The Evolution of Nest Site Specialization and its Macroevolutionary Consequences in Passerine Birds

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Abstract. – A long-standing hypothesis in evolutionary biology is that the evolution of resource specialization can lead to an evolutionary dead end, where specialists have low diversification rates and limited ability to evolve into generalists. However, in recent years, advances in comparative methods investigating trait-based differences associated with diversification have enabled more robust tests of this idea and have found mixed support. Here we test the evolutionary dead end hypothesis by estimating net diversification rate differences associated with nest site specialization among 3,224 species of passerine birds. In particular, we test whether the adoption of hole-nesting, a nest site specialization that decreases predation, results in reduced diversification rates relative to nesting outside of holes. Further, we examine whether evolutionary transitions to the specialist hole-nesting state have been more frequent than transitions out of hole-nesting. Using diversification models that accounted for background rate heterogeneity and different extinction rate scenarios, we found that hole-nesting specialization was not associated with diversification rate differences. Furthermore, contrary to the assumption that specialists rarely evolve into generalists, we found that transitions out of hole-nesting occur more frequently than transitions into hole-nesting. These results suggest that interspecific competition may limit adoption of hole-nesting, but that such competition does not result in limited diversification of hole-nesters. In conjunction with other recent studies using robust comparative methods, our results add to growing evidence that evolutionary dead ends are not a typical outcome of resource specialization.

Keywords: diversification, hidden-state models, cavity nesting, passerines, resource specialization

Resource specialization, where species use a narrower range of resources compared to related taxa, is a common phenomenon in evolution. There are many reasons resource specialization (hereafter specialization) can evolve. For example, specialists can benefit from reduced competition, or from the avoidance of predators or parasites (Futuyma and Moreno 1988; Bernays 1989; Schluter 2000). Once evolved, specialization can have diverse consequences for macroevolutionary dynamics. Historically, specialization often has been considered an evolutionary dead end, resulting in both reduced net diversification relative to generalists, and difficulty evolving from specialist to generalist. This difficulty may arise when adaptation to specialized resources occurs across numerous traits (Futuyma and Moreno 1988), and, when coupled with changes in resource availability, can lead to elevated extinction rates in specialists. Additionally, reduced diversification of specialists could result if the number of available niches in the specialized state is low.

Consistent with the evolutionary dead end view of specialists, the prevailing historical viewpoint was that specialists evolve from generalist ancestors (Schluter 2000). Prior studies have found mixed support for this viewpoint, with inferences from phylogenetic comparative methods showing that generalists regularly evolve from specialists in many clades (Day et al. 2016; Sexton et al. 2017; Villastrigo et al. 2021). These results indicate that the dominant direction of evolutionary transitions involving specialization should be treated as an open question for any specialization scenario.

More recent studies have also noted the potential for specialization to lead to increased diversification through multiple mechanisms. First, the release of specialists from the effects of competition and/or predation could trigger periods of niche-filling

diversification (Schluter 2000), leading to higher rates of speciation in specialists compared to generalists. Further, in circumstances where specialization releases specialists from competition or predation (Futuyma and Moreno 1988), specialists may experience greater population persistence than generalists, which could increase diversification rates by decreasing extinction rates or by increasing rates of allopatric speciation via the longer survival of nascent lineages (Harvey et al. 2019), or both. Finally, specialist lineages could also have higher diversification rates if more specialized lineages have more fragmented distributions or lower rates of dispersal (Gavrilets et al. 2000; Birand et al. 2012).

New diversification models and statistical developments have the potential to alter our conclusions on the macroevolutionary consequences of specialization. A growing awareness of type I error (Davis et al. 2013; Rabosky and Goldberg 2015), the importance of large sample sizes for statistical power (Davis et al. 2013; Day et al. 2016), and the misspecification of the null hypothesis for the original state-dependent diversification models (Beaulieu and O'Meara 2016; Rabosky and Goldberg 2017; Caetano et al. 2018) have produced a wave of new studies using robust statistical approaches on the question of whether specialization is linked to diversification. The majority of studies after Day et al.'s (2016) review on specialization have found either no association between specialization and diversification (e.g. Alhajeri and Steppan 2018; Crouch and Ricklefs 2019; Villastrigo et al. 2020) or have found that specialization is associated with higher diversification rates (e.g. Conway and Olsen 2019; Otero et al. 2019; Tonini et al. 2020), with few studies indicating specialization leads to an evolutionary dead end (e.g. Cyriac and Kodandaramaiah 2018; Day et al. 2016). These

new results seem to indicate that specialization rarely leads to evolutionary dead ends. While these newer studies are using trees with more tips and more transitions to specialist states, which can help disentangle consequences of specialization in diversification (Davis et al. 2013), moving forward it is necessary to integrate new methods that incorporate heterogeneity in the diversification process unlinked from specialization (Beaulieu and O'Meara 2016).

Here we examine the evolution of specialization of nesting microhabitat and its macroevolutionary consequences using nesting site data for 3,224 species of passerine birds. Passerines provide a unique opportunity to examine the importance of hole-nesting as an example of resource specialization that may drive diversification patterns using phylogenetic comparative methods. First, there are over 6,000 species of passerines, and hole-nesting behavior has evolved multiple times across the clade, providing numerous transitions to estimate rates of diversification linked to trait evolution (Davis et al. 2013). Second, passerines can be grouped into three general nesting habits: open-cup nesters, dome nesters and hole nesters (e.g. Wallace 1868; Martin 1995; Collias 1997). Open-cup and dome nesters are relatively unspecialized in their nesting substrate compared to hole-nesters; however, dome nesters also have reduced predation rates compared to open-cup nesters (Oniki 1979; Linder and Bollinger 1995; Auer et al. 2007; Martin et al. 2017). Therefore, our approach of comparing adoption of these three nest types enables us to assess the potential effects of nest site specialization versus escape from predation on diversification rates (Fig. 1).

Nest site selection is a critically important aspect of avian habitat because nest failure rates are high in birds (Nice 1957), and predation is the main cause of nestling

mortality (Ricklefs 1969; Martin 1993). Adoption of hole-nesting has the advantage of protecting offspring from predation, and non-excavating hole nesters show an approximate 43% reduction in nest failure compared to non-hole nesters (Martin 1995). Thus, the main ecological consequence of hole-nesting specialization is that it provides release from predation. Hole-nesting represents resource specialization for two reasons: the great majority of species that nest in holes do so obligately, and exclusive use of holes greatly restricts the substrates that are suitable for nest building.

Hole-nesting is associated with the evolution of a suite of traits, such as increased nesting period, brighter egg coloration, and larger clutch sizes, that may make it difficult to transition out of hole-nesting (Martin and Li 1992; Kilner 2006). Moreover, the great majority of hole-nesting passerines cannot excavate their own nest holes, and competition for this limited resource is intense (Newton 1994). While hole-nesting likely evolves as a response to predation pressure, the intense competition for nesting sites decreases nest hole availability (Cockle et al. 2011). This competition may limit ecological opportunity for hole-nesting lineages despite the reduction in predation pressure. Further, reliance on holes for nesting may increase extinction risk when environmental changes reduce nest hole availability, particularly if transitioning from hole-nesting is difficult. This combination of characteristics makes hole-nesting in passerines a form of specialization that could be an evolutionary dead end. However, as hole-nesting decreases nest predation, it is also possible that adopting hole-nesting could lead to greater population persistence over time which, in turn, could lead to reduced extinction rates or increased rates of allopatric speciation (Harvey et al. 2019).

Thus it is also possible that hole-nesting lineages could experience greater diversification rates than the more generalist nonhole-nesting lineages.

