

1 **Polyploidy and elevation contribute to opposing latitudinal gradients in**
2 **diversification and species richness in lady ferns (Athyriaceae)**

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35 **Summary**

36 • In ferns, the temperate-tropical sister clades *Athyrium* and *Diplazium* present an
37 opportunity to study a latitudinal contrast in diversification dynamics.

38 • We generated a taxonomically expanded molecular chronogram and used
39 macroevolutionary models to analyze how diversification rates have changed through
40 time, across lineages, and in concert with changes in elevation and ploidy. We tested a
41 novel model of cladogenetic state-change in which polyploidy can arise as an
42 infraspecific polymorphism, with diversification parameters distinct from those of
43 pure diploids and polyploids.

44 • Both *Athyrium* and *Diplazium* accelerated their diversification near the Oligocene-
45 Miocene transition. In *Diplazium*, the rate shift is older, with subsequent net
46 diversification somewhat slower and suggestive of diversity-dependence. In *Athyrium*,
47 diversification is faster and associated with higher elevations. In both clades,
48 polyploids have the highest rate of net accumulation but lowest (negative) net
49 diversification, while the converse is true for polymorphic species; diploids have low
50 rates of both net accumulation and diversification.

51 • Diversification in *Athyrium* may have responded to ecological opportunities in
52 expanding temperate habitats during the Neogene, especially in mountains, while the
53 pattern in *Diplazium* suggests saturation in the tropics. Neopolyploids are generated
54 rapidly, primarily through accelerated cladogenesis in polymorphic species, but are
55 evolutionary dead ends.

56 **Key words:** Athyriaceae, elevation, latitudinal gradient, macro-evolution, SSE
57 models, polyploidy

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69 **Introduction**

70 How does the tempo (rate) and mode (process) of lineage diversification vary across
71 latitude? Consideration of this question has largely focused on explaining the
72 latitudinal gradient in species richness, especially with respect to how temperate-
73 tropical contrasts in time-integrated area, temperature and metabolic rate, and
74 ecological opportunity might influence the rate of speciation (reviewed in Schluter,
75 2015). A general prediction is that, all else being equal, tropical clades should be
76 older, and/or have higher net diversification, compared to temperate clades (Wiens &
77 Donoghue, 2004; Mittelbach *et al.*, 2007). In this context, temperate-tropical sister
78 clades that otherwise share similar ecologies offer opportunities to study the correlates
79 and potential drivers of diversification across latitudes over a common timescale.

80 One such opportunity presents itself in the lady-fern family Athyriaceae, in which
81 the primarily temperate genus *Athyrium*, including allied genera, is sister to the
82 primarily tropical *Diplazium* (Wei *et al.*, 2013, 2018). These clades respectively
83 include about 220 and 350 species that occur in habitats that range from wet and
84 shaded streambanks at lower altitudes to dry and open grasslands higher up (Tryon &
85 Tryon, 1982; Wang *et al.*, 2013). Both clades are more or less globally distributed,
86 with the majority of species in tropical and temperate Asia (Wang *et al.*, 2013) and
87 the tropical Andes (Tryon & Tryon, 1982), and each has been the subject of recent
88 systematic and biogeographic studies (Wei *et al.*, 2013, 2015, 2018). Here, we
89 generate a more comprehensive dataset for comparative analyses of diversification: in
90 particular, how the timing, extent, and phylogenetic distribution of rate heterogeneity
91 differ in these clades, and what this reveal about their responses to potential drivers of
92 diversification.

93 In considering such drivers, a common theme that emerges from prior studies of
94 fern diversification is ecological opportunity. Seminal work at broad taxonomic and
95 deep temporal scales emphasized how fern lineages, particularly epiphytes,
96 proliferated ‘in the shadow of angiosperms’, i.e. in novel habitats created as the latter
97 ascended in the late Cretaceous/early Cenozoic (Schneider *et al.*, 2004; Schuettpelz &
98 Pryer, 2009; but see Testo & Sundue, 2016). More recently, birth–death models fitted
99 to phylogenies and the fossil record showed evidence for diversity-dependent

100 origination, interpreted as reflecting opportunistic niche-filling, and extinction driven
101 by environmental change (Lehtonen *et al.*, 2017). For example, in Polypodiaceae,
102 faster diversification is correlated with changes in elevation, a proxy for habitat type,
103 suggesting that niche shifts drive lineage proliferation (Sundue *et al.*, 2015). In the
104 case of *Athyrium-Diplazium*, one might predict that diversification in *Athyrium* was
105 accelerated by ecological opportunities arising during the expansion of temperate
106 habitats during the Neogene, compared to *Diplazium*, which might be in the later
107 stages of diversity-dependent clade growth, reflecting its persistence in more
108 continuously stable tropical environments. Both clades might show responses to
109 elevation, reflecting their respective colonization of temperate and tropical mountains,
110 such as temperate Hengduan Mountains and the tropical Kinabalu and Andes.

111 Another potential driver of diversification that is not directly related to latitude, but
112 demands attention due to its prevalence in ferns, is polyploidy (e.g. Schneider *et al.*,
113 2017). There is evidence that more than 50% of the species in the *Athyrium-*
114 *Diplazium* clade are polyploid (e.g. Praptosuwiryo, 2008; Bir & Verma, 2010). The
115 effect of polyploidy on plant diversification has received much attention from both
116 theoretical and empirical perspectives (e.g. Mayrose *et al.*, 2011; Soltis *et al.*, 2016;
117 reviewed in Vamosi *et al.*, 2018). In particular, polyploid formation as a mode of
118 speciation motivated the development of cladogenetic state-dependent diversification
119 models, in which the rate of speciation coincident with state change is parameterized
120 (Mayrose *et al.*, 2011; Zhan *et al.*, 2016; see also Goldberg & Igić, 2012). These have
121 since been used to show that neopolyploids tend to be evolutionary 'dead ends'
122 (Mayrose *et al.*, 2011; Arrigo & Barker, 2012) due to effects such as gene imbalance
123 of the sex chromosomes during the meiosis (Orr, 1990), increased abortion in
124 heteroploid hybrids (Ramsey & Schemske, 2002), and inefficiency of selection for
125 multi-copy genes in polyploids (Wright, 1969). We wish to know if this holds true in
126 the diversification dynamics of *Athyrium* and *Diplazium*.

127 Previous studies of neopolyploidy using cladogenetic state-change models only
128 considered two states for species, diploid and polyploid (Zhan *et al.*, 2016). However,
129 infraspecific variation in ploidy is common in plants (Wood *et al.*, 2009); in the
130 *Athyrium-Diplazium* clade, 5–10% of species have polyploid individuals recorded
131 within one or more diploid populations (Walker, 1966; Tryon & Tryon, 1982;
132 Takamiya *et al.*, 1999, 2000; Takamiya & Ohto, 2001; Praptosuwiryo, 2008; Bir &

133 Verma, 2010). Such cases of polymorphism may represent incipient speciation via
134 polyploidization. To test this idea, we constructed cladogenetic state-change models
135 that include three states, diploid, polyploid, and a polymorphic diploid/polyploid state,
136 with free parameters assigned to the rate at which polymorphic species give rise to
137 new neopolyploids via cladogenesis events.

138 Our study can be summarized as a temperate-tropical contrast in diversification
139 dynamics, focusing on how sister clades have responded to common potential drivers
140 of diversification. Specifically, our aims are to reconstruct a time-scaled global
141 phylogeny of the *Athyrium-Diplazium* clade, estimate variation in diversification rates
142 through time, and infer the effects of elevation and polyploidy. Our intent is to shed
143 light on latitudinal differences in the tempo and mode of diversification, and
144 contribute to a more general understanding of the latitudinal diversity gradient.

145

146 **Materials and Methods**

147 **Taxon sampling and molecular data**

148 Our sampling strategy followed the most recent systematic studies of Athyriaceae
149 (Wei *et al.*, 2013, 2015, 2018; PPG I, 2016). In this study, the circumscription of
150 *Athyrium* includes four closely related genera (*Anisocampium*, *Athyrium*, *Cornopteris*
151 and *Pseudathyrium*) and ten sections (sects. *Athyrium*, *Biserrulata*, *Dissitifolia*,
152 *Mackinnoniana*, *Otophora*, *Polystichoides*, *Rupestria*, *Spinulosa*, *Wallichiana* and
153 *Yokoscentia*) (Supporting Information Table S1). Since no sectional classification in
154 the *Diplazium* clade (especially in subgenus *Callipteris*), we tentatively subdivided
155 *Diplazium* into 11 clades (CHI, DIP, LEP, MET, MON, NEO, PSE, SEA, SIB, SPE
156 and UNK) according to their morphological similarity, phylogenetic relationship and
157 biogeographical affinity (Table S1).

158 In total, 85 out of *c.* 220 species of *Athyrium* (39%) and 129 out of *c.* 350 species of
159 *Diplazium* (37%) on the global scale were sampled, including representatives from all
160 taxonomic subdivisions or infrageneric groups covering the entire geographical
161 distribution range and ecological habitats of these two genera. Voucher information is
162 listed in Table S2. We included 26 representatives from Aspleniaceae, Athyriaceae,
163 Blechnaceae, Cystopteridaceae, Desmophlebiaceae, Diplaziopsidaceae,
164 Dryopteridaceae, Hemidictyaceae, Onocleaceae, Rhachidosoraceae, Thelypteridaceae
165 and Woodsiaceae as outgroups and to provide appropriate nodes for fossil calibrations.

166 We assembled a dataset of eight chloroplast regions (*atpA*, *atpB*, *matK*, *rbcL*,
167 *rpl32-trnP*, *rps4*, *rps4-trnS*, *trnL-F*) with a total aligned length of 8297 bp. We used
168 1129 previously published sequences and generated 336 new sequences (Table S2).
169 DNA extraction, amplification, sequencing, and alignment protocols followed Wei *et*
170 *al.* (2013, 2018).

