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# Pulled Diversification Rates, Lineage-Through-Time Plots and Modern Macroevolutionary Modelling

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## Abstract

Estimating time-dependent rates of speciation and extinction from dated phylogenetic trees of extant species (timetrees), and determining how and why they vary is key to understanding how ecological and evolutionary processes shape biodiversity. Due to an increasing availability of phylogenies, a growing number of process-based methods relying on the birth-death model have been developed in the last decade to address a variety of questions in macroevolution. However, this methodological progress has regularly been criticised such that one may wonder how reliable the estimations of speciation and extinction rates are. In particular, using lineage-through-time (LTT) plots, a recent study (Louca and Pennell, 2020) has shown that there are an infinite number of equally likely diversification scenarios that can generate any timetree. This has led to questioning whether or not diversification rates should be estimated at all. Here we summarize, clarify, and highlight technical considerations on recent findings regarding the capacity of models and inferences to disentangle diversification histories. Using simulations we demonstrate the characteristics of pulled diversification rates and their utility. We recognize the recent findings are a step forward in understanding the behavior of macroevolutionary modelling, but they in no way suggest we should abandon diversification modelling altogether. On the contrary, the study of macroevolution using phylogenies has never been more exciting and promising than today. We still face important limitations in regard to data availability and methodological shortcomings, but by acknowledging them we can better target our joint efforts as a scientific community.


*Key words*: Birth-death models, extinction, speciation, phylogenetics

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## Introduction

A major goal in evolutionary biology is to understand the large-scale processes that have shaped biodiversity patterns through time. One important way to investigate this is by modelling species diversification using speciation and extinction, which can vary over time and among groups. It is commonplace to find areas, or clades, in phylogenetic trees that accumulate lineages faster than others. Diversification models often aim to explain this variation in diversification patterns by associating bursts of speciation or extinction with factors such as time (Höhna et al., 2016b), lineages (Rabosky, 2014), character traits (Maddison et al., 2007), or the environment (Morlon et al., 2016).

The growing number of large phylogenetic trees that capture a significant proportion of living species provide increasing power and resolution for such studies (Jetz et al., 2012; Smith and Brown, 2018; Upham et al., 2019). Furthermore, the availability of a wide variety of methods and software (e.g. BAMM (Rabosky, 2014), -SSE models (Maddison et al., 2007), RPANDA (Morlon et al., 2016), MEDUSA (Alfaro et al., 2009)) have made diversification studies increasingly popular in the last decade. Approaches that can link diversification to a particular process or trait are among the most appealing to researchers in the field because they enable us to test long-standing hypotheses in evolutionary biology and ecology including those related to the evolution of key innovations (Silvestro et al., 2014), the colonisation of new areas (McGuire et al., 2014), the effect of elevation (Lagomarsino et al., 2016; Quintero and Jetz, 2018) and the latitudinal diversity gradient (Rolland et al., 2014; Pulido-Santacruz and Weir, 2016; Rabosky et al., 2018; Igea and Tanentzap, 2020).

A recent study (Louca and Pennell, 2020) (abbreviated to LP) demonstrates how one approach, based on lineage-through-time (LTT) plots, cannot reliably estimate rates of speciation and extinction over time using extant timetrees. LP show how results of this approach can be misleading and provide potential solutions to the issues raised by proposing new summary statistics. This publication has provoked a response from the

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community (Morlon et al., 2020) and stimulated considerable discussion, with some going so far as to suggest that speciation and extinction cannot be estimated using phylogenetic trees (Pagel, 2020). As a result, this study has called into question the meaning of diversification rate estimates generated from any analytical framework. Here, we aim to outline the major concepts discussed in LP in an accessible way, targeting a broad audience. We then put the results and conclusions of LP into historical context and explore how the implications of this study apply to macroevolutionary modelling today.

## Modelling diversification rates

A typical workflow for diversification rate modelling using molecular phylogenetic trees is as follows. DNA sequence data are obtained for species in a study group, which are then used to estimate species relationships in the form of a phylogenetic tree. Typically, this phylogenetic tree contains only extant species, and it is time-calibrated using ages derived from different sources including fossils (Sauquet, 2013) (note, however, that fossils are usually only used for calibration and tree shape estimation and not incorporated in subsequent estimation of diversification rates). The output of this process is referred to as an extant timetree. Once a tree has been generated, a birth-death model is fitted to explain patterns of diversification in the tree.

The simplest birth-death models assume that each branch of a phylogenetic tree shares the same rate of "birth" (speciation) events, as well as "death" (extinction) events (Nee et al., 1994; Nee, 2006; Ricklefs, 2007; Morlon et al., 2011). The speciation rate (or $\lambda(t)$) is the rate at which lineages arise at time $t$ (or more precisely during a small time interval, $dt$, between $t$ and $t + dt$, formally called infinitesimal birth rate). Likewise, extinction rate (or $\mu(t)$) is the rate at which lineages disappear. Under this simple framework $\lambda$ and $\mu$ are constant over time and the same across all clades. In addition, not all extant taxa are included in the phylogenetic tree and the percentage of lineages present is known as the sampling fraction (or $\rho$). By making use of all of these parameters, a

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birth-death model allows us to investigate whether the net diversification rate, defined as $r(t) = \lambda(t) - \mu(t)$, has varied over time or among clades (Morlon et al., 2011; Rabosky, 2014; Maliet et al., 2019; Barido-Sottani et al., 2020) and ultimately uncover the processes that have given rise to extant biodiversity in the study group.

## A summary of the main concepts and findings in Louca and Pennell (2020)

### *The deterministic Lineage Through Time plot*

The approach used by LP relies on the Lineage Through Time (LTT) plot (Nee et al., 1992) (Fig. 1), which shows how extant lineages (i.e. only those existing in the present-day) accumulated over time using a phylogenetic tree. Each point in an LTT corresponds to a change in the number of lineages from the root of a phylogenetic tree at $t = 0$ to the present day at $t = T$ (Fig. 1a). This perspective is typically used when looking at evolution from the coalescent standpoint. Alternatively, as in LP, time can be counted backwards as an age (using the symbol $\tau = T - t$), starting from the present day at $\tau = 0$ until $\tau = T$ at the origin of the clade (Fig. 1b). This approach is often used when plotting phylogenetic trees. For easier interpretation and consistency with LP, we will generally consider timescale as age, or $\tau$, throughout this manuscript.

Simply put, when a clade diversifies faster, the slope of the LTT becomes steeper, but when diversification slows, the slope of the LTT levels off. When only extant lineages are considered, as in LP, LTT plots will never exhibit a drop in total lineage diversity over time, that is, the slope will never be negative. However, this does not mean that extinction does not have an effect on the shape of the LTT (Nee, 2006). By examining the shape of the LTT plot we can begin to understand how diversification rates fluctuated over the history of a clade (Ricklefs, 2007) and develop evolutionary hypotheses on why these fluctuations occurred.

To study general properties of phylogenetic trees, a model of the branching process is used. Several models are available, but the birth-death model remains the most widely

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used, and is easily interpreted (Nee, 2006). The birth-death model is a continuous-time Markov chain where at any given age ($\tau$) we can calculate the probability of speciation (birth of a lineage) or extinction (death of a lineage) happening. The stochastic nature of the model means that multiple simulations will result in different histories of diversification. For stochastic models like the birth-death model we can calculate their expected value, either by averaging over multiple realisations (simulations) or by approximating it with a set of continuous equations, yielding a deterministic model. Such a model directly yields the expected value one would get by averaging over infinitely many realisations, thus it is deterministic because it is fully defined by the parameters, that is, no uncertainty from stochasticity is involved. This latter approach is taken by LP who model the birth-death process as a set of differential equations, which is advantageous because these equations can be solved analytically.

