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Chapter VI: Detecting the dependence of diversification on multiple traits from phylogenetic trees and trait data

Multiple traits and diversification

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Abstract

Species diversification may be determined by many different variables, including the traits of the diversifying lineages. The diversitree framework contains methods to detect the dependence of diversification on these traits. For the analysis of traits with multiple states, diversitree offers MuSSE (Multiple States-dependent Speciation and Extinction). However, MuSSE and other state-dependent speciation and extinction models have been shown to yield false positives, because they cannot separate differential diversification rates from dependence of diversification on the observed traits. The recently introduced method HiSSE (Hidden State-dependent Speciation and Extinction) resolves this problem by allowing a hidden state to affect diversification rates. Unfortunately, this framework does not allow traits with more than two states, and, perhaps more interestingly, the simultaneous action of multiple traits on diversification. Here, we introduce an R package (SecSSE: Several examined and concealed States-dependent Speciation and Extinction) that combines the features of HiSSE and MuSSE to simultaneously infer state-dependent diversification across two or more traits or states while accounting for the role of a possible concealed trait. Moreover, SecSSE also has improved functionality compared to its two 'parents'. First, it allows for an observed trait being in two or more states simultaneously, which is useful for example when a taxon is a generalist or when the exact state is not precisely known. Second, it provides the correct likelihood when conditioned on non-extinction, which has been incorrectly implemented in diversitree and HiSSE. To illustrate our method, we apply SecSSE to 7 previous studies that used MuSSE, and find that in 5 out of 7 cases, the conclusions drawn based on MuSSE were premature. We test with simulations whether SecSSE sacrifices statistical power to avoid the high type I error problem of MuSSE, but we find that this is not the case: for the majority of simulations where the observed traits affect diversification, SecSSE detects this.

KEYWORDS: macroevolution; evolving traits; speciation rates; phylogenetic tools

Introduction

Differences in rates of speciation and extinction might be associated with differences in the diversifying species' traits (strict-sense species selection, Jablonski 2008). Phylogenies, together with the trait distribution on the tips, contain information on this. To test whether a trait plays a role in species diversification, a suite of methods that merge phylogenetic and trait information has been developed. The State-dependent Speciation and Extinction (SSE) family of models (the 'diversitree' framework) assumes a model of trait evolution along the branches of a phylogeny and an effect of the state of a trait on speciation and extinction. For instance, for a trait that can take more than two categorical states, diversitree offers MuSSE (Multiple States-dependent Speciation and Extinction, Fitzjohn 2012). The procedure starts by assigning probabilities of each trait state based on the information on extant species' traits, and then goes root-ward, along the branches and nodes using the parameters (speciation, extinction and trait evolution rates) to compute the probability of states at the crown of the tree, which is the likelihood of the model given the phylogeny and tip trait data. The optimization of this computed likelihood allows us to find which parameter combination most likely explains the data.

These SSE methods generally look at variation in diversification rates across trait states and when applied to empirical datasets, a significant influence of trait states on speciation has often been found (e.g., Arbuckle and Speed 2015; Marki et al. 2015). However, Maddison and Fitzjohn (2014) noted that the SSE inference framework may be flawed because of pseudoreplication and codistribution of characters: phylogenetically related species are expected to be more similar in their traits, precisely because of their evolutionary relatedness, and not necessarily because their trait value causes a radiation. Rabosky and Goldberg (2015) confirmed that indeed the traditional SSE framework has a high type I error rate. If there is a shift in diversification rates, an SSE analysis will likely attribute it to the variation in trait states, even if the trait in question has little to do with this shift.

To resolve these problems, Rabosky and Huang (2016) developed a permutation approach where speciation rates for each lineage are estimated using only the reconstructed phylogenetic tree. Then, sets of species with similar rates are shuffled and the correlation with the trait values is calculated. Repeating this procedure several times produces a distribution of null correlations to which the empirical correlation is compared. In this approach, trait evolution is ignored. Recently, Rabosky and Goldberg (2017) proposed a nonparametric method which considers the difference between the averages of per-tip speciation rates associated with each state (this is their test statistic). Independently, the evolution of a neutral trait is simulated (given some model) on the empirical phylogeny. This assigns trait states to the species at the tips of the phylogeny. For each simulated dataset, the test statistic is calculated and the frequency distribution of these values is used for comparison with the observed test statistic in a statistical hypothesis-testing fashion. They found that their method reduces the Type I error rate, but its power to detect trait-dependent diversification is lower than BiSSE. Although such nonparametric approaches are valid to look for association between a given trait in extant species and rates of diversification, one might want

to jointly assess how a trait evolves over time and whether and to what extent this leads to the rise and fall of lineages. In line with the conceptual foundation of the SSE framework, Beaulieu and O’Meara (2016) proposed HiSSE (Hidden State-dependent Speciation and Extinction), a new method to avoid false positives. This method explicitly allows for the possibility that the process of diversification is not related to the focal trait, but rather to some unknown, hidden, trait. In other words, under a traditional SSE analysis, a clade with non-homogeneous diversification rates will be linked to the observed trait variation. In contrast, in HiSSE a hidden-state model may be selected when diversification is heterogeneous but this variation cannot be attributed to the focal trait. This does not mean that there is a single trait responsible for the observed branching patterns, but just that differences in diversification cannot solely be explained by variation in the observed trait. Beaulieu and O’Meara (2016) found that HiSSE is able to distinguish between models with and without an effect of the observed trait on diversification in most of their simulations.