The evolutionary dead end hypothesis assumes that it is easy to adopt hole-nesting habits, but difficult to transition out of them because of secondary adaptations that restrict evolutionary transitions (Collias and Collias 1984). In passerines, most hole nesters are secondary (i.e., they cannot make new cavities, and thus they rely on what is already in the environment), and so adoption of hole-nesting is mainly a behavioral shift not requiring extensive morphological modification (although see above for examples of life history traits associated with hole-nesting). This could make it relatively easy to evolve hole-nesting behavior. However, the intense competitive environment that hole nesters face could mean that hole-nesting niches are already saturated, making it a more difficult habit to adopt. To examine these possibilities, we not only assess the impact of nest type on diversification rates, but we also calculate transition rates among them.

We employ a graphical modeling approach (Jordan 2004) using Bayesian statistics to infer parameters of state-dependent speciation and extinction models (SSE models), and diversification-free models (Mkn models) coded in RevBayes software (Höhna et al. 2016). The large dataset and Bayesian framework enable us to avoid a number of common pitfalls for similar studies on diversification. Additionally, we adopt a systematic approach to specifying prior distributions to model extinction rates that allows us to assess the consistency of estimates and inference across a spectrum of macroevolutionary diversification scenarios. Finally, after determining the best model, we used it to reconstruct ancestral states of nest types to visualize the pattern of

evolutionary history for the trait and compare with previous ancestral state reconstructions.

MATERIALS AND METHODS

Data

Our nesting data is a slightly adapted version of a dataset originally assembled for McEntee et al. (2018). This dataset was generated by scoring the nesting behavior of the passerine species using descriptions from the Handbook of the Birds of the World Alive, (Del Hoyo et al. 2017, last accessed 30 June 2016, hereafter HBW). Specifically, species' nests were scored as either open-cup, dome, hole, or some combination of two of these three nest types for the few species that show flexible nest type use. "Hole" refers to any nest built inside a tree cavity, rock crevice, or earthen bank. The small number of brood parasites were excluded from analyses. McEntee et al. (2018) were able to score the nest types for approximately three quarters of the 5,912 passerine species from the information in the HBW species descriptions.

Next, we matched the passerine species in the nesting dataset to the species' names on the tips of the avian supermatrix phylogeny of Burleigh et al. (2015), one of the largest phylogenetic trees of birds constructed exclusively from molecular data. This tree was time-calibrated using penalized likelihood in r8s (Sanderson 2003) with twenty fossil calibrations from throughout the avian phylogeny (Baiser et al. 2018). We identified all cases in which a species in the Burleigh et al. (2015) phylogenetic tree, which used the Clements taxonomy, did not have a corresponding species with the exact same name in the dataset from the HBW Alive/BirdLife International taxonomy. This was due to either missing nesting data or a taxonomic mismatch. For taxonomic

mismatches, we examined the taxonomic history for these species in Avibase (https://avibase.bsc-eoc.org/), and when appropriate, changed the species name in the nest dataset to match the phylogenetic tree (Burleigh et al. 2015). Taxa treated as subspecies in the HBW taxonomy (2015) but as taxa in Burleigh et al. (2015) were not included in our analyses because they did not occur as tips in the phylogenetic tree. We then trimmed the phylogenetic tree to include only species in the nesting dataset from HBW. The resulting data set has the nesting state for 3,224 passerine species. Of these, 1943 species had dome nests (D), 722 had cup nests (C), and 458 had hole nests (H). Among species with multiple nest types, 60 were cup or hole nesters (CH), 29 were dome or cup nesters (DC), and 13 were dome or hole nesters (DH). In the few instances (less than 1% of internal nodes) where this tree was not bifurcating (required for the diversification analyses described below) because nodes were collapsed in the r8s analysis, we resolved bifurcations randomly using the function `multi2di` from the R package `ape` (Paradis and Schliep 2019).

Diversification models with different numbers of states

Using the three states assigned to the tips of the 3,225-taxon phylogenetic tree (3,224 passerines plus a single species representing the parrot outgroup), we defined four state-dependent speciation and extinction models (SSEs). The first multistate SSE model, called MuSSE-3 here, uses three main states: dome D, cup C, and hole H (Fig. 2a), each with their own speciation and extinction rate (Fig. 2a). MuSSE-3 also has transition rates between each state (q_{ij} with $i, j = D, C, H$, and $i \neq j$) governing the rate of evolution from one nest type to another. All transitions between nest types are

possible. Taxa that have multiple nest types are coded in the input data as belonging to multiple states simultaneously. For example, a bird taxon reported to have nested in both hole and dome nests is coded in the input data as (H, D). For taxa (tips) with two nest types, the likelihood calculation includes the product of two transition probabilities from the most recent common ancestor to each potential state in the tips instead of a single transition probability from the most recent common ancestor to one single state.

The second model is a six state SSE called MuSSE-6 in which the six main states represent not only dome D, cup C, and hole H nest types, but also all the pairwise combinations of multiple nest types (dome and cup (DC), dome and hole (DH), and cup and hole (CH), Fig. 2a) to account for those species that show flexibility in nest type use. MuSSE-6 includes speciation and extinction rate parameters linked only to the D, C, and H states. Transition rates between all states are possible except transitions between those states representing two different nest types and the single nest type state not including either of these nest types (e.g. state DC does not connect with state Hsince that represents a double transition, Fig. 2b). The MuSSE-6 model helped us identify the most appropriate way to score states when taxa can use more than one nest type.

The third and fourth models are the hidden state extensions of the MuSSE-3 and MuSSE-6 respectively. The hidden state extension is necessary to avoid null hypothesis misspecification since the null hypothesis should include background heterogeneity in the diversification rate that is not linked to the trait of interest (fulfilled by character-independent models thoroughly discussed in Beaulieu and O'Meara (2016), and Caetano et al. (2018). For both the three-state model and the six-state

model, we expanded the three single nest type states to six with subscripts A or B.These models are the MuHiSSE-3 and MuHiSSE-6 respectively (Fig. 2).

Diversification models with different assumptions about extinction rates

Fossil records for passerines suggest rapid turnover of diversity over time, with high species diversity and diversification rates (Jetz et al. 2012; Marshall 2017) despite the extinctions of entire early passerine lineages (Hieronymus et al. 2019; Ksepka et al. 2019), high turnover of passerines within Europe between the Oligocene and the present (Manegold 2008; Bochenski et al. 2021), and the impact of late Pleistocene species extinctions (Steadman et al. 2015; Oswald and Steadman 2018). Diversification models using phylogenetic trees with only extant taxa often appear to underestimate the rates of extinction (Höhna et al. 2011; Louca and Pennell 2021), producing error in many diversification analyses (Stadler 2013). This presents a potential problem in the passerines because of the fossil evidence that turnover has been high (see also Marshall's third law of paleobiology: the average extinction rate approximately equals the average origination rate; Marshall 2017).

