1   **The Evolution of Nest Site Specialization and its Macroevolutionary**

2   **Consequences in Passerine Birds**

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Zenil-Ferguson, R.; McEntee, J.P.; Burleigh, J.G.; and Duckworth, R.A. - Nest type linked to diversification

19   *Abstract.* – A long-standing hypothesis in evolutionary biology is that the evolution of

20   resource specialization can lead to an evolutionary dead end, where specialists have

21   low diversification rates and limited ability to evolve into generalists. However, in recent

22   years, advances in comparative methods investigating trait-based differences

23   associated with diversification have enabled more robust tests of this idea and have

24   found mixed support. Here we test the evolutionary dead end hypothesis by estimating

25   net diversification rate differences associated with nest site specialization among 3,224

26   species of passerine birds. In particular, we test whether the adoption of hole-nesting, a

27   nest site specialization that decreases predation, results in reduced diversification rates

28   relative to nesting outside of holes. Further, we examine whether evolutionary

29   transitions to the specialist hole-nesting state have been more frequent than transitions

30   out of hole-nesting. Using diversification models that accounted for background rate

31   heterogeneity and different extinction rate scenarios, we found that hole-nesting

32   specialization was not associated with diversification rate differences. Furthermore,

33   contrary to the assumption that specialists rarely evolve into generalists, we found that

34   transitions out of hole-nesting occur more frequently than transitions into hole-nesting.

35   These results suggest that interspecific competition may limit adoption of hole-nesting,

36   but that such competition does not result in limited diversification of hole-nesters. In

37   conjunction with other recent studies using robust comparative methods, our results add

38   to growing evidence that evolutionary dead ends are not a typical outcome of resource

39   specialization.

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

41   specialization

42   Resource specialization, where species use a narrower range of resources

43   compared to related taxa, is a common phenomenon in evolution. There are many

44   reasons resource specialization (hereafter specialization) can evolve. For example,

45   specialists can benefit from reduced competition, or from the avoidance of predators or

46   parasites (Futuyma and Moreno 1988; Bernays 1989; Schluter 2000). Once evolved,

47   specialization can have diverse consequences for macroevolutionary dynamics.

48   Historically, specialization often has been considered an evolutionary dead end,

49   resulting in both reduced net diversification relative to generalists, and difficulty evolving

50   from specialist to generalist. This difficulty may arise when adaptation to specialized

51   resources occurs across numerous traits (Futuyma and Moreno 1988), and, when

52   coupled with changes in resource availability, can lead to elevated extinction rates in

53   specialists. Additionally, reduced diversification of specialists could result if the number

54   of available niches in the specialized state is low.

55   Consistent with the evolutionary dead end view of specialists, the prevailing

56   historical viewpoint was that specialists evolve from generalist ancestors (Schluter

57   2000). Prior studies have found mixed support for this viewpoint, with inferences from

58   phylogenetic comparative methods showing that generalists regularly evolve from

59   specialists in many clades (Day et al. 2016; Sexton et al. 2017; Villastrigo et al. 2021).

60   These results indicate that the dominant direction of evolutionary transitions involving

61   specialization should be treated as an open question for any specialization scenario.

62   More recent studies have also noted the potential for specialization to lead to

63   increased diversification through multiple mechanisms. First, the release of specialists

64   from the effects of competition and/or predation could trigger periods of niche-filling

65   diversification (Schluter 2000), leading to higher rates of speciation in specialists

66   compared to generalists. Further, in circumstances where specialization releases

67   specialists from competition or predation (Futuyma and Moreno 1988), specialists may

68   experience greater population persistence than generalists, which could increase

69   diversification rates by decreasing extinction rates or by increasing rates of allopatric

70   speciation via the longer survival of nascent lineages (Harvey et al. 2019), or both.

71   Finally, specialist lineages could also have higher diversification rates if more

72   specialized lineages have more fragmented distributions or lower rates of dispersal

73   (Gavrilets et al. 2000; Birand et al. 2012).

74   New diversification models and statistical developments have the potential to

75   alter our conclusions on the macroevolutionary consequences of specialization. A

76   growing awareness of type I error (Davis et al. 2013; Rabosky and Goldberg 2015), the

77   importance of large sample sizes for statistical power (Davis et al. 2013; Day et al.

78   2016), and the misspecification of the null hypothesis for the original state-dependent

79   diversification models (Beaulieu and O'Meara 2016; Rabosky and Goldberg 2017;

80   Caetano et al. 2018) have produced a wave of new studies using robust statistical

81   approaches on the question of whether specialization is linked to diversification. The

82   majority of studies after Day et al.'s (2016) review on specialization have found either no

83   association between specialization and diversification (e.g. Alhajeri and Steppan 2018;

84   Crouch and Ricklefs 2019; Villastrigo et al. 2020) or have found that specialization is

85   associated with higher diversification rates (e.g. Conway and Olsen 2019; Otero et al.

86   2019; Tonini et al. 2020), with few studies indicating specialization leads to an

87   evolutionary dead end (e.g. Cyriac and Kodandaramaiah 2018; Day et al. 2016). These

88   new results seem to indicate that specialization rarely leads to evolutionary dead ends.

89   While these newer studies are using trees with more tips and more transitions to

90   specialist states, which can help disentangle consequences of specialization in

91   diversification (Davis et al. 2013), moving forward it is necessary to integrate new

92   methods that incorporate heterogeneity in the diversification process unlinked from

93   specialization (Beaulieu and O'Meara 2016).

94   Here we examine the evolution of specialization of nesting microhabitat and its

95   macroevolutionary consequences using nesting site data for 3,224 species of passerine

96   birds. Passerines provide a unique opportunity to examine the importance of hole-

97   nesting as an example of resource specialization that may drive diversification patterns

98   using phylogenetic comparative methods. First, there are over 6,000 species of

99   passerines, and hole-nesting behavior has evolved multiple times across the clade,

100   providing numerous transitions to estimate rates of diversification linked to trait evolution

101   (Davis et al. 2013). Second, passerines can be grouped into three general nesting

102   habits: open-cup nesters, dome nesters and hole nesters (e.g. Wallace 1868; Martin

103   1995; Collias 1997). Open-cup and dome nesters are relatively unspecialized in their

104   nesting substrate compared to hole-nesters; however, dome nesters also have reduced

105   predation rates compared to open-cup nesters (Oniki 1979; Linder and Bollinger 1995;

106   Auer et al. 2007; Martin et al. 2017). Therefore, our approach of comparing adoption of

107   these three nest types enables us to assess the potential effects of nest site

108   specialization versus escape from predation on diversification rates (Fig. 1).

109   Nest site selection is a critically important aspect of avian habitat because nest

110   failure rates are high in birds (Nice 1957), and predation is the main cause of nestling

111 mortality (Ricklefs 1969; Martin 1993). Adoption of hole-nesting has the advantage of

112 protecting offspring from predation, and non-excavating hole nesters show an

113 approximate 43% reduction in nest failure compared to non-hole nesters (Martin 1995).

114 Thus, the main ecological consequence of hole-nesting specialization is that it provides

115 release from predation. Hole-nesting represents resource specialization for two

116 reasons: the great majority of species that nest in holes do so obligately, and exclusive

117 use of holes greatly restricts the substrates that are suitable for nest building.

118 Hole-nesting is associated with the evolution of a suite of traits, such as

119 increased nesting period, brighter egg coloration, and larger clutch sizes, that may

120 make it difficult to transition out of hole-nesting (Martin and Li 1992; Kilner 2006).

