

1 Asynchronous rates of lineage, phenotype, and niche diversification in a continental-scale  
2 adaptive radiation

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24 **Abstract**

25 Rapidly diversifying clades are central to the study of diversification dynamics. This central  
26 importance is perhaps most apparent when rapid evolution occurs across several axes of  
27 diversification (*e.g.*, lineage, phenotype, and niche); such clades facilitate investigations into the  
28 interplay between adaptive and non-adaptive diversification mechanisms. Yet, empirical  
29 evidence from rapidly evolving clades remains unclear about the relationships, if any, across  
30 diversification axes. This is especially apparent regarding the timing of diversification rate shifts.  
31 We address this knowledge gap through comparisons of the rate and timing of lineage,  
32 phenotypic, and niche diversification in *Penstemon*, a rapidly-evolving angiosperm genus. We  
33 find that diversification rate shifts in *Penstemon* are asynchronous; while we identify a burst and  
34 subsequent slowdown in lineage diversification rate ~2.0-2.5 MYA, shifts in phenotypic and  
35 niche diversification rates either lagged behind temporally or did not occur at all. We posit that  
36 this asynchronicity in diversification rate shifts is the result of initial niche-neutral diversification  
37 followed by adaptive, density-dependent processes. Our findings contribute to a growing body of  
38 evidence that asynchronous shifts in diversification rates may be common and question the  
39 applicability of expectations for diversification dynamics across disparate empirical systems.

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## 47 **Introduction**

48           Key to the study of diversification dynamics – how and why speciation and extinction  
49 occur, and vary, across time and space – is understanding the interplay between species’  
50 phenotypes and the environment in which they live. Of particular interest to biologists in this  
51 respect are clades which exhibit great degrees of variation in the rate of net lineage  
52 diversification (speciation minus extinction) through time, as identifying potential biotic and  
53 abiotic factors responsible for shifts in the rate of lineage diversification is integral to our  
54 understanding of the evolutionary processes generating biological diversity (Morlon, 2014).  
55 While there are numerous processes that can affect diversification dynamics, in many conceptual  
56 models, the primary sources driving diversification rate variation have an ecological basis  
57 (Aguilée et al. 2018; Aristide & Morlon, 2019).

58           Perhaps the most well-known of these processes is adaptive radiation: the proliferation of  
59 ecological roles and adaptations in different species within a lineage (Givnish, 1997). Adaptive  
60 radiation does not require the rapid accumulation of species *per se*, and in fact, rapid shifts in  
61 rates of diversification (‘explosive diversification’) are often incorrectly synonymized with  
62 adaptive radiation (Givnish, 2015). However, it is often the case that the two processes are  
63 linked, such that the diversification into many ecologically specialized forms via adaptive  
64 radiation also confers a sharp and temporally localized increase in the rate of lineage  
65 diversification (Gavrilets & Losos, 2009; Rundell & Price, 2009; Moen & Morlon, 2014; Martin  
66 & Richards, 2019). Integral to adaptive radiation theory is the concept of ecological opportunity,  
67 or the abundance of accessible resources underused by competing taxa (Schluter, 2000).  
68 Championed as a prerequisite for adaptive radiation by Simpson (1953), the importance of  
69 ecological opportunity stems from the idea that an abundance of accessible resources, made

70 available through, for example, changes in environmental conditions, or the evolution of novel  
71 phenotypes, can facilitate ecological specialization and ultimately the formation of new species  
72 (Simpson, 1953; Wellborn & Langerhans, 2015; Stroud & Losos, 2016). And, like adaptive  
73 radiation, while ample ecological opportunity does not necessarily lead to an increase in clade-  
74 wide diversification rates, bursts in lineage diversification rates attributed to speciation driven by  
75 ecological opportunity are frequently observed in empirical studies (*e.g.*, Burbrink & Pyron,  
76 2010; Arakaki et al. 2011; García-Navas et al. 2018).

77         Typically, a shift in the rate of lineage diversification occurs soon after an ecological  
78 opportunity arises, producing an ‘early burst’ pattern indicative of adaptation to available niche  
79 space (Gavrilets & Losos, 2009; Yoder et al. 2010; Gillespie et al. 2020). This early burst pattern  
80 of lineage diversification can be accompanied by a burst in the rate of phenotypic diversification  
81 as well (Burbrink & Pyron, 2010; García-Navas et al. 2018). Reduced rates of phenotypic and  
82 lineage diversification are sometimes observed subsequent to early bursts (Rundell & Price,  
83 2009; Moen & Morlon, 2014; Martin & Richards, 2019), and in cases where decreases in  
84 diversification rates are apparent, they tend to be attributed to density-dependent factors from  
85 increased competition between species as available niche space fills (Gavrilets & Vose, 2005;  
86 Ingram et al. 2012; Aguilée et al. 2018; Aristide & Morlon, 2019). However, the prevalence of  
87 diversification rate slowdowns in empirical systems, at least for phenotypic evolution, has been  
88 increasingly brought into question (Harmon et al. 2010; Slater & Friscia, 2019; Gillespie et al.  
89 2020). Furthermore, there has been little consensus regarding the relative timing of shifts in  
90 lineage, phenotypic, and niche diversification rates. While some studies have documented close  
91 associations between the timing of lineage diversification rates and rates of phenotypic (*e.g.*,  
92 Rabosky et al. 2013; García-Navas et al. 2018) and niche (*e.g.*, Kozak & Wiens, 2010; Title &

93 Burns, 2015) evolution, many others report a lack of association, or a decoupling of  
94 diversification rates (*e.g.*, Adams et al. 2009; Folk et al. 2018; Testo & Sundue, 2018; Crouch &  
95 Ricklefs, 2019; Boucher et al. 2020). This, coupled with mixed support for the ecological  
96 opportunity hypothesis at all in continental- or global-scale radiations (*e.g.*, Liedtke et al. 2016;  
97 Maestri et al. 2017; Folk et al. 2018; García-Navas et al. 2018), makes it unclear whether there is  
98 any general relationship between lineage, phenotypic, and niche diversification dynamics in  
99 rapidly radiating clades, especially with respect to the relative timing of rate shifts. Such  
100 uncertainty about the relationship between rates of lineage, phenotypic, and niche diversification  
101 means that expectations for a clade having potentially experienced adaptive radiation,  
102 particularly a continental radiation, are unclear. Studies of such clades, therefore, would  
103 contribute greatly to our understanding of diversification dynamics and the processes which  
104 generate biological diversity.

105         Here, we address this gap in our understanding of diversification dynamics through  
106 comparisons of the rate and relative timing of lineage, phenotypic, and niche diversification in a  
107 continental radiation of plants, *Penstemon* Schmidel (Plantaginaceae). Commonly known as the  
108 beardtongues, the genus *Penstemon* has nearly 300 described species, and represents the most  
109 speciose genus of angiosperms endemic to North America (Wolfe et al. 2006; Freeman, 2019).  
110 *Penstemon* exhibits an exceptional array of phenotypic diversity in floral and vegetative  
111 characters, with the degree floral diversity in particular suggesting a substantial history of  
112 selective pressure from pollinators (Straw, 1966). Species of *Penstemon* occupy a wide variety of  
113 ecological niches, with several examples of edaphic specialization (*e.g.*, sand dunes, oil shales,  
114 and calcareous soils), although most species prefer semi-disturbed, xeric habitats (Wolfe et al.  
115 2006; Wolfe et al. 2021). This tendency toward habitat specialization and the limited geographic

116 distributions that often accompany it has resulted in conservation concerns for many *Penstemon*  
117 species (*e.g.*, Wolfe et al. 2014, 2016; Rodriguez-Peña et al. 2018; Stone et al. 2019, 2020;  
118 Zacarías-Correa et al. 2020). Phylogenetic studies of *Penstemon* have revealed diversification  
119 patterns consistent with a rapid evolutionary radiation, including difficulty with estimating  
120 relationships between clades early in the evolutionary history of the genus, likely due to the  
121 effects of incomplete lineage sorting (Wolfe et al. 2006; Wessinger et al. 2016, 2019), and an  
122 early, rapid burst in the rate of lineage diversification coincident with glacial activity during the  
123 Pleistocene (Wolfe et al. 2021). Consequently, the degree of phenotypic diversity and niche  
124 specialization, and the presence of an early burst pattern of lineage diversification coincident  
125 with environmental changes at a global scale, has resulted in the hypothesis that *Penstemon*  
126 represents a continental example of a rapid adaptive radiation (Wolfe et al. 2006, 2021).  
127 However, to date, no analysis of the relative timing of shifts in lineage, phenotypic, and niche  
128 diversification has been conducted in the context of the *Penstemon* radiation.

