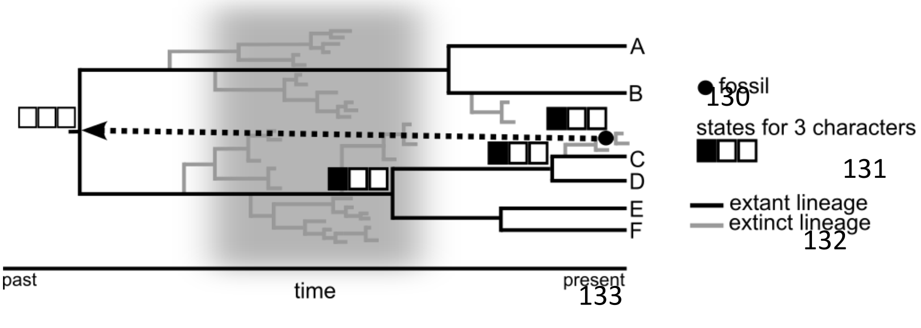

Figure 1. *Summary of the extinct lineage effect and character evolution effect. The displayed tree reflects the true evolutionary history of clade A-F (including extinct lineages shown in grey). Fossils belonging to clade A-F, but not to any nested clade within A-F are more likely to be sampled during the interval that is shaded grey because there are more extinct lineages. This is the extinct lineage effect. Morphological data enables the fossil (black circle) to be identified as belonging to clade C-F. Following the common approach of using fossils to calibrate the stem node of the clade to which they are identified as belonging to, the fossil is used to calibrate the stem node of clade C-F. Note that the fossil is not used to calibrate the stem node of clade C-D (the clade to which it actually belongs). This is because there is insufficient morphological character data to assign the fossil more precisely to this clade. This is the character evolution effect.*

Here, I characterise these two effects with simulations. Specifically, I explore how these effects influence the expected delay between the time of origin of a clade and the occurrence of its first fossil. This is a key quantity in node calibration, which I hereafter refer to as the *clade fossil delay*. I then discuss the potential implications of these effects for divergence time estimation and evolutionary analyses.

EXTINCT LINEAGE EFFECT

To explore this effect, branching processes were simulated in R using the *sim.bd.taxa* function from TreeSim (Stadler 2019). Branching processes had 10 extant species and either speciation rate (λ) = 0.1 and extinction rate (μ) $= 0$ (a pure birth or Yule process), or λ = 1 and μ = 0.9 (a birth-death branching process with the same net diversification rate as the Yule process). Fossils were simulated along the branches of these birth death branching processes at three different rates (ψ), 0.01, 0.001, or 0.0001. The age of the oldest simulated fossil within each extant clade was then recorded, alongside the difference between this fossil age and the age of its respective clade (i.e. the clade fossil delay). In the Yule simulation this simply involves assigning fossils to clades in the simulated branching process. In the birth-death simulation, fossils are assigned to clades following removal of extinct lineages from the birth-death branching process. In both cases, the fossil is automatically assigned to the “correct” clade. These simulations enable the extinct lineage effect to be quantified in the context of simple phylogenetic trees.

In a further simulation, a branching process was simulated that was affected by a mass extinction event. The timing of the mass extinction event was at 50% of the age of the simulated branching process, and caused 90% of lineages to go extinct. Fossils were simulated and assigned to a clade as set out above. However, results were only recorded for the clade fossil delay for fossils belonging to the clade incorporating all extant species in the simulated branching process. This provided a direct way to assess the impact of the mass extinction event, which is simulated to occur at the same time relative to the age of the overall clade.

Across these simulations, the presence of extinct lineages consistently increases the clade fossil delay (Fig. 2a-c compared to maroon lines Fig. 2d-i). This pattern occurs to such an extent that the probability of sampling a fossil increases through successive time intervals significantly after the origin of the clade. This occurs in simulations with a constant μ (Fig.2d-f), but the effect is especially strong in the simulation with a mass extinction event (Fig.2g-i). The effect is also stronger when ψ is lower, although it does occur for all values of ψ that were trialled (which span 2 orders of magnitude).