121 Moreover, the great majority of hole-nesting passerines cannot excavate their own nest

122 holes, and competition for this limited resource is intense (Newton 1994). While hole-

123 nesting likely evolves as a response to predation pressure, the intense competition for

124 nesting sites decreases nest hole availability (Cockle et al. 2011). This competition may

125 limit ecological opportunity for hole-nesting lineages despite the reduction in predation

126 pressure. Further, reliance on holes for nesting may increase extinction risk when

127 environmental changes reduce nest hole availability, particularly if transitioning from

128 hole-nesting is difficult. This combination of characteristics makes hole-nesting in

129 passerines a form of specialization that could be an evolutionary dead end. However, as

130 hole-nesting decreases nest predation, it is also possible that adopting hole-nesting

131 could lead to greater population persistence over time which, in turn, could lead to

132 reduced extinction rates or increased rates of allopatric speciation (Harvey et al. 2019).

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133 Thus it is also possible that hole-nesting lineages could experience greater

134 diversification rates than the more generalist nonhole-nesting lineages.

135   The evolutionary dead end hypothesis assumes that it is easy to adopt hole-

136 nesting habits, but difficult to transition out of them because of secondary adaptations

137 that restrict evolutionary transitions (Collias and Collias 1984). In passerines, most hole

138 nesters are secondary (i.e., they cannot make new cavities, and thus they rely on what

139 is already in the environment), and so adoption of hole-nesting is mainly a behavioral

140 shift not requiring extensive morphological modification (although see above for

141 examples of life history traits associated with hole-nesting). This could make it relatively

142 easy to evolve hole-nesting behavior. However, the intense competitive environment

143 that hole nesters face could mean that hole-nesting niches are already saturated,

144 making it a more difficult habit to adopt. To examine these possibilities, we not only

145 assess the impact of nest type on diversification rates, but we also calculate transition

146 rates among them.

147   We employ a graphical modeling approach (Jordan 2004) using Bayesian

148 statistics to infer parameters of state-dependent speciation and extinction models (SSE

149 models), and diversification-free models (Mkn models) coded in RevBayes software

150 (Höhna et al. 2016). The large dataset and Bayesian framework enable us to avoid a

151 number of common pitfalls for similar studies on diversification. Additionally, we adopt a

152 systematic approach to specifying prior distributions to model extinction rates that

153 allows us to assess the consistency of estimates and inference across a spectrum of

154 macroevolutionary diversification scenarios. Finally, after determining the best model,

155 we used it to reconstruct ancestral states of nest types to visualize the pattern of

156   evolutionary history for the trait and compare with previous ancestral state

157   reconstructions.

158   MATERIALS AND METHODS

159   *Data*

160   Our nesting data is a slightly adapted version of a dataset originally assembled

161   for McEntee et al. (2018). This dataset was generated by scoring the nesting behavior

162   of the passerine species using descriptions from the Handbook of the Birds of the World

163   Alive, (Del Hoyo et al. 2017, last accessed 30 June 2016, hereafter HBW). Specifically,

164   species' nests were scored as either open-cup, dome, hole, or some combination of two

165   of these three nest types for the few species that show flexible nest type use. "Hole"

166   refers to any nest built inside a tree cavity, rock crevice, or earthen bank. The small

167   number of brood parasites were excluded from analyses. McEntee et al. (2018) were

168   able to score the nest types for approximately three quarters of the 5,912 passerine

169   species from the information in the HBW species descriptions.

170   Next, we matched the passerine species in the nesting dataset to the species'

171   names on the tips of the avian supermatrix phylogeny of Burleigh et al. (2015), one of

172   the largest phylogenetic trees of birds constructed exclusively from molecular data. This

173   tree was time-calibrated using penalized likelihood in r8s (Sanderson 2003) with twenty

174   fossil calibrations from throughout the avian phylogeny (Baiser et al. 2018). We

175   identified all cases in which a species in the Burleigh et al. (2015) phylogenetic tree,

176   which used the Clements taxonomy, did not have a corresponding species with the

177   exact same name in the dataset from the HBW Alive/BirdLife International taxonomy.

178   This was due to either missing nesting data or a taxonomic mismatch. For taxonomic

179   mismatches, we examined the taxonomic history for these species in Avibase

180   (https://avibase.bsc-eoc.org/), and when appropriate, changed the species name in the

181   nest dataset to match the phylogenetic tree (Burleigh et al. 2015). Taxa treated as

182   subspecies in the HBW taxonomy (2015) but as taxa in Burleigh et al. (2015) were not

183   included in our analyses because they did not occur as tips in the phylogenetic tree. We

184   then trimmed the phylogenetic tree to include only species in the nesting dataset from

185   HBW. The resulting data set has the nesting state for 3,224 passerine species. Of

186   these, 1943 species had dome nests (D), 722 had cup nests (C), and 458 had hole

187   nests (H). Among species with multiple nest types, 60 were cup or hole nesters (CH),

188   29 were dome or cup nesters (DC), and 13 were dome or hole nesters (DH). In the few

189   instances (less than 1% of internal nodes) where this tree was not bifurcating (required

190   for the diversification analyses described below) because nodes were collapsed in the

191   r8s analysis, we resolved bifurcations randomly using the function `multi2di` from the

192   R package `ape` (Paradis and Schliep 2019).

193   *Diversification models with different numbers of states*

194   Using the three states assigned to the tips of the 3,225-taxon phylogenetic tree

195   (3,224 passerines plus a single species representing the parrot outgroup), we defined

196   four state-dependent speciation and extinction models (SSEs). The first multistate SSE

197   model, called MuSSE-3 here, uses three main states: dome D, cup C, and hole H (Fig.

198   2a), each with their own speciation and extinction rate (Fig. 2a). MuSSE-3 also has

199   transition rates between each state (q_{ij} with $i, j = D, C, H$, and $i \neq j$) governing the rate

200   of evolution from one nest type to another. All transitions between nest types are

201   possible. Taxa that have multiple nest types are coded in the input data as belonging to

202   multiple states simultaneously. For example, a bird taxon reported to have nested in

203   both hole and dome nests is coded in the input data as (H, D). For taxa (tips) with two

204   nest types, the likelihood calculation includes the product of two transition probabilities

205   from the most recent common ancestor to each potential state in the tips instead of a

206   single transition probability from the most recent common ancestor to one single state.

207   The second model is a six state SSE called MuSSE-6 in which the six main

208   states represent not only dome D, cup C, and hole H nest types, but also all the

209   pairwise combinations of multiple nest types (dome and cup (DC), dome and hole (DH),

210   and cup and hole (CH), Fig. 2a) to account for those species that show flexibility in nest

211   type use. MuSSE-6 includes speciation and extinction rate parameters linked only to the

212   D, C, and H states. Transition rates between all states are possible except transitions

213   between those states representing two different nest types and the single nest type

214   state not including either of these nest types (e.g. state DC does not connect with state

215   H since that represents a double transition, Fig. 2b). The MuSSE-6 model helped us

216   identify the most appropriate way to score states when taxa can use more than one nest

217   type.

218   The third and fourth models are the hidden state extensions of the MuSSE-3 and

219   MuSSE-6 respectively. The hidden state extension is necessary to avoid null

220   hypothesis misspecification since the null hypothesis should include background

221   heterogeneity in the diversification rate that is not linked to the trait of interest (fulfilled

222   by character-independent models thoroughly discussed in Beaulieu and O'Meara

223   (2016), and Caetano et al. (2018). For both the three-state model and the six-state

10

224   model, we expanded the three single nest type states to six with subscripts A or

225   B.These models are the MuHiSSE-3 and MuHiSSE-6 respectively (Fig. 2).

226

227   *Diversification models with different assumptions about extinction rates*

228   Fossil records for passerines suggest rapid turnover of diversity over time, with

229   high species diversity and diversification rates (Jetz et al. 2012; Marshall 2017) despite

230   the extinctions of entire early passerine lineages (Hieronymus et al. 2019; Ksepka et al.