In a Bayesian framework, one way to incorporate knowledge about higher extinction rates is by using a prior distribution that accommodates independent information about the magnitude of the rates. Therefore, in our three-state, six-state, and hidden-state models, we defined prior distributions for the extinction rates with enhanced lower bounds, and we systematically increased these lower bounds across model fits (Table A1). We specified these lower bounds by making extinction rates' lower bound to be dependent on speciation rates. In mathematical form, we defined this

process by using the linear function for extinction rates as $\mu_i = A * \lambda_i + \delta$ where $A = 0.7, 0.8, 0.9, 1, 1.1, 1.2$, for all the states $i = D, C, H$. Therefore, the extinction rates are a linear combination of random variables. The speciation rates λ_i have a log-normal prior distribution with hyperparameters ($\mu_\lambda = ln\left(\frac{3225}{2}\right), \sigma_\lambda = 1.12$) and the second random variable of the linear function, represented by the parameter δ, is distributed as $Exp\ (2)$. Defining extinction rate prior distribution as a linear combination of random variables, allowed us to fix the value A as the lower bound for the extinction fraction $a_i = \frac{\mu_i}{\lambda_i}$ (we use the same definition of extinction fraction as (Beaulieu and O'Meara 2016) while permitting extra variability in the diversification rates, so that extinction rates are not fully a deterministic function of speciation rates. For example, the extinction rate of taxa with dome nests that is at least 80% of the speciation rate is defined with the equation $\mu_D = 0.8 * \lambda_D + \delta$. In this example the posterior estimation of the extinction fraction for state Dome ($a_D = \frac{\mu_D}{\lambda_D}$) is forced to be at least 80%, but the extinction fraction can still be larger than the bound of 0.8 thanks to random variable δ (i.e. $a_D = \frac{\mu_D}{\lambda_D} > 0.8$). In most of our models, prior distributions for extinction rates were defined equally (i.e. $\mu_D = \mu_C = \mu_H = A * \lambda_D + \delta$). In one model, we specified $\mu_H < \mu_D < \mu_C$ by making $A = 0.6, 0.7$, and 0.8 respectively to verify that: 1) our inferences weren't driven by this specification of the prior; and 2) that likelihood is informative given our dataset (supplementary Fig. S11).

Testing the role of diversification in nest type evolution

To test if nest type evolution is linked to the diversification of passerines, we calculated the marginal log-likelihoods for the MuSSE-3 and the character independent

model (CID-3). It is important to notice that in the CID-3 notation the 3 refers to the number of hidden states that have different diversification rates, and not necessarily the number of states of the main trait, to compare against the correct null hypothesis as discussed in Caetano et al. (2018). We calculated Bayes factors (Kass and Raftery 1995) to compare CID-3 against the MuSSE-3 model. To approximate the marginal log-likelihood we calculated 18 stepping stones using the methodology from Xie et al. (2011). We calculated the difference between the log-marginal likelihoods of CID-3 and MuSSE-3 models using the statistic κdefined as$\kappa = ln(P(X|CID-3)) - ln(P(X|MuSSE-3))$, where X represents our state sample and the phylogenetic tree. If κ has a value larger than 1, then the CID-3 model is preferred. The MuSSE-3 model is preferred when $\kappa < -1$. The test statistic is inconclusive when κ has a value in the interval (-1, 1). We did not calculate marginal log-likelihood for a CID-6 model to compare against the MuHiSSE-3 because CID-6 would require the estimation of too many parameters (36 in total: 12 diversification rates, 6 transition rates, 12 hidden state transition rates parameters, and 6 frequency parameters for the root), and, as discussed in the results, we found strong evidence that nest type is not linked to diversification using the much simpler CID-3 and the MuSSE-3.

Ancestral state reconstruction using diversification-free models

It was important to verify the most probable state at the root since previous studies have found that hole is the most likely state at the root, suggesting that all passerines descend from an already specialized nest type. The results showed that nest type evolution is not linked to the diversification process (see Results section).

Therefore, we defined both three-state (Mk3, Fig. 2a) and six-state (Mk6, Fig. 2b) Markov models without diversification parameters (hereafter, "diversification-free models") to reconstruct the ancestral nest type in passerines. We calculated the marginal ancestral state reconstruction at each of the internal nodes. Since our reconstructions use a Bayesian framework, in each of the nodes we plot the state with the maximum *a posteriori* value of the marginal posterior distribution (Fig. 5), as previously done in Freyman and Höhna (2018), and Zenil-Ferguson et al. (2019) implemented in RevBayes (Höhna et al. 2016). For all of our estimations, we treated the root as a stochastic vector whose posterior distribution needed to be jointly estimated along with the diversification and transition parameters (see numbers of parameters in Fig. 2). For the Musse-3 model, the stochastic vector we chose at the root was composed of three frequencies that sum up to 1 (for dome, cup, and hole respectively) and that stochastic vector had a Dirichlet distribution with parameter $(1/3,1/3,1/3)$ as the prior distribution. We calculated the posterior distribution for this stochastic vector via our MCMC algorithms.

Implementation of models and inferences

All the diversification and diversification-free models in Fig. 2 were implemented as graphical models in RevBayes (Höhna et al. 2016). We customized and ran a Markov chain Monte Carlo (MCMC; Metropolis et al. 1953; Hastings 1970) algorithm to sample the posterior distribution of each model. For all the models (diversification and diversification-free), we assumed that the state value at the root was unknown, and we estimated the posterior distribution of the frequencies at the root. The prior distribution

of the root values was defined via a Dirichlet distribution with uniform frequencies across the number of states for each model. Convergence and effective sample sizes of at least 250 for every parameter in the MCMC were assessed using Tracer (Rambaut et al. 2018) and two chains were run per model to verify convergence. Each MCMC run was performed in the HiPerGator cluster at the University of Florida, and took an average of 240 hours to converge. All our implementations and results are available at https://github.com/roszenil/nestdivmodel.

RESULTS

Diversification is not linked to nest type

While our MuSSE models found associations between nest type and diversification rates, our MuHiSSE models showed that these associations were spurious. This is because the addition of hidden states accounts for the possibility that diversification is driven by unmeasured factors other than nest type. In particular, for the MuSSE-3 model of nest evolution, we found that hole nesters had faster net diversification (defined as speciation minus extinction) than cup nesters, and the smallest net diversification was associated with dome nesters (Fig. 3a). This result was also true when the prior of extinction fraction was faster ($a = \mu/\lambda = 0.8$, Fig. 3a), or when the states were coded using the MuSSE-6 (Fig. 3a). This result also holds for all enhanced extinction fractions ($0 << a = \mu/\lambda < 1$) (Fig. A1). When fitting the hidden state models MuHiSSE-3 and MuHiSSE-6 models (Fig. 3b), we found that the three-state posterior distributions of net diversification rates completely overlap within A and within B while being different between A and B, indicating that diversification rate

differences across the phylogenetic tree are due to hidden states (Fig. 3b). These results were consistent when we allowed MuHiSSE-3 to have an extinction fraction of at least 0.8 (Fig. 3b). Furthermore, when calculating Bayes factors comparing the CID-3 (diversification rates independent of nest type) model against the MuSSE-3 (diversification rates depend on nest type), we found that the CID-3 model is preferred over the MuSSE-3 ($\kappa = -6033.72 - (-7772.40) = 1738.67 > 1$). Altogether, there is strong evidence that the heterogeneity observed in passerine diversification rates is not linked to nest type.

Evolving out of holes is faster than evolving back into them

Since nest type is not linked to the diversification process, we turn to understand the dynamics under the diversification-free Mk3 model. The posterior distribution of transition rates from the Mk3 model shows that the rate of transitioning from hole to cup was the fastest (mean $q_{32} = 9.1x10^{-3}$, 95% credible interval $(6.5x10^{-3}, 0.01)$), followed by the transition from hole to dome (mean $q_{31} = 6.5x10^{-3}$, 95% credible interval $(3.7x10^{-3}, 8.2x10^{-3})$, Fig. 4A). Transition rates from either dome or cup to hole nests were similar, and both were slower than the transition rates out of hole nests (dome to hole transition rates have a mean $q_{13} = 1.6x10^{-3}$, and 95% credible interval $(6.6x10^{-4}, 2.8x10^{-3})$; cup to hole transition rates have a mean $q_{23} = 1.2x10^{-3}$, credible interval $(7.3x10^{-4}, 2.5x10^{-3})$, shown in Fig. 4a. This pattern was also found in the Mk6 model (Fig. 4b) which further revealed that transitioning from hole-nesting to use of two different nest types (DH or CH) is rare (Fig. 4b, credible intervals for these rates contain zero).