For any branching process simulated with extinction, the corresponding tree with extinct lineages removed has a different distribution of branch lengths compared to a branching process simulated with no extinction (a Yule tree). For example, a birth-death tree with extinct lineages removed will have longer branches toward the root of the tree. This results in longer time intervals between the origin time of each extant clade and could therefore explain the result in Figure 2 - i.e. the larger the time interval between the origin of each clade, the greater the potential of sampling fossils that are significantly younger than the clade (regardless of extinct lineages). Therefore, fossils were also simulated on the birth-death trees and mass extinction trees *after* (rather than before) removal of extinct lineages. The impact of the extinct lineage effect could therefore be isolated.

In this subsequent simulation, the key pattern discussed above (i.e. an increased clade fossil delay in the birth-death and mass extinction simulation) is significantly reduced (Fig. 2d-h, blue lines). The extinct lineage effect therefore appears to cause much of the delay in the expected time to sample fossils for clades.

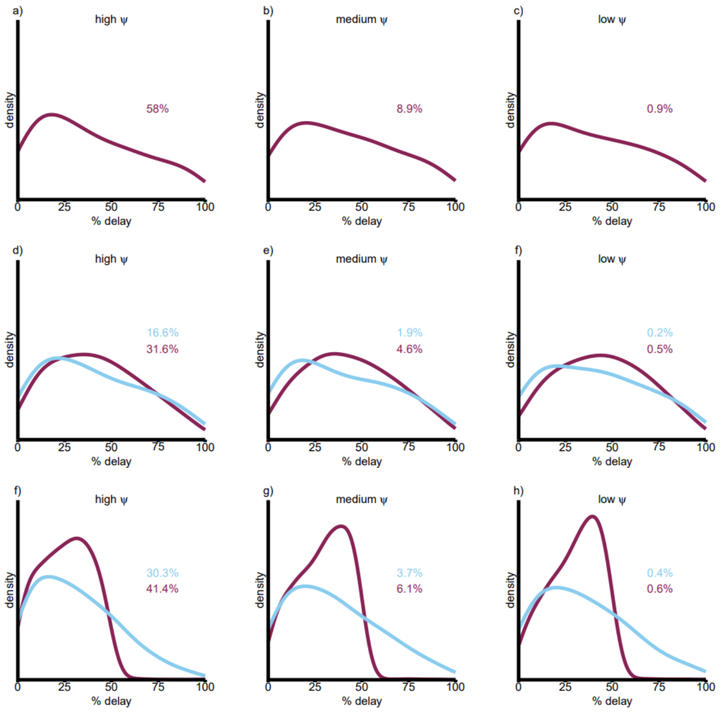

***Figure 2.** The extinct lineage effect. In each case the line is the probability density of the clade fossil delay following 500 replicate simulations. In a-c. there are no extinct lineages in the simulated branching process, in d-f. there is a birth-death branching process with a constant extinction rate, and in f-h. there is a mass extinction event at 50% of the age of the clade. In d-h. the blue line shows the probability density when fossils are only simulated along extant lineages, whilst the maroon line shows the probability density when fossils are simulated along extinct and extant lineages. The % values (written, not on the axis) indicate the percentage of simulated branching processes for which fossils were simulated (i.e. when ψ is low no fossil is simulated on many of the trees), with the colour corresponding to whether or not the fossils were simulated along all lineages or just extant lineages.*

CHARACTER EVOLUTION EFFECT

To explore the character evolution effect, Yule processes and fossils were simulated similarly to the description above. Simulated fossils were then assigned to clades based on synapomorphies they shared with clades in the simulated Yule process, rather than automatically being assigned to the correct clade. Therefore, binary (morphological) characters were simulated along the branches of each Yule process using the *simSeq* function from *phangorn* (Schliep 2011) and synapomorphies for each clade were identified based on the character states of extant tips. Where a fossil possessed 90% of the synapomorphies for a clade it was assigned to that clade. This means some of the simulated fossils are not assigned to any clade. Therefore, to ensure a sufficient number of fossils are actually assigned to clades, higher values for ψ are used in these simulations (0.05, 0.01, and 0.002). Fossils were then assigned to the stem node of the clade to which they were identified as sharing synapomorphies with (thus recapitulating how node calibration is undertaken in empirical datasets). The age of the oldest fossil assigned to each node relative to the actual node age was recorded and treated as the fossil clade delay. As well as exploring different values for ψ, simulations were performed with either 8, 40, or 2000 binary characters, and a substitution rate (κ) for the binary characters of either 0.002, 0.01, or 0.05. Additional analyses were also performed where fossils were automatically assigned to the correct clade as a reference point to compare the impact of limited character data. In this second set of simulations, μ was set to 0 in all cases to isolate the character evolution effect without a confounding impact from the extinct lineage effect. Meanwhile, different numbers of tips were used (10, 25, or 100) because this variable is hypothesised to affect the strength of the character evolution effect (as set out in the introduction).