129 We built data sets of ecological niche and phenotypic trait variables, and, using a  
130 modified version of the time-calibrated *Penstemon* phylogeny inferred in Wolfe et al. (2021),  
131 analyzed macroevolutionary patterns of lineage, phenotypic, and niche diversification in  
132 *Penstemon*. Using these data, we aim to answer the following questions: (1) Do shifts in the rates  
133 of phenotypic and niche diversification coincide with the shift in lineage diversification rate? (2)  
134 Do rates of lineage diversification depend on the states of phenotypic or environmental  
135 variables? (3) Does the timing of diversification coincide with potential increases in ecological  
136 opportunity due to changes in glacial activity and global temperature?

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## 138 **Methods**

139 *Data generation*

140           The phylogeny used in this study is a modified version of the time-calibrated phylogeny  
141 inferred in Wolfe et al. (2021). Briefly, this phylogeny was constructed from 43 nuclear loci  
142 generated from a targeted amplicon sequencing approach described in Blischak et al. (2014), and  
143 inferred with *RAxML* v8.2.10 (Stamatakis, 2014). Divergence times were calibrated with  
144 previously inferred divergence events in the Lamiales (Vargas et al. 2014) in *BEAST* v2.6.0  
145 (Bouckaert et al. 2019) using a relaxed log-normal clock model (Drummond et al. 2006); these  
146 estimates were then used as secondary calibration bounds to date the phylogeny using *treePL*  
147 (Smith & O’Meara, 2012). More details about the methodology used to produce the time-  
148 calibrated *Penstemon* phylogeny can be found in Wolfe et al. (2021).

149           Species occurrence data were collected for each species of *Penstemon* that was both in  
150 the constructed phylogeny and available on GBIF ([gbif.org](http://gbif.org)). GBIF coordinates were downloaded  
151 on June 26, 2020. For ten species (listed in Supplemental Table 1), we added coordinates from  
152 the primary literature and from herbarium records accessed through the SEINet portal  
153 ([swbiodiversity.org/seinet/](http://swbiodiversity.org/seinet/)). We retained all occurrence points accurate to at least three decimals  
154 for both latitude and longitude, and removed occurrences which had identical coordinates or  
155 were clearly outside the species’ described range. Finally, we included only taxa for which there  
156 were at least three occurrences in the data set following these curation measures.

157           To build the data set to evaluate the evolution of ecological niche space, we first  
158 extracted 28 environmental layers capturing features of the climate, soil, landcover, and topology  
159 at 30s resolution. These layers included: 19 temperature and precipitation variables (bioclim  
160 variables from [worldclim.org](http://worldclim.org)), elevation (from [worldclim.org](http://worldclim.org)), slope, aspect, and six landcover  
161 classes from [earthenv.org/landcover](http://earthenv.org/landcover). Values for slope and aspect were calculated in R, using the

162 elevation raster file. The six landcover classes correspond to the percentage landcover of  
163 evergreen/deciduous needleleaf trees, evergreen broadleaf trees, deciduous broadleaf trees,  
164 mixed/other trees, shrubs, and herbaceous vegetation. For each GBIF occurrence point,  
165 environmental conditions were extracted in R, and for each taxon, median values of each  
166 predictor were used in downstream analyses. Environmental data were then ordinated via  
167 phylogenetic principal components analysis (pPCA) using the ‘*phyl.pca*’ function on the  
168 correlation matrix in the R package *phytools* (Revell, 2012), forming a composite variable  
169 summarizing species’ niche space. All downstream analyses using both the niche data set and the  
170 phylogeny were performed with a pruned phylogeny including only taxa for which niche data  
171 were collected.

172         Phenotypic traits for species were collected primarily from species descriptions in the  
173 Flora of North America (Freeman, 2019). For species not included in the Flora (mainly those  
174 endemic to Mexico), we accrued data through a combination of primary species’ descriptions  
175 and measurements of specimens from herbarium collections. For these species (listed in  
176 Supplemental Table 1), we downloaded images of herbarium specimens via the SEINet portal,  
177 the Steere Herbarium at the New York Botanical Gardens, and the National Autonomous  
178 University of Mexico (UNAM) Herbarium and made virtual measurements of focal phenotypic  
179 characters with *ImageJ* (Schneider et al. 2012). We collected both categorical and continuous  
180 traits: the full list of traits can be found in Supplemental Table 2. For continuous traits, we  
181 recorded the midpoint value, after outlier measurements were discarded. For categorical traits,  
182 we coded each descriptor sequentially, starting from zero. For species with multiple or  
183 ambiguous categorical characters, a single trait value was selected at random. We used the R  
184 package *StatMatch* (D’Orazio, 2012) to compute a phenotypic distance matrix from Gower’s



185 Distance metric (Gower, 1971), calculated with the correction proposed by Kaufman and  
186 Rousseeuw (1990). All downstream analyses using both the phenotypic data set and the  
187 phylogeny were performed with a pruned phylogeny including only taxa for which phenotypic  
188 data were collected. To ordinate the phenotypic data, we conducted generalized  
189 multidimensional scaling (PCOA) on the distance matrix using the ‘pcoa’ function in the R  
190 package *ape* (Paradis & Schliep, 2019), using the Cailliez correction (Cailliez, 1983). Because  
191 PCOA does not produce traditional loadings in the same sense that PCA does, we produced an  
192 alternative measure of variable importance for each PCOA axis. We did so by regressing the first  
193 axis of the ordinated data with each uncorrected variable and dividing the  $R^2$  for each variable by  
194 the sum of all  $R^2$  values, producing a relative measure of variable importance. We also  
195 constructed these values for the niche data set, to facilitate comparisons of niche and phenotype  
196 variable importance.

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### 198 *Ancestral state reconstruction and phylogenetic generalized least-squares*

199 We performed ancestral state reconstruction on the ordinated phenotypic and niche data  
200 sets using the *phylopars* package (Bruggeman et al. 2009) in R. We fit models of continuous trait  
201 evolution under Brownian motion (BM), Ornstein-Uhlenbeck (OU) and Early Burst (EB)  
202 models, and chose the best model using the corrected Akaike information criterion (AICc;  
203 Akaike, 1973). Then, using the reconstruction from the best model, we calculated the degree of  
204 the trait shift at each node by finding the absolute difference between the reconstructed values at  
205 a focal node and its parent node. Major shifts were identified as values in the 95<sup>th</sup> percentile of  
206 trait shifts.