Hole-nesting is the most probable state at the root

The Mk3 model reconstructs hole-nesting as the state with the highest probability at the most recent common ancestor of the Passeriformes we found that the maximum *a posteriori* for the root's marginal posterior probability was 0.77 for hole-nesting when the tree included the parrot *Strigops habroptila* as the outgroup. This probability decreased to 0.51 when the tree only contained passerines and no outgroup (states hole and cup make the other 0.49 of probability). The posterior distribution for the stochastic vector at the root had as its maximum the vector (Dome=0.30, Cup=0.25, Hole=0.45).

DISCUSSION

Over the years, there have been diverse perspectives on the link between ecological specialization and evolutionary diversification (Vamosi et al. 2014). On the one hand, ecological specialization has been viewed as an evolutionary dead end because it leads to evolutionary changes in traits that are difficult to reverse, which can leave specialized taxa at higher risk of extinction when conditions change. On the other hand, other studies have emphasized the links between specialization and increased diversification (Losos et al. 1994; Schluter 2000) because a narrower niche could increase diversification either directly, if niche shifts are associated with multiple speciation events (Yoder et al. 2010), or indirectly, because of differences in dispersal, connectivity, population persistence, and/or range size of specialist and generalist species (Greenberg and Mooers 2017; Harvey et al. 2019). Testing the outcomes of specialization on diversification rates has been difficult because macroevolutionary

models require datasets with a large number of independent origins of specialization (Day et al. 2016).

Here, using state-dependent diversification models in a large tree of passerines, we found that hole-nesting specialization does not differ from cup and dome-nesting in diversification rates. We also found that transition rates into hole-nesting were low compared to transitions out of hole-nesting (Fig. 4), and that the most probable state for the root of all passerines was hole-nesting. These three results highlight the lack of support for the hypothesis that specialization leads to an evolutionary dead end. Our results, combined with a number of recent studies addressing this hypothesis (Day et al. 2016; Cyriac and Kodandaramaiah 2018), raise the question of whether the link between diversification rates and ecological specialization is truly variable across taxa and traits, or whether newer phylogenetic comparative methods and larger data sets are allowing us to test hypotheses more rigorously. Interestingly, in our study, which uses a large dataset with numerous transitions to the specialized hole-nesting state, we found that, in the absence of accounting for hidden states, adoption of hole-nesting behavior led to higher, not lower, diversification rates (Fig. 3a). However, when we included hidden states, and thus accounted for the possibility that other, unmeasured variables are driving the relationship, we found no difference in diversification rates across the three nesting types (Fig. 3b). This comparison emphasizes that phylogenetic studies of specialization that do not account for hidden states may need to reevaluate their null hypotheses of diversification. More generally, our results, in combination with recent tests of the evolutionary dead end hypothesis, suggest that there is growing evidence

that specialization rarely leads to an evolutionary dead end (Stern et al. 2017; Villastrigo et al. 2020).

The ecological consequences of specialization can be diverse – specialization can, in some cases, decrease competition for resources and, in other cases, increase it. For example, many studies have focused on diet or host specialization, which frequently reduce competition over food or breeding resources (Vamosi et al. 2014). However, specialization can also enable species to escape predation (e.g. Singer et al. 2019) and, in such cases, may actually increase competition for scarce, protective resources. Such is the case for hole-nesting birds, where an important consequence of hole-nesting is that it strongly reduces the risk of nest predation, an important driving force behind life history evolution in birds (Martin 1995). In our study, we were able to distinguish between the influence of ecological specialization *per se* versus one specific ecological consequence of specialization – reduced predation. Dome-nesting birds also show decreased predation rates (Oniki 1979; Linder and Bollinger 1995; Auer et al. 2007; Martin et al. 2017), but they are relatively unspecialized in nesting substrates compared to hole-nesters. However, neither hole-nesting nor dome-nesting were associated with elevated diversification rates. This suggests that, in passerine birds, reduced nest predation rates do not have a strong influence on diversification dynamics and, while escaping predation can often lead to ecological release (Herrmann et al. 2021), this does not necessarily translate into increased ecological opportunity or subsequent adaptive diversification. Moreover, reduced predation can also increase species' ability to persist and, hence, decrease extinction risk. Yet, a change in abundance of nesting holes, such as declines in tree cavities, has been shown to lead to heightened risk of

extinction (Duckworth and Badyaev 2007). Thus, the lack of a relationship with diversification rates may reflect a balance between these various ecological consequences of adopting hole-nesting.

Another factor that may account for inconsistency across studies in the links between specialization and macroevolutionary dynamics is the extent to which specialization results in further evolution of specific traits. Ecological specialization is a narrowing of an organism's niche, and so results from a change in how an organism interacts with its environment. Futuyma and Moreno (1988) point out that specialization, at a minimum, only requires a behavioral shift, and specialized taxa vary in the extent that there is subsequent evolution of other traits. Thus, it is possible that whether specialization is an evolutionary dead end or not may be strongly linked to the extent of secondary adaptation that follows it. In the case of hole-nesting birds, specialization is largely a behavioral shift, although it does lead to evolutionary changes in many life history and breeding traits (see below). Thus, it may be that hole-nesting specialization does not lead to an evolutionary dead end because of the relative ease of reversing these traits (Fig. 4). In the future, studies that explicitly compare multiple types of specialization with distinct ecological consequences and distinct influences on trait evolution would shed light on their relative importance for macroevolutionary dynamics.

The low rate of evolutionary transitions into, relative to out of, hole-nesting (Fig. 4) provides further evidence against the evolutionary dead end hypothesis and instead suggests that evolving ecological specialization can be challenging. In the case of hole-nesters, becoming ecologically specialized involves facing increased interspecific competition, as holes are sought not only by other birds but also by other vertebrates

(Newton 1994). Thus, competition may prevent evolutionary transitions towards specialization. Evolutionary transitions from hole-nesting to open-cup nesting, which occur at a relatively high rate, involve different evolutionary challenges, such as life history specialization. Open-cup nesting species have exceptionally high nestling growth rates, which appear to be an adaptation to increased predation risk (Ricklefs 1979). Moving from the relaxed selection regime on growth rates associated with hole-nesting to the strong selection regime on growth rates in open-cup nests would seem to act as a filter that would limit this transition. However, our results suggest that, at least in passerine birds, evolving such life history shifts is easier than evolving the ability to deal with the intense competition of hole-nesting.

We also found evidence that it was easier for birds to transition from hole to cup rather than hole to dome. Most hole nesters build an open-cup style nest, rather than a dome, within the cavity (Price and Griffith 2017). Our finding that transitions to open-cup nesting were easier compared to transitions to dome may simply reflect that transitions from hole-nesting occurred more often in taxa that were already building open-cup nests within their holes. Thus, for most hole nesters, transitioning to dome-nesting may be more difficult because it would require two steps: leaving cavities and changing how the nest is built. While in the minority, there are several clades, particularly at the base of the passerine tree, that build dome style nests within cavities (Price and Griffith 2017). Future studies comparing transitions to dome and open-cup nests based on whether hole-nesting species are already building a dome or cup style nest within their cavity would add further insight into the mechanisms behind these different transition rates.