While HiSSE represents a significant improvement in the toolbox for analysis of state-dependent diversification, it only allows for a single binary observed trait, not for traits with multiple states, or multiple traits. Furthermore, HiSSE requires full trait information for all tips in the tree, which may not always be available. Finally, HiSSE contains an error in the conditioning on survival, which is also present in the other models of diversitree. Here, we introduce SecSSE (Several examined and concealed States-dependent Speciation and Extinction) that combines the features of HiSSE and MuSSE to simultaneously infer state-dependent diversification across two or more traits or states while accounting for the role of a possible concealed trait. We provide an R package of the same name. It correctly conditions on survival but the incorrect conditioning is also available in the package for comparison. Furthermore, it can handle data with missing or partial trait information for some species. For instance, consider a species whose precise state is unknown but some knowledge exists so that some states are left out. This can be specified in SecSSE. This feature is not only useful for missing or partial data but it is also suitable for assigning taxa to more than one state simultaneously (e.g., polymorphic species). We then revisit seven studies where particular trait states were found to be linked to differential speciation rates using MuSSE. These studies were published before the problems with the SSE had been identified, and their findings have not yet been screened for false positives. Lastly, we rigorously assess the type I and II errors of SecSSE by simulations.

Materials and methods

SecSSE

SecSSE uses the same framework as HiSSE, but allows for multiple examined (observed) and multiple concealed (hidden) states. We simply extend the differential equations for D_{ij} and E_{ij} in the diversitree framework to all possible examined states i and concealed states j . This means that the user has to specify a speciation and extinction rate for all possible state combinations, and a

transition rate matrix for all possible transitions. For the latter we recommend that any structure in transition rates between examined states is also adopted in concealed states, for example, we advise to use the same number of concealed states as the number of examined states, and when certain transitions are not allowed between examined states, we recommend that transitions between concealed states are also forbidden. This is simply to reduce the otherwise very large number of parameters, and to avoid that conclusions are based on the presence or absence of this structure rather than on the influence of the examined or concealed states on diversification rates.

SecSSE differs from the diversitree models and HiSSE in the way conditioning on non-extinction of the crown lineages is computed. Diversitree/HiSSE calculates the D_i and E_i for every trait state i at the root. Then the unconditional likelihood L is computed as a weighted average over the D_i values:

$$L = \sum_i D_i w_i \quad (1)$$

where weights w_i are typically the (normalized) D_i themselves, i.e.,

$$w_i = \frac{D_i}{\sum_i D_i} \quad (2)$$

The likelihood conditioned on non-extinction of the crown lineages according to diversitree/HiSSE is obtained by dividing the unconditional likelihood, Eq. (1), by the weighted sum of non-extinction probabilities multiplied by the speciation rate at the root (because we are looking at a tree, Nee et al. 1994):

$$L = \frac{\sum_i D_i w_i}{\sum_i w_i \lambda_i (1 - E_i)^2} \quad (3)$$

In other words, the likelihood is first summed, with weights, over the various traits and then conditioned. However, we argue that the conditioning should occur first, and then the weighted sum over the various traits should be taken:

$$L = \sum_i \frac{D_i w_i}{\lambda_i (1 - E_i)^2} \quad (4)$$

This is because we first want to compute the full conditional likelihood given that the trait at the root is in state i , and then apply the weights we want to attach to each root state. These weights can be freely chosen by the user, depending on how much confidence one has about the root state. In Bayesian terms, the weights can be regarded as the prior distribution over the trait states. As stated above, diversitree/HiSSE uses the normalized D_i themselves as weights (Eq. (2)) and hence solely bases the weights on the data. This is similar to how posterior Bayes factors are defined: rather than integrating the likelihood over the prior (which is how conventional Bayes factors are defined), posterior Bayes factors integrate over the posterior (Aitkin 1991). In the same spirit one could argue that the weights should be

$$w_i = \frac{\frac{D_i}{\lambda_i(1-E_i)^2}}{\sum_i \frac{D_i}{\lambda_i(1-E_i)^2}} \quad (5)$$

In our applications we used the likelihood of Eq. (4) with weights as in Eq. (2). The R package SecSSE also offers the option to use the likelihood of Eq. (1) and Eq. (3), or the weights of Eq. (5).

In BiSSE/HiSSE, the initial probabilities of trait states at the tips are either 0 or 1, depending on the state the species have. For the multistate case, SecSSE assigns an initial probability of 1 to all those states that can be taken by a species and 0 for the rest. This configuration of initial probabilities at the tips is fully consistent with the likelihood calculation.