207 To investigate evolutionary associations between ecological niche space and phenotype,  
208 we performed phylogenetic generalized least squares regression (PGLS). PGLS generates slope  
209 and intercept estimates which account for interspecific autocorrelation of residuals due to  
210 species' shared phylogenetic histories. Because our goal was to evaluate the potential impact of  
211 environmental predictor variables on the phenotype, we conducted all PGLS analyses with the  
212 first axis of the phenotype PCOA (phenotype summary statistic) as the response variable, and for  
213 predictor variables, we used the first axis of the niche pPCA (niche summary statistic) and  
214 individual uncorrected environmental variables. For each predictor variable, we performed PGLS  
215 under Pagel's  $\lambda$  model, which is a generalization of the assumption of Brownian Motion (Pagel,  
216 1997). In this model,  $\lambda$  can range from 0 to 1; when  $\lambda = 0$ , the analysis is equivalent to ordinary  
217 least squares regression, and when  $\lambda = 1$ , the analysis is equivalent to phylogenetic independent  
218 contrasts (residual errors are Brownian). All PGLS analyses were conducted with the *nlme*  
219 package in R (Pinheiro et al. 2021).

220

### 221 *Macroevolutionary rates analyses*

222 We used *BAMM* (Rabosky, 2014) to assess rates of net lineage diversification through  
223 time. Prior settings were derived from the R package *BAMMtools* (Rabosky et al. 2014). We ran  
224 the *BAMM* analysis for 50 million MCMC steps, in four chains, sampling every 10,000 steps.  
225 We set the minimum clade size for shift inference equal to two (no terminal branch shifts), and  
226 set the global sampling fraction to 0.9. Otherwise, parameter values were left at their default  
227 values, or were inferred from *BAMMtools*. During post-processing, the first 10% of samples  
228 were discarded as burn-in. We also used the *BAMM* trait model to assess the rate of both  
229 phenotypic and niche diversification through time. The first axes of the ordinated phenotypic and

230 niche data sets were used as input, and we used *BAMMtools* to generate prior settings as with the  
231 diversification analysis. For the phenotypic data, we ran the analysis for 2 billion steps, sampling  
232 every 100,000 steps, and for the niche data, we ran the analysis for 250 million steps, sampling  
233 every 10,000 steps. The first 50% and 10% of samples for the phenotypic and niche data,  
234 respectively, were discarded as burn-in during post-processing. All other settings followed those  
235 described for the *BAMM* diversification analysis. To examine variation in rates of diversification  
236 in the context of particular clades, we also generated rate-through-time plots for eleven clades on  
237 the basis of their importance in the context of *Penstemon* taxonomy.

238         We used a regression approach to assess potential relationships between  
239 macroevolutionary rates and global temperature. To do so, we first obtained global temperature  
240 estimates over the past ~3.5 million years, which were derived from a temperature anomaly data  
241 set (de Boer et al. 2014). We then generated a *BAMM* rates-through-time matrix for each of the  
242 three *BAMM* analyses, obtaining estimates of the average rate diversification metric at 100  
243 discrete points in time (roughly 1000-year time slices). Values for the rate diversification metric  
244 were then paired with global temperature estimates to the nearest 1,000-year interval. We then  
245 performed regression analyses with linear, exponential, and quadratic regression, and selected  
246 the best model for each diversification metric using AIC.

247         We used *ES-sim* (Harvey & Rabosky, 2018) to test for relationships between trait  
248 variation and variance in lineage diversification. We performed this test for both phenotypic and  
249 niche data sets, using the first axis of each ordinated data set as input. We also ran *ES-sim* on  
250 each individual continuous phenotypic and environmental trait, to test relationships between a  
251 particular trait and rates of lineage diversification.

252

## 253 **Results**

### 254 *Data generation*

255           In total, our data collection and processing efforts generated a phenotypic data set for 280  
256 taxa and a niche data set for 277 taxa (from 14,495 GBIF occurrences). The taxa included in both  
257 data sets are identical, except for three taxa (*P. debilis*, *P. wendtiorum*, and *P. tracyi*) not  
258 included in the niche data set because of a lack of GBIF occurrences owed to the rarity of these  
259 species and (for *P. debilis* and *P. tracyi*) their protected status due to conservation concerns.  
260 Relative measures of variable importance for the ordinated phenotypic and niche data sets can be  
261 found in Tables 1 and 2, respectively. The phenotypic traits with a corrected  $R^2 > 0.05$   
262 (analogous to loadings in a traditional PCA) were inflorescence type, leaf length, flower  
263 presentation, lower topography, throat expansion, plant form, and the presence/absence of basal  
264 leaves (Table 1). For the niche data set, variables meeting this criterion were mostly measures of  
265 temperature and precipitation seasonality, and the percentage of herbaceous vegetation (Table 2).  
266 Species with higher values for the niche summary statistic tend to be at higher elevations with  
267 more herbaceous landcover, more temperature seasonality (but lower minimum, maximum, and  
268 mean temperatures), and less precipitation seasonality (with less overall precipitation, but  
269 comparatively more precipitation during the dry season).

270

### 271 *Ancestral state reconstruction and phylogenetic generalized least-squares*

272           The best ancestral state reconstruction model for the ordinated phenotypic data set was  
273 the BM model, which had an AICc score slightly more than 2 units less than the next closest  
274 models and comprised over half of the model weight (Table 1). Our method for identifying shifts  
275 in ancestral phenotype indicated that two of the three largest shifts in phenotype occur at the base

276 of clades containing subgenus *Dasanthera* and subgenus *Penstemon* section *Caespitosi* (Figure  
277 1). These two clades also occupy a similar phenotypic space with respect to the ordinated  
278 phenotypic data set. Values of the phenotype summary statistic are similar between these  
279 species, representing some of the lowest values in the genus (Figure 1). This reflects the fact that  
280 species in these clades tend to exhibit a shrub or subshrub growth form with no basal leaves, and  
281 with corolla throats expanded on the upper side. These characters were determined to be among  
282 the most important to the PCOA (Table 1), making it unsurprising that species with similarities  
283 in these traits have similar values for the phenotype summary statistic.

284 For the niche data set, the best model for ancestral state reconstruction was the EB model  
285 (Table 2). However, this model was nearly indistinguishable from the BM model, with AICc  
286 scores for both models very similar. Both the BM and EB models had AICc scores close to 2  
287 units less than the OU model, and together, they comprised > 85% of the total model weight.  
288 Some of the largest shifts in ancestral niche space were located at the base of clades containing  
289 subgenus *Penstemon* sections *Fasciculus* and *Caespitosi* (Figure 2). This corresponds with a shift  
290 in geographic distribution for section *Fasciculus*; the species in this clade are among the most  
291 southernly-distributed *Penstemon* species, found almost exclusively in Mexico and Guatemala.  
292 The ancestral state reconstruction reflects this shift to habitats at lower elevations with less  
293 temperature variability (but higher overall temperatures) and more precipitation variability (with  
294 more overall precipitation, except during the dry season). There are a few other clade-wide shifts  
295 (e.g., at the base of section *Caespitosi*), but these may be artifacts produced by the ancestral state  
296 reconstruction method, such that large shifts in the trait estimate at a given node does not  
297 necessarily relate to the estimate of the trait along the terminal branch. In the case of section

298 *Caespitosi*, a shift from a positive to a negative value occurs at the node in question, but the  
299 majority of taxa in this clade exhibit a positive value for the niche summary statistic (Figure 2).

300 For each PGLS analysis, estimates of  $\lambda$  were high, with all point estimates of  $\lambda > 0.95$   
301 and confidence intervals capped at the upper parameter bound (Table 3). This suggests a strong  
302 phylogenetic signal to the data, such that the shared phylogenetic history between species  
303 strongly affects the error structure of the residuals, and that this error structure is similar to  
304 expectations under Brownian motion. Only two environmental variables had a significant effect  
305 on the phenotype summary statistic, suggesting that there is no significant relationship between  
306 the residuals of the regression between the phenotype summary statistic and the majority of the  
307 environmental variables tested, after accounting for expected phylogenetic covariance (Table 3).  
308 The two variables with significant effects were the percentage of evergreen broadleaf trees ( $\lambda =$   
309  $0.987$ , slope =  $-1.05 \times 10^{-2}$ ,  $p = 0.001$ ) and the amount of precipitation during the warmest  
310 quarter ( $\lambda = 0.983$ , slope =  $-3.05 \times 10^{-4}$ ,  $p = 0.038$ ).