171

172 **Divergence time estimation**

173 We inferred the posterior distribution of chronograms using BEAST 1.8.4 (Drummond
174 & Rambaut, 2007) with the alignment partitioned by protein coding regions and
175 intergenic spacers. Preliminary analyses rejected a strict molecular clock, so we used
176 the uncorrelated lognormal relaxed clock model with a birth–death prior on tree shape.
177 Eight independent runs of 30 million generations, sampling every 1000 generations,
178 were carried out on the Cyberinfrastructure for Phylogenetic Research (CIPRES)
179 Science Gateway (<http://www.phylo.org>; Miller *et al.*, 2010). The resulting log files
180 were combined (with the first 50% samples discarded as burn-in) using
181 LOGCOMBINER 1.8.4 (Drummond & Rambaut, 2007) and checked in TRACER 1.6 to
182 make sure the effective sampling sizes for most of the relevant estimated parameters
183 were well above 200. The tree files were resampled from each run using the same
184 burn-in strategy in LOGCOMBINER. The maximum clade credibility (MCC) topology
185 with a posterior probability limit of 0.5 and mean branch lengths was summarized
186 using TREEANNOTATOR 1.8.4 (Drummond & Rambaut, 2007).

187 Four calibration points were used. For the root node the prior was a Normal
188 distribution with a mean age of 107.29 Myr and a SD of 10 (with 95% highest
189 posterior density [HPD]: 90.84–123.7 Myr) to cover the range of published split times
190 from 91 Myr to 125 Myr including the 95% HPD intervals (Schneider *et al.*, 2004;
191 Schuettpelz & Pryer, 2009; Rothfels *et al.*, 2015; but see Testo & Sundue, 2016). We
192 used the Paleocene fossil of *Onoclea* (Rothwell & Stockey, 1991) to constrain the
193 minimum age of the Onocleaceae crown group with a lognormal prior distribution
194 with an offset of 54.5 Myr and mean of 1.0 (with 95% HPD: 55.02–68.58 Myr). We
195 used earliest fossil record of *Woodwardia*, known from Paleocene deposits of North
196 America, to constrain the crown node of Blechnaceae (Collinson, 2001), using the
197 same prior settings. We assigned the *Diplazium*-like fossil reconstructions
198 (*Makotopteris princetonensis*, Stockey *et al.*, 1999; *Dickwhitea allenbyensis*, Karafit

199 *et al.*, 2006) found in the Middle Eocene deposits of British Columbia in North
200 America to the stem lineage of *Diplazium* (Wei *et al.*, 2015), yielding a lognormal
201 prior with an offset of 36.5 Myr and mean of 2.0 (with 95% HPD: 37.93–74.78 Myr)
202 for the crown node of the *Athyrium-Diplazium* clade.

203

204 **State-independent diversification analysis**

205 We used Bayesian Analysis of Macroevolutionary Mixtures (BAMM) 2.2.2 (Rabosky,
206 2014) to estimate rates of speciation (λ) and extinction (μ) through time and across
207 clades on the MCC tree, pruned to the ingroup *Athyrium-Diplazium* clade. To account
208 for incomplete sampling, we estimated the sampling fractions of infrageneric groups
209 (Table S1) from our own taxonomic knowledge and the literature (e.g. Ching, 1964;
210 Kato, 1977; Tryon & Tryon, 1982; Hsieh, 1986; Tryon & Stolze, 1991; Zhang, 1992;
211 Stolze *et al.*, 1994; Wang, 1997; Mickel & Smith, 2004; Y.C. Liu *et al.*, 2011; Wang
212 *et al.*, 2013; Wei *et al.*, 2013, 2018). Four MCMC analyses were run for 3 million
213 generations each, sampled every 1000 generations. Each run was checked to ensure
214 that the effective sample size (ESS) exceeded 200, and the first 10% of samples were
215 discarded as burn-in. Results were summarized using BAMMTOOLS (Rabosky *et al.*,
216 2014). To evaluate the best model generated by BAMM (compared with a null model
217 with no diversification rate shifts), we relied on Bayes Factors calculated with the
218 COMPUTEBAYESFACTOR function of BAMMTOOLS.

219

220 **State-dependent diversification analyses**

221 We tested two variables for an association with diversification: mean elevation and
222 ploidy. Data were obtained from a variety of sources, including online databases
223 (Global Plants, <https://plants.jstor.org>; Tropicos, <http://www.tropicos.org>; IPCN,
224 <http://www.tropicos.org/Project/PCN>; CCDB,
225 <http://ccdb.tau.ac.il/Pteridophytes/Athyriaceae>), herbarium specimens, and cytological
226 reports (e.g. Mehra & Bir, 1960; Walker, 1966, 1973; Tryon & Tryon, 1981;
227 Takamiya *et al.*, 1999, 2000; Takamiya & Ohta, 2001; Praptosuwiryo, 2008; Bir &
228 Verma, 2010), and are available in Table S3.

229 To test the hypothesis of net diversification accelerated by divergence in elevation
230 (Sundue *et al.*, 2015), we used BAMM to estimate the rates of elevation change (β) at
231 the tips of the MCC tree, and performed phylogenetic generalized least squares

232 (PGLS) regression of β on tip values of $r = \lambda - \mu$ using GEIGER (Harmon *et al.*, 2008).
233 In addition, we tested for a direct effect of elevation using the quantitative-state
234 speciation-extinction (QuaSSE) model (FitzJohn, 2010). We considered seven
235 candidate relationships between elevation (log-transformed) and λ : 1) λ is constant
236 and independent of elevation; 2) linear; 3) sigmoid; 4) unimodal, represented by a
237 vertically offset Gaussian function; and another three models (linear, sigmoid, and
238 unimodal) with a directional tendency (Table S4). We ran each model on the entire
239 ingroup clade as well as separately on *Athyrium* and *Diplazium* using DIVERSITREE
240 0.9-10 (FitzJohn, 2012). We compared models using the Akaike information criterion
241 (AIC).

242 To explore the effect of ploidy on diversification we used the cladogenetic state
243 change speciation-extinction (ClaSSE) model (Goldberg & Igic, 2012). Species were
244 scored as 1 (polymorphic, i.e. diploid with records of intermingled polyploids), 2
245 (diploid), 3 (polyploid), or NA (unknown). We considered reports of $2n = 80$ for
246 *Athyrium* and $2n = 82$ for *Diplazium* as diploid. All other species data were multiples
247 of these numbers (e.g. $2n = 160$, $2n = 123$, $2n = 164$, $2n = 205$, $2n = 246$, $2n = 328$)
248 and were thus scored as polyploids (Table S3). We constructed a variety of models
249 with up to seven cladogenetic rates, denoted as a triplet of values for the ancestral and
250 descendant states: λ_{111} , λ_{112} , λ_{113} , λ_{123} , λ_{222} , λ_{223} , λ_{333} , three extinction rates (μ_1 , μ_2 , μ_3),
251 and four transition rates (q_{12} , q_{13} , q_{21} , q_{23}) (Fig. 1). We disallowed anagenetic change
252 from polyploidy (3) to diploidy (1, 2) because no diploidization events were reported
253 according to previous cytological studies and our CHROMEVOLE analysis (see results).
254 After some preliminary analyses, we settled on six candidate models: 1) a full model
255 with all 13 free parameters; 2) a null model with three parameters, in which
256 diversification is state-independent and anagenetic change is symmetric; 3) a
257 ‘cladogenetic’ model with 11 parameters and a single rate of anagenetic change; 4) an
258 ‘anagenetic’ model with five parameters and a single rate for all speciation and
259 extinction events; 5) an ‘extinction’ model with five parameters and 1 rate each for
260 speciation and anagenetic change; and 6) a ‘cladogenetic-anagenetic’ model with 11
261 parameters, and a single rate for extinction (Fig. 1; Table S5). Models were run on the
262 whole ingroup clade and separately on each genus, and compared using AIC.
263 Parameters of the best-fit models were estimated using MCMC for 100,000
264 generations in DIVERSITREE.

265 To test the reliability of our ClaSSE analysis given that nearly 40% of the sampled
266 species have unknown ploidy, we simulated 100 trees of the same size as our ingroup
267 clade using the maximum likelihood parameter values of the best-fit model. For each
268 tree we ‘impoverished’ the tip states by randomly setting 40% to the unknown state,
269 NA, and performed model selection using the candidate pool as described above. We
270 also compared the parameters estimated from the impoverished data to their
271 simulation values. All procedures were carried out using DIVERSITREE 0.9-10.

272

273 **Reconstruction of polyploidy evolution**

274 To infer the evolutionary history of polyploidy along the phylogeny, we carried out
275 an ancestral state reconstruction using CHROMEVOLE 2.0 (Glick & Mayrose, 2014)
276 implemented in RASP 4.0 (Yu *et al.*, 2015). To shorten the calculation time, we used
277 the ‘Auto_run’ option, which automatically optimized all the parameters for
278 chromosome evolution models, including rates of single ascending dysploidy (γ),
279 descending dysploidy (δ), whole-genome duplication (ρ) and demi-polyploidy (μ_d).
280 After model optimization, 10,000 simulations were performed using both maximum
281 likelihood and Bayesian approaches to summarize the final reconstruction result.

282

283 **Results**

284 **Divergence time estimation**

285 Our molecular dating analysis yielded age estimates that are broadly congruent with
286 those of previous studies with the variations less than 10 Myr (Schneider *et al.*, 2004;
287 Schuettpeitz & Pryer, 2009; Rothfels *et al.*, 2015; Wei *et al.*, 2015; but see Testo &
288 Sundue, 2016 using a different strategy of divergence time estimation based on a time
289 calibrated phylogeny) (Fig. S1). Those that differed tended to be older (e.g. the crown
290 node of the *Diplazium* clade, 47.9 Myr [95% HPD: 41.1–59.4 Myr] in the present
291 study; 41.7 Myr [95% HPD: 33.6–49 Myr] in Wei *et al.*, 2015). This could be due, at
292 least in part, to our much denser sampling of *Athyrium* and *Diplazium* (214 species
293 versus 92 in Wei *et al.*, 2015).