Revisiting MuSSE Analyses with SecSSE

We searched for studies where trait-dependent diversification was analyzed with MuSSE, with potentially elevated type I errors. We used the Web of Science and Google Scholar search terms ‘MuSSE’ and ‘diversification’. We used only data sets with three examined trait states for computational reasons, and discarded publications for which the analyses were not clearly replicable (e.g. ambiguity in settings, or missing information). We obtained seven studies from very different taxonomic groups (resulting in highly variable crown ages, 34–269 Myr.), including ants (Insecta: Formicidae, Burchill and Moreau 2016, number of tips $n = 116$), pupfish (Actinopterygii: Cyprinodontidae, Helmstetter et al. 2016, $n = 114$), corvid birds (Aves: Corvoidea, Marki et al. 2015, $n = 763$), ruminants (Mammalia: Ruminantia, Cantalapiedra et al. 2013, $n = 197$), epiphytic ferns (Plantae: Polypodiaceae, Sundue et al. 2015, $n = 417$), grunters (Teleostei: Terapontidae, Davis et al. 2016, $n = 38$), and amphibians (Amphibia, Arbuckle and Speed 2015, $n = 2683$). State-dependent diversification rates were reported in all seven studies. In most studies, extinction and transition rates were not examined, or were very low (in the case of extinction), so we assumed a single value for the transition rates and a single, trait-independent, value for the extinction rates. We adopted the model settings of the original MuSSE analysis as much as possible, which involved the use of a variable sampling fraction in four studies (Arbuckle and Speed 2015; Burchill and Moreau 2016; Davis et al. 2016; Helmstetter et al. 2016). In the case of Helmstetter et al. (2016), we included all taxa present in the tree but missing from the trait data file and assigned them the value NA (equal chance of inclusion in all states), rather than removing them from the tree as done in the original study. In the case of Arbuckle and Speed (2015), we chose to assign conspicuously-colored taxa with unknown function to either the conspicuously-colored or the camouflaged trait states (but not the polymorphic state) rather than creating a separate fourth state for them as in the original study. In both cases, we report results based on the original methodology in Appendix 1. For all studies we obtained the phylogenetic tree and associated trait data from the publication or the authors.

We used the convention introduced in HiSSE to denote observed states with numbers and hidden/concealed states with letters. For example, λ_{1A} denotes the speciation rate when the lineage is in a state with observed trait value 1 and concealed trait value A. We defined three models with different settings for the speciation rates. In the Constant Rates model (CR, hereafter), all species have the same speciation rate λ regardless of their trait state ($\lambda_{1A} = \lambda_{2A} = \lambda_{3A} = \lambda_{1B} = \lambda_{2B} = \lambda_{3B} = \lambda_{1C} = \lambda_{2C} = \lambda_{3C} = \lambda$), equivalent to a regular constant-rates birth-death model. In the Examined-Trait-Independent speciation model (ETI) speciation rates are allowed to vary only between concealed states ($\lambda_{1A} = \lambda_{2A} = \lambda_{3A} \neq \lambda_{1B} = \lambda_{2B} = \lambda_{3B} \neq \lambda_{1C} = \lambda_{2C} = \lambda_{3C}$). In the Examined-Trait-Dependent speciation model (ETD), speciation rates are allowed to vary only between the examined states ($\lambda_{1A} = \lambda_{1B} = \lambda_{1C} \neq \lambda_{2A} = \lambda_{2B} = \lambda_{2C} \neq \lambda_{3A} = \lambda_{3B} = \lambda_{3C}$). Additionally, we used a model with only two concealed states, where we allowed speciation rates to vary between concealed states but not the examined states i.e., an ETI model but with only 2 concealed states ($\lambda_{1A} = \lambda_{2A} = \lambda_{3A} \neq \lambda_{1B} = \lambda_{2B} = \lambda_{3B}$). We distinguish between the two ETI models by labelling them as ETI2 and ETI3 where the number refers to the number of concealed states.

To avoid being trapped in a local optimum during the Maximum Likelihood search, we used five different sets of initial parameter values, one in which we used estimates from the standard birth-death model, one in which we halved these values, one in which we doubled these values, one in which we only changed μ to 0.02, and one in which we used the mean of the estimated speciation rates reported in the original studies. Finally, we performed model selection based on Akaike weights (Wagenmakers and Farrell 2004), to select the best-performing model for each study.

Performance of SecSSE Analysis

To assess the robustness of our conclusions on model selection, we carried out a three-step procedure for each empirical dataset. 1) Using the parameters that maximize the likelihood for each of the three models (CR, ETI and ETD) we simulated 100 datasets (phylogenies and traits; details below) that are structurally similar to the empirical data. 2) We ran SecSSE analyses under the three models for each simulated data set. 3) Finally, we compared models and counted the instances where the generating model was proven to be the best model according to Akaike weight.

For the simulations, we considered a trait with three examined states and three concealed states, as used for the parameter inference on the empirical data (i.e., 1, 2, 3 and A, B, C respectively), leading to a nine-state system (i.e., 1A, 2A, 3A, 1B, 2B, 3B, 1C, 2C, 3C). The simulation starts with two species with the same trait state; this state can shift to the other states at a transition rate Q . Our simulation is conditioned on survival of these two species; if one of the species becomes extinct, the simulation is discarded. Speciation and extinction rates depend on trait states and, in line with the MuSSE framework, a shift in a trait state does not involve speciation, nor does speciation involve a change in trait state. The simulation is performed for a period of time equal to the crown age of the empirical data. A phylogeny is then reconstructed from the record of speciation and extinctions during the simulation. Concealed states are merged, so we end up with

only 3 examined states. We performed SecSSE inference on the simulated data using the same models as for the empirical data. We excluded the dataset of Helmstetter et al. (2016) from our performance analysis because their phylogeny was reconstructed at the genus level and their low sampling fraction (10%). We also had to exclude Arbuckle and Speed (2015) study, because the high estimates of the speciation rate for some trait states (Table 1) combined with a very old crown age (Amphibia - 269 Myr.) caused our simulations to sometimes produce trees that were so large that they were computationally unmanageable.