231   2019), high turnover of passerines within Europe between the Oligocene and the

232   present (Manegold 2008; Bochenski et al. 2021), and the impact of late Pleistocene

233   species extinctions (Steadman et al. 2015; Oswald and Steadman 2018). Diversification

234   models using phylogenetic trees with only extant taxa often appear to underestimate the

235   rates of extinction (Höhna et al. 2011; Louca and Pennell 2021), producing error in

236   many diversification analyses (Stadler 2013). This presents a potential problem in the

237   passerines because of the fossil evidence that turnover has been high (see also

238   Marshall's third law of paleobiology: the average extinction rate approximately equals

239   the average origination rate; Marshall 2017).

240   In a Bayesian framework, one way to incorporate knowledge about higher

241   extinction rates is by using a prior distribution that accommodates independent

242   information about the magnitude of the rates. Therefore, in our three-state, six-state,

243   and hidden-state models, we defined prior distributions for the extinction rates with

244   enhanced lower bounds, and we systematically increased these lower bounds across

245   model fits (Table A1). We specified these lower bounds by making extinction rates'

246   lower bound to be dependent on speciation rates. In mathematical form, we defined this

247   process by using the linear function for extinction rates as $\mu_i = A * \lambda_i + \delta$ where $A =$

248    $0.7, 0.8, 0.9, 1, 1.1, 1.2$, for all the states $i = D, C, H$. Therefore, the extinction rates are a

249   linear combination of random variables. The speciation rates λ_i have a log-normal prior

250   distribution with hyperparameters $(\mu_\lambda = ln\left(\frac{3225}{2}\right), \sigma_\lambda = 1.12)$ and the second random

251   variable of the linear function, represented by the parameter δ, is distributed as Exp (2).

252   Defining extinction rate prior distribution as a linear combination of random variables,

253   allowed us to fix the value A as the lower bound for the extinction fraction $a_i = \frac{\mu_i}{\lambda_i}$ (we

254   use the same definition of extinction fraction as (Beaulieu and O'Meara 2016) while

255   permitting extra variability in the diversification rates, so that extinction rates are not fully

256   a deterministic function of speciation rates. For example, the extinction rate of taxa with

257   dome nests that is at least 80% of the speciation rate is defined with the equation $\mu_D =$

258    $0.8 * \lambda_D + \delta$. In this example the posterior estimation of the extinction fraction for state

259   Dome $(a_D = \frac{\mu_D}{\lambda_D})$ is forced to be at least 80%, but the extinction fraction can still be larger

260   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

261   models, prior distributions for extinction rates were defined equally (i.e. $\mu_D = \mu_C =$

262    $\mu_H = A * \lambda_D + \delta$). In one model, we specified $\mu_H < \mu_D < \mu_C$ by making $A = 0.6, 0.7$, and

263   0.8 respectively to verify that: 1) our inferences weren't driven by this specification of the

264   prior; and 2) that likelihood is informative given our dataset (supplementary Fig. S11).

265

266   *Testing the role of diversification in nest type evolution*

267   To test if nest type evolution is linked to the diversification of passerines, we

268   calculated the marginal log-likelihoods for the MuSSE-3 and the character independent

269   model (CID-3). It is important to notice that in the CID-3 notation the 3 refers to the

270   number of hidden states that have different diversification rates, and not necessarily the

271   number of states of the main trait, to compare against the correct null hypothesis as

272   discussed in Caetano et al. (2018). We calculated Bayes factors (Kass and Raftery

273   1995) to compare CID-3 against the MuSSE-3 model. To approximate the marginal log-

274   likelihood we calculated 18 stepping stones using the methodology from Xie et al.

275   (2011). We calculated the difference between the log-marginal likelihoods of CID-3 and

276   MuSSE-3 models using the statistic κ defined as $\kappa = ln(P(X|CID-3)) - ln(P(X|MuSSE-$

277    $3))$, where X represents our state sample and the phylogenetic tree. If κ has a value

278   larger than 1, then the CID-3 model is preferred. The MuSSE-3 model is preferred when

279    $\kappa < -1$. The test statistic is inconclusive when κ has a value in the interval (-1, 1). We

280   did not calculate marginal log-likelihood for a CID-6 model to compare against the

281   MuHiSSE-3 because CID-6 would require the estimation of too many parameters (36 in

282   total: 12 diversification rates, 6 transition rates, 12 hidden state transition rates

283   parameters, and 6 frequency parameters for the root), and, as discussed in the results,

284   we found strong evidence that nest type is not linked to diversification using the much

285   simpler CID-3 and the MuSSE-3.

286

287   *Ancestral state reconstruction using diversification-free models*

288   It was important to verify the most probable state at the root since previous

289   studies have found that hole is the most likely state at the root, suggesting that all

290   passerines descend from an already specialized nest type. The results showed that

291   nest type evolution is not linked to the diversification process (see Results section).

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292   Therefore, we defined both three-state (Mk3, Fig. 2a) and six-state (Mk6, Fig. 2b)

293   Markov models without diversification parameters (hereafter, "diversification-free

294   models") to reconstruct the ancestral nest type in passerines. We calculated the

295   marginal ancestral state reconstruction at each of the internal nodes. Since our

296   reconstructions use a Bayesian framework, in each of the nodes we plot the state with

297   the maximum *a posteriori* value of the marginal posterior distribution (Fig. 5), as

298   previously done in Freyman and Höhna (2018), and Zenil-Ferguson et al. (2019)

299   implemented in RevBayes (Höhna et al. 2016). For all of our estimations, we treated the

300   root as a stochastic vector whose posterior distribution needed to be jointly estimated

301   along with the diversification and transition parameters (see numbers of parameters in

302   Fig. 2). For the Musse-3 model, the stochastic vector we chose at the root was

303   composed of three frequencies that sum up to 1 (for dome, cup, and hole respectively)

304   and that stochastic vector had a Dirichlet distribution with parameter $(1/3, 1/3, 1/3)$ as

305   the prior distribution. We calculated the posterior distribution for this stochastic vector

306   via our MCMC algorithms.

307

308   *Implementation of models and inferences*

309   All the diversification and diversification-free models in Fig. 2 were implemented

310   as graphical models in RevBayes (Höhna et al. 2016). We customized and ran a

311   Markov chain Monte Carlo (MCMC; Metropolis et al. 1953; Hastings 1970) algorithm to

312   sample the posterior distribution of each model. For all the models (diversification and

313   diversification-free), we assumed that the state value at the root was unknown, and we

314   estimated the posterior distribution of the frequencies at the root. The prior distribution

315   of the root values was defined via a Dirichlet distribution with uniform frequencies

316   across the number of states for each model. Convergence and effective sample sizes of

317   at least 250 for every parameter in the MCMC were assessed using Tracer (Rambaut et

318   al. 2018) and two chains were run per model to verify convergence. Each MCMC run

319   was performed in the HiPerGator cluster at the University of Florida, and took an

320   average of 240 hours to converge. All our implementations and results are available at

321   https://github.com/roszenil/nestdivmodel.