294

295 **Diversification analyses: BAMM, QuaSSE and ClaSSE**

296 The BAMM analysis indicated significant increases in net diversification rate in both
297 *Athyrium* and *Diplazium* (Figs 2a and S2; Table S6). In *Athyrium*, two shifts were

298 inferred at 16.86 Myr and 5.02 Myr, with the latter leading to a rate 17 times higher
299 than the root rate (0.044–0.76 events Myr⁻¹ per lineage; Fig. 2a). In *Diplazium* one
300 shift was inferred at 23.43 Myr (Fig. 2a). Rates-through-time (RTT) plots revealed
301 contrasting diversification dynamics between *Athyrium* and *Diplazium* following their
302 initial shifts (Fig. 2b). In *Athyrium*, the net diversification rate increased continuously,
303 while in *Diplazium* the rate plateaued to a level about half that of *Athyrium* at the
304 present.

305 Diversification is associated with elevation itself but not its evolutionary rate. The
306 PGLS regression of the rate of change in elevation on net diversification was
307 nonsignificant ($r^2 = 0.286$, $P = 0.379$). By contrast, the best-fit QuaSSE model ($w_i =$
308 0.88; Table S4) for the *Athyrium-Diplazium* clade included a unimodal relationship
309 between λ and elevation, with λ highest between 1440 m to 3500 m and constant μ
310 (Fig. 2a). There was a negative directional tendency in *Athyrium* (-0.091) and the
311 entire ingroup clade (-0.086), but a relatively low negative directional tendency in
312 *Diplazium* (-0.0013) (Table 1). Separate analyses of *Athyrium* and *Diplazium* showed
313 differences in their unimodal curves for λ . In *Diplazium* the peak is at a lower rate and
314 elevation, and the curve closely matches the frequency distribution of species by
315 elevation, while in *Athyrium* the peak is higher in rate and elevation, and the curve is
316 shifted right relative to the species' frequency distribution (Fig. 3). The best-fit model
317 for *Athyrium* includes a negatively valued directional tendency parameter, but the one
318 for *Diplazium* does not (Table 1).

319 Our ClaSSE analyses yielded strong support for ploidy-dependent diversification
320 dynamics. The 'cladogenetic' model, with 10 free parameters for speciation and
321 extinction but no differences in q was selected by AIC (Fig. 4a,b,c,d; Table 2). With
322 this model, rates of speciation in polyploid ($\lambda_3 = 0.7054$ events Myr⁻¹ per lineage) and
323 polymorphic species ($\lambda_1 = 1.388$ events Myr⁻¹ per lineage) exceed rates for diploids
324 ($\lambda_2 = 0.1993$ events Myr⁻¹ per lineage), with the total rate being highest for
325 polymorphic species (Fig. 4a,d; Table 2). Extinction rates are low for diploid and
326 polymorphic species but high for polyploids (Fig. 4c); as a result, the net
327 diversification rate of the polymorphic state is the highest, the rate for diploids is
328 lower but positive, and the rate for polyploids is lowest and slightly negative (Fig. 4e).
329 However, the net accumulation rate of polyploids is highest, followed by diploids and
330 polymorphic species (Fig. 4f). In our simulation analysis of the effect of missing data

331 on model selection, 93% of replicates recovered the correct cladogenetic model (Fig.
332 S3; Table S7), indicating that in this case, model selection is not obviously biased by
333 40% missing data.

334 Our CHROMEVOL analysis inferred no dysploidization or diploidization events, and
335 at least 15 polyploidization events indicating that all polyploid species in *Athyrium*
336 and *Diplazium* are neopolyploids (Fig. S4).

337

338 **Discussion**

339 **Latitudinal contrasts in diversification between temperate *Athyrium* and tropical** 340 ***Diplazium***

341 Our dated phylogeny and BAMM analysis showed that species diversity in the
342 primarily temperate genus *Athyrium* has accumulated relatively recently, with an
343 increasing net rate through the Neogene, compared to its primarily tropical sister
344 clade *Diplazium*, which showed dynamics more suggestive of diversity-dependence.
345 Similar temperate-tropical rate contrasts that run counter to the latitudinal diversity
346 gradient have been found in fishes, birds, mammals, and seed plants (Weir & Schluter,
347 2007; Schluter, 2015; Spriggs *et al.*, 2015), and contradict the hypothesis that higher
348 diversity in the tropics is the result of faster per-lineage diversification (e.g.
349 Mittelbach *et al.*, 2007). The *Athyrium-Diplazium* contrast may reflect how
350 diversification has responded at a coarse scale to waxing and waning ecological
351 opportunities in temperate and tropical habitats, respectively. In this view, the
352 relatively recent and rapid radiation in *Athyrium* was driven by increasing niche
353 availability in expanding temperate habitats, while for *Diplazium*, the contraction of
354 tropical forests had the opposite effect, as global cooling began in the Late Oligocene
355 (Zachos *et al.*, 2001; Morley, 2003). The plateau of net diversification rate in
356 *Diplazium* suggests that the clade is closer to ecological saturation than *Athyrium*, in
357 which the upward trend in rate suggests a clade in the early stages of rapid growth,
358 further from equilibrium (Fig. 2b). These dynamics are consistent with the idea that
359 species diversity is predicted by area, and other proxies for carrying capacity,
360 integrated over time (Fine & Ree, 2006; Jetz & Fine, 2012). In addition, the relatively
361 high turnover rate μ/λ in *Athyrium* (Fig. S5) may be due to more severe climatic
362 oscillations in the temperate zone than in the comparatively stable tropics over the
363 past 20 Myr or so (Tiffney & Manchester, 2001; Morley, 2003), consistent with the

364 latitudinal gradient in turnover found in previous studies (Jablonski *et al.*, 2006; Weir
365 & Schluter, 2007; but see Rabosky *et al.*, 2015). This high rate of species turnover, in
366 turn, suggests a rapid species replacement through non-adaptive evolution in
367 *Athyrium* (Rundell & Price, 2009).

368 Additional support for the idea that diversification dynamics in *Diplazium* are
369 closer to an equilibrium state than *Athyrium* is provided by our QuaSSE analysis of
370 elevation. In *Diplazium* the inferred response of speciation rate to elevation closely
371 matches the frequency distribution of species along elevation (also is reflected by a
372 low negative directional tendency; Table 1), suggesting a stable state, while in
373 *Athyrium* the rate curve is right-shifted: speciation is fastest at elevations higher than
374 the peak of species richness (Fig. 3). The relative lack of high-elevation species of
375 *Athyrium* could be explained partly by the inferred negative directional tendency in
376 the model – i.e. species originated at high elevations but evolved anagenetically
377 toward lower elevations – but it seems more plausible that *Athyrium* has simply
378 colonized mountains too recently for the number of high-elevation species to reach
379 equilibrium. We would expect such a lag time to be lengthened by the relatively high
380 extinction rate inferred for *Athyrium* versus *Diplazium* (Table 1).

381 Our analyses of elevation stand in contrast to those in the epiphytic fern family
382 Polypodiaceae, in which faster diversification was positively correlated with the rate
383 of change in elevation, not elevation itself, suggesting a process of adaptive
384 divergence and niche-filling along elevation gradients (Sundue *et al.*, 2015). We were
385 unable to replicate this result from our data, possibly because of insufficiently dense
386 taxon sampling. Nevertheless, our results suggest that diversification is accelerated by
387 occurring in mountains, especially for *Athyrium*. However, it is worth noting that even
388 within *Diplazium*, the highest net diversification was inferred in the lone Andean
389 clade (Fig. 2a). Left open are questions about how and why, such as to what degree is
390 speciation accelerated by non-adaptive processes (such as allopatric genetic drift)
391 versus those involving adaptation? More focused studies of possible mechanisms are
392 needed. One might look for interactions between traits with environmental tolerances
393 and mountain buildings in tropical and temperate zones (Xing & Ree, 2017; Hughes
394 & Atchison, 2015). This has been shown in studies of other plant groups have found
395 that the evolution of traits such as growth form, leaf size, pollination syndromes, and
396 metabolic pathways may be linked to faster diversification in mountains where

397 seasonally cold and arid habitats are dominant (Arakaki *et al.*, 2011; Drummond *et al.*,
398 2012; Roquet *et al.*, 2013; Schwery *et al.*, 2015; Lagomarsino *et al.*, 2016).

399

400 **Polyploidy-driven diversification in the *Athyrium-Diplazium* clade**

401 In ferns, several traits, including growth habit, leaf size, leaf morphology and
402 gametophyte morphology, have been studied for their effect on diversification
403 (Sundue *et al.*, 2015; Ramírez-Barahona *et al.*, 2016; Testo & Sundue, 2018), but
404 polyploidy, despite its ubiquity and precedent in being implicated in diversification
405 (Wood *et al.*, 2009), has thus far escaped intensive quantitative analysis. While the
406 macroevolutionary consequences of polyploidy remain a subject of debate (e.g.
407 Mayrose *et al.*, 2011, 2015; Zhan *et al.*, 2014; Soltis *et al.*, 2014), our inference of
408 rapid turnover with negative net diversification in polyploids (Fig. 3d) is consistent
409 with the ‘extinction-risk’ hypothesis, that neopolyploids suffer deleterious genetic
410 effects (Wright, 1969; Orr, 1990; Ramsey & Schemske, 2002), and thus are often
411 evolutionary dead ends (Mayrose *et al.*, 2011; Arrigo & Baker, 2012). This effect is
412 counterbalanced by the high net diversification rate of polymorphic species (Fig. 4d),
413 which rapidly produce polyploids through cladogenesis events and thereby contribute
414 to polyploids having the highest net accumulation rate (Fig. 4e). Rapid and recurrent
415 neopolyploidy in *Athyrium* and *Diplazium* (Fig. S4) is distinct from *Asplenium*, which
416 is characterized by a dominance of paleopolyploids (Schneider *et al.*, 2017). Taken
417 together, these results imply that although neopolyploids in *Athyrium* and *Diplazium*
418 tend to be short-lived, they play a key role in diversification dynamics (Vamosi *et al.*,
419 2018).