LP refer to an LTT generated by such models as a deterministic LTT or dLTT, which corresponds here to the expected LTT generated by trees with given speciation and extinction rates. Empirical LTTs generated using extant timetrees can be compared to model-generated dLTTs (where $\lambda$ and $\mu$ are known) to disentangle, on average, how speciation and extinction have influenced patterns of diversity over time. To do this, the probability of the data given the model, or the likelihood, is calculated and compared across different models to select the one that fits best. Importantly, LP showed that, when $\lambda$ and $\mu$ are shared across the tree, the likelihood can be fully written as a function of the observed LTT and the dLTT. Typically, as the parameters of the models are modified, the likelihood will change, the model becoming more or less likely. The best-fitting model can then be selected, representing our best hypothesis for how and to what extent speciation and extinction rates varied over time.

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### *Model congruence and congruence classes*

One might expect that every dLTT is represented by a single model - a single set of histories or continuous functions for $\lambda(t)$ and $\mu(t)$ - but LP show that this is not the case. Instead, different models can produce the same dLTT. These models may have very different patterns of speciation and extinction over time, yet, if different models produce the same dLTT then they will also share the same likelihood for any given LTT. This makes it impossible to choose the model that is the best representative of the evolutionary history of the clade in question. Models that generate the same dLTT fall into the same "congruence class". These congruence classes contain an infinite number of models with different parameter values that all produce the same dLTT. LP explain that when trying to select the best model we often start with a relatively small set of allowed models that we test. An example of such a difference is whether speciation rate is fixed, or allowed to vary over time. LP suggest that instead of selecting the model closest to the true process, we are instead selecting the model closest to the congruence class that includes the true process (see Fig. 3 in LP). In extreme cases, the best fitting model could thus be further from the true process than a model with a lower likelihood, just because the former is included in the congruence class and the latter is not. However, LP concede that because we only assess a limited set of models, it is unlikely that we encounter models belonging to the same congruence class, but it is nevertheless possible. The consequence of multiple, equally likely models with different speciation and extinction rates is that these rates cannot be determined. This is a statistical phenomenon known as unidentifiability - the likelihood is the same for multiple parameter values making it impossible to choose one over another.

### *Unidentifiability*

In macroevolutionary modelling we might be interested to know how both $\lambda$ and $\mu$ have changed over time (Alfaro et al., 2009). However, the unidentifiability issue outlined above means that we would not be able to ascertain the true parameter values of the

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models that generate our dLTTs. Another well-known example of this in macroevolution is the unidentifiability of $\alpha$ and $\theta$ from Ornstein-Uhlenbeck models of trait evolution (Ho and Ané, 2014). This problem is not unique to macroevolutionary models, and, in fact, stems from a basic mathematical issue Rannala (2002); Ponciano et al. (2012).

Consider a simple example of the concept in which we want to determine the parameter values for $x$ and $y$. For each value of $x$ in equation 0.1 below, we can find a $y$ that satisfies this equation - and there are an infinite number of equally likely possibilities. It is only when we add more information (in the form of equation 0.2) that we can determine the unique pair of values for $x$ and $y$. Put simply, a solution can be found only if you possess at least the same number of equations as unknowns. In this case the unidentifiability is caused by overparameterization - there is an excess of parameters such that the model cannot estimate the values of any of them.

$$2x + y = 7 \tag{0.1}$$

$$3x + 2y = 12 \tag{0.2}$$

Though the LTT is generated through the use of many different observations and elements (DNA, fossils for time-calibration, extant species sampling) it is represented by a single curve made up of one observation at any given point in time that represents the number of lineages in a clade (Fig. 1). Fitting a model to an LTT is like fitting two parameters ($\lambda$ and $\mu$) for the slope ($\lambda - \mu$), which gives you only one value. This problem has been highlighted previously (Nee, 2006), where $\lambda - \mu$ is the net diversification rate. If we try to estimate $\lambda$ and $\mu$ separately we find it very difficult (Fig. 2a,b) but we are much more precise when estimating $\lambda - \mu$ (Fig. 2c). Estimates of $\lambda$ and $\mu$ are highly correlated (see MCMC analyses, Fig. 2d) and we find a flat surface in the likelihood where different pairs of values for $\lambda$ and $\mu$ are equally likely (Fig. 2e), signifying unidentifiability.

This issue of unidentifiability means that for any $\lambda$ there exists a $\mu$ that yields the

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same dLTT. However, as LP show, if the rates vary over time, it is possible to choose almost any function for $\lambda(t)$, and obtain a function $\mu(t)$ that produces the same dLTT. So, LP go beyond the classical unidentifiability issues for the parameters of a given model (as in the example above) and extend it to the space of models itself. For example, using a very large angiosperm phylogenetic tree (Smith and Brown, 2018), LP showed that the observed LTT is congruent with two opposing scenarios (Fig. 2 in LP): either a continuous increase or a continuous decline in both speciation and extinction rates through time (though the resulting diversification rates of these two scenarios are very similar). Therefore, if we observe a rapid increase in the number of lineages in our LTT (assuming complete sampling) we cannot determine if it was caused by a decrease in extinction rate, or an increase in speciation rate. If we want to use models to explain LTTs then our variables are inadequate and we must look towards other possible solutions.

### *Pulled rates and their interpretation*

A consequence of using extant timetrees is that LTT plots will likely underestimate the number of lineages at any given time because our trees are missing species (Silvestro et al., 2018). Species can be missing for two reasons: (1) they went extinct or (2) they were not sampled. However, these two factors will have differing effects on the LTT and our estimates of diversification rates. The idea that extinction can affect estimates of diversity when using data from only extant species may seem counter-intuitive, but a marked effect has been shown (Nee, 2006). Extinction must occur in the past. Lineages that originated recently have had less time to go extinct (Nee et al., 1994; Ricklefs, 2007), so the effect of extinction on our estimates using only extant species is reduced towards the present. This leads to an increase in the rate of lineage accumulation towards the present, as the effect of extinction decreases, which occurs even when rates are constant, as seen in Figure 3f. Conversely, incomplete sampling of a group occurs up to the present day and more strongly affects the recent history (Phillimore and Price, 2008), as the deeper nodes in the

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phylogeny can be reconstructed with only a few species. The relative importance of extinction and sampling completeness will influence whether the PSR departs from $\lambda$ more in the past or in the present. In summary, the presence of extinction will cause us to underestimate speciation rate further in the past, because the number of extinct species increases as we consider more time, while incomplete sampling will lead to underestimates of speciation rates that are more recent (Ricklefs, 2007).

LP bring these two ideas together and define the 'pulled' speciation rate (PSR or $\lambda_p$) i.e. the speciation rate modified by extinction and sampling fraction. At a given time, PSR is the estimated speciation rate multiplied by 1 minus the probability that a lineage is missing from the tree due to extinction or incomplete sampling, $E$. We will not go into details regarding the calculation of $E$ here, but further information can be found in supplementary materials of LP. PSR is shown in the following equation

$$\lambda_p(\tau) = \lambda(\tau)(1 - E(\tau)) \tag{0.3}$$

So, if all species are in the tree and there is no extinction (i.e. the probability of missing lineages, or $E$, is 0) then the PSR is equal to the (un-pulled) speciation rate. Any increase in extinction rate or the number of unsampled lineages (i.e. $E > 0$) will cause PSR to drop, or be 'pulled', below speciation rate (Figs. 2,4). The lower the extinction rate and the higher the sampling fraction, the closer the estimates of PSRs will be to speciation rate. The effect of a shift in speciation rate on PSR is demonstrated in Figure 4.