322

323   RESULTS

324   *Diversification is not linked to nest type*

325   While our MuSSE models found associations between nest type and

326   diversification rates, our MuHiSSE models showed that these associations were

327   spurious. This is because the addition of hidden states accounts for the possibility that

328   diversification is driven by unmeasured factors other than nest type. In particular, for

329   the MuSSE-3 model of nest evolution, we found that hole nesters had faster net

330   diversification (defined as speciation minus extinction) than cup nesters, and the

331   smallest net diversification was associated with dome nesters (Fig. 3a). This result was

332   also true when the prior of extinction fraction was faster ($a = \mu/\lambda = 0.8$, Fig. 3a), or

333   when the states were coded using the MuSSE-6 (Fig. 3a). This result also holds for all

334   enhanced extinction fractions ($0 << a = \mu/\lambda < 1$) (Fig. A1). When fitting the hidden

335   state models MuHiSSE-3 and MuHiSSE-6 models (Fig. 3b), we found that the three-

336   state posterior distributions of net diversification rates completely overlap within A and

337   within B while being different between A and B, indicating that diversification rate

338   differences across the phylogenetic tree are due to hidden states (Fig. 3b). These

339   results were consistent when we allowed MuHiSSE-3 to have an extinction fraction of at

340   least 0.8 (Fig. 3b). Furthermore, when calculating Bayes factors comparing the CID-3

341   (diversification rates independent of nest type) model against the MuSSE-3

342   (diversification rates depend on nest type), we found that the CID-3 model is preferred

343   over the MuSSE-3 ($\kappa = -6033.72 - (-7772.40) = 1738.67 > 1$). Altogether, there is

344   strong evidence that the heterogeneity observed in passerine diversification rates is not

345   linked to nest type.

346

347   *Evolving out of holes is faster than evolving back into them*

348   Since nest type is not linked to the diversification process, we turn to understand the

349   dynamics under the diversification-free Mk3 model. The posterior distribution of

350   transition rates from the Mk3 model shows that the rate of transitioning from hole to cup

351   was the fastest (mean $q_{32} = 9.1x10^{-3}$, 95% credible interval $(6.5x10^{-3}, 0.01$)),

352   followed by the transition from hole to dome (mean $q_{31} = 6.5x10^{-3}$, 95% credible

353   interval $(3.7x10^{-3}, 8.2x10^{-3})$, Fig. 4A). Transition rates from either dome or cup to hole

354   nests were similar, and both were slower than the transition rates out of hole nests

355   (dome to hole transition rates have a mean $q_{13} = 1.6x10^{-3}$, and 95% credible interval

356    $(6.6x10^{-4}, 2.8x10^{-3})$; cup to hole transition rates have a mean $q_{23} = 1.2x10^{-3}$, credible

357   interval $(7.3x10^{-4}, 2.5x10^{-3})$, shown in Fig. 4a. This pattern was also found in the Mk6

358   model (Fig. 4b) which further revealed that transitioning from hole-nesting to use of two

359   different nest types (DH or CH) is rare (Fig. 4b, credible intervals for these rates contain

360   zero).

361

Hole-nesting is the most probable state at the root

363   The Mk3 model reconstructs hole-nesting as the state with the highest probability

364   at the most recent common ancestor of the Passeriformes we found that the maximum

365   *a posteriori* for the root's marginal posterior probability was 0.77 for hole-nesting when

366   the tree included the parrot *Strigops habroptila* as the outgroup. This probability

367   decreased to 0.51 when the tree only contained passerines and no outgroup (states

368   hole and cup make the other 0.49 of probability). The posterior distribution for the

369   stochastic vector at the root had as its maximum the vector (Dome=0.30, Cup=0.25,

370   Hole=0.45).

371   DISCUSSION

372   Over the years, there have been diverse perspectives on the link between

373   ecological specialization and evolutionary diversification (Vamosi et al. 2014). On the

374   one hand, ecological specialization has been viewed as an evolutionary dead end

375   because it leads to evolutionary changes in traits that are difficult to reverse, which can

376   leave specialized taxa at higher risk of extinction when conditions change. On the other

377   hand, other studies have emphasized the links between specialization and increased

378   diversification (Losos et al. 1994; Schluter 2000) because a narrower niche could

379   increase diversification either directly, if niche shifts are associated with multiple

380   speciation events (Yoder et al. 2010), or indirectly, because of differences in dispersal,

381   connectivity, population persistence, and/or range size of specialist and generalist

382   species (Greenberg and Mooers 2017; Harvey et al. 2019). Testing the outcomes of

383   specialization on diversification rates has been difficult because macroevolutionary

384   models require datasets with a large number of independent origins of specialization

385   (Day et al. 2016).

386   Here, using state-dependent diversification models in a large tree of passerines,

387   we found that hole-nesting specialization does not differ from cup and dome-nesting in

388   diversification rates. We also found that transition rates into hole-nesting were low

389   compared to transitions out of hole-nesting (Fig. 4), and that the most probable state for

390   the root of all passerines was hole-nesting. These three results highlight the lack of

391   support for the hypothesis that specialization leads to an evolutionary dead end. Our

392   results, combined with a number of recent studies addressing this hypothesis (Day et al.

393   2016; Cyriac and Kodandaramaiah 2018), raise the question of whether the link

394   between diversification rates and ecological specialization is truly variable across taxa

395   and traits, or whether newer phylogenetic comparative methods and larger data sets are

396   allowing us to test hypotheses more rigorously. Interestingly, in our study, which uses a

397   large dataset with numerous transitions to the specialized hole-nesting state, we found

398   that, in the absence of accounting for hidden states, adoption of hole-nesting behavior

399   led to higher, not lower, diversification rates (Fig. 3a). However, when we included

400   hidden states, and thus accounted for the possibility that other, unmeasured variables

401   are driving the relationship, we found no difference in diversification rates across the

402   three nesting types (Fig. 3b). This comparison emphasizes that phylogenetic studies of

403   specialization that do not account for hidden states may need to reevaluate their null

404   hypotheses of diversification. More generally, our results, in combination with recent

405   tests of the evolutionary dead end hypothesis, suggest that there is growing evidence

Zenil-Ferguson, R.; McEntee, J.P.; Burleigh, J.G.; and Duckworth, R.A. - Nest type linked to diversification

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

407   et al. 2020).

408   The ecological consequences of specialization can be diverse – specialization

409   can, in some cases, decrease competition for resources and, in other cases, increase it.

410   For example, many studies have focused on diet or host specialization, which frequently

411   reduce competition over food or breeding resources (Vamosi et al. 2014). However,

412   specialization can also enable species to escape predation (e.g. Singer et al. 2019) and,

413   in such cases, may actually increase competition for scarce, protective resources. Such

414   is the case for hole-nesting birds, where an important consequence of hole-nesting is

415   that it strongly reduces the risk of nest predation, an important driving force behind life

416   history evolution in birds (Martin 1995). In our study, we were able to distinguish

417   between the influence of ecological specialization *per se* versus one specific ecological

418   consequence of specialization – reduced predation. Dome-nesting birds also show

419   decreased predation rates (Oniki 1979; Linder and Bollinger 1995; Auer et al. 2007;

420   Martin et al. 2017), but they are relatively unspecialized in nesting substrates compared

421   to hole-nesters. However, neither hole-nesting nor dome-nesting were associated with

422   elevated diversification rates. This suggests that, in passerine birds, reduced nest

423   predation rates do not have a strong influence on diversification dynamics and, while

424   escaping predation can often lead to ecological release (Herrmann et al. 2021), this

425   does not necessarily translate into increased ecological opportunity or subsequent

426   adaptive diversification. Moreover, reduced predation can also increase species' ability

427   to persist and, hence, decrease extinction risk. Yet, a change in abundance of nesting

428   holes, such as declines in tree cavities, has been shown to lead to heightened risk of

19

429   extinction (Duckworth and Badyaev 2007). Thus, the lack of a relationship with

430   diversification rates may reflect a balance between these various ecological

431   consequences of adopting hole-nesting.