420 Intraspecific variation in ploidy is common in plants, and many case studies have
421 examined the origins of polyploidy at the population level, especially in genera of
422 Asteraceae such as *Tragopogon* (Symonds *et al.*, 2010), *Artemisia* (Pellicer *et al.*,
423 2010), *Helianthus* (Bock *et al.*, 2014), as well as in fern genera *Asplenium* (e.g. Dyer
424 *et al.*, 2012) and *Dryopteris* (e.g. Ekrt & Koutecký, 2016), and *Pteris* (Chao *et al.*,
425 2012). This study is the first to explicitly model how such polymorphic species may
426 contribute to diversification dynamics at a macroevolutionary scale, and so it seems
427 noteworthy to find that despite occurring at low frequency, their net diversification
428 rates are in fact higher than pure diploids or polyploids. This is largely driven by λ_{113} ,
429 the rate at which a polymorphic ancestor splits into polymorphic and polyploid

430 descendants (Fig 4a). Mechanistically, it seems reasonable to predict that this
431 parameter reflects how newly formed polyploids in a population are likely to be
432 reproductively isolated from their diploid progenitors, and thus form a new lineage.
433 Intraspecific polyploidy can predispose species to be better adapted to harsher
434 conditions in novel environments (Levin, 1983; Ramsey & Schemske, 2002; te Beest
435 *et al.*, 2012). These results could be framed as a ‘ploidy-polymorphism compensation’
436 hypothesis, wherein polymorphic species play a central role in the maintenance of
437 neopolyploid diversity. The *Athyrium-Diplazium* clade is not likely to be unique in
438 supporting this hypothesis; we predict that other lineages such as *Asplenium*
439 (*Aspleniaceae*) and *Dryopteris* (*Dryopteridaceae*) and *Pteris* (*Pteridaceae*), in which
440 autopolyploidy, hybridization and apomixis are prevalent (Chao *et al.*, 2012; H.M.
441 Liu *et al.*, 2012), may have similar compensatory dynamics mitigating the high
442 extinction risk of polyploids.

443 Why might polyploids have high speciation rates (Fig. 4a)? It is possible that some
444 factors (a novel physical trait or a property of the environment) associated with
445 polyploidization may play a role (Zhan *et al.*, 2016; Vamosi *et al.*, 2018). A few case
446 studies have linked polyploidization to trait evolution and diversification (Schranz *et*
447 *al.*, 2011; Tank *et al.*, 2015). In ferns, polyploids may facilitate long-distance
448 dispersal via tolerance of inbreeding, i.e. gametophytic selfing, increasing the success
449 rate of single-spore colonization (e.g. Tryon, 1985; Testo *et al.*, 2015; Sessa *et al.*,
450 2016). This in turn might favor colonization of different ecological niches, and thus
451 increase the probability for peripatric speciation. In *Deparia*, the species-rich genus in
452 *Athyriaceae* that is sister to *Athyrium-Diplazium*, polyploid species are characterized
453 by increased dispersal abilities and greater range expansion than sexual diploids (Kuo
454 *et al.*, 2016). This seems to be true as well for *Athyrium* and *Diplazium*, in which
455 polyploid species are often found more broadly distributed than their diploid relatives
456 (Tryon & Tryon, 1982; Takamiya *et al.*, 1999, 2000; Takamiya & Ohta, 2001; Bir &
457 Verma, 2010). It thus seems necessary for future studies to target other traits
458 potentially linked polyploidy that could enhance dispersal ability.

459

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470

471 **Author contributions**

472 X.-C.Z. and R.W. conceived the idea of this study; R.W. performed the experiments;
473 M.A.S. provided important Neotropical materials; R.W. and R.H.R. analyzed data;
474 and R.W., R.H.R., and M.A.S. wrote the paper, with significant contribution from X.-
475 C.Z.

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727

728 **Supporting Information**

729 Additional Supporting Information may be found online in the Supporting
730 Information tab for this article:

731

732 **Fig. S1** Chronogram of the *Athyrium-Diplazium* clade with four calibration points.

733 **Fig. S2** Positions of regime shifts in the maximum a posteriori configuration inferred
734 from the Bayesian analysis of macro-evolutionary mixtures (BAMM).

735 **Fig. S3** Rate simulations and their estimates of confidence intervals for each of the
736 states under 100 replicates of simulated trees and random assignment of 'NA' to tip
737 states in ClaSSE.

738 **Fig. S4** CHROMEVOL inferences for the *Athyrium-Diplazium* clade.

739 **Fig. S5** Rate-through-time plot of rates of speciation, extinction and species turnover
740 (termed ' μ/λ ') obtained from BAMM.

741 **Table S1** Species sampling fraction used in macroevolution analyses.

742 **Table S2** Taxa, voucher information and GenBank accession numbers of specimens
743 used in this study.

744 **Table S3** Traits information and coding in QuaSSE, CHROMEVOL and ClaSSE.

745 **Table S4** Model selection and results of QuaSSE on the entire ingroup clade as well
746 as separately on *Athyrium* and *Diplazium*.

747 **Table S5** Model selection and results of ClaSSE based on the entire ingroup clade as
748 well as separately on *Athyrium* and *Diplazium*.

749 **Table S6** Bayes factor of different diversification rate models fitted on the *Athyrium-*
750 *Diplazium* dataset.

751 **Table S7** ClaSSE model simulation based on 100 simulated trees with random
752 assignment of missing information to tips.

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773 **Table 1** Parameter comparison of the best-fit model of quantitative state speciation and extinction (QuaSSE) analysis based on the entire ingroup
 774 clade as well as separately on *Athyrium* and *Diplazium*. λ_0 , rate at lowest values of the log elevation for sigmoid and unimodal models; λ_1 ,
 775 maximum speciation rate for the unimodal models; x_{mid} , inflection point of the sigmoid or the place of the maximum for modal models; s^2 , width
 776 (variance) of the Gaussian function; μ , rate of extinction; σ^2 , Brownian diffusion rate of trait evolution.

Rates and Parameters	<i>Athyrium</i> clade	<i>Diplazium</i> clade	The entire ingroup clade
	Unimodal with directional tendency	Unimodal with no tendency	Unimodal with directional tendency
λ_0	1.00e-04	0.0322	0.0303
λ_1	0.8484	0.4197	0.5643
μ	0.5375	0.1617	0.3315
x_{mid}	7.83	6.965	7.712
s^2	0.1954	0.1066	0.4128
σ^2	0.0482	0.0524	0.0481
Directional	-0.091	-0.0013	-0.086

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786 **Table 2** Parameter comparisons of the best-fit model in cladogenetic state change speciation and extinction (ClaSSE) analysis based on the
 787 entire ingroup clade as well as separately on *Athyrium* and *Diplazium*. Both containing diploidy and polyploidy is coded as 1; diploidy is coded
 788 as 2; polyploidy is coded as 3. λ , speciation rate; μ , rate of extinction; q , transition rate between two states. All rates are presented with median
 789 values and 95% highest posterior density (HPD) intervals in brackets based on 100 000 MCMC optimization.

	<i>Athyrium</i> clade	<i>Diplazium</i> clade	The entire ingroup clade
Rates	Best-fit model: Cladogenetic speciation and extinction ($\lambda_{111} \neq \lambda_{222} \neq \lambda_{333} \neq \lambda_{112} \neq \lambda_{113} \neq \lambda_{123} \neq \lambda_{223}$, $\mu_1 \neq \mu_2 \neq \mu_3$, $q_{12} = q_{21} = q_{13} = q_{23}$)		
λ_{111}	0.5221 [0.2089–1.3218]	0.2128 [0.0951–0.4404]	0.334 [0.1902–0.6158]
λ_{222}	0.1758 [0.0822–0.3604]	0.0898 [0.0467–0.1573]	0.1326 [0.0741–0.2332]
λ_{333}	0.9462 [0.1513–2.727]	0.7056 [0.2735–1.279]	0.7054 [0.2575–1.299]
λ_{112}	0.1758 [0.01–0.6164]	0.0864 [0.0045–0.3042]	0.0836 [0.0061–0.229]
λ_{113}	1.6779 [0.261–4.305]	0.9392 [0.3241–2.023]	0.8974 [0.4328–1.7195]
λ_{123}	0.173 [0.008–0.6014]	0.063 [0.0032–0.2097]	0.073 [0.0038–0.2145]
λ_{223}	0.3929 [0.032–1.485]	0.0707 [0.0028–0.4607]	0.0667 [0.0029–0.3541]
μ_1	0.3658 [0.023–1.263]	0.0672 [0.0027–0.3211]	0.1658 [0.0107–0.49]
μ_2	0.2075 [0.022–0.4686]	0.0258 [0.001–0.1228]	0.0809 [0.0054–0.2366]
μ_3	1.427 [0.5903–3.247]	0.7513 [0.3637–1.336]	0.829 [0.4372–1.432]
q	0.0421 [0.004–0.1926]	0.0276 [0.0076–0.0704]	0.0255 [0.0081–0.0614]

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796 **Figure Legends:**

797 **Fig. 1** The cladogenetic-state change speciation and extinction (ClaSSE) models with
798 cladogenetic and anagenetic changes. (a) The full model including cladogenetic
799 diversification and anagenetic state change is depicted. In the diagram, lineages
800 presented by both diploid and polyploid populations have state 1, and those with only
801 diploids have state 2, and those with only polyploids have state 3. Each speciation
802 event gives rise to two daughters (as shown by arrows), either in the same state (at
803 rates λ_{111} , λ_{222} , and λ_{333}) or in different states (λ_{112} , λ_{113} , λ_{123} , and λ_{223}). Thus, changes
804 in ploidy can occur through the anagenetic (q_{12} , q_{13} , q_{21} , and q_{23}) or cladogenetic
805 pathway (λ_{112} , λ_{113} , λ_{123} , and λ_{223}). Extinction rates in each state are represented by μ_1 ,
806 μ_2 and μ_3 . The rates in gray indicate equal rates, respectively. (b) The null model with
807 equal speciation, extinction and transition rates, respectively. (c) The ‘cladogenetic’
808 model. (d) The ‘anagenetic’ model. (e) The ‘Extinction’ model. (f) The ‘cladogenetic-
809 anagenetic’ model.