Similarly, LP also present pulled diversification rate (PDR or $r_p$). This parameter is similar to the net diversification rate ($\lambda - \mu$) but, as above, is modified by another term. This new term is the relative ($\frac{1}{\lambda}$) rate of change in speciation rate over time ($\frac{d\lambda}{d\tau}$). This causes the pulled diversification rate to lag behind the unpulled rate. The PDR can be represented by the following equation :

$$r_p = \lambda - \mu + \frac{1}{\lambda} \cdot \frac{d\lambda}{d\tau} \tag{0.4}$$

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The perspective in which time is viewed is critical when interpreting PDR. When reading time backwards (using $\tau$) diversification decreases from present ($\tau = 0$)to the past and PDR does so faster than expected (plus sign in equation (0.4)), going "too far" before stabilizing to the ancestral value. The first part of this equation is the net diversification rate, $\lambda - \mu$, which is thus corrected by a 'pull' corresponding to the rate of change of the speciation rate. The 'pull' of PDR is actually a delay in the response of this parameter when compared to diversification rate. This is in contrast to the 'pull' of PSR, which refers to a reduction in the estimated value of PSR relative to $\lambda$. This has some unexpected consequences if speciation rate rapidly increases as the PDR will rapidly decrease before starting to increase (Fig. 5a) as speciation rate stabilises. We note that LP also defined a pulled extinction rate, (PER or $\mu_p$), which is similar to PDR and we do not go into details about its calculation here (see LP, Louca et al. (2018) for further details).

The difference between the true diversification rate and an estimated PDR can be likened to a race between an amateur and a professional race car driver. The professional driver, representing the true diversification rate in our analogy, hits the apex of each corner, going smoothly around a racetrack until the finish line. The amateur, representing PDR, will eventually arrive at the finish line, but may exceed track limits a few times when doing so because of their poor reactions. However, if the track is simply a straight line both will perform equally well. This is because the PDR is equal to the diversification rate ($r = \lambda - \mu$) whenever $\lambda$ is constant in time ($\frac{d\lambda}{d\tau} = 0$), but differs from $r$ when $\lambda$ varies with time (see Technical considerations below for more details).

LP show mathematically that there is only one of each pulled rates (PSR, PDR, PER) per congruence class. These pulled rates are useful because a single congruence class can be represented by a single set of pulled rates, rather than the infinite number of possibilities of speciation and extinction rates. This is because pulled rates are compound parameters that are, as shown in Louca et al. (2018), constructed from the LTT, using its slope and the rate of change of the slope (curvature). LP show that these are related to $\lambda$

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and $\mu$: each pulled rate depends on both speciation and extincton. The shape of the dLTT plot is fully determined by any two of the rates, and the remaining rate can be calculated from the other two.

With these new variables we can begin to ask questions such as: has diversification been constant over time? Pulled rates can be estimated using many commonly used models of diversification (Louca and Pennell, 2020). The PSR is the speciation rate one would get by constraining extinction to be 0 and assuming complete species sampling. For PDR this involves making the speciation rate time-independent (i.e. speciation rate is constant over time). In summary, PSR provides information about how speciation rate changes over time while taking into account past extinction and the proportion of lineages sampled. PDR provides a slightly delayed estimate of $r$ with extreme responses to rapid changes in $\lambda$. While the PSR can be very different from the underlying speciation and extinction rates, the PDR is close to the net diversification rate as long as speciation rate does not change too rapidly. A scenario with multiple, extremely rapid changes over a short time scale (e.g. Fig. 5d) may be biologically possible, but from an empirical point of view one would not fit models with many changes in small time intervals.

## Technical considerations

### *How continuously can speciation and extinction rates vary?*

In their approach LP consider speciation and extinction to be a continuous property of a species, which can have instantaneous values at any time. When speciation and extinction are modelled as continuous processes, a change in the rates is immediately visible in the dLTT plot. In real trees, however, we can only observe discrete events: either a branch splits, goes extinct, or it continues. When working with a large phylogenetic tree and many species, the LTT is smooth and the slope and curvature, which are necessary for the estimation of the pulled rates, can be reliably estimated. However, many studies attempt to estimate diversification rates with relatively small numbers of species (e.g.

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<1000). When the tree used is small, the LTT plot resembles an irregular staircase, and although one can calculate the slope by using sufficiently large time intervals, the calculation of the curvature will be less reliable. So, one should not forget that allowing for continuous rate variation is less applicable in those cases where the number of species considered is small. The consequence of this is that rate variation can be difficult to detect using birth-death models, particularly in small phylogenetic trees (Condamine et al., 2019).

In practice (with a limited number of species), what precision is reasonable to aim for in the characterization of variation in diversification rate? Considering step-wise changes, a straightforward limit is the total number of speciation events in a tree with $n$ extant species, which is in $n-1$ speciation events. Above this limit some changes will not be detectable because the number of parameters will exceed the number of observations. Another limit concerns the speed of change, which is applicable even if the number of parameters is limited. For example, consider sinusoidal variations with frequency $f$, where only one parameter is to be inferred. Noting that the number of speciation events per unit time is $\lambda n$, this sets an upper limit to $f$ that can be detectable with the data. This also shows that rapid changes are more difficult to infer as $n$ decreases and highlights the limitations of inferring pulled rates (or any rates inferred using continuous approximation) with small phylogenetic trees. A reasonable approach is thus to consider relatively simple, but biologically meaningful, scenarios without implausibly rapid rate variations. If so, $r_p$ will be (often very) close to $r$.

*The delay in PDR is a result of the lag time between extinction and speciation*

Consider a simple case with no extinction ($\mu = 0$) so that changes in $r$ that only come from changes in $\lambda$. If so, $r = \lambda$ but $r_p$ is not exactly $\lambda$ because of temporal variations in $\lambda$ (the term $\frac{1}{\lambda} \cdot \frac{d\lambda}{d\tau}$ in equation (0.4)). LP suggest that "*the pulled diversification rate can be interpreted as the effective net diversification rate if $\lambda$ was time-independent*". In our example, this means replacing a scenario where $\mu$ is constant (at 0) and $\lambda$ varies with a

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scenario where $\lambda$ is constant and $\mu$ varies. LP explain this from the point of view of holding speciation rate constant and using extinction rate to account for changes in diversification rate. To get the same changes in the LTT using changes in $\mu$ instead of changes in $\lambda$ (which is kept constant by construct), a delay is needed to wait for species to arise, before they can go extinct. The difficulty with this, as initially noted by Nee et al. (1992), is that there is a slight delay between the effect of speciation and the effect of extinction.