Fossils automatically assigned to the correct node - With fossils automatically assigned to (the stem node of) the correct clade, the fossil clade delay is broadly distributed, regardless of the size of the simulated branching process. However, the delay is larger for smaller clades (Fig.3a). This is because regardless of the size of the simulated branching process, the absolute branch lengths are identical, but the branch lengths relative to the age of the clades to which fossils are assigned is larger in the smaller simulated branching processes. This increases the potential for fossils to be significantly younger relative to the age of their clade (which causes larger fossil clade delays because this is a relative measure).

Variable quantities of data - With a large number of simulated morphological characters, the pattern differs considerably (Fig.3b). In the smaller simulated branching processes in particular (10 and 25 tips), there are proportionally fewer larger delays, although far fewer trees have any fossil assigned to a node (Fig.3b). By contrast, in the larger simulated branching process, the proportion of large delays actually increases (i.e. the distribution represented by the maroon line shifts rightwards in Fig.3b compared to Fig.3a).

In the small branching process with a large quantity of data, the dramatic reduction in the percentage of clades that have fossils assigned to them occurs because a high percentage of clades have a large number of apparent synapomorphies (99.6% of clades have synapomorphies, and the average number of synapomorphies for these clades is 56). Fossils therefore need a very large number of synapomorphies to be assigned to a clade. Importantly, a very large number of these apparent synapomorphies are erroneous. For example, when ancestral character states at nodes are also taken into account when identifying synapomorphies (and not just tip states), the average number of synapomorphies per clade drops to 11 (i.e. there are character state changes within the clade despite all the extant members having the same state). This is directly relevant for assigning fossils to clades, given that fossils will tend to possess character combinations more similar to ancestral nodes, and explains why there is often a failure to assign any fossil to a clade.

Meanwhile, the reduction in the proportion of large delays in the small branching process with large quantities of data occurs because large numbers of synapomorphies are particularly concentrated on clades with long stem branches (Fig.3c). Long stem branches will lead to large fossil clade delays given they cause fossils to be assigned to relatively older nodes. With large amounts of data, fossils are far less likely to be assigned to clades with long stem branches because they have so many, often erroneous, synapomorphies.

In the large simulated branching process with a large quantity of character data there are relatively fewer synapomorphies and the total number of clades with synapomorphies is reduced dramatically (around 2.75% of clades, with an average of under 5 synapomorphies). In such a scenario, fossils are more likely to be assigned to more general clades than to which they actually belong, thus reducing the proportion of simulations with very short fossil clade delays (Fig. 3a-b).

Nonetheless, it is notable that with the large simulated branching process the pattern remains fairly constant regardless of the quantity of data (Fig. 3b, d-e). This may appear surprising because with a large quantity of data there is a much higher proportion of clades with synapomorphies (around 2.75% of clades with the highest quantity of data compared to 0.05% of clades with the lowest quantity of data) which would imply that fossils would be more readily assigned to narrower clades. However, as with the small branching process, many of the apparent synapomorphies with large quantities of data are erroneous given that when the synapomorphies are identified when also considering ancestral sequences the percentage of clades with synapomorphies falls to 0.77. These erroneous synapomorphies are also particularly concentrated on younger clades (when ancestral sequences are taken into account the average age of clades with synapomorphies increases from 23.8, to 26.2), adding a further barrier to precisely assigning fossils precisely to young clades when analysing large quantities of data.