We also explored the performance of SecSSE with several parameter combinations. We ran five sets of simulations with differences in speciation and extinction rates (i.e. different speciation or extinction rates for the various traits states) and the number of states (a three-state or a five-state trait). We simulated 100 datasets. Finally, we ran a SecSSE analysis on all these data sets, again with the three models we described above, and compared them using Akaike weights in order to evaluate how often the generating model is preferred over the other two.

Results

Empirical Data Sets

We detected a significant effect of a concealed trait in five out of seven empirical data sets (Table 1). For these five studies, a model with three concealed states always outperformed a model with only two concealed states. Speciation rates in models without concealed state were identical or similar to rates reported in the original studies, except the two cases where we had to adjust model settings making diversification rates incomparable (Arbuckle and Speed 2015, Helmstetter et al. 2016; see Methods). Extinction rates were in most cases close to zero. We will now discuss the results for each data set in more detail.

Burchill and Moreau (2016) focused mainly on transition rates between three different colony sizes (low, medium, high) across 400 ant species in 118 genera. They also provided estimates of colony size-dependent speciation rates, however, and find rates that vary approximately threefold across the three size categories ($\lambda = 0.01-0.03$). Their results indicate that small colonies have the lowest rates, and medium colonies the highest. Our re-analysis suggests that there is no significant evidence for colony size being responsible for speciation rate variation.

Helmstetter et al. (2016) found support for the idea that diversification rates in viviparous cyprinodontiform fishes are about threefold higher than in their oviparous annual or non-annual counterparts. These diversification rates are mainly driven by differences in speciation rates, as extinction rates do not differ between the three groups. Our re-analysis indicates that the model with variation between examined states was most likely, supporting these findings.

In the case of corvid birds, Marki et al.'s (2015) results point towards increased net diversification rates (again, mainly as a result of elevated speciation rates, because extinction rates are similar

across states) in pair-breeding birds, relative to cooperatively-breeding or uniparentally breeding birds. Although their most likely model is one including free transition rates (and fixed extinction rates), they also ran a model including one fixed transition rate (as we did) and still found that speciation rates are higher in pair-breeders. Speciation rates in the model including free transition rates are only slightly higher (0.128) than in the model with fixed transitions (0.125). Our re-analysis finds no support for an influence of breeding system on speciation rates because a model with variation across concealed states is most likely.

Cantalapiedra et al. (2013) used three different models to explore the relationship between speciation rates and ruminant feeding mode (browser, grazer, and a mix between both), allowing variability in transition rates. They found that ruminants with mixed feeding mode had the highest speciation rate, followed by grazers, and browsers. We did not find feeding mode-dependent speciation rates. Allowing variation in transition rates may accommodate a shift in speciation rates. However, we expect that including free transition rates in our analyses would not influence our inferred speciation rates, because our estimated speciation rates in the ETI model are nearly identical to those of Cantalapiedra et al.

Sundue et al. (2015) ran a number of analyses on an epiphyte dataset, to study whether diversification rates differ between plants with simple, 1-pinnate or 2-3-pinnate leaf patterns. We replicated their three-state analysis with SecSSE. The authors found that 1-pinnate plants are associated with higher net diversification rates than simple (next highest rates), and 2-3-pinnate leaves are. Although Sundue et al. tested for susceptibility to type I error rates in their data set using a neutral, simulated trait as suggested by Rabosky and Goldberg (2015), they did not detect spurious effects of leaf shape on diversification patterns. However, we find that a model including variable concealed states is most likely.

Davis et al. (2016) concluded that in terapontid fishes, herbivores have considerably higher net diversification rates than either omnivores or carnivores. Our re-analysis with SecSSE points toward a model with one speciation rate across all states; Davis et al (2016) acknowledged that MCMC plots of their rates overlap considerably due to power issues, which is in line with our findings.

Finally, Arbuckle and Speed (2015) investigated the influence of coloration on amphibian diversification. They find higher net diversification rates for conspicuously-colored taxa than for cryptically colored or polymorphic taxa (both have similar rates). Extinction is close to zero across states. SecSSE found no significant difference in rates across examined states. While Arbuckle and Speed (2015) assigned a fourth state to taxa with uncertain coloration strategies (multiple strategies used by one species), we kept the three-state set-up and used our new feature (trait state uncertainty) to incorporate this partial information. We believe this does not influence the outcome of the SecSSE analysis, because taxa with uncertain coloration strategies represented only 2% of total taxa in the tree ($n = 2683$).