Ancestral State Reconstruction

In all our ancestral state reconstructions we found that hole-nesting is the most probable state at the root of the passerines. When including the parrot *Strigops habroptilus* as the outgroup, the maximum of the marginal posterior distribution of the node had a probability of 0.77 for hole-nesting. Without this outgroup, we still recovered hole-nesting with a maximum a posteriori of 0.51 (see figure S12 in supplementary information). These two reconstructions represent significant evidence in favor of the specialized state hole-nesting at the origin of passerines. Previous work has emphasized that hole-nesting, dome-nesting, and open-cup nesting all appear to have occurred early in the history of passerines (Collias 1997; Price and Griffith 2017; Fang et al. 2018; McEntee et al. 2018), with Collias (1997) suggesting that the nest type of the earliest passerine might be unknowable because of the apparent rapid evolution of nest type in early passerines. Our ancestral state reconstruction, using an approach where we included strong species-level sampling and assessed whether differing diversification rates had to be accounted for (Maddison 2006), tips the balance in favor of hole-nesting as the ancestral state for the common ancestor of extant passerines. Notably, this result contrasts with the ancestral state reconstruction of (Fang et al. 2018), which included more sampling outside of passerines and less species-level sampling within passerines. However, in our study, support for hole-nesting as the ancestral state serves to underscore how radically the evolution of hole-nesting as a specialization fails to meet the generalists-to-specialists view of evolution; rather than a dead end, all passerine diversity emerged from a specialist ancestor.

498 *Modeling the diversification consequences of specialization*

499 State-dependent diversification models are a flexible tool to test whether
500 specialization is linked to diversification rates. A recent ongoing discussion in the field of
501 macroevolution centers on the impossibility of estimating speciation and extinction rates
502 from phylogenetic trees with only extant taxa using birth and death stochastic models
503 with time-dependent parameters (Louca and Pennell 2020). The non-identifiability of
504 parameters in the time-dependent diversification models, and the possibility of multiple
505 congruent likelihoods across time-dependent models may affect the state-dependent
506 diversification models presented here. State-dependent diversification models are
507 simply a special case of the time-dependent models explored in Louca and Pennell
508 (2020). However, taking into consideration the nest type and using informative priors to
509 represent different extinction scenarios show the potential for recovering the direction of
510 differences between states, even if point estimates for speciation and extinction rates
511 are not the same across these scenarios (Fig. 4, and Fig. A1), as suggested by Louca
512 and Pennell (2020). Our main findings under the assumptions of enhanced extinction
513 suggest that the relative differences among states are possible to infer despite the non-
514 identifiability between different extinction histories. Therefore, it is important when
515 applying state-dependent diversification models to clarify whether the goal is to obtain
516 point estimates for speciation or extinction rates, or to find relative differences in the
517 history of diversification linked to the states. In this study, we were interested in the
518 latter, and our results suggest that finding relative differences of diversification between
519 states can be performed in a systematic fashion with consistent results.

Overall, we were able to rigorously test different hypotheses and extinction scenarios because of the size of the tree, the number of transitions between nest types, and the large proportion of passerines in the sample (> 51%). Studies of specialization linked to diversification might conclude spurious associations of specialization and diversification when independent transitions to and from the specialized state are too few (Uyeda et al. 2018), tree size is not large enough (Davis et al. 2013), and sampling fraction is small (Chang et al. 2020). Historically, standards for the quality rather than the quantity of the sample needed to fit SSE models have been lacking, so it is important that future studies of state-dependent diversification be aware of these issues.

CONCLUSIONS

Our macroevolutionary analysis failed to find any link between specialization of nest type and diversification rates under different extinction scenarios, suggesting that there is little support for the evolutionary dead end hypothesis in this case study. Moreover, contrary to this hypothesis, we also found that transitions from the specialized state were relatively easy compared to transitions toward the more generalist states and that the root state of passerines is most probably hole-nesting. Our results suggest that the ecological consequences of resource specialization, whether due to escape from competition or predation, might be key to understanding its macroevolutionary consequences. This work adds to other recent studies that have found little support for the evolutionary dead end idea, suggesting that evolution of resource specialization is more evolutionarily labile than previously thought. We suggest that future studies of this question would benefit from explicit comparison of resource specialization that vary in their ecological consequences, as well as the extent of trait

evolution necessary for specialization. Such studies would enable a greater understanding of the mechanisms that underlie variable links between specialization and macroevolutionary dynamics.

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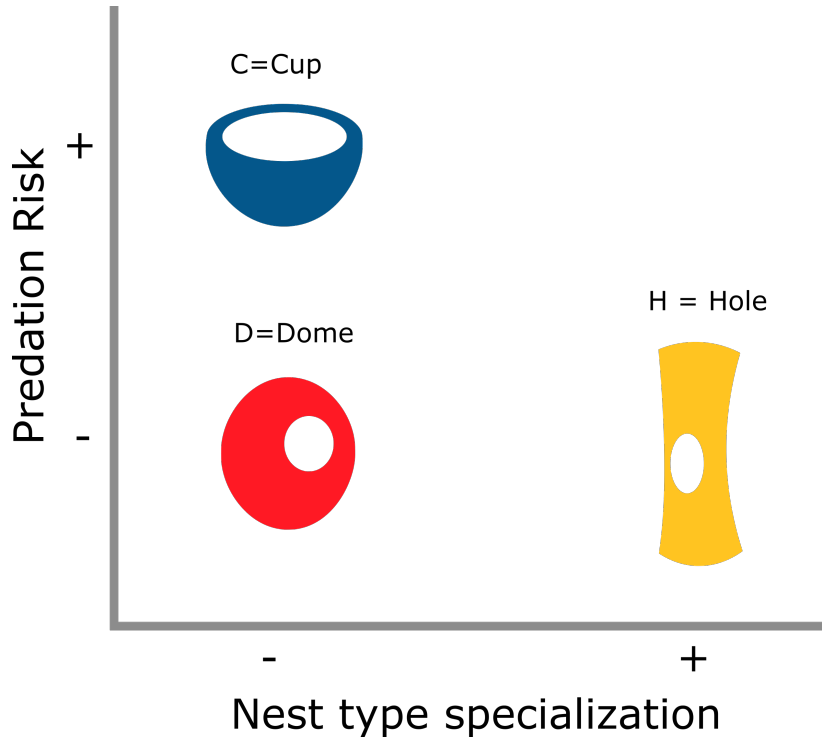
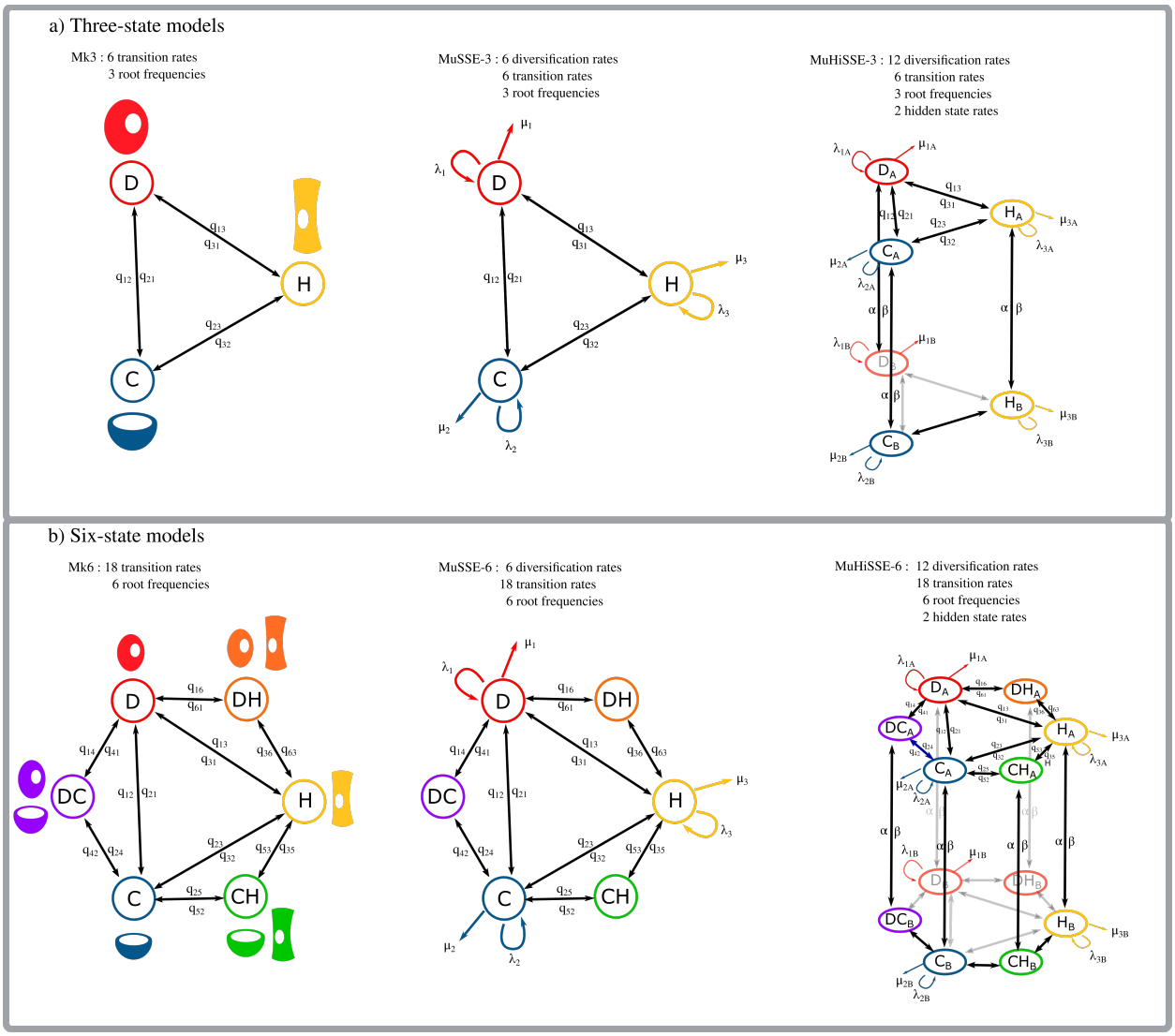