With smaller quantities of character data and for smaller simulated branching processes, there is a shift back toward larger delays compared to with large quantities of data (Fig. 3d-e, compared to Fig. 3b). In this situation, far fewer synapomorphies are identified (in the small branching process only 21% of clades, which have on average just over 1 synapomorphies) and clades with long stem lineages are less likely to be penalised (compared to analysing large quantities of data in small trees – Figure 3b). Indeed, in this situation, clades subtended by long branches are often likely to be the only clades with synapomorphies to which fossils can be assigned. For example, in the small tree with 8 simulated characters, stem branch lengths for clades with synapomorphies are almost twice as long as those without synapomorphies ($t\ test\ p < 0.001$). Therefore, in this situation fossils are *more* likely to be assigned to clades with long stem branches.

Variable ψ - With low ψ, increasing the size of the simulated branching process dramatically increases the fossil clade delay (Fig. 3f). With larger branching processes, there are proportionally more younger lineages (relative to the overall age of the clade), which increases the probability that younger fossils are simulated. Also, in larger branching processes the character data enables less precision with respect enabling fossils to be assigned to clades because a lower proportion of clades have any synapomorphies. This increases the probability that fossils that belong to younger clades are assigned to older and more general clades. As ψ increases, the delay decreases, especially when the simulated branching process is large (Fig. 3d, g). The change with respect to a low ψ occurs because fossils are simulated at a sufficient rate that the larger number of fossils simulated in younger clades becomes irrelevant. There are already a large number of older fossils simulated on earlier branches in the branching process. Meanwhile the difference between the small and large simulated branching processes with a high ψ exists because the largest simulated branching processes are also the oldest. Therefore, even though fossils are simulated shortly after the origin of the branching process in each case, the delay, relative to the clade age, is less in the larger branching process that contains older clades.

Variable κ - The impact of increasing κ, (especially from low to medium) is somewhat similar to the effect with ψ, although the impact of low κ is less pronounced (Fig. 3d, h-i). However, the reasons for the pattern are quite different. For example, for a low κ and a large branching process there is a higher percentage of clades with synapomorphies compared to with an intermediate κ (5.5% compared to 0.22%). This can be explained by the fact that with lower κ, there is less chance of substitutions at the same site evolving independently in different lineages, and a lower chance of reversals to the ancestral state in clades for which a transition to the derived state occurred on the stem branch. Intuitively, one might expect a higher percentage of clades with synapomorphies to lead to fossils being assigned to narrower clades (and thus lower the fossil clade delay). However, with a low κ, the average length of the stem branch for clades with synapomorphies is significantly longer ($t\ test\ p < 0.001$). Given fossils are assigned to the stem node of their respective clades, these longer stem branches increase the fossil clade delay. By contrast, with a high κ and in the large simulated branching process, there are rarely synapomorphies for any clade. As such, no fossil was ever assigned to a clade in all the replicate simulations (Fig. 3i). In contrast to the large simulated branching process, the effect of different levels of κ was far less pronounced in the small simulated branching processes. Nonetheless, one notable pattern in the small branching process with high κ is that a significant number of fossils were wrongly assigned to clades that are younger and more specific than those to which they actually belong (around 27% of fossils).