Overall, our confidence in the new conclusions for the seven datasets is high: the results of our simulation analysis show that when SecSSE suggests that trait state is not related to diversification dynamics and a constant rates model is not appropriate either (i.e., when the ETI model is selected as the best one), this is indeed the case for 96 % of the simulations for study 1, 98.9 % for study 3, 98.8 % for study 4 and 96.6 for study 5 (Fig. 1).

Robustness of SecSSE Analysis

In general, our simulation results show that SecSSE is reasonably robust to type I error. In the robustness analysis for the empirical studies, we found that SecSSE incorrectly suggested that speciation depends on the examined trait between 8 and 15% of all cases (Figure 1). In other words, for 8-15 out of 100 simulated datasets, SecSSE picked the ETD model as the best performing when ETD was not in fact the generating model. This relatively low type I error is not accompanied by low power to detect trait-dependent diversification: when the generating model has dependence on the examined trait, it is correctly chosen on average 84.6% of the cases (Fig. 1).

In the additional analysis on simulations with various parameter settings, for the majority of the simulated datasets, SecSSE correctly selected the generating model as the most likely according to the AIC values (Table 2). When speciation is variable between states, the least favorable results are obtained when extinction is elevated ($\mu = 0.1$) or the difference between speciation rates is rather small (parameter sets 2 and 1 respectively). However, when extinction is zero and the difference in speciation rates is higher (set 3), accuracy increases: the generating ETI model is correctly selected in the 78% of the cases. In this same set of simulations, SecSSE correctly selected trait-dependent diversification in the 93% of all the cases when the ETD was the generating model. Similar to the analysis for empirical data, we found that SecSSE erroneously selects trait dependent-diversification as the most likely scenario for 14% of the simulated datasets. When trait states had the same speciation rate but differed in extinction rates, SecSSE correctly selects ETD in the 63% of the cases.

Discussion

We have introduced a generalization of the concealed-state framework that can be applied to traits with multiple states or multiple traits. We used it to re-evaluate trait-dependent diversification for seven studies where a MuSSE-type analysis, i.e. ignoring concealed traits, had been done. Our analysis revealed that the conclusions of these studies are not fully supported. We have also shown that SecSSE, like HiSSE, avoids high rates of false positives.

Simulations showed that when extinction is high or differences in diversification are due to variation in extinction rates across states, the reliability of SecSSE decreases. This is not a

surprising finding: high extinction rate generally makes parameter estimations difficult with a diversity-independent model (Etienne et al. 2012), because extinction erases the signature of macroevolutionary processes on reconstructed phylogenies (Rabosky 2010). We observed that with high extinction rates, SecSSE tends to erroneously choose a constant-rates model more often than when extinction is zero, i.e. the power of SecSSE is compromised. However, type I error (inferring dependence of speciation on examined traits when it does not exist) remains relatively low. It is often the case that SecSSE points at a constant-rates model as the best performing model when in fact an ETI is the generating model. This indicates that for certain parameter combinations, the method might be insensitive to detect shifts in diversification rates. However, we note that SecSSE's purpose was to test dependence of diversification on traits. If the purpose is to find deviations from a constant-rate model, there are other, more appropriate, approaches (e.g. BAMM, Rabosky 2014; DDD, Etienne and Haegeman 2012; MEDUSA, Alfaro et al. 2009).

Phylogenies and trait data contain information on trait-dependent diversification, but if type I errors are to be avoided, the macroevolutionary dynamics must often be strongly and solely influenced by the trait to be detected. When we simulated trait dependence, all diversification rates were linked to species traits, every single shift in trait state influenced the rate of speciation and for those simulations, and SecSSE correctly chose a trait-dependent diversification model most of the times. We argue that for empirical datasets, SecSSE can find evidence of trait-dependent diversification only when all the diversification events are associated with the trait state. If the trait in question is only partially responsible for branching patterns in the phylogeny, SecSSE might not find support for a link between trait state and macroevolutionary dynamics. In this sense, the concealed-state framework is conservative. When a SecSSE analysis reveals a link between a trait and diversification, we can be quite certain that this is indeed the case. Thus, we foresee that future studies on macroevolution may fail to find a signal of trait dependence *unless* trait evolution and diversification dynamics are strongly coupled.

The traits used in five of the revisited studies were claimed to be linked to diversification because a shift in character state would represent a morphological innovation, enabling the exploitation of new areas in niche space. We think that the suitability of these traits as candidates for trait-dependence is questionable. It is not clear how the use of new niches is translated into variation in geographic range expansion, which is a species' feature directly linked to probabilities of speciation/extinction (Gaston 1998; Pigot et al. 2010). Perhaps more importantly, BiSSE, MuSSE and SecSSE assume that a shift in character state does not cause a lineage split. However, a change in a trait linked to ecological opportunity might lead to ecological diversification, violating a core assumption in these models. One option is to develop a concealed-trait version of the ClaSSE model proposed by Goldberg and Igić (2012) where cladogenetic changes are allowed. This is an interesting avenue for further study.