Figure 1. Nesting habits of passerine taxa can be organized based on a trade-off between specialization and predation risk. The most specialized nest type is hole that has the smallest predation risk, followed by dome with also a lower risk but relatively unspecialized in its nesting substrate. Finally, open-cups have the highest predation risk but are also considered unspecialized.



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742 **Figure 2.** Models of nest type evolution. In panel a) three models are depicted: Mk3
743 which assumes all possible pairwise transitions for three types of nest (dome (D), cup
744 (C), and hole (H)); MuSSE-3 which assumes the same transitions but adds
745 diversification parameters to each of the states, and MuHiSSE-3 that adds the
746 possibility of heterogeneity in the diversification process different from the diversification
747 attributable to the state. In panel b) similar models to the ones found in panel (a) are
748 defined with three states that represent nest type (D, C, H) with the addition of three

more states that characterize those species with two nest types (dome-cup(DC), dome-hole (DH), cup-hole (CH)).

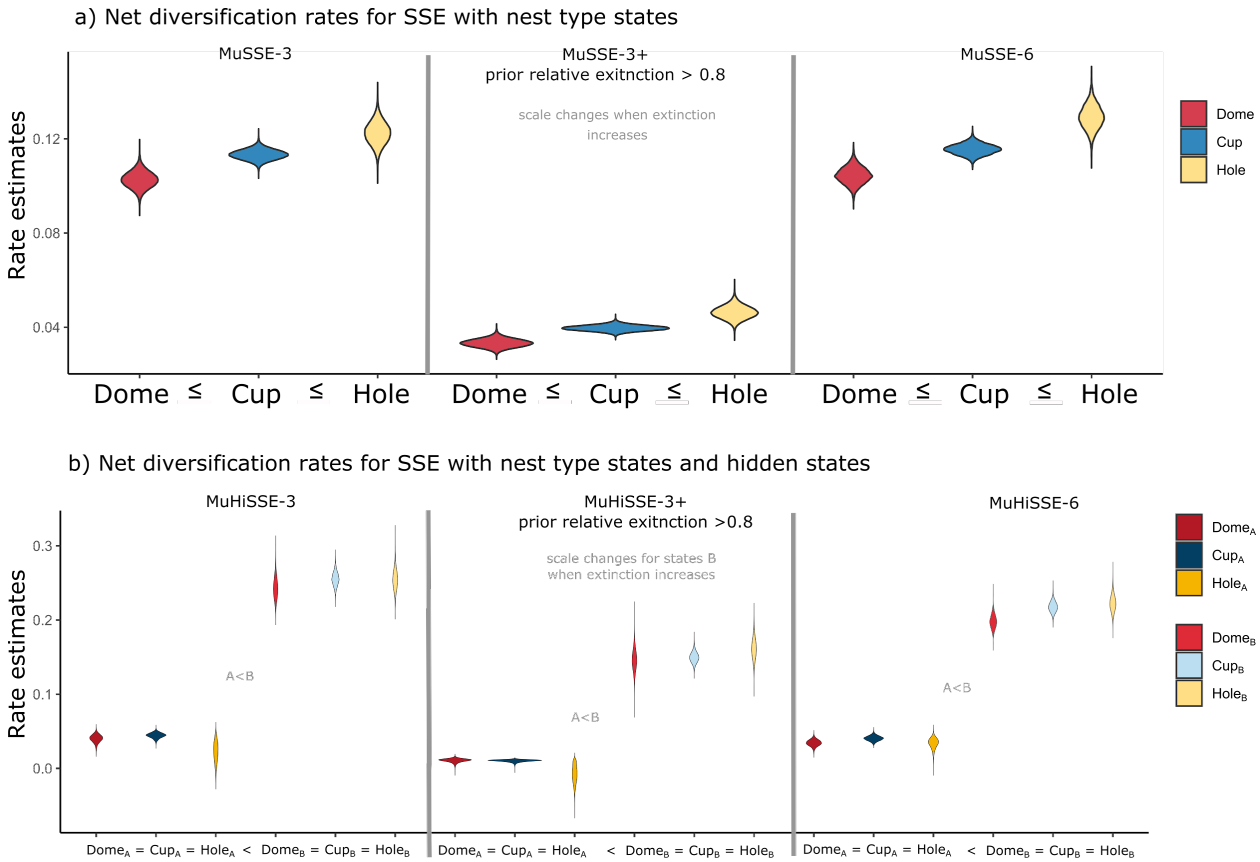

Figure 3. Net diversification rate estimates (speciation and extinction) for a) multistate dependent diversification models (MuSSE), and b) multistate and hidden state dependent diversification models (MuHiSSE). In models in (a) we consistently estimated that hole nesters have faster net diversification rates than dome nesters, and that cup nesters had intermediate rate estimates. This finding was consistent when we assumed the extinction fraction to be at least 0.8 (MuSSE-3 model + prior extinction fraction > 0.8), or adding more intermediate states (MuSSE-6 model). The most noticeable change is that increasing the extinction fraction (MuSSE-3 model+ extinction fraction >

0.8) decreases net diversification estimates (y-axis), but the relative differences between states are maintained. For models in (b) we observe that all diversification differences are due to the differences in hidden state (A<B) rather than nest type (Dome=Cup=Hole for A and B states), which is strong evidence against the direct influence of nest type on the speciation and extinction of passerines. Even increasing the extinction fraction (MuHiSSE-3 + prior extinction fraction > 0.8) or adding more intermediate states (MuHiSSE-6) yields the same results.