As mentioned previously, lineages that originated more recently have had less time to go extinct. In a constant birth-death process, this is only visible in recent history: the slope of the LTT is $r = \lambda - \mu$ during most of the past but increases to $\lambda$ for very recent times where the stationary behaviour has not yet been reached. However, this phenomenon is not unique to very recent times - it will also occur whenever there is a change in speciation rate. Ultimately, this is the cause of the difference between PDR and $r$. For example, a massive increase in the number of lineages caused by a burst of speciation means that many new lineages become available to go extinct over a short time period. However, the rate of extinction is still determined by how many lineages there were prior to the burst. As time continues, these numerous new species will begin to go extinct, meaning that extinction rate will increase to "catch up" to speciation rate and reach a new stationary point. This effect is stronger when $\lambda$ varies rapidly (i.e. high $\frac{1}{\lambda} \cdot \frac{d\lambda}{d\tau}$). Conversely, speciation cannot occur in a lineage after it has gone extinct, so there is no similar lag caused by changes in extinction rate. This is also why variation in extinction rate would not cause PDR to deviate from $r$ (Fig. 5b).

## Discussion

A recent study by Morlon et al. (2020) presents an alternative point of view that opposes the conclusions in LP. They focus on how a hypothesis-based framework allows us to overcome many of the issues that are raised in LP. Indeed, we are limiting our set of models to be tested to only those that represent our hypotheses about the factors shaping

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diversification in a given group. We are not often interested in determining the precise values of speciation and extinction rate but rather how different diversification scenarios summarised by models containing $\lambda$ and $\mu$ explain patterns in a phylogenetic tree. The criticisms put forward by Morlon et al. (2020) will stimulate important discussion about key points that must be considered when using diversification models. We extend this discussion by highlighting several key points that must be considered in addition.

### *Uses and limitations of LTTs*

LTTs are a simplistic way to visualize and summarise a time-calibrated phylogenetic tree, ignoring information related to branch lengths, tree topology and extinct species (Morlon et al., 2011). However, under the assumption of $\lambda$ and $\mu$ being shared across all species LP showed that the LTT contains the complete information about the underlying branching process (See also Lambert and Stadler (2013)). This simplicity provided the opportunity for LP to show mathematically how LTTs can lead to misinterpretation. However, these issues are not new to macroevolutionary biology. A review by Nee (2006) clearly demonstrated how an LTT may change when extinction is present alongside speciation (birth-death), as opposed to speciation alone (pure-birth), summarising theory from previous work (Nee et al., 1992, 1994; Harvey et al., 1994). If the growth of an extant timetree is represented as an LTT on a semi-log scale (i.e. lineage number is logarithmic, time is not, see Fig. 1) we would expect the trend to be linear under a pure birth process (with constant speciation and no extinction). If extinction is introduced then the LTT would deviate from this linearity. When both rates are constant and greater than 0, the curve is expected to be linear over most of its history, but as time reaches the present the rate of lineage accumulation will increase (i.e. the LTT slope will become steeper), as shown in Figure 3a. With no prior knowledge of the parameters, this could be because of increasing speciation rate towards the present (Fig. 3b), instead of decreasing effect of extinction (Fig. 3). It is important to keep in mind that we are dealing

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with a phylogenetic tree made up of entirely extant species. The unobserved branches of species that went extinct (and are therefore not in the extant timetree) do not contribute to the LTT, making the estimated lineage accumulation rate lower in the past (or 'pulling' it down). Nee et al. (1994) highlighted this issue 20 years ago in the context of models where diversification rates were constant over time and now LP have provided an important extension of this idea to models that allow for rates to vary through time. The well-known limitations of LTTs for inferring speciation and extinction rates have continued to be addressed in other studies (Ricklefs, 2007; Vamosi et al., 2018; Rabosky and Lovette, 2008; Crisp and Cook, 2009) since Nee et al. (1994), most recently in LP. This begs the question: what other previously proposed ideas remain hidden in the literature that could be useful to macroevolutionary modelling?

### *Diversification rates vary among clades*

The conclusions of LP imply that we can test hypotheses about whether diversification rates deviate from constancy over time using pulled rates. We would be unable to pin this on changes in speciation or extinction rate, but would get a sense of how variable diversification has been. This would be useful for testing whether diversification in particular clades has remained constant or been subject to large shifts in diversification (e.g. mass extinctions) but not when diversification rate has shifted in a subclade (e.g. due to the evolution of a key innovation). The first use of pulled rates was in Louca et al. (2018), where they studied bacterial diversification, stating "*Our findings suggest that, during the past 1 billion years, global bacterial speciation and extinction rates were not substantially affected during the mass extinction events seen in eukaryotic fossil records.*" This might suggest that nothing particularly extraordinary happened in the macroevolutionary dynamics of bacteria in the last billion years. However, it is important to note that the models used in Louca et al. (2018) (and Louca and Pennell (2020)) do not allow rates to vary among clades. The rates estimated using such clade-homogeneous

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models will correspond to the average rates over time in the entire study group, therefore missing out on any variation among clades - for example any difference in diversification rates between those species that use terrestrial vs marine environments (Louca et al., 2018). Given the importance of subclades in driving inferred diversification patterns (see (Morlon et al., 2011; Rabosky, 2020)), this may mean that we miss out on important and interesting dynamics when using pulled rates. Louca et al. (2018) touch on this point themselves: "*It is possible that diversification within individual bacterial clades may have been influenced by eukaryotic radiations and extinctions, and that these cases are overshadowed when considering all bacteria together.*" Given the diversity of life on Earth, it is unrealistic to assume that major events would have had the same effect on all lineages of a large, cosmopolitan clade, with vast amounts of genetic, morphological and ecological variation. The same criticism could be levelled at LP's use of a large phylogenetic tree of angiosperms (Smith and Brown, 2018) that contains more than 65,000 of the roughly 300,000 known species, ranging from small ephemeral plants like *Arabidopsis thaliana* to gigantic, long-lived trees such as *Eucalyptus regnans*. Furthermore, a large amount of research has shown that diversification rates have varied significantly among flowering-plant clades (e.g. (O'Meara et al., 2016; Igea et al., 2017; Vamosi et al., 2018; Onstein, 2019; Soltis et al., 2019; Zenil-Ferguson et al., 2019; Magallón et al., 2019)).

Fortunately, the assumption of homogeneous rates among clades is not common in modern approaches. For instance, Bayesian Analysis of Macroevolutionary Mixtures (BAMM) (Rabosky, 2014) is one of several methods (Alfaro et al., 2009; Morlon et al., 2016; Höhna et al., 2016a; Maliet et al., 2019; Barido-Sottani et al., 2020) that relaxes the assumption that all lineages share the same evolutionary rates at a given point in time (Rabosky, 2017). This is a key difference from the models used by LP because it allows lineages to differ in their rates of speciation and extinction. With BAMM, the entire phylogeny could be described using a model similar to what is used in Louca and Pennell (2020), or alternatively, it could be described using multiple processes that explain rates of

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diversification on different parts of the tree. These non-homogeneous diversification rates may help to alleviate the unidentifiability issues demonstrated by LP by taking into account additional information included in tree topology and branch lengths.

Another model commonly used to estimate and compare diversification rates among clades is the Binary-State Speciation and Extinction (BiSSE) model (Maddison et al., 2007), part of a family of models known as the state-dependent models of diversification (-SSE models (Ng and Smith, 2014; O'Meara and Beaulieu, 2016; Beaulieu and O'Meara, 2016; Caetano et al., 2018)). These models are extensions of the birth-death model that also include information about character states of extant species. They estimate ancestral states at each node of the phylogenetic tree, as well as rates of transition between character states. LP state that the likelihood functions of SSE models are too complex to be addressed in their manuscript, but suggest that the same problems they uncover probably still apply. The increased complexity of likelihood calculations LP refer to is because BiSSE makes use of the full tree topology (Maddison et al., 2007), rather than just the timing of branching events as in the LTT (Nee et al., 1994). LP further suggest that it remains unclear how the dependence on character states (which, if removed, collapses equations in BiSSE to those shown in Nee et al. (1994)) affects the unidentifiability issue they raise. In the original BiSSE paper (Maddison et al., 2007), two important and relevant assumptions were made:

1. Sampling fraction is assumed to be 100%
2. Speciation, extinction and transition rate are constant per character state.