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811 **Fig. 2** Results of the Bayesian analysis of macro-evolutionary mixtures (BAMM) and
812 quantitative-state speciation and extinction (QuaSSE) analyses. (a) Chronorate plot
813 with branches colored by speciation rate (events Myr^{-1} per lineage) as indicated by
814 the scale bar, representing a summary of the full post-burn-in Markov chain Monte
815 Carlo (MCMC) sample of the BAMM analysis. Red circles indicate the positions of
816 regime shifts in the maximum *a posteriori* (MAP) configuration. Numbers beneath
817 the shifts indicate the marginal probability of a shift occurring along that branch. Log
818 elevation is shown by the horizontal bar for each species. The vertical dashed lines
819 indicate the approximate ranges of altitudes in which elevated speciation rates were
820 inferred by QuaSSE analysis, and extant species whose elevation falls in this range
821 have their data colored red. (b) Rate-through-time plots for speciation rate (events
822 Myr^{-1} per lineage) with 95% confidence interval indicated by yellow shaded areas.
823 Red, the rate across the phylogeny (‘background rate’); blue, the rate of *Athyrium*
824 clade; green, the rate of *Diplazium* clade.

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826 **Fig. 3** Relationship between the elevation and speciation rates as inferred from
827 QuaSSE analysis, with comparison to the elevational species richness histograms of
828 the *Athyrium* clade, the *Diplazium* clade and the whole phylogeny, respectively. Red,

829 the rate across the phylogeny; blue, the rate of *Athyrium* clade; green, the rate of
830 *Diplazium* clade.

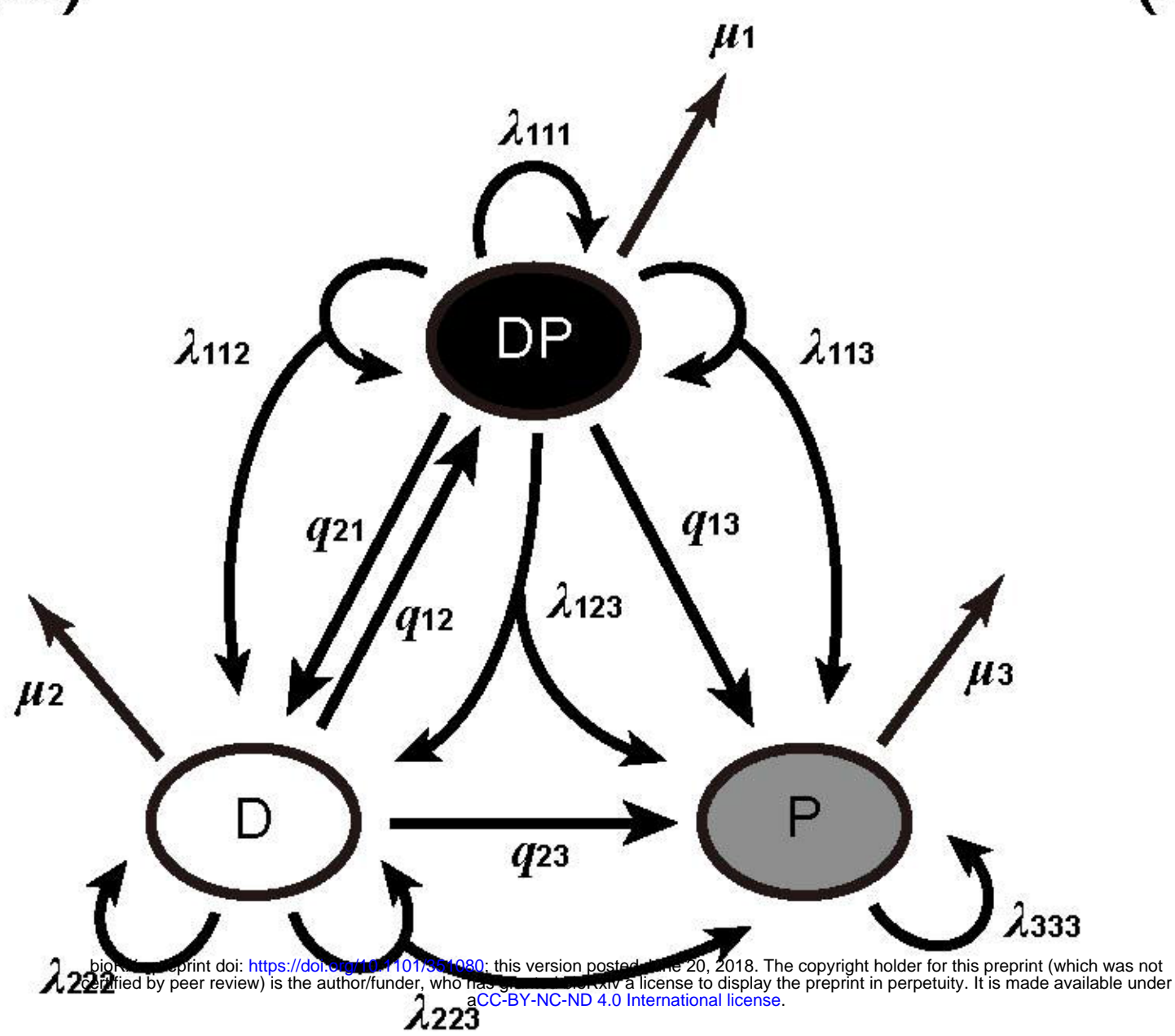
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832 **Fig. 4** Posterior distributions of macroevolutionary rates under the best-fitting model
833 of the ClaSSE analysis based on the MCC tree, allowing cladogenetic speciation and
834 extinction of diploids and polyploids. (a) The cladogenetic speciation rates of diploid-
835 polyploid mediated speciation events. (b) The extinction rates of three ploidy states. (c)
836 The transition rate of all anagenetic change (equal). (d) Summary of speciation rates
837 of each state. (e) The net diversification rates of ploidy in cladogenetic speciation. (f)
838 The net accumulation rates of ploidy in cladogenetic speciation.

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(a)

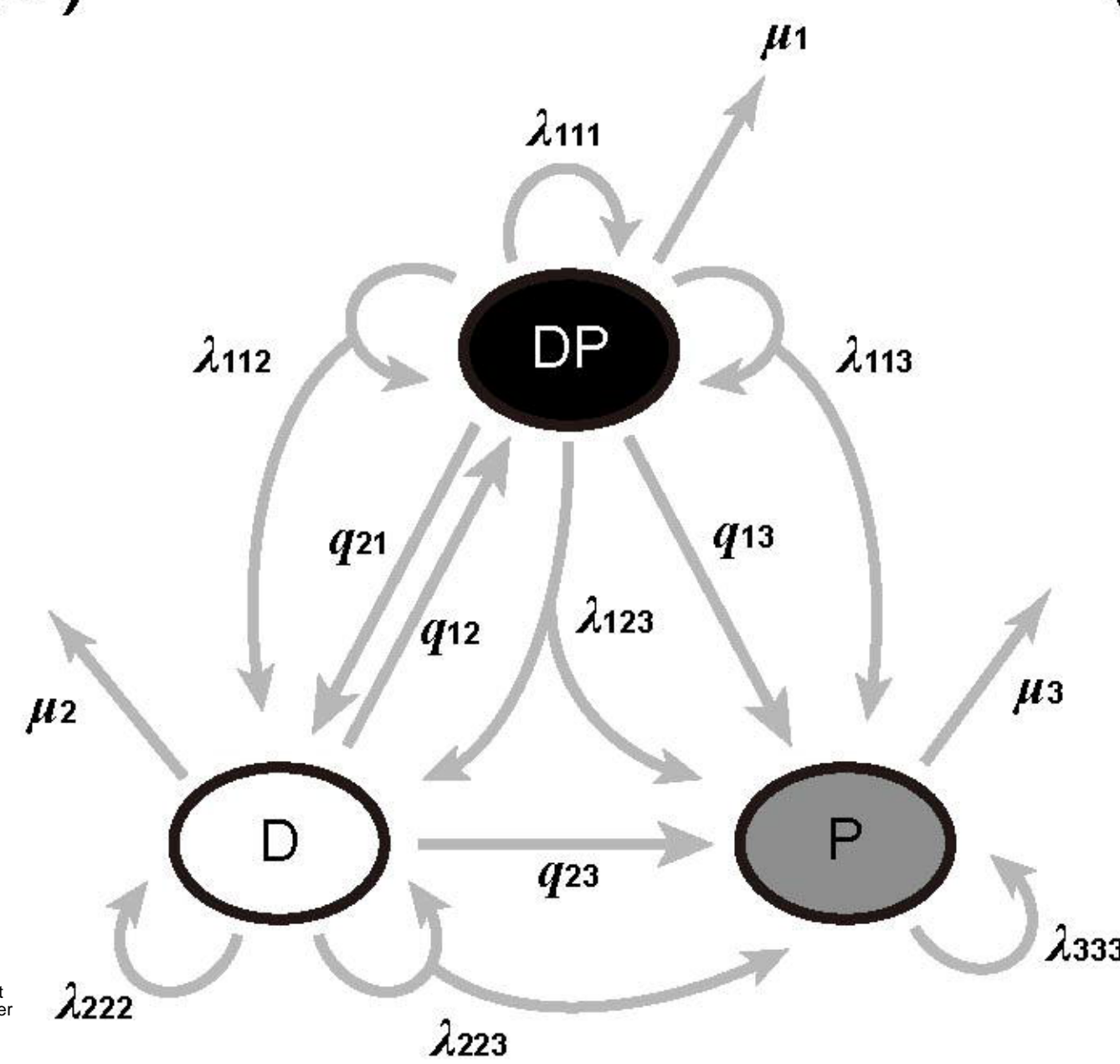
Full model



No. of parameters: 14

(b)