311

### 312 *Macroevolutionary rates analyses*

313 For all three evolutionary rates analyses in *BAMM*, neither the estimates of effective  
314 sample size (ESS) or visualization of trace plots suggested failed MCMC chain convergence. For  
315 the lineage diversification analysis, the best model was one with a single rate shift (Supplemental  
316 Table 3). Of the credible set of shift configurations, the single best shift configuration  
317 (proportion of posterior samples,  $f$ , = 0.88) suggests a single burst in the rate of lineage  
318 diversification about 2.0-2.5 MYA (Figure 3a). This shift includes all major clades of *Penstemon*  
319 except subgenus *Dasanthera*, *P. personatus*, *P. scapoides*, and *P. caesius*. Subsequent to this  
320 burst, the rate of diversification slowed continuously to the present day, where estimates of

321 speciation rates are the lowest (Figure 3b). Our results very closely resemble those found in  
322 Wolfe et al. (2021) and are consistent with the hypothesis that *Penstemon* represents a recent and  
323 rapid evolutionary radiation (Wolfe et al. 2006).

324 Analyses of rate shifts in phenotypic diversification were inconclusive, with Bayes  
325 Factors continuing to increase with larger numbers of shifts, and posterior probabilities spread  
326 across shift configurations (Supplemental Table 4). Plots of clade-specific diversification rates  
327 show that sections *Fasciculus* and *Spectabiles* exhibit a burst in phenotypic diversification, while  
328 section *Caespitosi* shows a slowdown of phenotypic diversification (Supplemental Figure 1).

329 Analyses of niche diversification were more informative. Although Bayes Factors continued to  
330 increase as the number of shifts increased, posterior probabilities place most weight on 0-3 shifts  
331 with a posterior probability of 0.42 for zero shifts and 0.28 for a single shift affecting two species  
332 (Supplemental Table 5). Plots of clade-specific diversification rates did not reveal differences  
333 between focal taxonomic groups, suggesting that our data do not point clearly to any particular  
334 shifts in niche diversification rate, although the presence of such shifts cannot be ruled out  
335 (Supplemental Figure 1).

336 Regressions between global temperature and lineage diversification rate indicated the  
337 quadratic model as the best fit, with > 99% of the AIC model weight, and a correlation  
338 coefficient (Kendall's  $\tau$ ) of 0.33 (Supplemental Table 6). This model suggests that lineage  
339 diversification rates were highest at intermediate global temperatures (Figure 4). The regressions  
340 between global temperature and phenotypic diversification produced somewhat equivocal  
341 results; the exponential and quadratic regressions gave very similar AIC scores, with roughly  
342 equal model weights for both, and correlation coefficients ( $\tau$ ) were also very similar  
343 (Supplemental Table 6). However, plots of both models support the same positive correlation

344 between global temperature and phenotypic diversification rates. Regressions between global  
345 temperature and niche diversification were similar to those for phenotypic diversification; the  
346 exponential model is a slightly better fit than the quadratic model, and the correlation  
347 coefficients ( $\tau$ ) are very similar (Supplemental Table 6), but plots of both models suggest a  
348 positive correlation between global temperature and niche diversification rates. We therefore  
349 present only the best models for the phenotypic and niche regression analyses (Figure 4), despite  
350 only miniscule differences between the best and second-best models.

351 Results of all significant *ES-sim* analyses can be found in Table 4. These analyses did not  
352 reveal statistically significant relationships between variance in either the phenotype or niche  
353 summary statistic and variance in rates of lineage diversification. However, tests on unordinated  
354 phenotypic and environmental variables produced significant results for plant height, annual  
355 mean temperature (bioclim variable 1), and mean temperature of the coldest quarter (bioclim  
356 variable 11). These results suggest a correlative relationship between mean temperature, plant  
357 height, and the rate of lineage diversification, such that taller plants, and warmer annual and  
358 winter temperatures, lead to higher rates of lineage diversification.

359

## 360 **Discussion**

361 We analyzed macroevolutionary patterns of lineage, phenotypic, and niche diversification  
362 in *Penstemon*, a large angiosperm genus that has recently undergone a continental-scale rapid  
363 evolutionary radiation. Our results indicate that although *Penstemon* experienced an early burst  
364 of lineage diversification rates, the peak of which occurred approximately 2.0-2.5 MYA (Figure  
365 3), there is no clear evidence that this was accompanied by an increase in the rate of phenotypic  
366 or niche evolution. Conversely, it appears that changes in phenotypic diversification rates likely



367 operate at a smaller taxonomic scale, changing in smaller clades, rather than genus-wide, and  
368 occurring well after the initial burst in lineage diversification rates (Supplemental Figure 1). We  
369 did, however, find evidence for higher rates of lineage diversification in warmer environments  
370 and in larger plants (Table 4), and it appears that, generally, rates of lineage, phenotypic, and  
371 niche diversification are correlated with global temperature (Figure 4). Our results contribute to a  
372 growing body of evidence suggesting that asynchronicity in diversification rate shifts may be  
373 common, and bring into question the general applicability of expectations for diversification  
374 dynamics, which are derived mainly from studies of island systems, to studies of continental  
375 radiations.

376

### 377 *Asynchronous shifts in rates of lineage, phenotypic, and niche diversification*

378 We observed a distinct tree-wide shift in the rate of net lineage diversification  
379 approximately 2.0-2.5 MYA (Figure 3) which is not evident in phenotypic or niche  
380 diversification. Rather, for niche evolution, most evidence points to no shifts in diversification  
381 rate at all (Supplemental Figure 1, Supplemental Table 5). For phenotype evolution, although the  
382 exact number of rate shifts is inconclusive (Supplemental Table 4), no single coincident shift is  
383 apparent, suggesting that changes in phenotypic diversification rate occur at a smaller taxonomic  
384 scale (intra-clade vs. entire genus) and lag temporally behind the shift in lineage diversification  
385 rate (Supplemental Figure 1). An important caveat about our results is that our summarizations  
386 of diversity in environmental and phenotypic space in *Penstemon* are not comprehensive. There  
387 are potentially important unmeasured phenotypic and ecological variables that, if added to our  
388 analyses, may indeed produce synchronous diversification rate shifts. Examples of this include  
389 floral volatiles integral to pollinator attraction (Parachnowitsch et al. 2012), interactions with

390 arbuscular mycorrhizae (Titus & del Moral, 1998), and a diverse array of secondary metabolites  
391 for defense against herbivory (*e.g.*, Kelly & Bowers, 2016, 2018). We highlight this caveat to  
392 acknowledge some of the limitations of our study, but also to emphasize another point; because  
393 finding an association (or not) between rates of diversification can occur irrespective of whether  
394 traits are functionally coupled, such findings should be interpreted with care (Uyeda et al. 2021).