432   Another factor that may account for inconsistency across studies in the links

433   between specialization and macroevolutionary dynamics is the extent to which

434   specialization results in further evolution of specific traits. Ecological specialization is a

435   narrowing of an organism's niche, and so results from a change in how an organism

436   interacts with its environment. Futuyma and Moreno (1988) point out that specialization,

437   at a minimum, only requires a behavioral shift, and specialized taxa vary in the extent

438   that there is subsequent evolution of other traits. Thus, it is possible that whether

439   specialization is an evolutionary dead end or not may be strongly linked to the extent of

440   secondary adaptation that follows it. In the case of hole-nesting birds, specialization is

441   largely a behavioral shift, although it does lead to evolutionary changes in many life

442   history and breeding traits (see below). Thus, it may be that hole-nesting specialization

443   does not lead to an evolutionary dead end because of the relative ease of reversing

444   these traits (Fig. 4). In the future, studies that explicitly compare multiple types of

445   specialization with distinct ecological consequences and distinct influences on trait

446   evolution would shed light on their relative importance for macroevolutionary dynamics.

447   The low rate of evolutionary transitions into, relative to out of, hole-nesting (Fig.

448   4) provides further evidence against the evolutionary dead end hypothesis and instead

449   suggests that evolving ecological specialization can be challenging. In the case of hole-

450   nesters, becoming ecologically specialized involves facing increased interspecific

451   competition, as holes are sought not only by other birds but also by other vertebrates

452 (Newton 1994). Thus, competition may prevent evolutionary transitions towards

453 specialization. Evolutionary transitions from hole-nesting to open-cup nesting, which

454 occur at a relatively high rate, involve different evolutionary challenges, such as life

455 history specialization. Open-cup nesting species have exceptionally high nestling

456 growth rates, which appear to be an adaptation to increased predation risk (Ricklefs

457 1979). Moving from the relaxed selection regime on growth rates associated with hole-

458 nesting to the strong selection regime on growth rates in open-cup nests would seem to

459 act as a filter that would limit this transition. However, our results suggest that, at least

460 in passerine birds, evolving such life history shifts is easier than evolving the ability to

461 deal with the intense competition of hole-nesting.

462 We also found evidence that it was easier for birds to transition from hole to cup

463 rather than hole to dome. Most hole nesters build an open-cup style nest, rather than a

464 dome, within the cavity (Price and Griffith 2017). Our finding that transitions to open-cup

465 nesting were easier compared to transitions to dome may simply reflect that transitions

466 from hole-nesting occurred more often in taxa that were already building open-cup nests

467 within their holes. Thus, for most hole nesters, transitioning to dome-nesting may be

468 more difficult because it would require two steps: leaving cavities and changing how the

469 nest is built. While in the minority, there are several clades, particularly at the base of

470 the passerine tree, that build dome style nests within cavities (Price and Griffith 2017).

471 Future studies comparing transitions to dome and open-cup nests based on whether

472 hole-nesting species are already building a dome or cup style nest within their cavity

473 would add further insight into the mechanisms behind these different transition rates.

474 *Ancestral State Reconstruction*

475   In all our ancestral state reconstructions we found that hole-nesting is the most

476   probable state at the root of the passerines. When including the parrot *Strigops*

477   *habroptilus* as the outgroup*,* the maximum of the marginal posterior distribution of the

478   node had a probability of 0.77 for hole-nesting. Without this outgroup, we still recovered

479   hole-nesting with a maximum a posteriori of 0.51 (see figure S12 in supplementary

480   information). These two reconstructions represent significant evidence in favor of the

481   specialized state hole-nesting at the origin of passerines. Previous work has

482   emphasized that hole-nesting, dome-nesting, and open-cup nesting all appear to have

483   occurred early in the history of passerines (Collias 1997; Price and Griffith 2017; Fang

484   et al. 2018; McEntee et al. 2018), with Collias (1997) suggesting that the nest type of

485   the earliest passerine might be unknowable because of the apparent rapid evolution of

486   nest type in early passerines. Our ancestral state reconstruction, using an approach

487   where we included strong species-level sampling and assessed whether differing

488   diversification rates had to be accounted for (Maddison 2006), tips the balance in favor

489   of hole-nesting as the ancestral state for the common ancestor of extant passerines.

490   Notably, this result contrasts with the ancestral state reconstruction of (Fang et al.

491   2018), which included more sampling outside of passerines and less species-level

492   sampling within passerines. However, in our study, support for hole-nesting as the

493   ancestral state serves to underscore how radically the evolution of hole-nesting as a

494   specialization fails to meet the generalists-to-specialists view of evolution; rather than a

495   dead end, all passerine diversity emerged from a specialist ancestor.

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497

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.

520   Overall, we were able to rigorously test different hypotheses and extinction

521   scenarios because of the size of the tree, the number of transitions between nest types,

522   and the large proportion of passerines in the sample (> 51%). Studies of specialization

523   linked to diversification might conclude spurious associations of specialization and

524   diversification when independent transitions to and from the specialized state are too

525   few (Uyeda et al. 2018), tree size is not large enough (Davis et al. 2013), and sampling

526   fraction is small (Chang et al. 2020). Historically, standards for the quality rather than

527   the quantity of the sample needed to fit SSE models have been lacking, so it is

528   important that future studies of state-dependent diversification be aware of these issues.

529   CONCLUSIONS

530   Our macroevolutionary analysis failed to find any link between specialization of

531   nest type and diversification rates under different extinction scenarios, suggesting that

532   there is little support for the evolutionary dead end hypothesis in this case study.

533   Moreover, contrary to this hypothesis, we also found that transitions from the

534   specialized state were relatively easy compared to transitions toward the more

535   generalist states and that the root state of passerines is most probably hole-nesting.

536   Our results suggest that the ecological consequences of resource specialization,

537   whether due to escape from competition or predation, might be key to understanding its

538   macroevolutionary consequences. This work adds to other recent studies that have

539   found little support for the evolutionary dead end idea, suggesting that evolution of

540   resource specialization is more evolutionarily labile than previously thought. We suggest

541   that future studies of this question would benefit from explicit comparison of resource

542   specialization that vary in their ecological consequences, as well as the extent of trait

543 evolution necessary for specialization. Such studies would enable a greater

544 understanding of the mechanisms that underlie variable links between specialization

545 and macroevolutionary dynamics.

546

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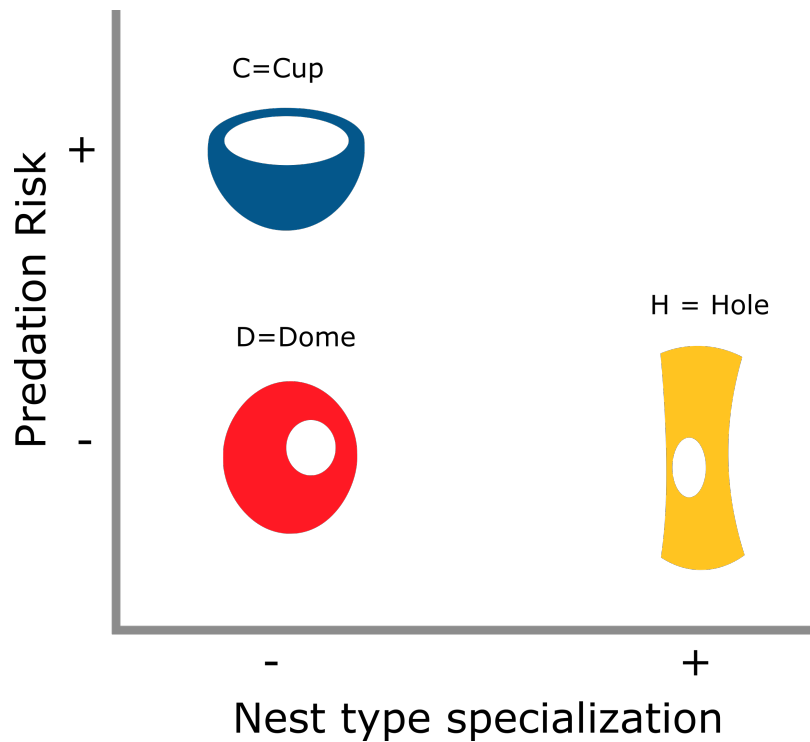
Figure 1. Nesting habits of passerine taxa can be organized based on a trade-off

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.