These may allow the BiSSE model to overcome (or pre-empt) some of the problems raised by LP. LP show that $\lambda$ equals PSR when sampling fraction is 100% and $\mu = 0$. The first of these was assumed in the original BiSSE model, though it has since been relaxed (FitzJohn et al., 2009). Extinction can easily be set to 0 in these models, which satisfies the second BiSSE assumption and allows estimation of $\lambda$. Similarly, PDR equals $r$ when $\lambda$ is constant, also an assumption in BiSSE. As a result, it appears that BiSSE models

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estimate rates of per-character state diversification that are similar or even analogous to pulled rates and may therefore be identifiable. Researchers using SSE models have noticed that estimated speciation and extinction rates are often correlated within states and therefore usually report net diversification rates. They also are often aware that the diversification rate might vary over time, and that the whole diversification history cannot be entirely captured in one value (or two values). LP's results suggest, however, that this is a rather robust approach: as the time-dependent variation in speciation and extinction rates cannot be uncovered reliably, one should consider the estimated diversification rate as a "pulled" rate. Nevertheless, we stress that the likelihood of time-dependent diversification models (as in LP) is not the same as the likelihood of state-dependent diversification models (-SSE models) and what is unidentifiable in the former does not say anything about identifiability in the latter.

It is unclear how lineage-dependent rates would affect model congruence, and how the additional information included when using models such as BiSSE and BAMM would affect the unidentifiability issues. However, what is clear is that the issues raised in LP cannot be readily applied to commonly used macroevolutionary approaches without further work to show that criticisms related to LTT-based approaches are applicable to these more complex models. Alternatively, even if unidentifiability issues remain in such models they may not be relevant in the questions the models were built to answer, for example those models that test for variation in diversification rates in association with particular clades or traits. In cases like these, it is not the precise values of rates that are important but instead whether rates in one group of lineages are higher than another.

Perhaps most importantly, this means that we should not forego building models that estimate diversification rates because one, simplistic approach has problems, but instead continue to improve them and build upon the work done in LP. A case in point is the issue of null model choice when using SSE models raised by Rabosky and Goldberg (2015). This criticism spurred on innovation that led to the development of models with

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hidden states (Beaulieu and O'Meara, 2016), which are now present in various new incarnations, e.g. (Caetano et al., 2018; Herrera-Alsina et al., 2019), of the SSE approach.

### *Pulled rates are difficult to interpret*

LP compared the usefulness of pulled rates to effective population size ($N_e$) in population genetics. Like $N_e$, diversification rates and pulled diversification rates are not real - they do not exist in nature. Instead their role is to help us interpret complex processes like diversification, and if they do not do this, then they lack utility. $N_e$ can be broadly defined as the number of breeding individuals in an idealised population (e.g. constant size, random mating) that would be able to explain the summary statistics in an observed population. $N_e$ is fairly intuitive and will react to biological phenomena in expected ways (e.g. under population structure (Whitlock and Barton, 1997) or non-random mating (Caballero and Hill, 1992)).

LP state that the variables they introduce are "easily interpretable". Their terminology, however, is not completely consistent nor coherent with more traditional uses, which can cause confusion. Given that $r = \lambda - \mu$ one might intuitively think that $r_p = \lambda_p - \mu_p$ but this is not the case - pulled rates are simply different ways of summarising congruence classes and each one is calculated using both speciation and extinction rates. PSR is reasonably intuitive, though given that extinction is also included it is more similar to a diversification rate than a speciation rate. Indeed, the PSR is defined as the slope of the LTT plot (Louca et al., 2018) (see Fig. 3f, 4f), which corresponds to the diversification rate in times far enough before present, and, in the case all extant species are included, to the speciation rate at present (Nee et al., 1992).

PDR, however, is much more difficult to interpret, perhaps initially because the 'pull' of PSR is not the same as the 'pull' of PDR. Whereas PSR decreases in value relative to $\lambda$, PDR is delayed in time relative to $r$ (Fig. 5) and could better be termed as 'delayed' rather than 'pulled'. We simulated a variety of diversification scenarios from

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simple to more complex (Fig. 5) and show that PDR and $r$ are similar in each case. However, PDR is not as intuitive as $r$ or $N_e$ - for example, drastic increases in $r$ can lead to sharp decreases in PDR (Fig. 5a). The inverted pattern PDR presents in this case would make it challenging to present in a clear and concise way. Given the added difficulty of its interpretation we question whether PDR provides us with a more useful estimate of the process of diversification than an estimate of $r$.

However, compared to other pulled rates, PDR could be especially useful, not as an effective parameter, like $N_e$, but as a reasonable approximation of the true $r$. Indeed, we noted above that when shifts in $\lambda$ are not too strong nor too rapid, PDR is close to $r$ (Fig. 4). Under non-parametric scenarios, trying to biologically interpret fine-grain variations in PDR would certainly lead to spurious conclusions. However, changes in PDR at a large scale are good proxies for large scale variation in $r$. This is clearly illustrated in Figure 4a where the main trend of the PDR is a recent increase in diversification, and in Figure 4d where the main trend is the stability of diversification.

Pulled rates can be estimated using only the shape of the LTT plot, without any further information, i.e. they are non-parametric estimates that do not suffer from the unidentifiability problems outlined previously. However, they cannot be directly interpreted in biologically meaningful terms; to estimate rates that are meaningful (e.g. $\lambda$, $\mu$ & $r$), one needs to make further assumptions such as constant rates of speciation and extinction over time.

### *On the use of models*

The discussion sparked by Louca and Pennell (2020) highlights an important issue: evolutionary biologists should be interested in the actual history of diversification of the clades they study, and the framework developed by Louca et al. (2018) shows how to do this using the shape of the LTT plot, without making strong assumptions about past speciation and extinction rates. This is classical knowledge, as we have argued; the slope of

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the LTT plot and variations therein contain information about the diversification history of the clade. Much of the debate, however, focuses on the ability to recover a "true" history of diversification. Indeed, the goal of a scientific study should be to find out what really happened, but it becomes confusing if one considers a simulated birth-death process as the "true" history. This birth-death process is determined by two parameters ($\lambda$ and $\mu$) that can vary over time. These parameters are supposed to correspond to the rate that a lineage splits into two lineages, or goes extinct. In reality, however, a species doesn't have a speciation and an extinction rate in the same way it has a geographic distribution and a population size. These rates only make sense when they are averaged over a number of species and a certain amount of evolutionary time. That is, they are descriptive statistics summarizing much more complex processes that are playing at the level of species, and that would eventually lead to speciation or extinction. Louca and Pennell (2020) convincingly show that one cannot estimate these statistics reliably from LTT plots, and propose statistics that can be estimated more reliably. That these alternative statistics do not exactly correspond to the parameters of the naive birth-death process is not a problem; the birth-death process is only a model of diversification, and not the truth about diversification itself. The framework built by Louca et al. (2018) and LP allows us to use the LTT to test whether the diversification rate was constant or not. If a researcher wants to know how speciation and extinction actually changed to give rise to this diversification history, they will have to use other methods.