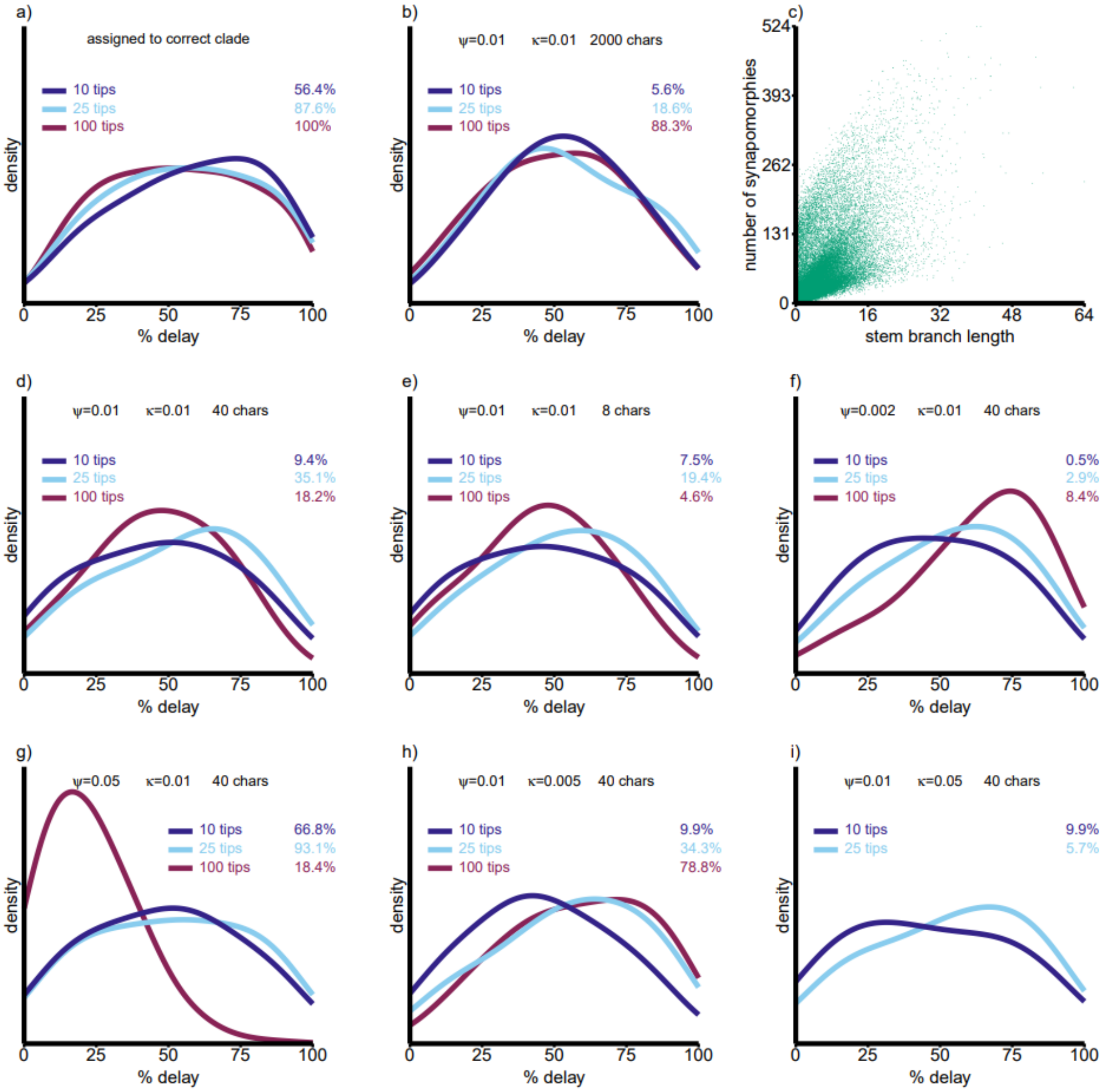

Figure 3. *The character evolution effect. In a-b and d-i the lines are the probability density for the clade fossil delay based on 500 replicate simulations with different numbers of tips in the branching process. The "% of replicates" indicates the percentage of simulated branching processes for which fossils were assigned to clades. The same colour coding is used as for the lines. Different parameter combinations are shown at the top of each plot. c. shows the relationship between the stem branch length and the number of synapomorphies identified for a clade with 2000 characters,* $\psi = 0.01$, and $\kappa = 0.01$.

POTENTIAL IMPLICATIONS FOR TIME-SCALING THE TREE OF LIFE

The simulations presented show that the extinct lineage effect and character evolution effect can both influence the fossil clade delay. This clearly has significant relevance to node calibration (by far the most common method of fossil calibration). As set out above, node calibration makes explicit assumptions about the fossil clade delay (Ho and Phillips 2009). Given divergence time estimates are highly sensitive to assumptions (Carruthers and Scotland 2021b), distortions of the fossil clade delay will necessarily affect divergence time estimates.