395         With this in mind, we note that similar patterns have been observed in other empirical  
396 studies. For example, lineage diversification without associated phenotypic diversification has  
397 been reported in a diverse array of taxonomic groups, including plethodontid salamanders  
398 (Adams et al. 2009), birds (Crouch & Ricklefs, 2019), and ferns (Testo & Sundue, 2018).  
399 However, lags in the timing of phenotypic diversification (*e.g.*, Folk et al. 2018) and niche  
400 diversification (*e.g.*, McCormack et al. 2010) are apparently less common. This discrepancy may  
401 be explainable if the initial burst in the rate of lineage diversification is not due to increased  
402 ecological opportunity (*i.e.*, niche-neutral), but subsequent diversification dynamics are indeed  
403 primarily density-dependent. Initial niche-neutral diversification can lead to a burst in the rate of  
404 lineage diversification without associated bursts in phenotypic or niche diversification rates  
405 (Aguilée et al. 2018; Folk et al. 2018). If, subsequent to this burst, increases in ecological  
406 opportunity enable ecological specialization and adaptation to novel environments, ecologically-  
407 driven diversification processes (*e.g.*, niche partitioning and divergence) can begin to supersede  
408 niche-neutral processes, leading to accelerations in phenotypic and niche diversification (Aguilée  
409 et al. 2018). Indeed, this logic was used to explain lags in phenotypic and niche diversification in  
410 the Saxifragales (Folk et al. 2018). Contrary, to our findings, however, Folk et al. (2018) did not  
411 observe a slowdown in Saxifragales lineage diversification rates. This was attributed to  
412 continued formation and expansion of novel habitat, consistent with ecological opportunity

413 caused from climate cooling and aridification after the mid-Miocene Climatic Optimum. While a  
414 slowdown is clearly evident in *Penstemon* (Figure 3), this is not in violation of theoretical  
415 expectations for density-dependent diversification dynamics; interspecific competition  
416 accelerates trait diversity, but not necessarily species richness, and in fact, competition can cause  
417 a slowdown in rates of lineage diversification even when there are no explicit ecological limits  
418 (Aristide & Morlon, 2019). Overall, our findings are consistent with initial niche-neutral  
419 diversification in *Penstemon*, followed by density-dependent diversification processes that  
420 simultaneously increased phenotypic diversity and led to a slowdown in lineage diversification  
421 rates.

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#### 423 *Geography, ecological opportunity, and the primary mode of speciation*

424 Although the initial burst in lineage diversification rates in *Penstemon* is consistent with  
425 niche-neutral diversification, we did also find evidence of a link between lineage diversification  
426 rate and ecological opportunity in the form of global temperature (Figure 4; Supplemental Table  
427 6). Relatedly, we found evidence for higher rates of lineage diversification in warmer  
428 environments and in larger plants (Table 4). If the initial burst in lineage diversification is  
429 primarily niche-neutral, why do there still appear to be ecological predictors of lineage  
430 diversification rates in *Penstemon*?

431 The timing of the burst in *Penstemon* lineage diversification rate corresponds to the onset  
432 of the Quaternary Period approximately 2.58 MYA (Head & Gibbard, 2015). With the  
433 Quaternary Period came severe environmental changes, including the development of ice sheets  
434 and the intensification of glaciation in the Northern Hemisphere (Head & Gibbard, 2015). A  
435 natural conclusion, then, would be to associate the shift in the rate of lineage diversification in

436 *Penstemon* with the increased ecological opportunity brought about by the cooling of the climate  
437 and the appearance of novel niches due to Quaternary glaciation. However, it is perhaps more  
438 likely that these relationships are a byproduct of the timing of the *Penstemon* radiation, and not a  
439 cause, *per se*. While ecological opportunity does increase during phases of rapid environmental  
440 change (Wellborn & Langerhans, 2015), and undoubtedly, Quaternary glaciation had profound  
441 effects on the geographic distributions and genomes of species (Hewitt, 2000), the lack of  
442 corresponding phenotypic and niche diversification in *Penstemon* suggests that although initial  
443 lineage diversification coincided with large-scale environmental change, speciation was still  
444 mostly occurring in environmental conditions similar to ancestral habitats. In this scenario, initial  
445 lineage diversification in *Penstemon* may have been a primarily geographic phenomenon:  
446 spurred by environmental changes, but only because those changes resulted in the formation of  
447 abundant habitat similar to habitats in which *Penstemon* species were already found.

448 Evidence from species distribution models in *Penstemon* subgenus *Dasanthera* lends  
449 some support for this scenario (Stone & Wolfe, 2021). For subgenus *Dasanthera*, which is sister  
450 to the rest of *Penstemon* and is not included in the lineage diversification rate shift, periods of  
451 peak glacial expanse likely brought about large increases in suitable habitat, particularly in the  
452 Columbia Basin and the Great Basin (Stone & Wolfe, 2021). These geographic regions, while  
453 ecologically similar to ancestral habitats, are now disjunct from current species' distributions,  
454 although a few narrowly endemic taxa (*i.e.*, *P. davidsonii* var. *praeteritus* and *P. fruticosus* var.  
455 *serratus*) still persist in isolated mountain ranges. A similar scenario for other *Penstemon*  
456 species, then, seems plausible, especially given the abundance of narrowly endemic species  
457 (most of which, consequently, are found in the Great Basin) and the lack of signal for shifts in  
458 niche diversification in our analyses (Supplemental Table 5; Supplemental Figure 1). Indeed, a

459 strong geographic component to speciation has been confirmed to be an important driver of  
460 diversification in *Penstemon*, with associations between founder-event speciation events and  
461 elevated rates of net lineage diversification pointing to a likely mechanism for the rapid radiation  
462 of the genus (Wolfe et al. 2021). The importance of these founder-event speciation events  
463 suggests that the dispersal of *Penstemon* species to previously unoccupied areas was key to the  
464 diversification of the genus (Wolfe et al. 2021). Likewise, the timing of these diversification  
465 bursts, coincident with pulses of glaciation during the Pleistocene, likely reflect repeated  
466 migration events into the Intermountain Region (Cronquist, 1978), and are consistent with  
467 expectations for species' responses to ecological opportunity in an adaptive radiation (Simpson,  
468 1953; Wolfe et al. 2021). With respect to the mechanism of speciation, a primarily niche-neutral  
469 process with a strong role of geography could be responsible for such patterns of lineage  
470 diversification. One possible example is the process of 'budding' speciation (*sensu* Anacker &  
471 Strauss, 2014), whereby larger-ranged progenitor species give rise to narrow-ranged species,  
472 often causing closely related species to be sympatric. Density-dependent processes could then  
473 follow this initial niche-neutral diversification, resulting in a slowdown of the overall lineage  
474 diversification process, increasing clade-specific rates of phenotypic diversification, and  
475 generating associations between species' phenotypes and the environment in which they live.  
476 Future studies on the geography of speciation in *Penstemon*, especially regarding asymmetry in  
477 the size of species' distributions and the degree of ecological and phenotypic overlap between  
478 close relatives, would be able to assess the support for this hypothesis.

479         Despite the apparent importance of niche-neutral processes in generating diversity in  
480 *Penstemon*, the influence of adaptive processes, particularly those related to floral evolution and  
481 the selective pressures induced by pollinators, should not be ignored. While the formation of

482 polyploid taxa is certainly an important diversification mechanism (*e.g.*, Keck, 1932, 1945), with  
483 virtually every *Penstemon* researcher since Pennell acknowledging the incredible diversity of  
484 floral shapes, sizes, and colors, it is clear that pollinator pressure has played a substantial role in  
485 the evolutionary history of this genus (Pennell, 1935; Straw, 1966; Wilson et al. 2004; Wolfe et  
486 al. 2006; Wessinger et al. 2019). Perhaps unsurprisingly, then, many studies have provided  
487 evidence of adaptation to different pollinators in *Penstemon* (*e.g.*, Straw, 1956, 1963; Crosswhite  
488 & Crosswhite, 1966), with transitions from insect- to hummingbird pollination especially  
489 common (Straw, 1963; Wilson et al. 2007; Wolfe et al. 2006, 2021). It is for this reason that the  
490 most likely density-dependent processes driving post-dispersal speciation to unoccupied niches  
491 involve adaptations to different pollinator types. This speciation process – relatively sluggish,  
492 compared to the multitude of founder-event speciation events associated with dispersal to  
493 unoccupied niches – would account for both the slowdown in lineage diversification rates  
494 (Figure 3) and clade-specific bursts in phenotypic diversification rates (Supplemental Figure 1)  
495 we have presented here. Supporting this claim, transitions from insect- to hummingbird  
496 pollination, while relatively common in *Penstemon*, have been associated with slowdowns in  
497 rates of diversification (Wessinger et al. 2019).