Zenil-Ferguson, R.; McEntee, J.P.; Burleigh, J.G.; and Duckworth, R.A. - Nest type linked to diversification
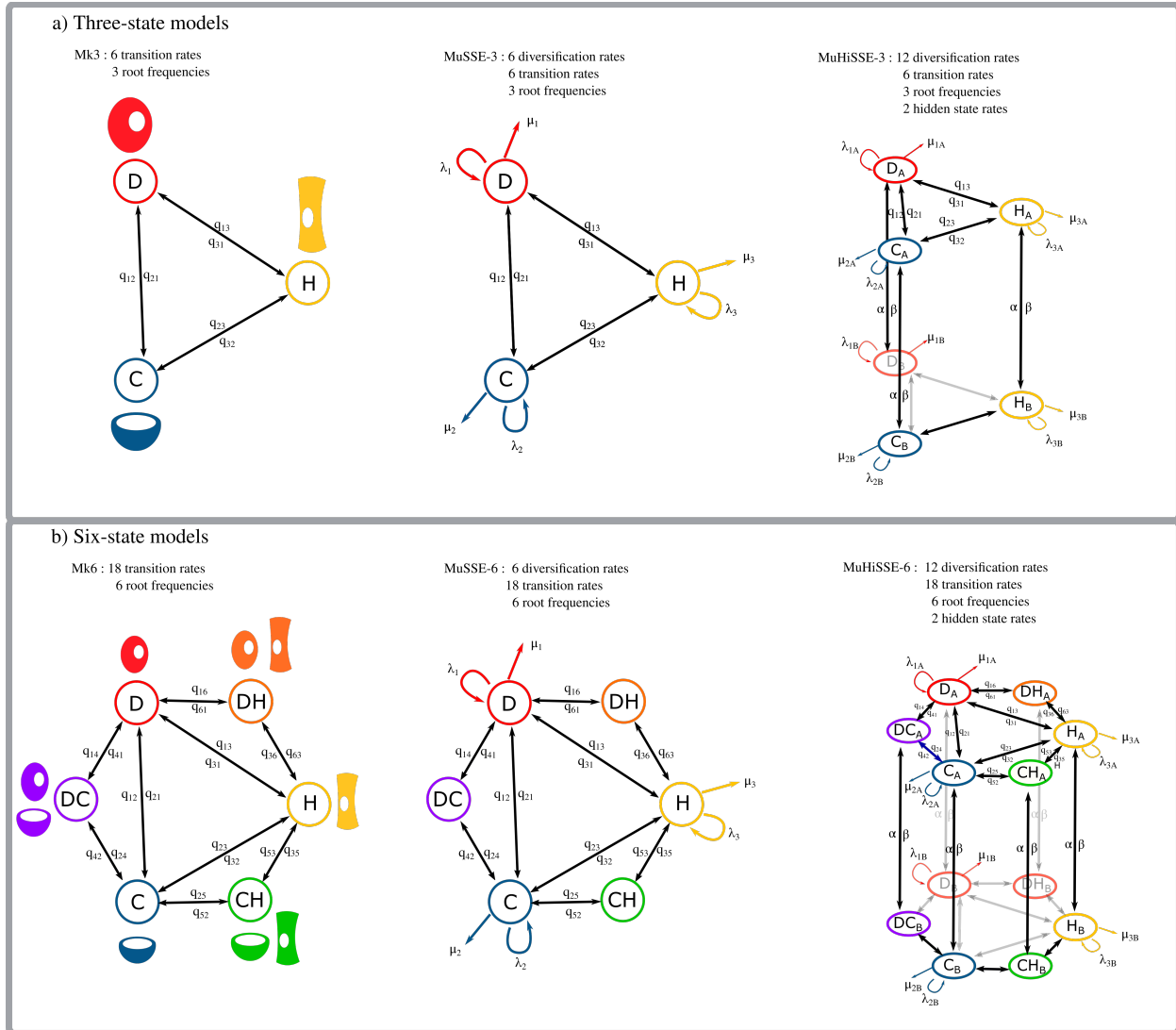
Figure 2. Models of nest type evolution. In panel a) three models are depicted: Mk3

which assumes all possible pairwise transitions for three types of nest (dome (D), cup

(C), and hole (H)); MuSSE-3 which assumes the same transitions but adds

diversification parameters to each of the states, and MuHiSSE-3 that adds the

possibility of heterogeneity in the diversification process different from the diversification

attributable to the state. In panel b) similar models to the ones found in panel (a) are

defined with three states that represent nest type (D, C, H) with the addition of three

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749   more states that characterize those species with two nest types (dome-cup(DC), dome-