## Conclusion

Louca and Pennell (2020) have pointed out key issues with how we approach macroevolutionary modelling, namely the inability to distinguish historical diversification scenarios under certain circumstances. Their formalization of the unidentifiability issues in LTT-based models is an important step forward that provides us with the mathematical tools to study the associated issues further. LP highlights the avenues we must consider

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and develop upon to ensure we do not make similar mistakes in the future. Whether variations in diversification rate are due to changes in speciation or extinction is certainly an interesting avenue of research, but LP have shown that exploring this would require much more than just fitting a model with speciation and extinction rates to an LTT. Indeed, more recent diversification models go beyond this by making use of additional information that the LTT lacks. Awareness and consideration of potential unidentifiability issues is important for macroevolutionary biologists going forward when they employ such models of diversification. However, it is important to note that LP does not show that speciation and extinction cannot be estimated with evolutionary trees (Pagel, 2020). Instead, they show that when using extant timetrees with a single, LTT-based approach, unidentifiability issues are encountered in the estimation of speciation and extinction rates, and that these problems can be circumvented by making use of pulled rates. Further work is needed to identify the extent to which the issues raised in LP apply to the more complex models of diversification used today. In the meantime it is important that the field continues to grow by using and building upon modern macroevolutionary methods, albeit with a critical eye.

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## Supplementary Material

Code associated with this manuscript is available from `http:/github.com/ajhelmstetter/pulled_rates`

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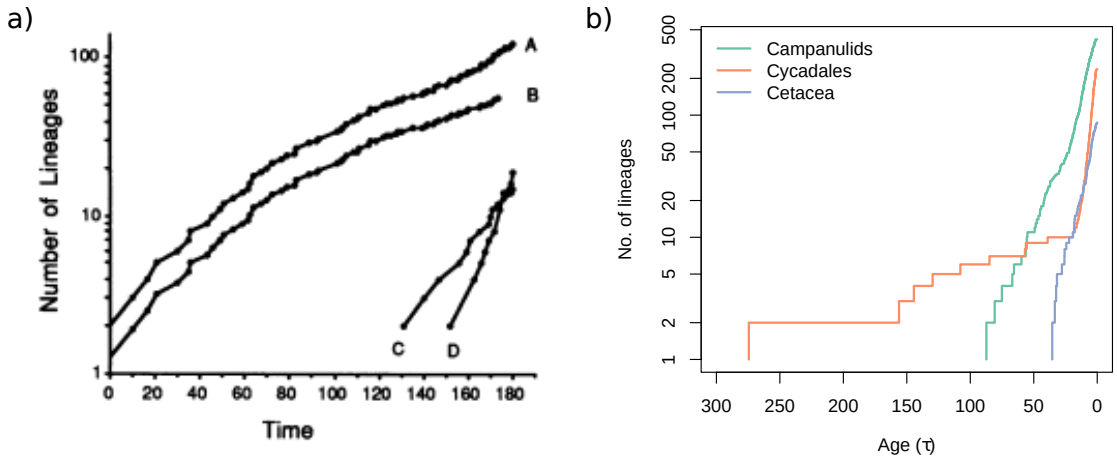


Fig. 1. (a) The first example of a lineage through time plot (LTT), taken from Nee et al. (1992) and based on a phylogenetic tree of birds. On the y-axis is the number of lineages (log scale) and the x-axis is time since origin (present on the right hand side of the graph). *"Each point corresponds to a change in the number of lineages. Line A, the pattern of origination of all 122 lineages; line B, same as A, but without the Passeri (line C) and the Ciconliformes (line D). Line B has been shifted downward to aid visual comparison. The diversification rate is quantified by the steepness of the slope."* In this panel, time is displayed going forward, from past to present as time since origin ($t$). (b) Three LTTs from modern phylogenetic trees of Campanulids (Beaulieu and Donoghue, 2013), Cycadales (Condamine et al., 2015) and Cetacea (Slater et al., 2010). In this panel, time is read backwards, from present to past as an age ($\tau$).

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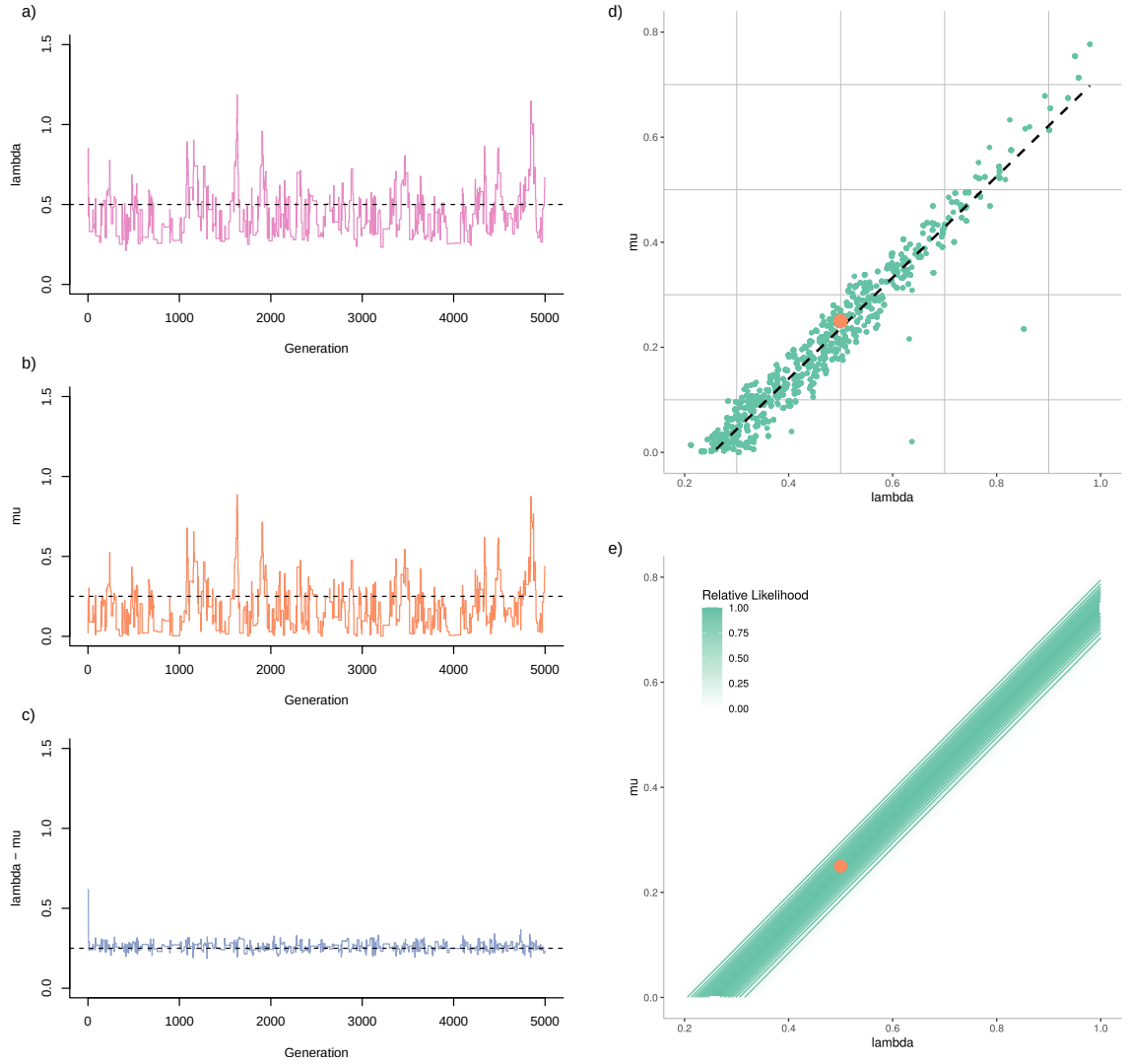