498         In light of this, it is important to consider the limitations of the analyses we have  
499 conducted in this study. By virtue of reducing the dimensionality of our data set into a single  
500 summary statistic, we capture less of the variance between species than is explained by the data  
501 set as a whole. By considering only the first principal component for niche and phenotype  
502 diversification as univariate traits, our analyses represent a biased sample of what is truly a  
503 multivariate pattern (Uyeda et al. 2015). While reducing the complexity of the data in this way is  
504 necessary to perform many of the analyses we have presented here, it also creates a dilemma we

505 must reconcile; it is possible – perhaps even likely – that the summary statistics employed in this  
506 study, despite explaining a large proportion of variance, may not be biologically meaningful  
507 summarizations of phenotypic and niche diversity in *Penstemon*. Future comparative studies  
508 interested in explaining diversification patterns in *Penstemon* may therefore benefit from  
509 generating a phenotypic data set more acutely focused on explaining the diversity of floral types  
510 evident in the genus.

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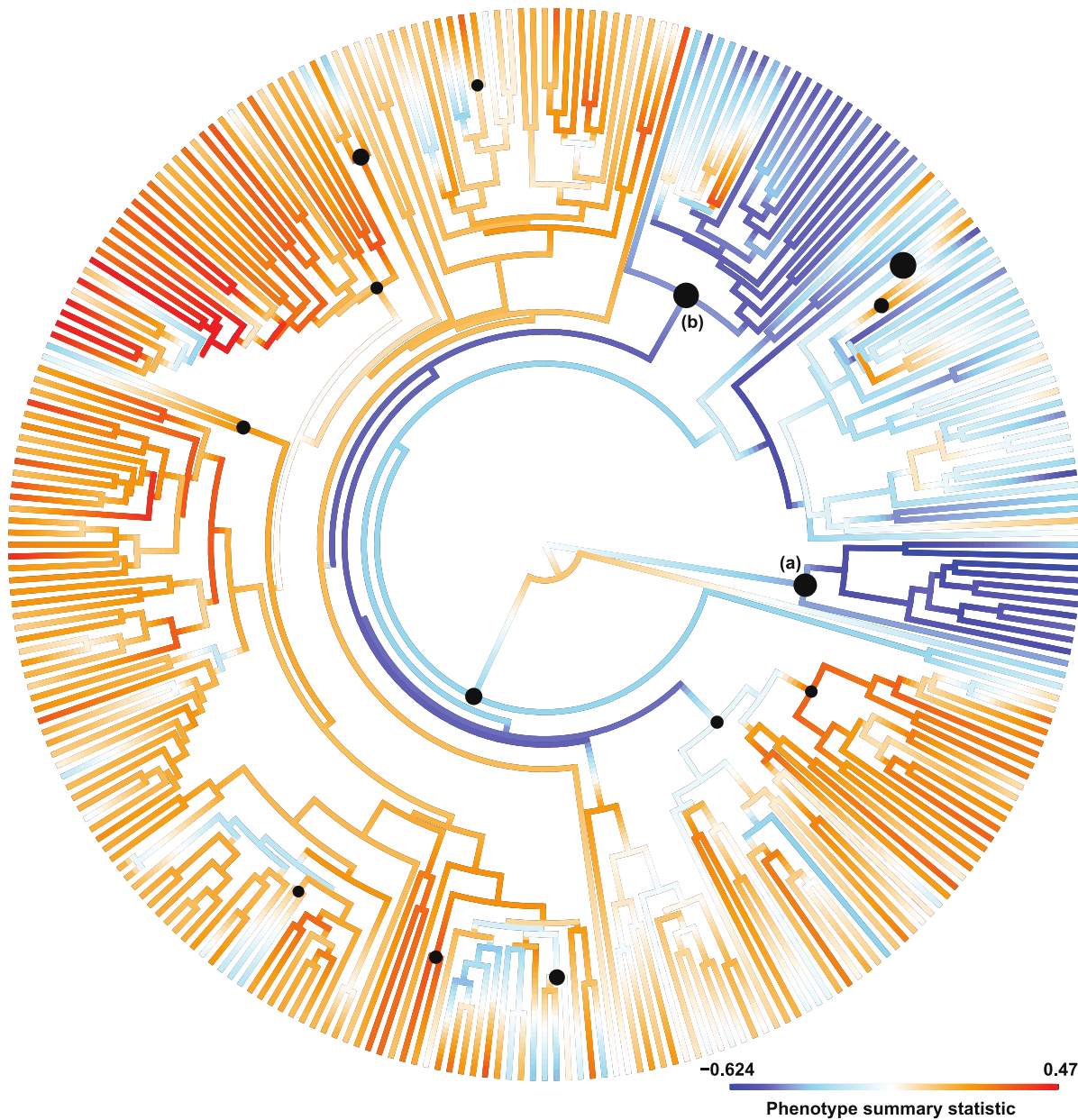
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528 **Figures and Tables**

529 *Figure 1.* Ancestral state reconstruction of the phenotype summary statistic. Labeled clades  
530 contain (a) subgenus *Dasanthera* and (b) section *Caespitosi*. Trait values represent scores of the  
531 first PC axis of ordinated phenotype data; cooler values indicate (generally) short plants with a  
532 shrub or subshrub growth form, no basal leaves, and with corolla throats expanded on the upper  
533 side. Black circles at nodes indicate the largest shifts (top 5%) in ancestral state estimates from  
534 the preceding node. Circles are scaled to the size of the shift in trait value.