750   hole (DH), cup-hole (CH)).
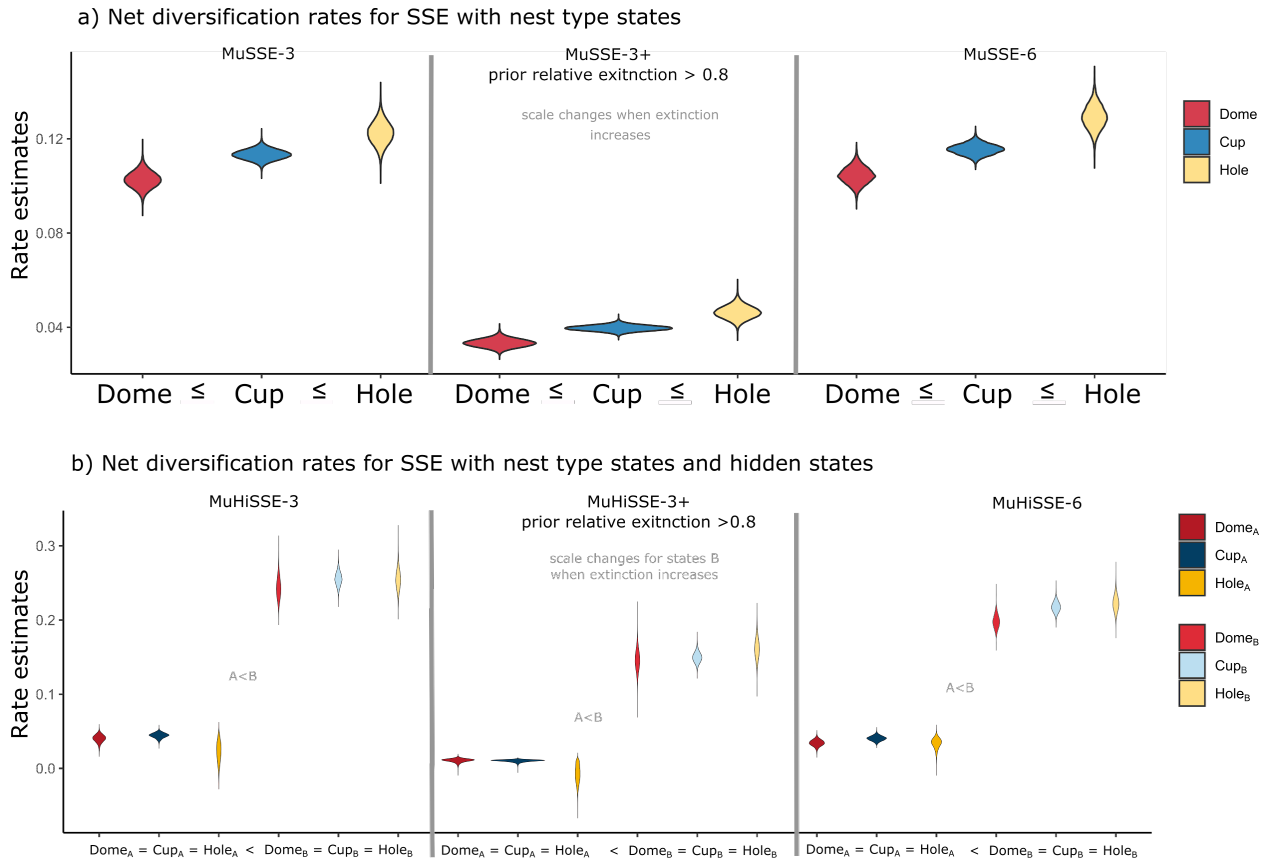
751

752   **Figure 3.** Net diversification rate estimates (speciation and extinction) for a) multistate

753   dependent diversification models (MuSSE), and b) multistate and hidden state

754   dependent diversification models (MuHiSSE). In models in (a) we consistently estimated

755   that hole nesters have faster net diversification rates than dome nesters, and that cup

756   nesters had intermediate rate estimates. This finding was consistent when we assumed

757   the extinction fraction to be at least 0.8 (MuSSE-3 model + prior extinction fraction >

758   0.8), or adding more intermediate states (MuSSE-6 model). The most noticeable

759   change is that increasing the extinction fraction (MuSSE-3 model+ extinction fraction >

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760   0.8) decreases net diversification estimates (y-axis), but the relative differences

761   between states are maintained. For models in (b) we observe that all diversification

762   differences are due to the differences in hidden state (A<B) rather than nest type

763   (Dome=Cup=Hole for A and B states), which is strong evidence against the direct

764   influence of nest type on the speciation and extinction of passerines. Even increasing

765   the extinction fraction (MuHiSSE-3 + prior extinction fraction > 0.8) or adding more

766   intermediate states (MuHiSSE-6) yields the same results.

767

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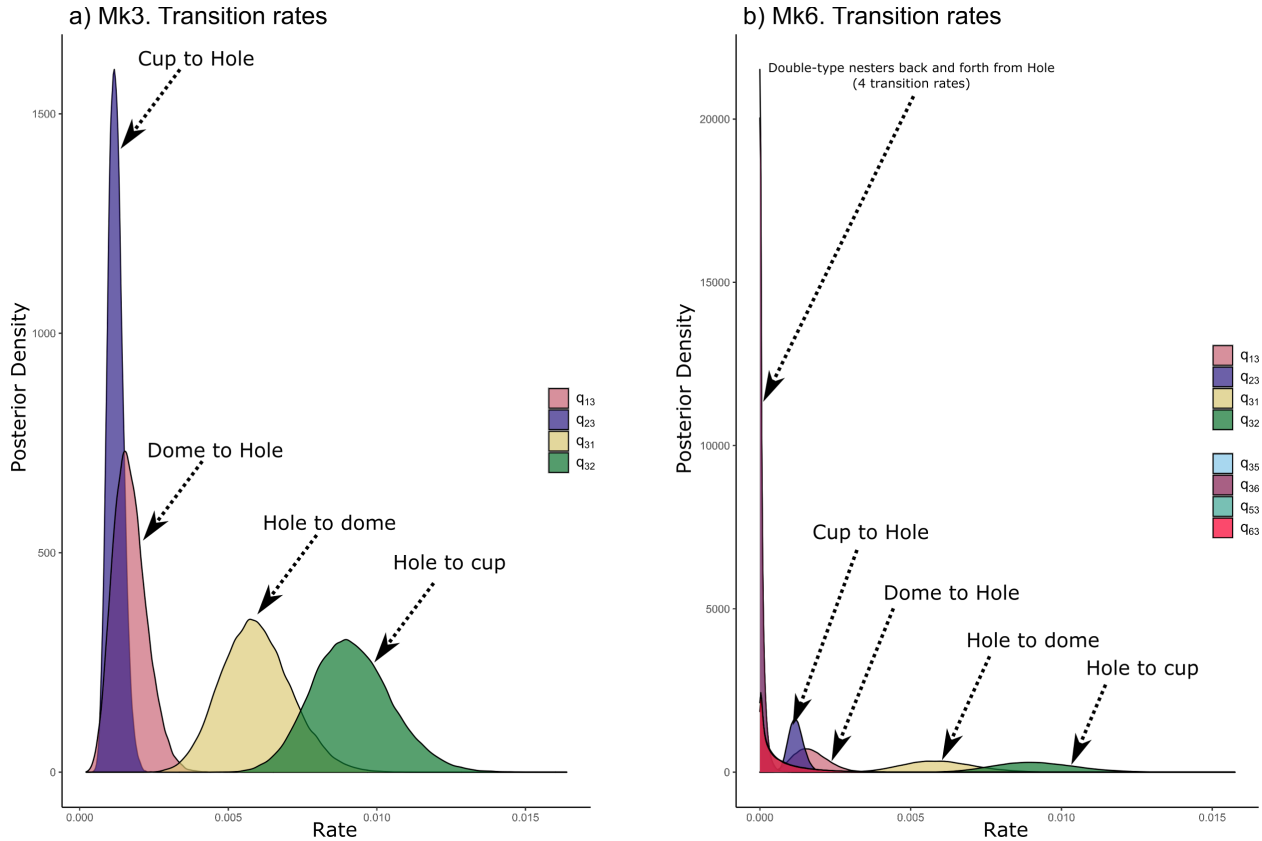
Figure 4. Transition rates between nest states for diversification-free models. In a) the model with three states (Mk3), the posterior distribution for the rate from hole to cup (q_{32}) is faster than any other rate to and from hole. For b) the model with six-states (Mk6), in which states with two nest 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).