The likelihood-free methods to detect trait-dependent diversification (Rabosky and Huang 2016; Rabosky and Goldberg 2017) might appear as a more attractive option over SSE models because

of their relatively simplicity and short computation time. However, there are four differences between these two approaches. First, in the nonparametric approaches, speciation rate does not vary along a branch according to shifts in trait states (unlike in SSE models), so the relationship between a trait and diversification rates can at most be correlational. In other words, when using such models, we could argue for an association between a trait and diversification rate, but we cannot claim causation. Second, there might be instances when diversification rates are not the main focus of research but the evolution of the trait itself. For instance, one could be interested in transition rates between the states of a trait which is linked to speciation. In this case, trait evolution cannot be analyzed separately from branching patterns, and only SecSSE is the right tool to use. Third, SecSSE allows the incorporation of additional biological information or the design of models with different constraints, for example some state transitions can be set as forbidden, which allows for testing a number of contrasting hypotheses. Finally, SecSSE is a likelihood-based framework, which is a sound and coherent statistical framework. Non-parametric approaches are somewhat more ad hoc, and often do statistics on statistics. Because both methods address very similar questions, they can be regarded as complementary to some extent (Zenil-Ferguson and Pennell 2017).

We have shown that when trait evolution is coupled to diversification rates, this does leave a signature on phylogenetic trees and extant species traits and that can be detected with SecSSE with relatively high power yet still low type I error. We see a promising development of models where different modes of speciation and trait evolution are explored to unravel the drivers of diversification.

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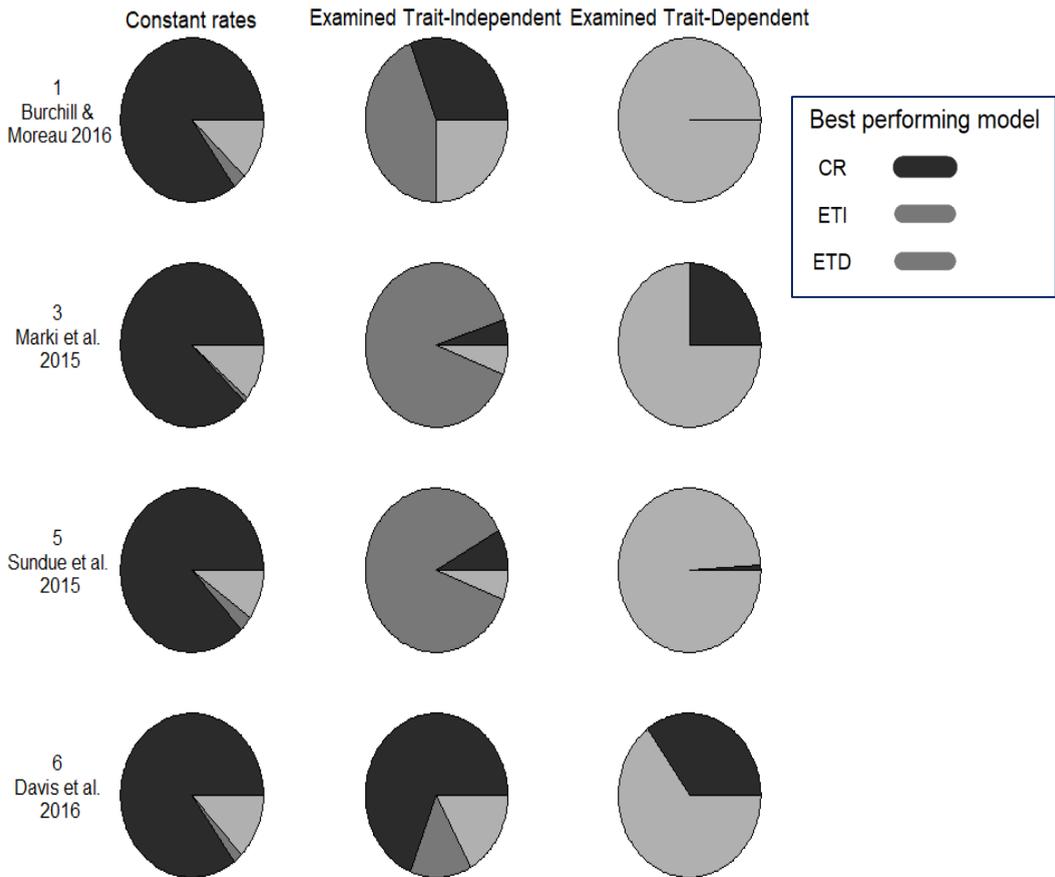


Fig. 1. Performance of SecSSE in model selection using simulated datasets that are structurally similar to the empirical datasets. One hundred datasets were simulated under constant-rate, trait-independent and trait-dependent diversification. SecSSE analyses were carried out under the same models for each simulated dataset. Each pie chart shows the proportion of cases in which each model was chosen by SecSSE as the best performing model (lowest AIC) under different generating models (columns).

Table 1. Estimates of speciation rate (λ), extinction rate (μ) and the rate of transition between trait states (Q) obtained by applying the maximum likelihood (ML) framework of SecSSE for 4 different models (constant-rate CR; trait-independent ETI with 2 and 3 concealed states; trait-dependent diversification ETD) to existing multistate data sets. Models are compared using Akaike weights (AIC_w). The references to the data sets are 1. Burchill & Moreau 2016, 2. Helmstetter et al. 2016, 3. Marki et al. 2015, 4. Cantalapiedra et al. 2014, 5. Sundue et al. 2015, 6. Davis et al. 2016, 7. Arbuckle & Speed 2015.