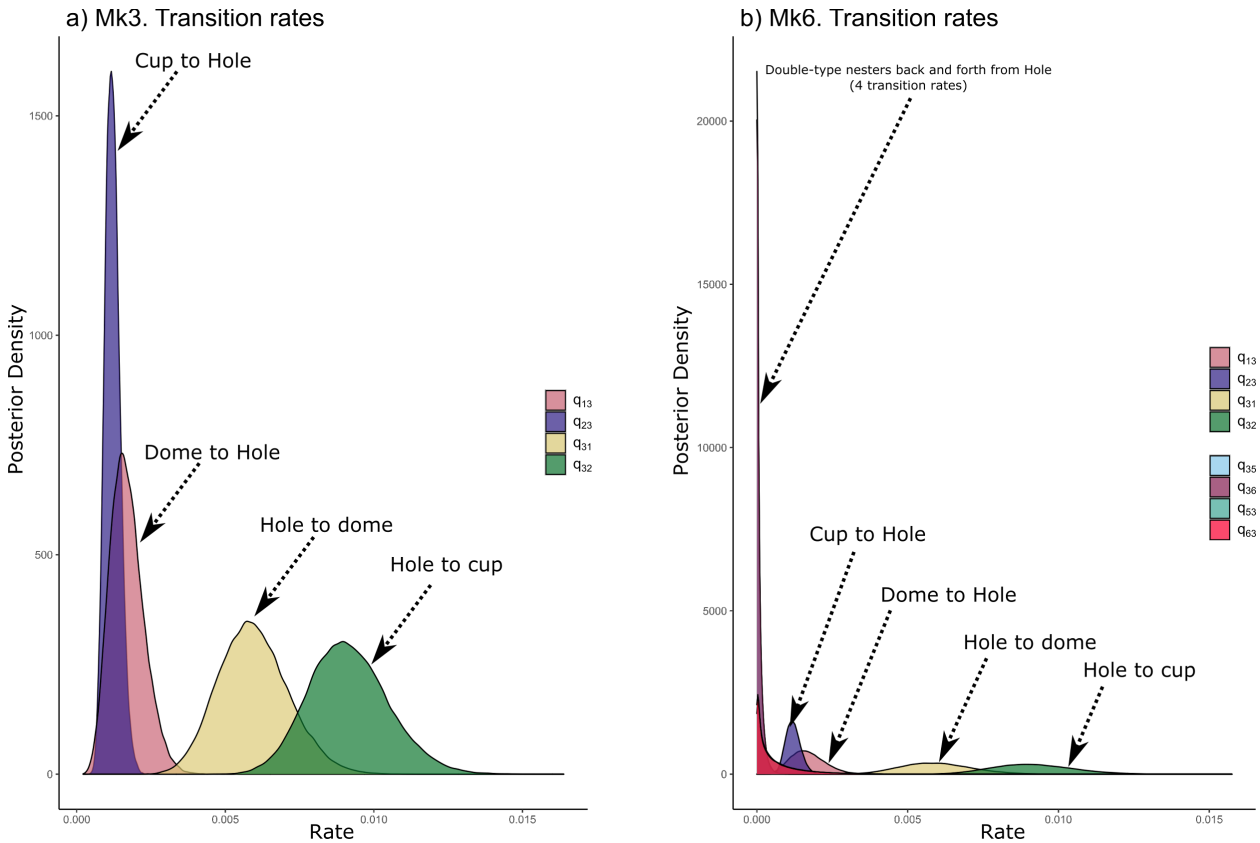

Figure 4. Transition rates between nest states for diversification-free models. In a) the model with three states (Mk3), the posterior distribution for the rate from hole to cup (q_{32}) is faster than any other rate to and from hole. For b) the model with six-states (Mk6), in which states with two nest types are included, we found that evolving out of hole is faster than into hole, with hole to cup faster than hole to dome. Transitions back to hole from cup or dome are less frequent, and evolving back and forth between states CH or DH to hole has the slowest rates (four rates overlap).

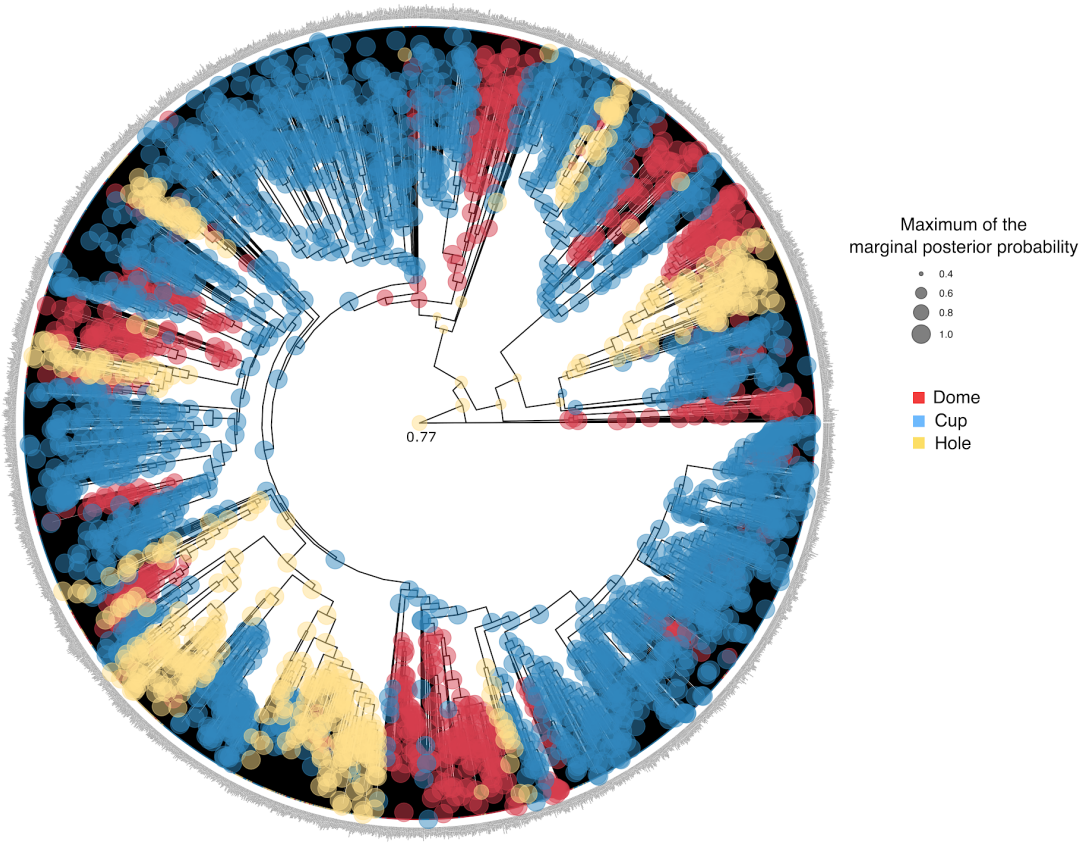

Figure 5. Ancestral state reconstruction for nest type using a Markov model with three states (Mk3). The size of a node represents the maximum probability value of the marginal posterior distribution of the node. The color of the node represents the nest type associated with the maximum posterior probability. This tree includes the hole-nesting *Strigops habroptila* as the outgroup.

787 APPENDIX

Table A1. Assumptions of extinction in different MuSSE-3 models.