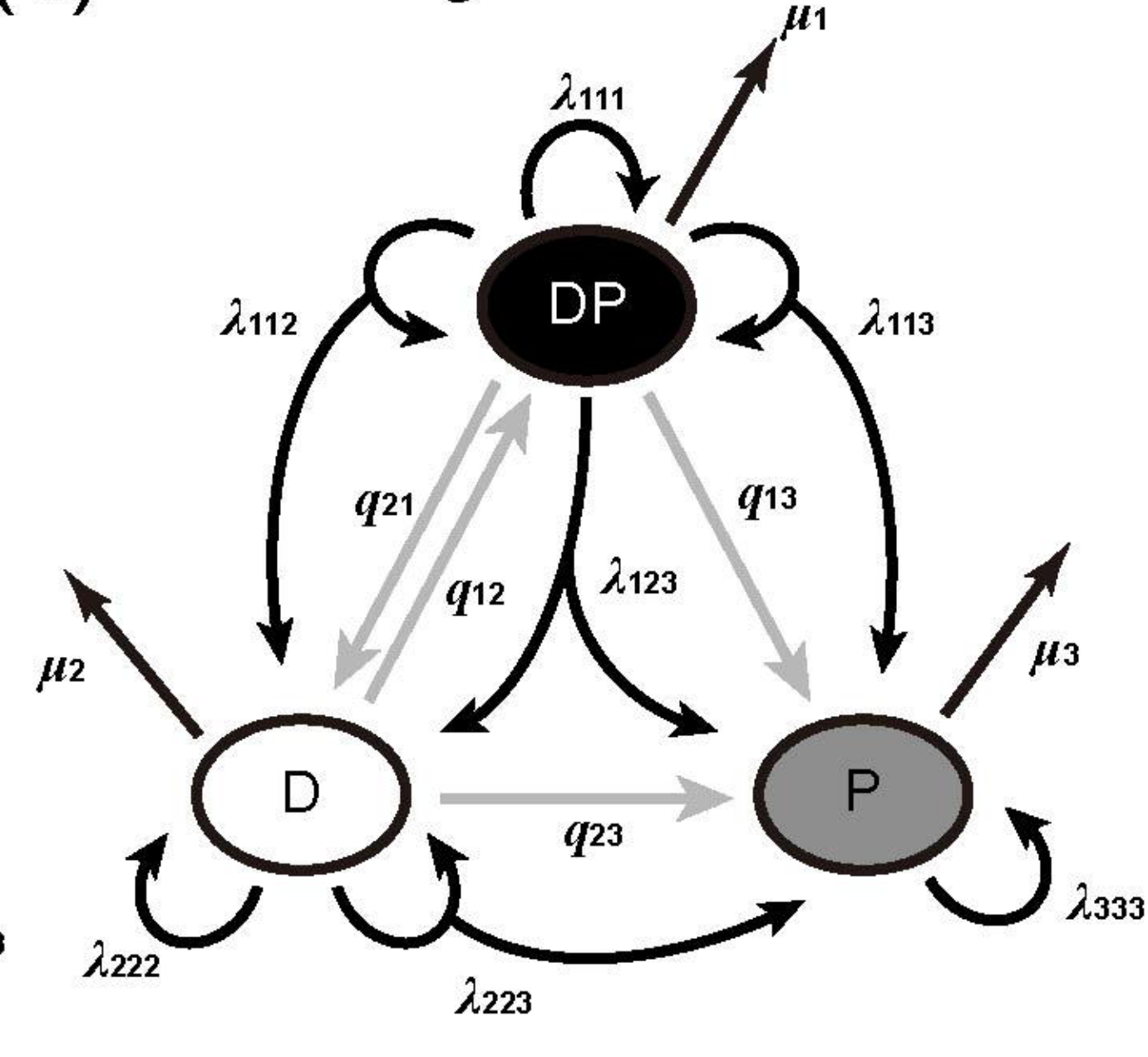
Null model



No. of parameters: 3

(c)

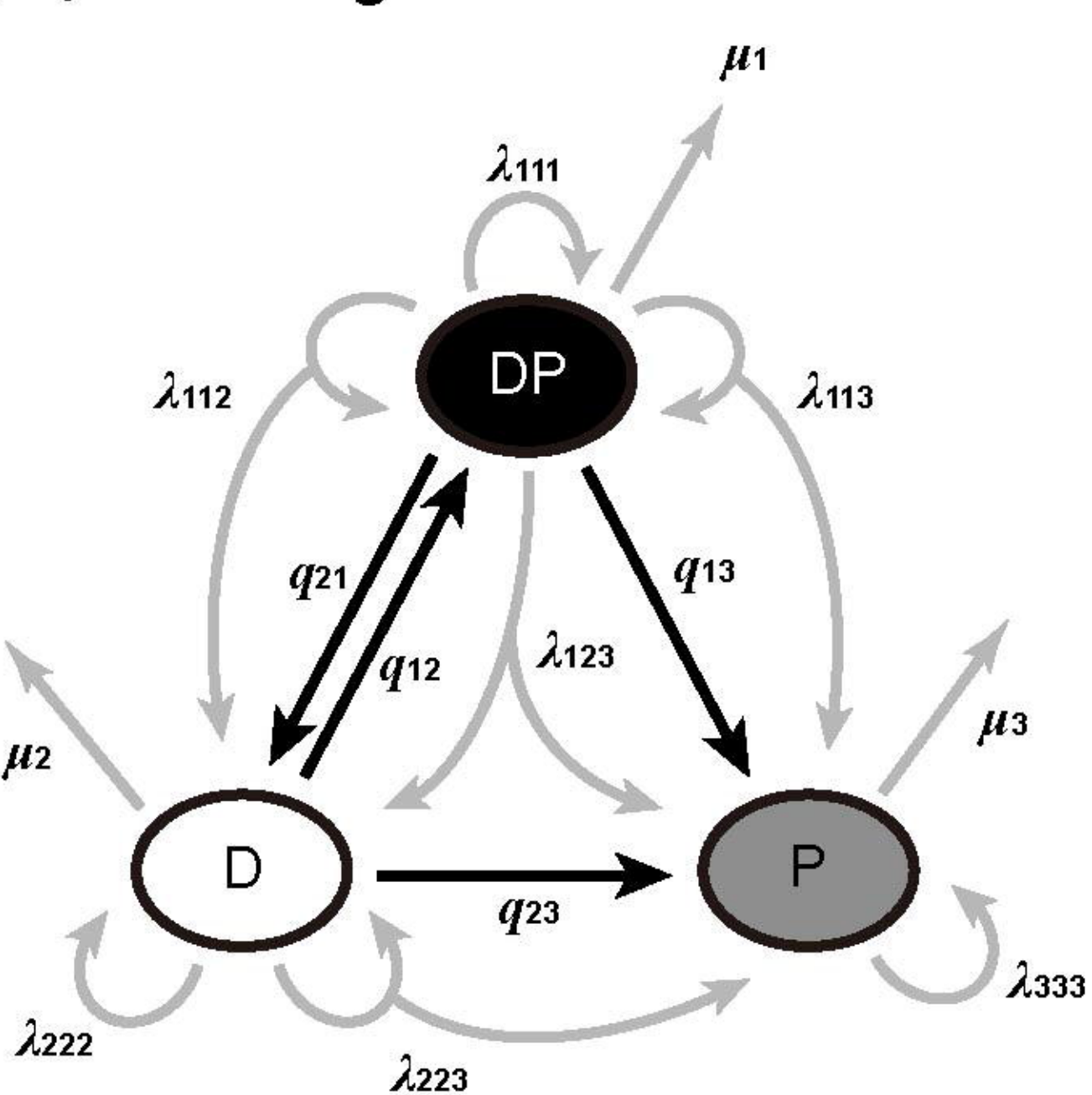
'Cladogenetic' model



No. of parameters: 11

(d)