Ref.	model	k	ML	AIC _w	λ_{1A}	λ_{2A}	λ_{3A}	λ_{1B}	λ_{2B}	λ_{3B}	λ_{1C}	λ_{2C}	λ_{3C}	μ	q		
1.	CR	3	-678.4369	0.00001	0.02130	0.02130	0.02130	0.02130	0.02130	0.02130	0.02130	0.02130	0.02130	0.02130	0.00001	0.00681	
	ETI2	4	-669.9529	0.02149	0.02694	0.02694	0.02694	0.00001	0.00001	0.00001	-	-	-	-	0.00003	0.00901	
	ETI3	5	-665.1353	0.97759	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.03172	0.03172	0.03172	0.00020	0.00869	
	ETD	5	-672.1128	0.00091	0.00698	0.03276	0.00191	0.00698	0.03276	0.00191	0.00698	0.03276	0.00191	0.00001	0.01357		
2.	CR	3	-503.1806	0.00001	0.24544	0.24544	0.24544	0.24544	0.24544	0.24544	0.24544	0.24544	0.24544	0.24544	0.17618	0.00161	
	ETI2	4	-492.1833	0.00007	0.08678	0.08678	0.08678	0.20506	0.20506	0.20506	-	-	-	-	0.00681	0.00162	
	ETI3	5	-487.0797	0.00398	0.20963	0.20963	0.20963	0.03859	0.03859	0.03859	0.09692	0.09692	0.09692	0.09692	0.00001	0.00161	
	ETD	5	-481.5579	0.99595	0.07721	0.13751	0.20837	0.07721	0.13751	0.20837	0.07721	0.13751	0.20837	0.07721	0.13751	0.20837	0.00001
3.	CR	3	-2611.514	0.00001	0.11845	0.11845	0.11845	0.11845	0.11845	0.11845	0.11845	0.11845	0.11845	0.11845	0.00001	0.00452	
	ETI2	4	-2579.453	0.00014	0.22487	0.22487	0.22487	0.09277	0.09277	0.09277	-	-	-	-	0.00001	0.00446	
	ETI3	5	-2569.565	0.99862	0.22825	0.22825	0.22825	0.09937	0.09937	0.09937	0.00001	0.00001	0.00001	0.00001	0.00001	0.00513	
	ETD	5	-2605.539	0.00001	0.14765	0.12441	0.08670	0.14765	0.12441	0.08670	0.14765	0.12441	0.08670	0.00001	0.00451		
4.	CR	3	-738.1913	0.00028	0.11169	0.11169	0.11169	0.11169	0.11169	0.11169	0.11169	0.11169	0.11169	0.11169	0.00001	0.00979	
	ETI2	4	-729.6217	0.54306	0.13238	0.13238	0.13238	0.02835	0.02835	0.02835	-	-	-	-	0.00001	0.00991	
	ETI3	5	-728.7980	0.45528	0.13871	0.13871	0.13871	0.02736	0.02736	0.02736	0.02736	0.02736	0.02736	0.02736	0.00001	0.01002	

	ETD	5	-734.5988	0.00138	0.08464	0.13182	0.11033	0.08464	0.13182	0.11033	0.08464	0.13182	0.11033	0.00001	0.00969
5.	CR	3	-1430.800	0.00026	0.13718	0.13718	0.13718	0.13718	0.13718	0.13718	0.13718	0.13718	0.13718	0.00001	0.00838
	ETI2	4	-1422.814	0.27857	0.11620	0.11620	0.11620	0.23611	0.23611	0.23611	-	-	-	0.00001	0.00774
	ETI3	5	-1420.8640.72031	0.24437	0.24437	0.24437	0.12514	0.12514	0.12514	0.04724	0.04724	0.04724	0.00001	0.00785	
	ETD	5	-1427.596	0.00086	0.14192	0.14026	0.05260	0.14192	0.14026	0.05260	0.14192	0.14026	0.05260	0.00001	0.00854
6.	CR	3	-163.70840.35352	0.12222	0.12222	0.12222	0.12222	0.12222	0.12222	0.12222	0.12222	0.12222	0.12222	0.04376	0.01907
	ETI2	4	-163.2112	0.21382	0.00001	0.00001	0.00001	0.11499	0.11499	0.11499	-	-	-	0.00319	0.01926
	ETI3	5	-162.8887	0.10860	0.12043	0.12043	0.12043	0.11985	0.11985	0.11985	0.00001	0.00001	0.00001	0.01770	0.01884
	ETD	5	-161.7954	0.32406	0.07164	0.13010	0.15989	0.07164	0.13010	0.15989	0.07164	0.13010	0.15989	0.00001	0.01658
7.	CR	3	-12400.77	0.00001	0.06757	0.06757	0.06757	0.06757	0.06757	0.06757	0.06757	0.06757	0.06757	0.01453	0.00110
	ETI2	4	-12203.33	0.00001	0.08143	0.08143	0.08143	0.04005	0.04005	0.04005	-	-	-	0.00001	0.01161
	ETI3	5	-12167.481.00000	0.08321	0.08321	0.08321	0.01693	0.01693	0.01693	0.04551	0.04551	0.04551	0.00001	0.00123	
	ETD	5	-12378.91	0.00001	0.00666	0.02759	0.07993	0.00666	0.02759	0.07993	0.00666	0.02759	0.07993	0.008776	0.00137