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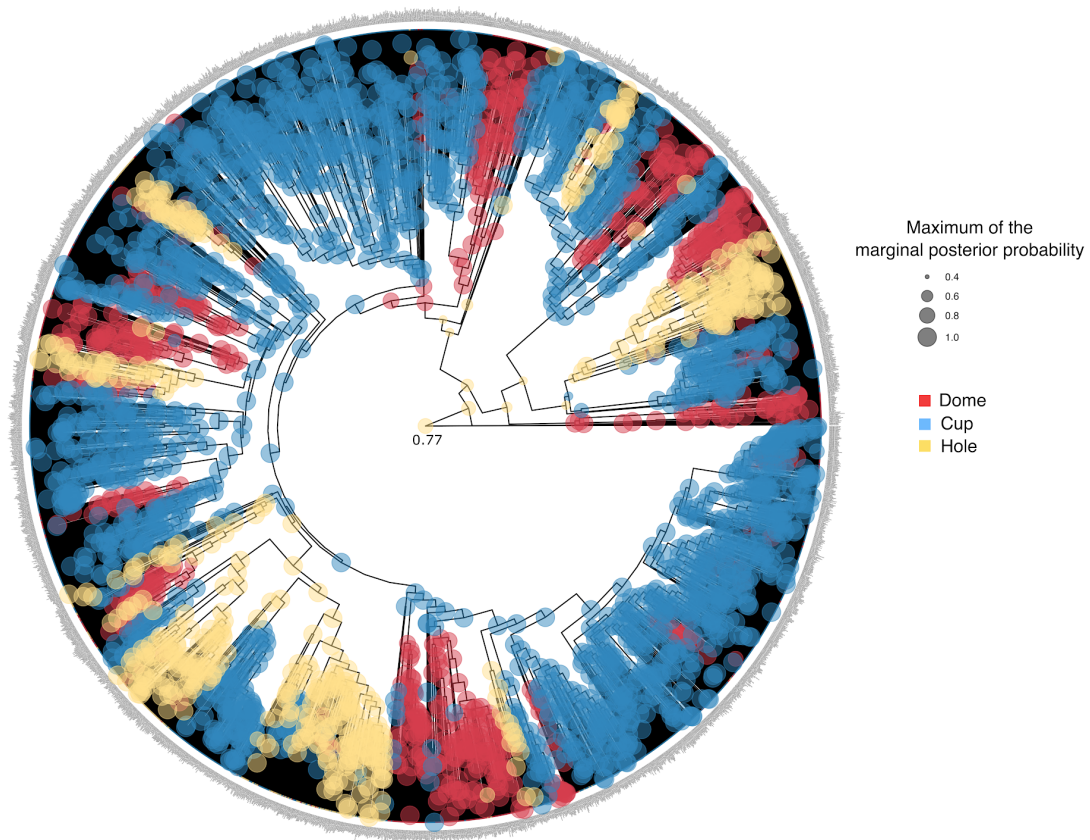
782   **Figure 5.** Ancestral state reconstruction for nest type using a Markov model with three

783   states (Mk3). The size of a node represents the maximum probability value of the

784   marginal posterior distribution of the node. The color of the node represents the nest

785   type associated with the maximum posterior probability. This tree includes the hole-

786   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 μ_λ 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.

788

789   In figure A1 we summarize the results of assumptions 1-6 from table A1. Here we can

790   observe that when it is assumed that the extinction fraction is greater than 0.7 but less

791   than 1, we recover the differences between states (Dome<Cup<Hole). When the

792   extinction fraction is at least 1 and the extinction rate is expected to be about as large

793   as speciation, then we found no differences in the posterior distributions of the three

794   states. Finally, when the extinction rate is assumed to be larger than the speciation rate

795   we obtain the reverse effect (Hole<Cup<Dome). These results show that despite our

796   prior assumptions about extinction rate, the hole-nesting taxa will have the greatest

797   influence in the direction of net diversification than cup or dome nesters. And finally, the

798   state dome always has the smallest effect on net diversification in absolute value.

799   These results might only be applicable to a trait that does not influence diversification,

800   as we have shown using hidden state and character independent models for nest type

801   (figure 2). Whether this pattern of finding similar differences in diversification is

802   sustained in the face of enhanced extinction holds for all traits in SSE models remains

803   to be tested.

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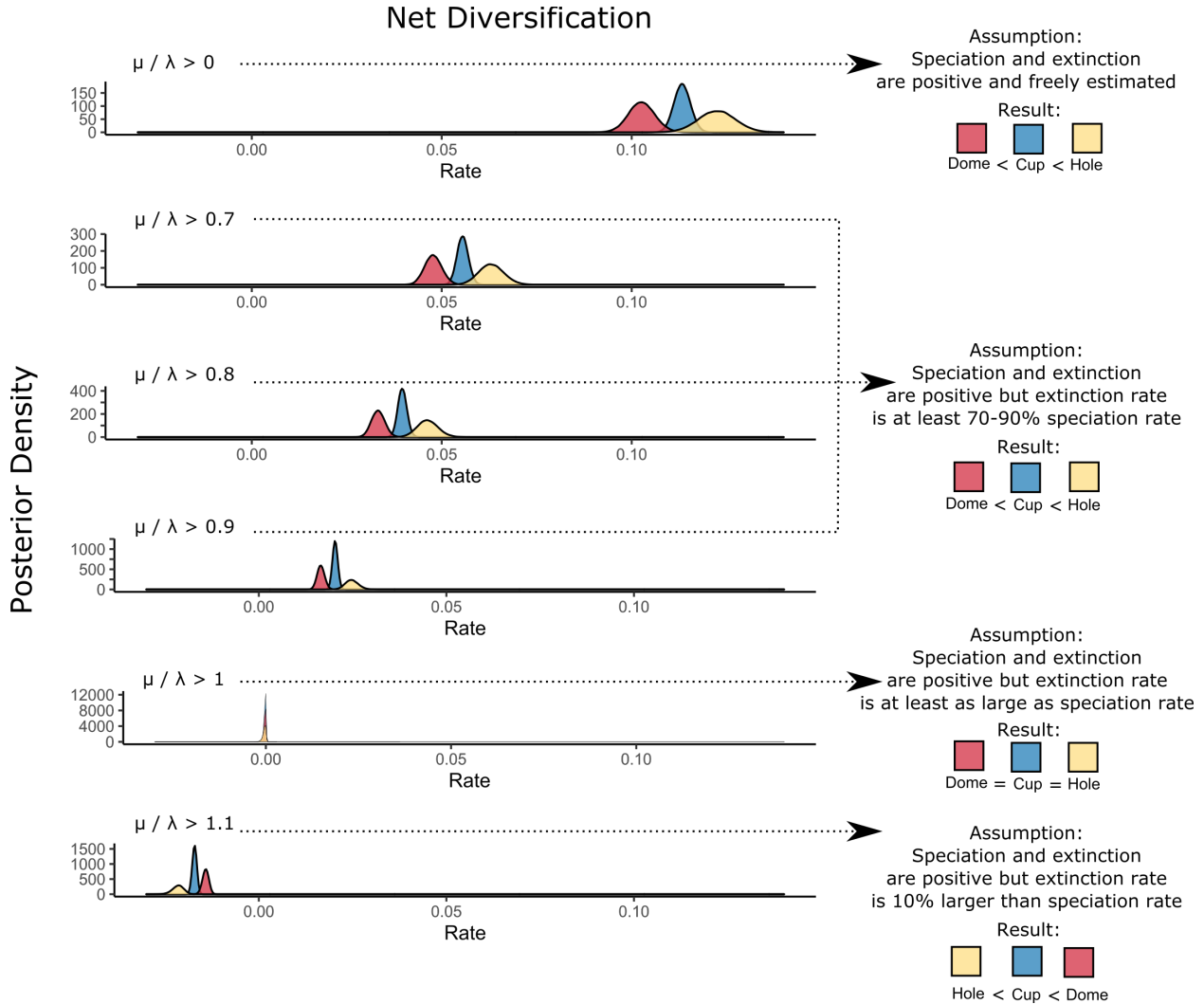
Figure A1. Different *a priori* assumptions about the minimum of extinction fraction ($a = \frac{\mu}{\lambda}$). When speciation and extinction are estimated freely ($a > 0$), the posterior distributions for net diversification show that the rate of diversification for hole is faster than cup, and both 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,

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814   that extinction is at least as fast as speciation for every state, where we found that

815   posterior distributions of net diversifications for every state overlap. Once we assumed

816   that extinction is much faster than speciation ($a > 1.1$), we found a mirror trend in the

817   inference with hole net diversification being the slowest, and dome being the fastest.

818