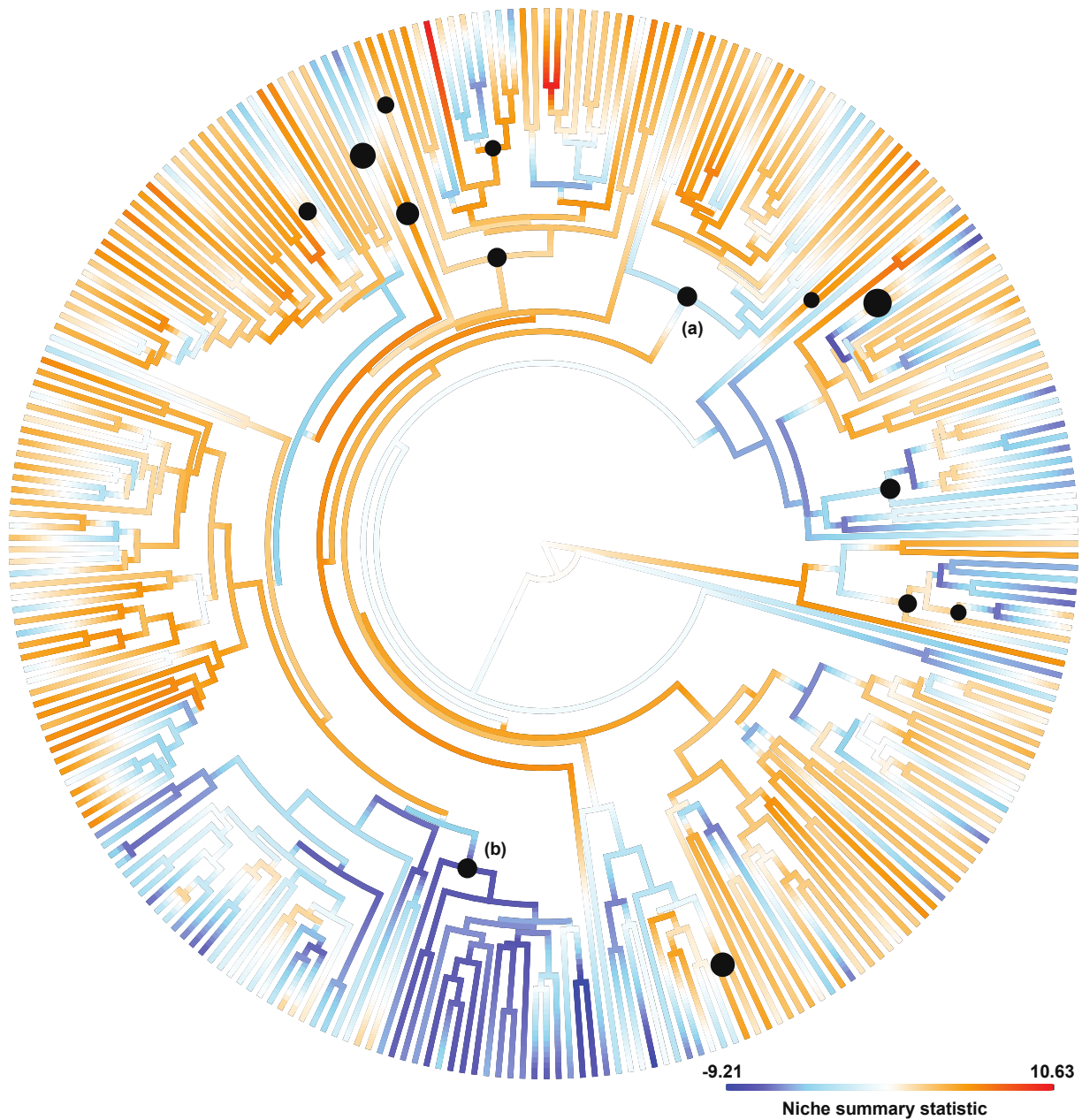


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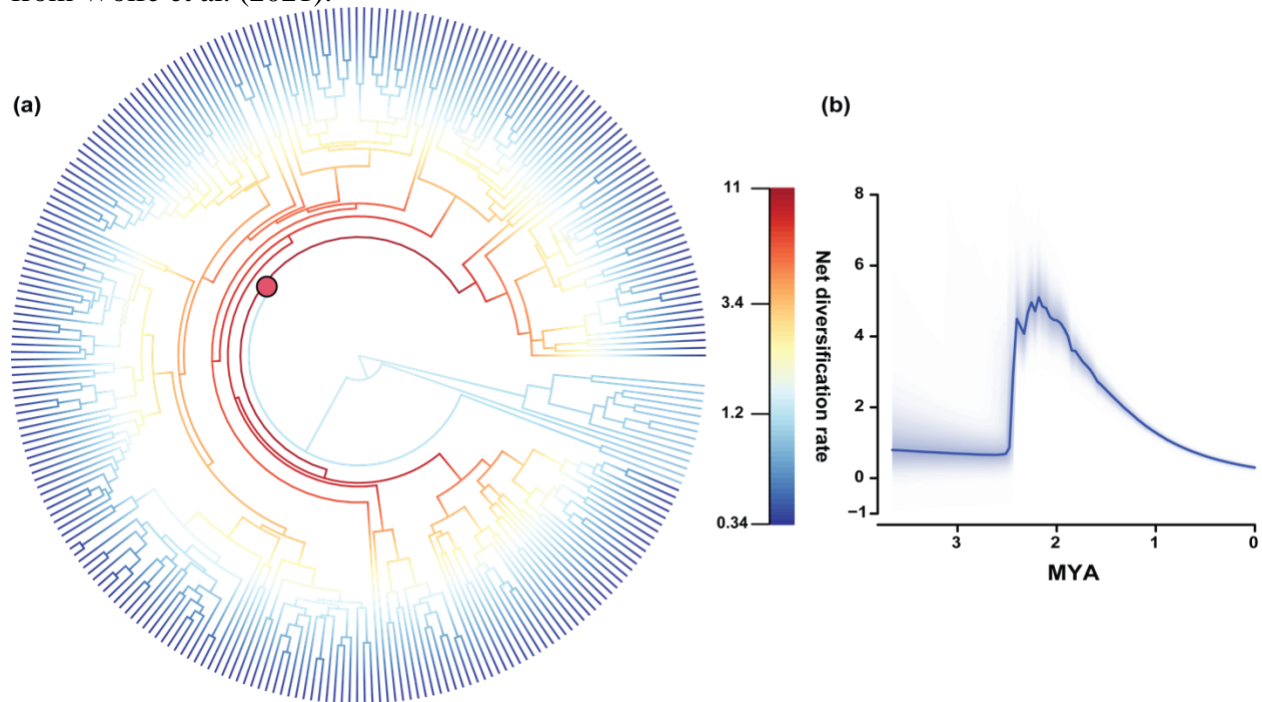
537 *Figure 2.* Ancestral state reconstruction of the niche summary statistic. Labeled clades contain  
538 sections (a) *Caespitosi* and (b) *Fasciculus*. Trait values represent scores of the first PC axis of  
539 ordinated niche data; warmer values indicate (generally) higher elevations with more herbaceous  
540 landcover, more temperature seasonality (but lower minimum, maximum, and mean  
541 temperatures), and less precipitation seasonality (with less overall precipitation, but  
542 comparatively more precipitation during the dry season). Black circles at nodes indicate the  
543 largest shifts (top 5%) in ancestral state estimates from the preceding node. Circles are scaled to  
544 the size of the shift in trait value.



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547 *Figure 3. Best shift configuration (a) and rates-through-time (RTT) plot (b) of *Penstemon**  
548 *lineage diversification rates in BAMM. (a) BAMM suggested a single shift in the rate of lineage*  
549 *diversification early in the evolutionary history of genus (red dot), followed by a subsequent*  
550 *slowdown of rates of lineage diversification. (b) Lineage diversification rates increased sharply*  
551 *between 2.0-2.5 MYA, declining steadily thereafter. Both findings are consistent with results*  
552 *from Wolfe et al. (2021).*



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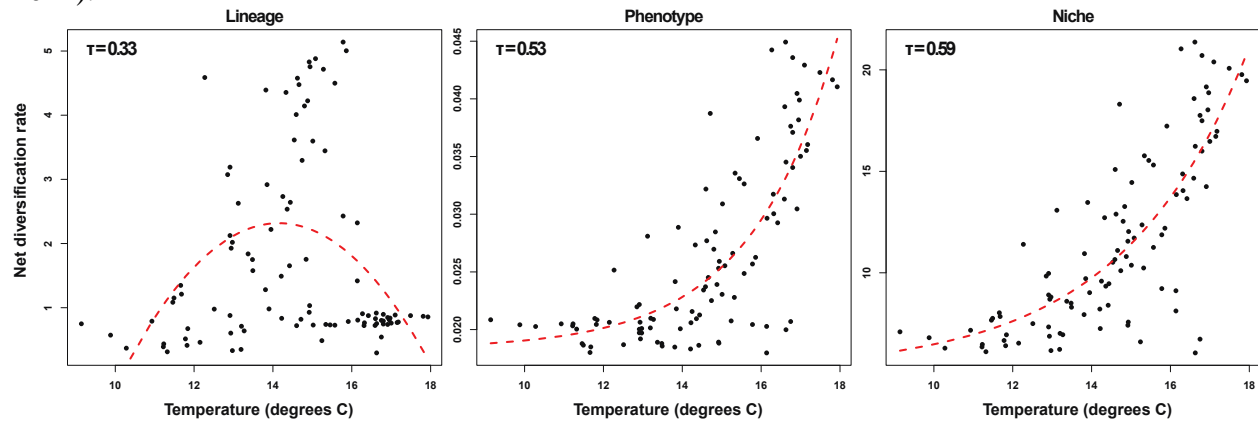
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564 *Figure 4.* Best regressions of global temperature with diversification rates. Global temperatures  
565 through time were inferred from climate model simulations of benthic delta-O-18 (de Boer et al.  
566 2014).