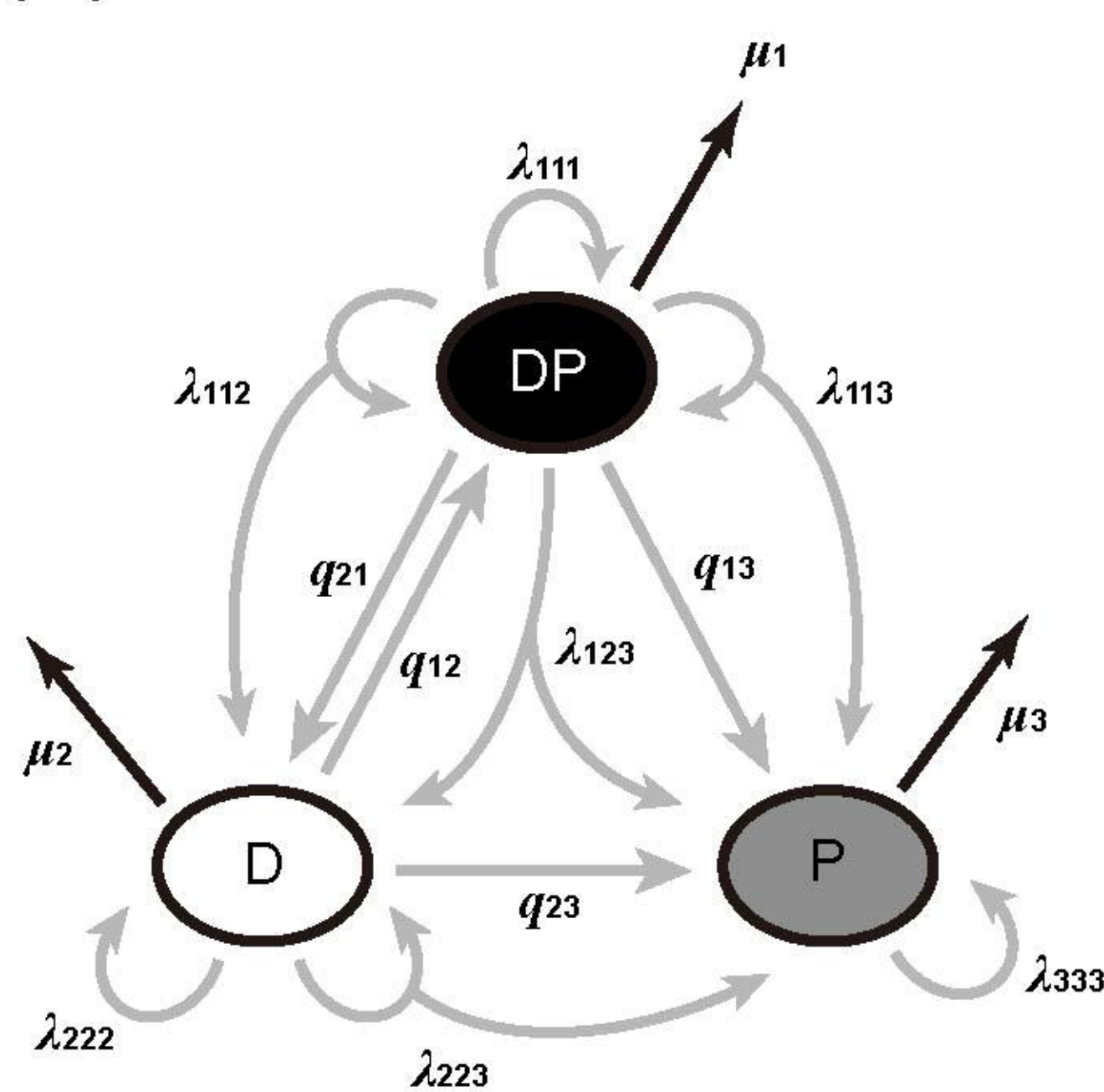
'Anagenetic' model



No. of parameters: 6

(e)

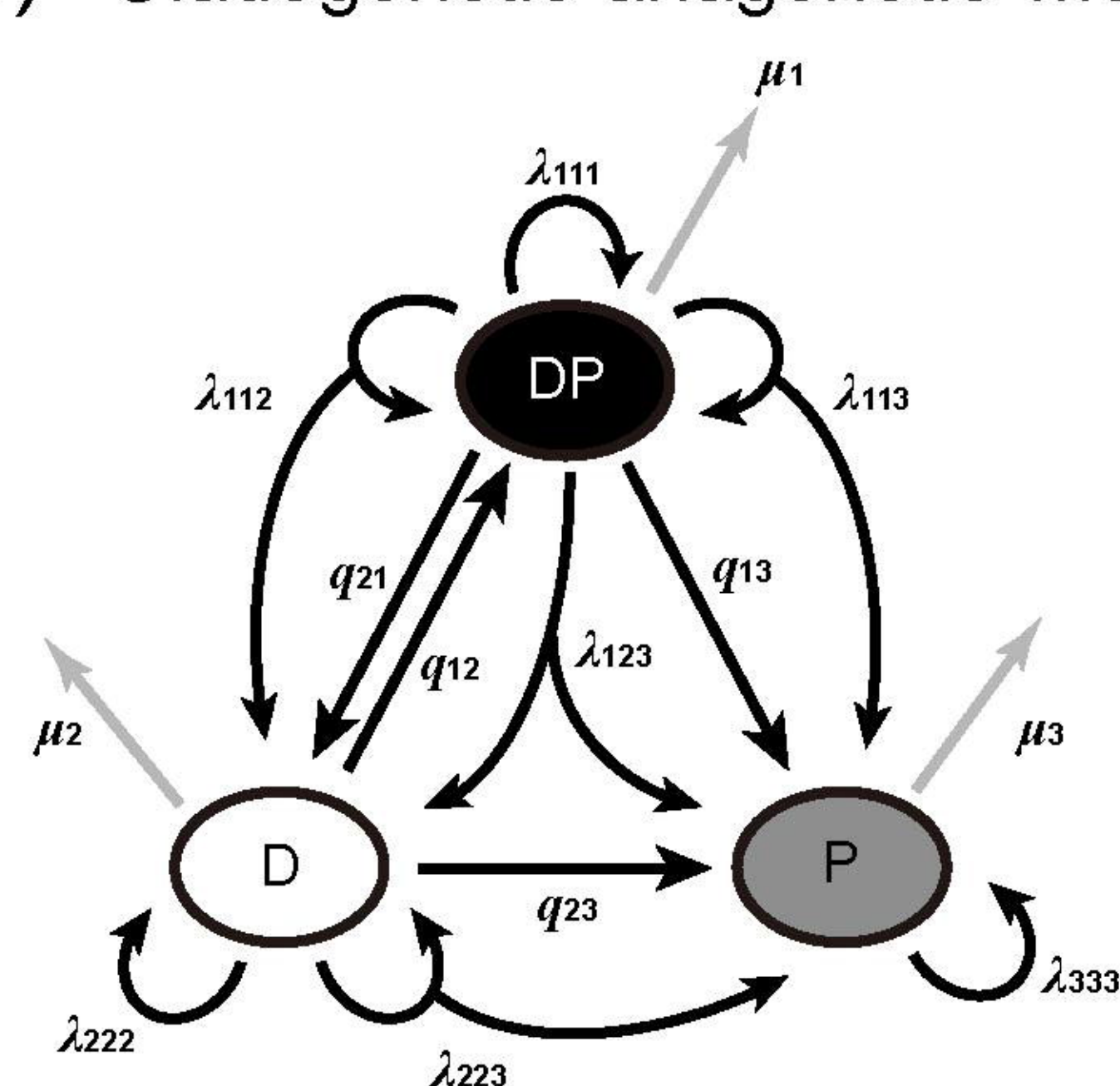
'Extinction' model



No. of parameters: 5

(f)

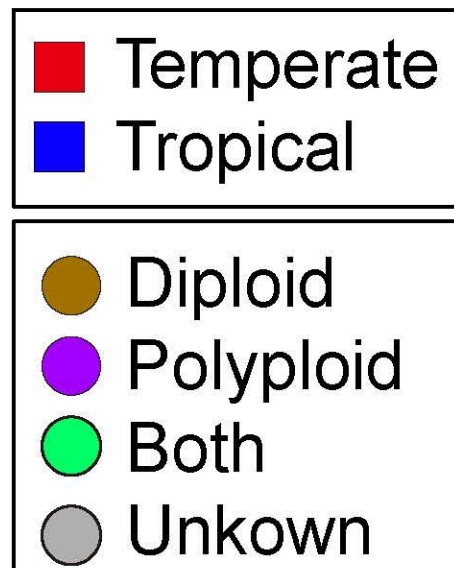
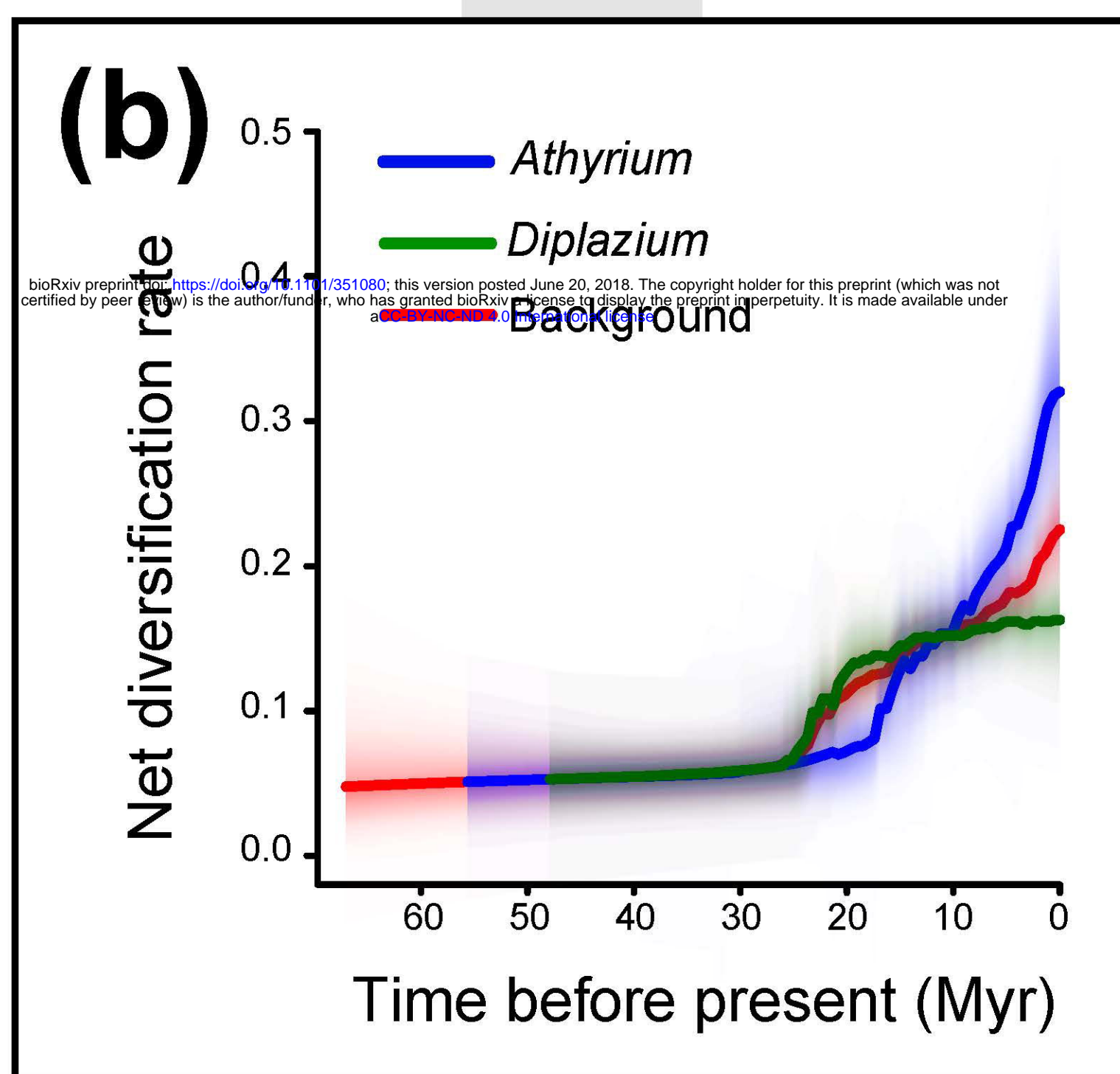
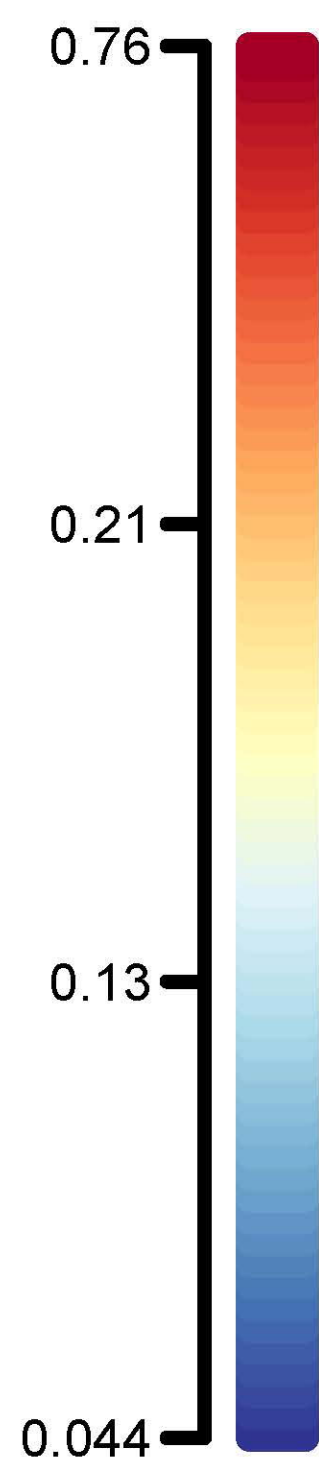
'Cladogenetic-anagenetic' model