For tip calibration, the relevance of these effects may be more varied. For the extinct lineage effect, a scenario where λ, μ and κ are constant may not pose problems. The phylogenetic relationships of the fossils and their degree of morphological divergence are estimated directly from the sequence data. Assuming κ is correctly estimated, this should mean that distortions to the relationship between clade ages and fossil ages are accounted for. However, where there is heterogeneity in either λ, μ, or κ, this lack of sensitivity to distortions brought about by the extinct lineage effect would likely break down. This is because there is little information with which to estimate heterogeneous rates parameters in divergence time estimation (Britton 2005; Carruthers and Scotland 2020; Carruthers and Scotland 2021).

For tip calibration and the character evolution effect, uncertainty in divergence time estimates is likely to increase. Consider a low ψ in a large phylogenetic tree (Fig. 3f). Confidence intervals would likely increase because the analysis will incorporate the inherent uncertainty in the phylogenetic relationships of (primarily young) fossils that this situation brings about. An effect equivalent to this is discussed a recent tip-dating study by Carruthers et al. (2025) who note that uncertainty in age estimates is significantly higher in clades with more fossils, and that this uncertainty stems from uncertainty in the phylogenetic relationships of the fossils.

Nonetheless, quantifying the multitude of scenarios in which the character evolution effect may or may not lead to erroneous divergence time estimates in tip calibration is far beyond the scope of this article. In an ideal world, where the correct model for morphological and molecular evolution is known, it is reasonable to assume that the character evolution effect will only increase the width of confidence intervals i.e. tip dating should account for phylogenetic uncertainty brought about by the character evolution effect without biases (as discussed above). However, such a scenario is never the case in empirical datasets. With incorrect or uncertain evolutionary rate models, and combined with the extinct lineage effect, one might expect significant error.

CONCLUSIONS

The purpose of this article is to identify interactions between molecular phylogenetic trees and the fossil record and to explore their implications for divergence time estimation. Two such effects have been identified, *the extinct lineage effect* and *the character evolution effect*. These effects are entirely distinct from taphonomic or geological biases that are often discussed in the context of the fossil record and divergence time estimation.

Both the extinct lineage effect and character evolution effect can influence the fossil clade delay. The extinct lineage effect becomes especially pronounced with low fossil sampling rates, and when there are very large numbers of extinct branches resulting from mass extinction events. In such situations, the fossil clade delay increases markedly. Meanwhile, the character evolution effect is especially pronounced in large phylogenetic trees with a low fossil sampling rate or a low rate of morphological evolution, and similarly increases the fossil clade delay. The character evolution effect is also significantly affected by the fact that different quantities of data, different evolutionary rates, and different tree sizes can profoundly affect the accuracy with which morphological synapomorphies are identified for clades. This in turn affects how fossils are assigned to nodes in phylogenetic trees.

The length of the fossil clade delay is a critical quantity for node calibration. The extinct lineage effect and character evolution affect are therefore highly relevant to divergence time analyses that use this approach. The implications of these affects for tip-calibration are likely to be more complex, but it is entirely plausible that they will profoundly affect divergence time estimates in this context.

Nonetheless, it is undoubtedly extremely difficult to determine precisely the extent to which these effects influence divergence time estimates in empirical datasets. Unlike simulations, we do not know the “true” age of any clade, and thus cannot quantify the difference in age between the clade and its oldest fossil. However, the phenomena underpinning these effects are simple and realistic enough to suggest they are widely relevant. Any clade with large amounts of extinct diversity that occurred significantly after its origin is likely to be affected by the extinct lineage effect. Meanwhile, any diversifying clade in which morphological data limits the resolution with which fossils can be assigned to subclades within it is likely to be affected by the character evolution effect.

As with any other problem in divergence time estimation, there is not necessarily a straightforward solution to this issue. One option is to perform analyses that attempt to account for these problems – for example by making very relaxed assumptions about clade ages in relation to fossil ages, or the amount of variation in substitution rates in the phylogenetic tree (Carruthers and Scotland 2023). However, such an approach can result in very wide confidence intervals that may be biologically unrealistic or difficult to interpret.