Assumption Extinction fraction	Extinction as a function of speciation	Prior distributions used*	Interpretation
1. NA	NA	$\lambda_i \sim LogN(\mu_\lambda, \sigma_\lambda)$ $\mu_i \sim LogN(\mu_\mu, \sigma_\mu)$	Speciation and extinction freely estimated
2. $\mu_i/\lambda_i \geq 0.7$ with $i = C, D, H$	$\mu_i = 0.7\lambda_i + \delta$	$\lambda_i \sim LogN(\mu_\lambda, \sigma_\lambda)$ $\delta \sim Exp(2)$ $i = C, D, H$	Extinction rate is at least 0.7 of speciation rate
3. $\mu_i/\lambda_i \geq 0.8$ with $i = C, D, H$	$\mu_i = 0.8\lambda_i + \delta$	$\lambda_i \sim LogN(\mu_\lambda, \sigma_\lambda)$ $\delta \sim Exp(2)$ $i = C, D, H$	Extinction rate is at least 0.8 of speciation rate
4. $\mu_i/\lambda_i \geq 0.9$ with $i = C, D, H$	$\mu_i = 0.9\lambda_i + \delta$	$\lambda_i \sim LogN(\mu_\lambda, \sigma_\lambda)$ $\delta \sim Exp(2)$ $i = C, D, H$	Extinction rate is at least 0.9 of speciation rate
5. $\mu_i/\lambda_i \geq 1.0$ with $i = C, D, H$	$\mu_i = \lambda_i + \delta$	$\lambda_i \sim LogN(\mu_\lambda, \sigma_\lambda)$ $\delta \sim Exp(2)$ $i = C, D, H$	Extinction rate is greater or equal to speciation rate
6. $\mu_i/\lambda_i \geq 1.1$ with $i = C, D, H$	$\mu_i = 1.1\lambda_i + \delta$	$\lambda_i \sim LogN(\mu_\lambda, \sigma_\lambda)$ $\delta \sim Exp(2)$ $i = C, D, H$	Extinction rate is at least 1.1 of speciation rate
7. $\mu_C/\lambda_C \geq 0.8$ $\mu_D/\lambda_D \geq 0.7$ $\mu_H/\lambda_H \geq 0.6$	$\mu_C = 0.8\lambda_C + \delta$ $\mu_D = 0.7\lambda_D + \delta$ $\mu_H = 0.6\lambda_H + \delta$	$\lambda_i \sim LogN(\mu_\lambda, \sigma_\lambda)$ $\delta \sim Exp(2)$ $i = C, D, H$	For cup, extinction rate is at least 0.8, for dome, is at least 0.7, and for hole, extinction rate is at least 0.6 of speciation rate

*For prior distributions of the models we used a log-Normal distribution for speciation (λ) with a mean parameter $\mu_\lambda$equal to the total number of lineages 3225 divided by 2, and standard deviation σ_λ of approximately 2. For the extra parameter (δ) we chose to model it using an exponential with rate 2. Other distributions were tested for that extra parameter and results were unaltered.

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In figure A1 we summarize the results of assumptions 1-6 from table A1. Here we can observe that when it is assumed that the extinction fraction is greater than 0.7 but less than 1, we recover the differences between states (Dome<Cup<Hole). When the extinction fraction is at least 1 and the extinction rate is expected to be about as large as speciation, then we found no differences in the posterior distributions of the three states. Finally, when the extinction rate is assumed to be larger than the speciation rate we obtain the reverse effect (Hole<Cup<Dome). These results show that despite our prior assumptions about extinction rate, the hole-nesting taxa will have the greatest influence in the direction of net diversification than cup or dome nesters. And finally, the state dome always has the smallest effect on net diversification in absolute value. These results might only be applicable to a trait that does not influence diversification, as we have shown using hidden state and character independent models for nest type (figure 2). Whether this pattern of finding similar differences in diversification is sustained in the face of enhanced extinction holds for all traits in SSE models remains to be tested.

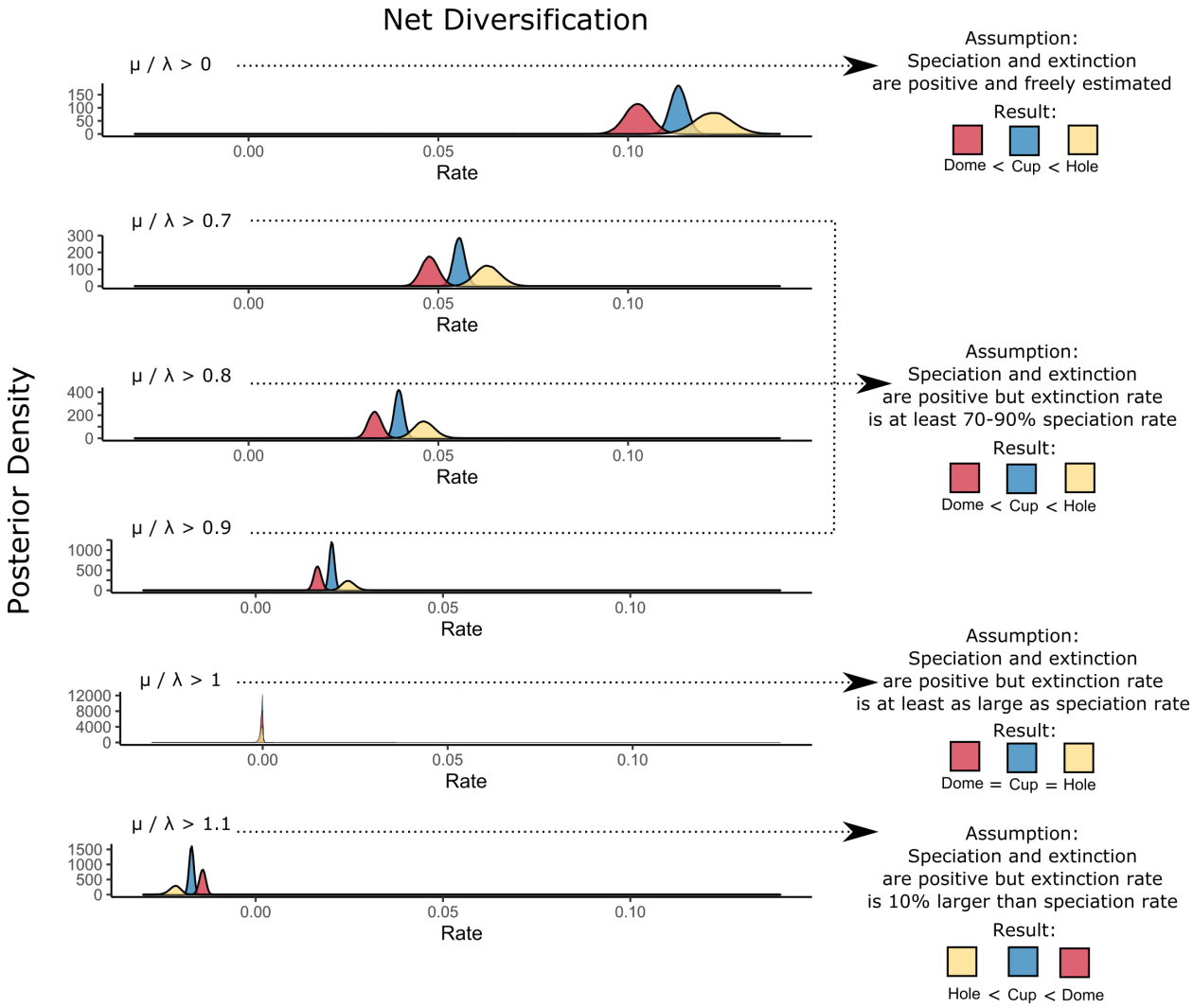

Figure A1. Different *a priori* assumptions about the minimum of extinction fraction ($a = \frac{\mu}{\lambda}$). When speciation and extinction are estimated freely ($a > 0$), the posterior distributions for net diversification show that the rate of diversification for hole is faster than cup, and both are faster than dome net diversification. This inference is supported even when we assume that extinction rate should be faster (in other words for cases where $a > 0.7, a > 0.8, a > 0.9$ for all states) but still smaller than speciation; however, this assumption changes the overall scale leading to smaller net diversification values. The inference from net diversification starts changing when we assumed $a > 1$, that is,

that extinction is at least as fast as speciation for every state, where we found that posterior distributions of net diversifications for every state overlap. Once we assumed that extinction is much faster than speciation $(a > 1.1)$, we found a mirror trend in the inference with hole net diversification being the slowest, and dome being the fastest.