Table 2. Performance of SecSSE in model selection using different rates of speciation and extinction. One hundred datasets were simulated under constant-rate (CR), trait-independent (ETI) and trait-dependent diversification (ETD). SecSSE analyses were carried out under the same models for each simulated dataset. Rows show the number of cases in which each model was chosen by SecSSE as the best performing model (lowest AIC). Sets 1, 2 and 3 show variation in speciation rate between the states of a three-state simulated trait whereas in set 4 a trait with five states was simulated. Set 5 shows variation in extinction rates across states. The cases where SecSSE correctly chose the generating model are shown in bold.

Generating model			Model used for inference		
			CR	ETI	ETD
Set 1	CR	$\lambda = 0.25; \mu = 0$	80	2	18
	ETI	$\lambda = 0.25, 0.1, 0.4; \mu = 0$	62	19	19
	ETD	$\lambda = 0.25, 0.1, 0.4; \mu = 0$	23	0	77
Set 2	CR	$\lambda = 0.35; \mu = 0.1$	81	0	19
	ETI	$\lambda = 0.45, 0.2, 0.5; \mu = 0.1$	65	17	18
	ETD	$\lambda = 0.45, 0.2, 0.5; \mu = 0.1$	10	0	90
Set 3	CR	$\lambda = 0.25; \mu = 0$	84	2	14
	ETI	$\lambda = 0.05, 0.2, 0.5; \mu = 0$	17	72	11
	ETD	$\lambda = 0.05, 0.2, 0.5; \mu = 0$	7	0	93
Set 4	CR	$\lambda = 0.25; \mu = 0$	91	0	9
	ETI	$\lambda = 0.1, 0.3, 0.4, 0.15, 0.6; \mu = 0$	22	65	13
	ETD	$\lambda = 0.1, 0.3, 0.4, 0.15, 0.6; \mu = 0$	9	0	91
Set 5	CR	$\mu = 0.1; \lambda = 0.4$	88	0	12
	ETI	$\mu = 0.1, 0.2, 0.3; \lambda = 0.4$	91	0	9
	ETD	$\mu = 0.1, 0.2, 0.3; \lambda = 0.4$	64	0	36

Appendix 1. Estimates of speciation rate (λ), extinction rate (μ) and the rate of transition between trait states (q) obtained by applying the maximum likelihood (ML) framework of SecSSE to Arbuckle & Speed 2015 dataset. Here we applied the new feature in SecSSE (trait state uncertainty) to keep a three-state trait (constant-rate CR3; trait-independent ETI3 with 2 and 3 concealed states; trait-dependent diversification ETD3) rather than considering 4 states as in Arbuckle and Speed's approach (CR4; ETI4 with 3 and 4 concealed states; ETD4). Models are compared using Akaike weights (AICw).

model	ML	AICw	λ_{1A}	λ_{2A}	λ_{3A}	λ_{4A}	λ_{1B}	λ_{2B}	λ_{3B}	λ_{4B}	λ_{1C}	λ_{2C}	λ_{3C}	λ_{4C}	λ_D^*	μ
CR3	-12397.21 0.00111	0.00001	0.06757	0.06757	0.06757	-	0.06757	0.06757	0.06757	-	0.06757	0.06757	-0.06757	-	-	0.01453
ETI3-2 0.00117	-12199.70	0.00001	0.08142	0.08142	0.08142	-	0.04003	0.04003	0.04003	-	-	-	-	-	-	0.00001
ETI3-3	-12163.77 0.00124	1.00000	0.08320	0.08320	0.08320	-	0.04550	0.04550	0.04550	-	0.01692	0.01692	0.01692	-	-	0.00001
ETD3	-12372.39 0.00137	0.00001	0.06638	0.02781	0.08047	-	0.06638	0.02781	0.08047	-	0.06638	0.02781	0.08047	-	-	0.00825
CR4	-12679.23 0.00096	0.00001	0.06757	0.06757	0.06757	0.06757	0.06757	0.06757	0.06757	0.06757	0.06757	0.06757	0.06757	0.06757	0.06757	0.01453
ETI4-3	-12448.68 0.00105	0.00002	0.01792	0.01792	0.01792	0.01792	0.04563	0.04563	0.04563	0.04563	0.08327	0.08327	0.08327	0.08327	-	0.00001
ETI4-4	-12436.87 0.00097	0.99997	0.10850	0.10850	0.10850	0.10850	0.01710	0.01710	0.01710	0.01710	0.04465	0.04465	0.04465	0.04465	0.07064	0.00021
ETD4	-12616.17 0.00119	0.00001	0.06164	0.04324	0.08433	0.01206	0.06164	0.04324	0.08433	0.01206	0.06164	0.04324	0.08433	0.01206	"	0.00013

* Note that λ_D in ETI4-4 represents the same rate for the 4 examined states and in model ETD4 represents the same rates as λ_{1A} , λ_{1A} , λ_{1A} and λ_{1A} .