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*Table 1. Model weights for Ancestral State Reconstruction and variable importance (phenotype)*

Model Testing			
	BM	OU	EB
AICc	-233.499	-231.495	-231.498
AICc weights	0.576	0.212	0.212
Variable Importance			
Variable	Intercept	R <sup>2</sup>	R <sup>2</sup> corrected
Staminode length (mm)	0.029	0.002	0.001
Tube length (mm)	0.055	0.004	0.001
Throat diameter (mm)	0.050	0.006	0.002
Flower shape	-0.049	0.015	0.005
Stamen location	-0.033	0.016	0.005
Height (cm)	-0.075	0.037	0.011
Corolla length (mm)	0.157	0.041	0.012
Staminode location	-0.044	0.050	0.015
Style length (mm)	0.207	0.094	0.028
Leaf width (mm)	-0.122	0.120	0.036
Lifespan	0.259	0.130	0.039
Anther dehiscence	-0.240	0.158	0.047
Inflorescence	-0.035	0.213	0.064
Leaf length (mm)	-0.276	0.225	0.067
Flower presentation	0.229	0.242	0.072
Lower topography	-0.046	0.336	0.100
Throat expansion	0.094	0.435	0.130
Form	0.110	0.594	0.177
Basal leaves	0.141	0.633	0.189

584 Values for AICc and AICc weights correspond to model tests for ancestral state reconstruction of  
585 the phenotype summary statistic. Intercept and R<sup>2</sup> values correspond to values from regressions  
586 to produce relative measures of variable importance for the niche summary statistic. Variables  
587 are listed in ascending order with respect to R<sup>2</sup>.  
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Table 2. Model weights for Ancestral State Reconstruction and variable importance (niche)

Model Testing			
	BM	OU	EB
AICc	1402.215	1404.219	1401.994
AICc weights	0.403	0.148	0.450
Variable Importance			
Variable	Intercept	R <sup>2</sup>	R <sup>2</sup> corrected
Slope	1.016	0.001	0.000
Aspect	1.236	0.001	0.000
% shrubs	0.895	0.002	0.000
Precip. of driest quarter	0.299	0.006	0.001
% deciduous broadleaf trees	0.844	0.012	0.002
% evergreen broadleaf trees	0.783	0.014	0.002
Precip. of driest month	-0.057	0.025	0.003
Mean temp. of wettest quarter	1.777	0.038	0.005
Mean diurnal temp. range	-7.644	0.049	0.006
% mixed/other trees	1.757	0.050	0.006
% needleleaf trees	1.564	0.051	0.007
Max temp. of warmest month	8.354	0.062	0.008
Precip. of warmest quarter	2.654	0.108	0.014
Mean temp. of warmest quarter	8.376	0.168	0.022
elevation	-3.411	0.171	0.022
Precip. of coldest quarter	2.562	0.204	0.026
Annual precip.	4.372	0.297	0.038
Mean temp. of driest quarter	3.815	0.433	0.056
Precip. of wettest quarter	4.465	0.452	0.058
% herbaceous vegetation	-2.176	0.458	0.059
Precip. of wettest month	4.683	0.472	0.061
Isothermality	18.126	0.506	0.065
Annual mean temp.	6.886	0.564	0.073
Precip. seasonality	7.004	0.583	0.075
Temp. seasonality	-13.212	0.652	0.084
Annual temp. range	-22.126	0.696	0.090
Mean temp. of coldest quarter	0.926	0.809	0.104
Min temp. of coldest month	-3.869	0.865	0.112

593 Values for AICc and AICc weights correspond to model tests for ancestral state reconstruction of  
 594 the niche summary statistic. Intercept and R<sup>2</sup> values correspond to values from regressions to  
 595 produce relative measures of variable importance for the niche summary statistic. Variables are  
 596 listed in ascending order with respect to R<sup>2</sup>.

Table 3. PGLS results

Variable	$\lambda$	95% confidence interval	Slope	<i>p</i> -value
Niche summary statistic	0.959	(0.868, 1)	1.45E-03	0.634
Elevation	0.960	(0.871, 1)	9.96E-06	0.547
Aspect	0.962	(0.873, 1)	-1.33E-04	0.377
Slope	0.962	(0.873, 1)	1.81E-03	0.720
% needleleaf trees	0.962	(0.873, 1)	5.46E-04	0.289
<b>% evergreen broadleaf trees</b>	0.987	(0.901, 1)	-1.05E-02	<b>0.001</b>
% deciduous broadleaf trees	0.965	(0.876, 1)	-5.11E-03	0.165
% mixed/other trees	0.965	(0.877, 1)	7.97E-04	0.350
% shrubs	0.961	(0.872, 1)	-2.27E-04	0.586
% herbaceous vegetation	0.960	(0.870, 1)	2.69E-04	0.691
Annual mean temp.	0.958	(0.867, 1)	-1.12E-03	0.668
Mean diurnal temp. range	0.963	(0.873, 1)	-8.86E-03	0.180
Isothermality	0.954	(0.861, 1)	-1.64E-03	0.420
Temp. seasonality	0.961	(0.870, 1)	-3.04E-07	0.997
Max temp. of warmest month	0.959	(0.869, 1)	-2.23E-03	0.444
Min temp. of coldest month	0.961	(0.870, 1)	-7.30E-05	0.972
Annual temp. range	0.965	(0.875, 1)	-1.17E-03	0.629
Mean temp. of wettest quarter	0.962	(0.873, 1)	5.13E-04	0.747
Mean temp. of driest quarter	0.962	(0.872, 1)	2.26E-04	0.867
Mean temp. of warmest quarter	0.959	(0.868, 1)	-1.70E-03	0.556
Mean temp. of coldest quarter	0.958	(0.866, 1)	-8.21E-04	0.702
Annual precip.	0.963	(0.873, 1)	-1.41E-05	0.692
Precip. of wettest month	0.963	(0.873, 1)	-1.18E-04	0.550
Precip. of driest month	0.961	(0.870, 1)	7.95E-05	0.936
Precip. seasonality	0.962	(0.872, 1)	-6.58E-04	0.212
Precip. of wettest quarter	0.963	(0.873, 1)	-3.91E-05	0.577
Precip. of driest quarter	0.962	(0.872, 1)	-4.59E-05	0.879
<b>Precip. of warmest quarter</b>	0.983	(0.896, 1)	-3.05E-04	<b>0.038</b>
Precip. of coldest quarter	0.961	(0.871, 1)	-4.87E-06	0.950

597 All PGLS analyses were conducted with the phenotype summary statistic as the dependent  
598 variable. Environmental variables listed in the first column constitute independent variables.  $\lambda$   
599 can range from 0 to 1; at  $\lambda = 0$ , the model simplifies into ordinary least squares regression, and at  
600  $\lambda = 1$ , the model simplifies into phylogenetic independent contrasts. Significant *p*-values ( $p <$   
601 0.05) are indicated in bold and correspond to the significance of the slope after accounting for  
602 expected covariance due to the phylogeny.  
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*Table 4. Significant ES-sim results*

Variable	$\rho$	R <sup>2</sup>	<i>p</i> -value
Annual mean temp.	0.307	0.094	0.039
Mean temp. of coldest quarter	0.296	0.088	0.047
Height (cm)	0.351	0.123	0.015

604  $\rho$  is the population Pearson correlation coefficient.

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