An alternative approach therefore, is to continue performing much more constrained analyses. These might be constrained in such a way as to meet a pre-conceived expectation. For example, many analyses of major clades such as angiosperms are constrained to meet pre-conceived expectations about the age of angiosperms from the fossil record, and such an approach is advocated in other groups (Magallón et al. 2015; Budd and Mann 2023; Zuntini et al 2024). Such approaches, when supported by extensive evidence from the palaeontological record are entirely reasonable (Budd and Mann 2023). Nonetheless, they do raise the question of to what extent divergence time analyses in such cases actually make an intellectual contribution to our understanding of evolution. For example, in the recent study of Zuntini et al. (2024) some degree of focus was given to an “early-burst” of angiosperm diversification. While there almost certainly is an early burst, we already knew this from the palaeobotanical record. What therefore has the time-calibrated phylogenetic tree actually contributed? In many further cases, when not considering the origin or early diversification of major clades such as flowering plants, animals, birds, or mammals, there is in any case very little auxiliary evidence on which to constrain divergence time analyses. Constraints placed on divergence time estimates in such cases are therefore often arbitrary. In such cases, either accepting much greater uncertainty, or being aware of the implications of alternative divergence time estimates for the conclusions drawn from the analyses is probably therefore preferable (e.g. Muñoz Rodríguez et al. 2018)

Both the extinct lineage effect and character evolution effect are underpinned by phenomena (extinction, character evolution, fossil sampling rate) that are incorporated into existing macroevolutionary modelling frameworks (e.g. birth death branching processes, models of morphological evolution). It is thus entirely plausible that one could construct a model in the future that accounts for these processes, and enables appropriate calibration densities, or minimum and maximum constraints to be defined. It is also the case that we have enough theoretical evidence and the computational tools to construct models for rates of molecular evolution that are more realistic than the limited selection currently available. For example, rates could be constrained by traits such as life-history that are known to be associated with substitution rates (Smith and Donoghue 2008; Smith and Beaulieu 2024). Unlike existing relaxed clock models, which often explore an arbitrary distribution of rates that is picked by the user for statistical convenience, such models would likely be more informative with respect to the rate on any given branch. This would likely down-weight the importance of fossil calibrations in divergence time estimation (Carruthers and Scotland 2021), improving the reliability of divergence time estimates even if the issues discussed here are not dealt with. Regardless of these details, further methodological development should enable divergence time estimation to make more meaningful contributions to evolutionary research.

REFERENCES

Baldwin BG, Sanderson MJ. 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proc Natl Acad Sci USA.* 95:9402-9406.

Berv JS, Field DJ. 2018. Genomic Signature of an Avian Lilliput Effect across the K-Pg Extinction. *Syst Biol.* 67:1-13.

Behrensmeyer AK, Kidwell SM, Gastaldo RA. 2000. Taphonomy and paleobiology. *Paleobiology.* 26:103-147.

Britton T. 2005. Estimating Divergence Times in Phylogenetic Trees Without a Molecular Clock. *Syst Biol*. 54: 500–507.

Brown JW and Smith SA. 2018. The past sure is tense: on interpreting phylogenetic divergence time estimates. *Syst Biol.* 67:340-353.

Budd GE and Mann RP. 2024. Two Notorious Nodes: A Critical Examination of Relaxed Molecular Clock Age Estimates of the Bilaterian Animals and Placental Mammals. *Syst Biol.* 73:223-234.

Carruthers T, Brockington SF, Maeda HA et al. *in review.* Evolution in arid environments: the role of betalains, succulence, and medullary bundles. *New Phyt.*

Carruthers T, Sanderson MJ, Scotland RW. 2020a. The Implications of Lineage-Specific Rates for Divergence Time Estimation. *Syst Biol*. 69:660–670.

Carruthers T, Muñoz-Rodríguez P, Wood JRI, Scotland RW. 2020b. The temporal dynamics of evolutionary diversification in Ipomoea. *Mol Phylogenetics Evol.* 106768.

Carruthers T and Scotland RW. 2020. Insights from empirical analyses and simulations on using multiple fossil calibrations with relaxed clocks to estimate divergence times. *Mol Biol Evol.* 37:1508-1529.

Carruthers T and Scotland RW. 2021a. Uncertainty in Divergence Time Estimation. *Syst Biol*. 70:855-861.

Carruthers T, Scotland RW. 2021b. The Implications of Interrelated Assumptions on Estimates of Divergence Times and Rates of Diversification. *Syst Biol.* 70:1181–1199.