Fig. 2. An example of unidentifiability issues encountered when trying to estimate values of two parameters ($\lambda$ & $\mu$) for the slope $\lambda - \mu$. We used a Bayesian Monte-Carlo Markov Chain approach to try to estimate the values of $\lambda$ and $\mu$ separately. We ran a chain for 5000 generations, sampling each generation. The traces for (a) $\lambda$ and (b) $\mu$ show a great deal of uncertainty in the parameter estimates compared to the estimates for (c) $\lambda - \mu$. True values are shown as black dashed lines in panels (a-c) and as orange circles in panels (d-e). We plotted $\lambda$ against $\mu$ and found that they two parameters were highly correlated (d). When then calculated the relative likelihood over a range of parameters values and found a flat ridge in the likelihood where different pairs of values for $\lambda$ & $\mu$ are equally likely - or unidentifiability (e).

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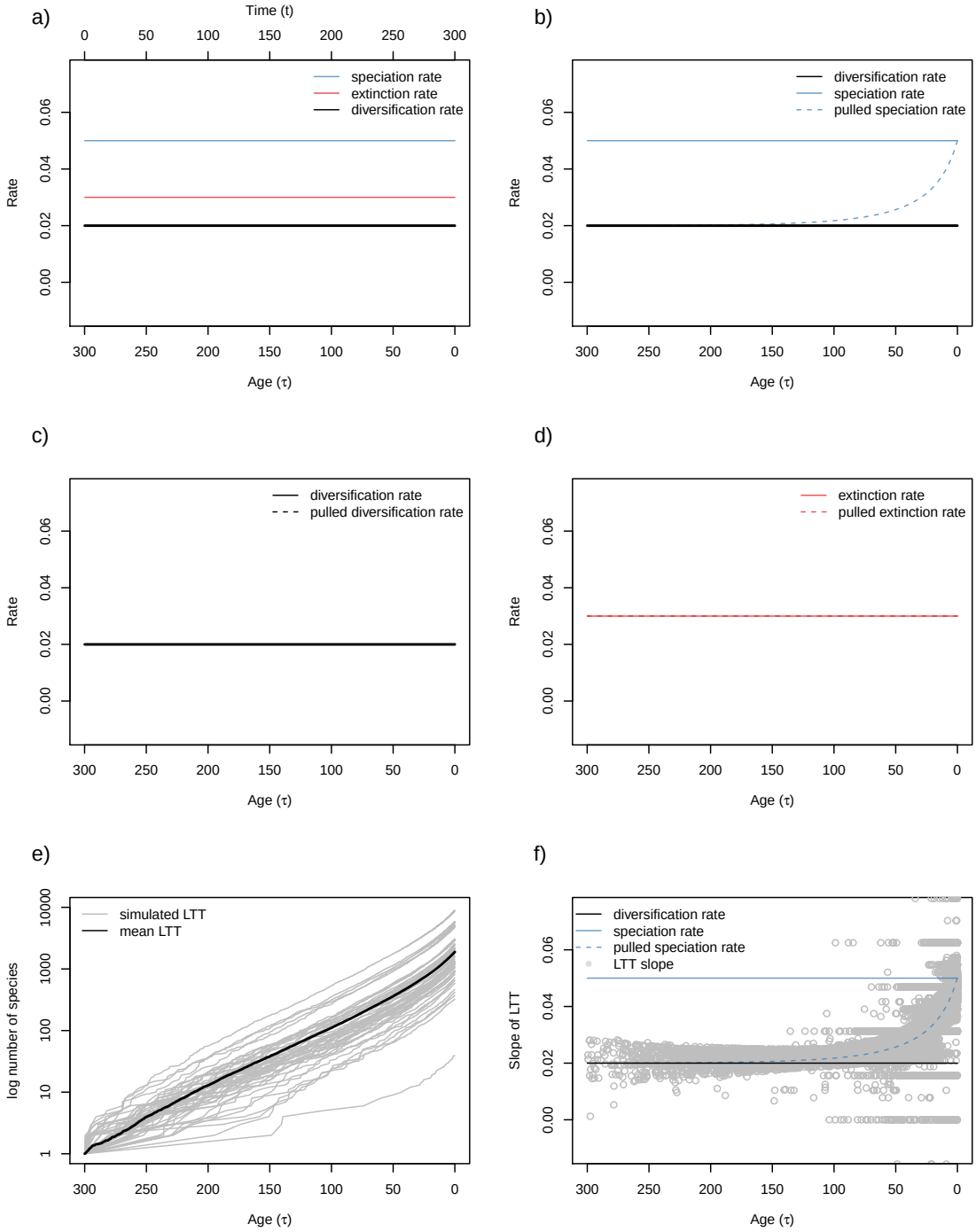


Fig. 3. A simple example of the relationship between constant diversification rates and corresponding pulled rates. Panel (a) shows values of speciation rate, extinction rate and diversification rate over time. An additional axis, at the top of panel (a) shows time going forward (t). Panel (b) shows how in the past, pulled speciation rate (PSR) is identical to the diversification rate (if sampling fraction = 1) while closer to the present PSR approaches speciation rate. The following two panels compare (c) $r$ & pulled diversification rate (PDR) and (d) compares $\mu$ & pulled extinction rate (PER). In these two cases the pulled rates are identical to the un-unpulled rates. Panel (e) shows 50 LTT plots (grey lines) simulated with the parameters used in panels (a-d) and the mean LTT (black line). Panel (f) shows the slopes of the LTTs in panel (e) over time, matching PSR and depicting the expected increase towards the present caused by the lack of effect of extinction - species do not have enough time to go extinct towards the present. An interactive version of this plot, in which parameters can be modified, can be found at https://ajhelmstetter.shinyapps.io/pulled_rates/.