Carruthers T and Scotland RW. 2023. Deconstructing age estimates for angiosperms. *Mol Phylogenetics Evol.* 107861.

Darwin C. 1859. On the Origin of Species. John Murray: London.

Heath TA, Huelsenbeck JP, Stadler T. 2014. The fossilized birth-death process for coherent calibration of divergence-time estimates. *Proc Natl Acad Sci USA.* 111:E2957-E2966.

Höhna S, Landis MJ, Heath TA et al. 2016. RevBayes: Bayesian phylogenetic inference using graphical models and an interactive model-specification language. *Syst Biol*. 65:726–736.

Ho SYW and Phillips MJ. 2009. Accounting for calibration uncertainty in phylogenetic estimation ofevolutionary divergence times. Syst Biol. 58:367-380.

Lagomarsino LP, Condamine FL, Antonelli A, Mulch A, Davis CC. The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). *New Phyt.* 210:1152-1154.

Magallón S, Hilu KW, Quandt D. 2013. Land plant evolutionary timeline: gene effects are secondary to fossil constraints in relaxed clock estimation of age and substitution rates. *Am J Bot.* 100:556-573.

Muñoz-Rodríguez P, Carruthers T, Wood JRI, Williams BRM, Weitemier K, Kronmiller B, Ellis D, Anglin NL, Longway L, Harris SA, Rausher MD, Kelly S, Liston A, Scotland RW. 2018. Reconciling conflicting phylogenies in the origin of sweet potato and dispersal to polynesia. *Curr Biol.* 28:1246-1256.

Muñoz-Rodríguez P, Carruthers T, Wood JRI et al. 2019. A taxonomic monograph of Ipomoea integrated across phylogenetic scales. *Nat Plants.* 5:1136–1144.

Ronquist F, Klopfstein S, Vilhelmsen L, Schulmeister S, Murray DL, Rasnitsyn AP. 2012a. A total-evidence approach to dating with fossils, applied to the early radiation of the hymenoptera. *Syst Biol*. 61:973–999.

Scotland RW, Olmstead RG, Bennet JR. 2003. Phylogeny reconstruction: the role of morphology. *Syst Biol.* 52:539-548.

Schliep KL. 2011. phangorn: Phylogenetic analysis in R. *Bioinf.* 27:592-593.

Smith SA and Beaulieu JM. 2024. *Ad fontes:* divergence time estimation and the age of angiosperms. *New Phyt.* 244:760-766.

Smith SA and Donoghue MJ. 2008. Rates of molecular evolution are linked to life-history in flowering plants. *Science.* 322:86-89.

Stadler T 2019. TreeSim: Simulating Phylogenetic Trees. R package version 2.4, https://CRAN.R-project.org/package=TreeSim.

Wortley AH and Scotland RW. 2006. The Effect of Combining Molecular and Morphological Data in Published Phylogenetic Analyses. *Syst Biol.* 55:677-685.

Title PO, Singhal S, Grundler MC et al. 2024. The macroevolutionary singularity of snakes. *Science.* 6685: 918-923.

Warnock RCM, Yang Z, Donoghue PCJ. 2012. Exploring uncertainty in the calibration of the molecular clock. *Biol Lett.* 8:156–159.

Weitemier K, Straubn SCK, Cronn RC, Fishbein M, Schmickl R, McDonnell A, Liston A. 2014. Hyb-Seq: Combining target enrichment and genome skimming for plant phylogenomics. *APPS.* 2:1400042.

Wilf P, Carvalho MR, Gandalfo MA, Ruben Cuneo N. 2017. Eocene lantern fruits from Gondwanan Patagonia and the early origins of Solanaceae. *Science*. 355:71–75.

Wilf P and Escapa IH. 2015. Green Web or megabiased clock? Plant fossils from Gondwanan Patagonia speak on evolutionary radiations. *New Phyt*. 207:283–290.

Zuntini A. et al. 2024. Phylogenomics and the rise of angiosperms. *Nature.* 629:843-850.

DATA AVAILABILITY

Code for all the analyses is here:

https://github.com/TomCarr/clade_fossil_delay/blob/main/README.md

CONFLICT OF INTEREST

The author has no conflicts of interest