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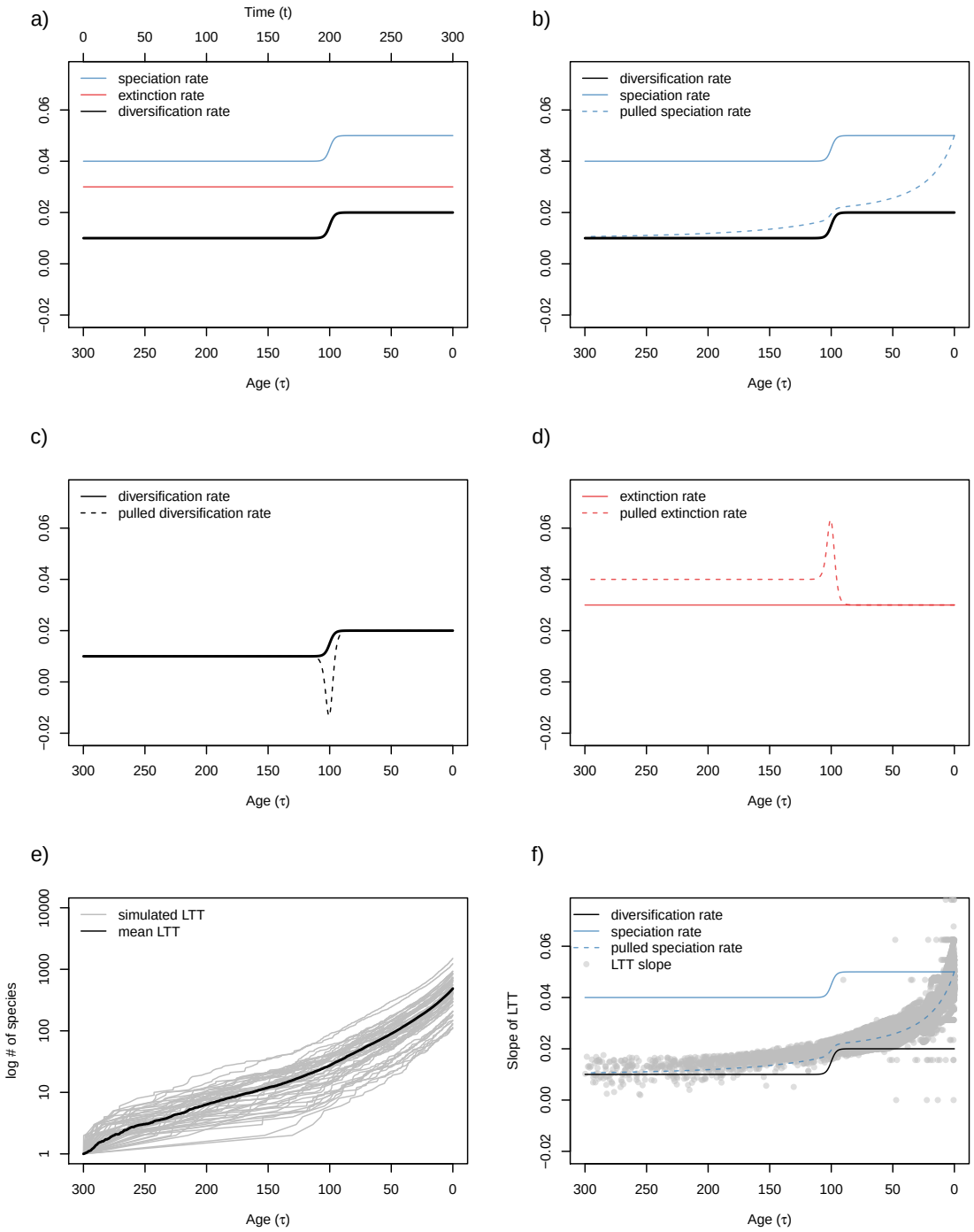


Fig. 4. A slightly more complex example of the relationship between constant diversification rates and corresponding pulled rates where a single shift - an increase in speciation rate - has taken place. Panel (a) shows values of speciation rate, extinction rate and diversification rate over time. An additional axis, at the top of panel (a) shows time going forward (t). Panel (b) shows the gradual change in PSR during the shift in speciation rate. Panel (c) compares $r$ and pulled diversification rate (PDR). The sudden increase in speciation rate causes PDR to decrease suddenly before recovering to the new diversification rate. Panel (d) compares extinction rate and pulled extinction rate (PER) and shows an inverse pattern to panel (c). Panel (e) shows 50 LTT plots (grey lines) simulated with the parameters used in panels (a-d) and the mean LTT (black line). Panel (f) shows the slopes of the LTTs in panel (e) over time, matching PSR and again depicting the expected increase towards the present caused by the lack of effect of extinction.

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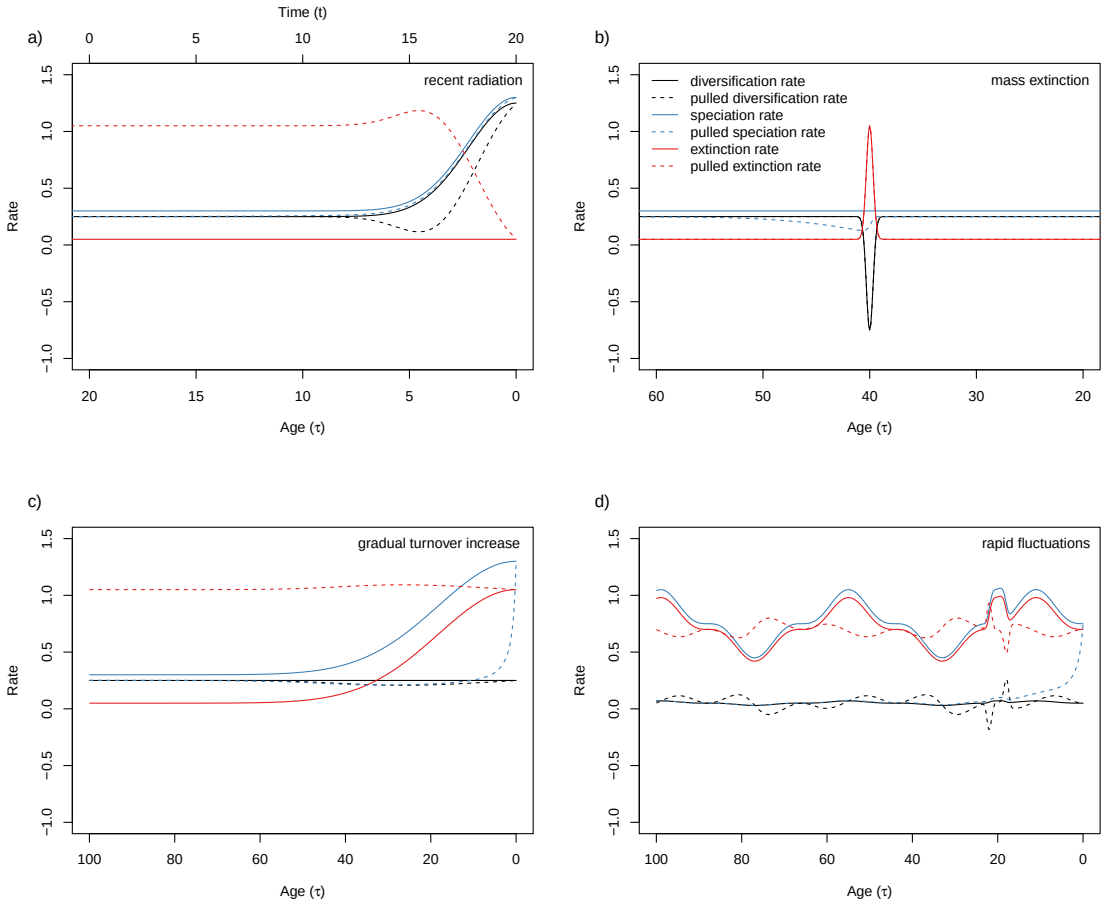


Fig. 5. Comparison of diversification rate and pulled diversification rate (PDR) under three simulated diversification scenarios that are commonly investigated (a-c) and a final, more complex scenario. Speciation rate, pulled speciation rate and extinction rate are also shown. Panel (a) shows a recent radiation where diversification rate and speciation rate sharply increase towards the present. An additional axis, at the top of panel (a) shows time going forward (t). Panel (b) shows a mass extinction event at 40 Ma in which extinction briefly but rapidly increases and then falls back to previous levels. Panel (c) shows a gradual increase in species turnover rate (both speciation and extinction increase slowly over time). Panel (d) shows a scenario where speciation and extinction rates are similar to each other but are in rapid fluctuation over time. This results in a relatively constant diversification rate and a rapidly fluctuating PDR that remains close to diversification rate ($r$).