No. of parameters: 12

(a)

C	Paleocene	Eocene	Oligocene	Miocene	P	Q
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70 60 50 40 30 20 10 0 5 6 7 8 9

Time before present (Myr) \log_e (Elevation)

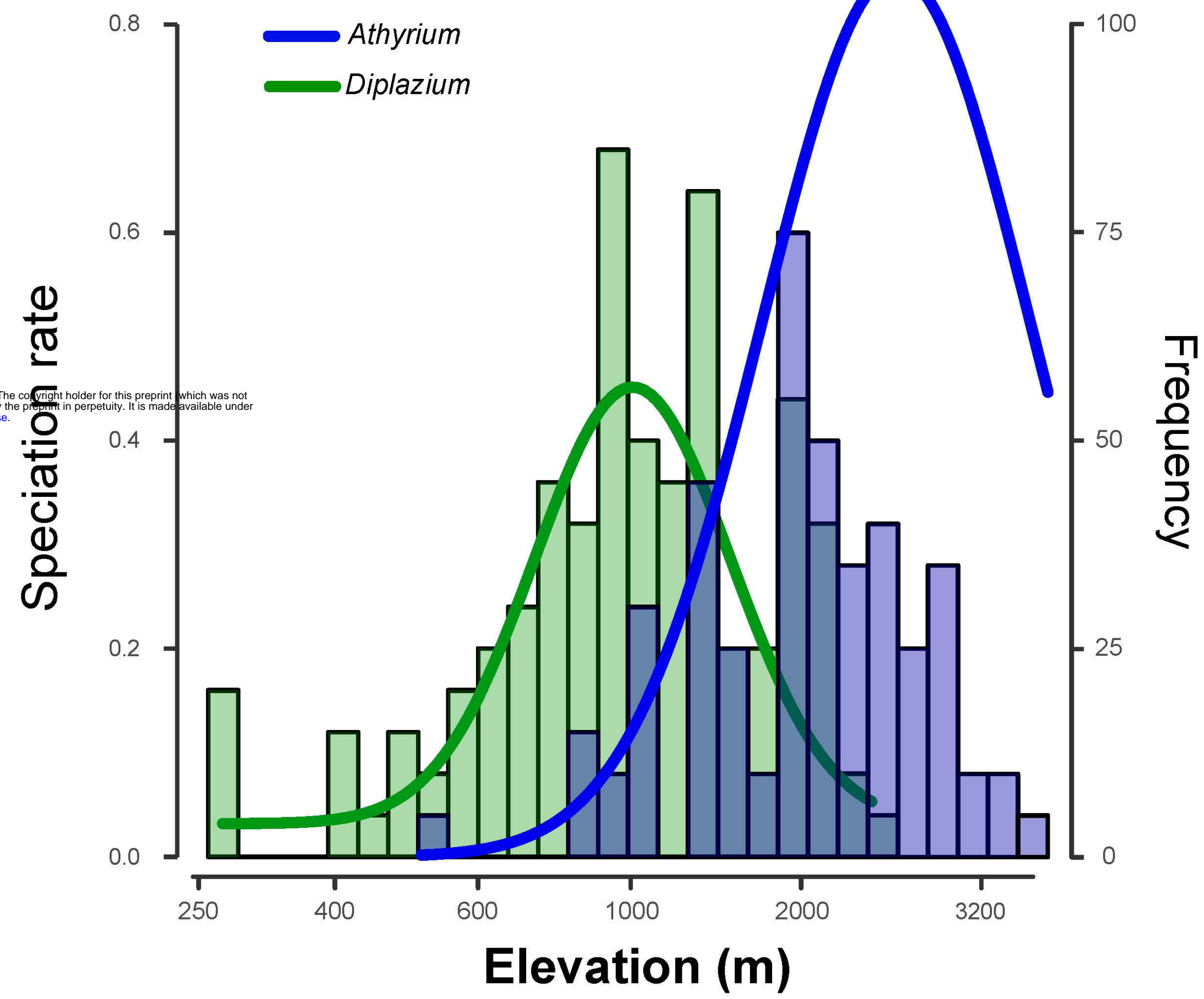
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0.486

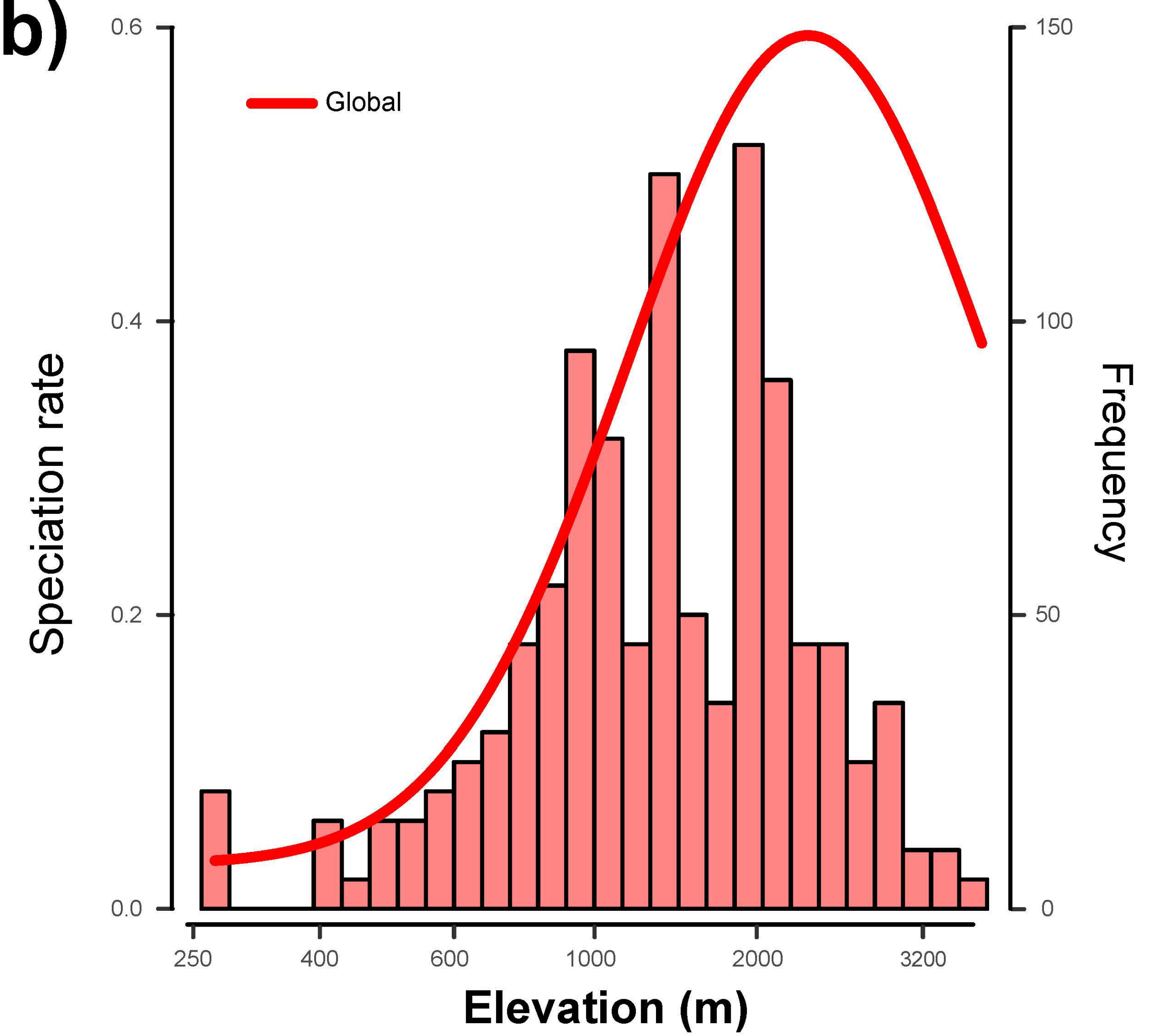
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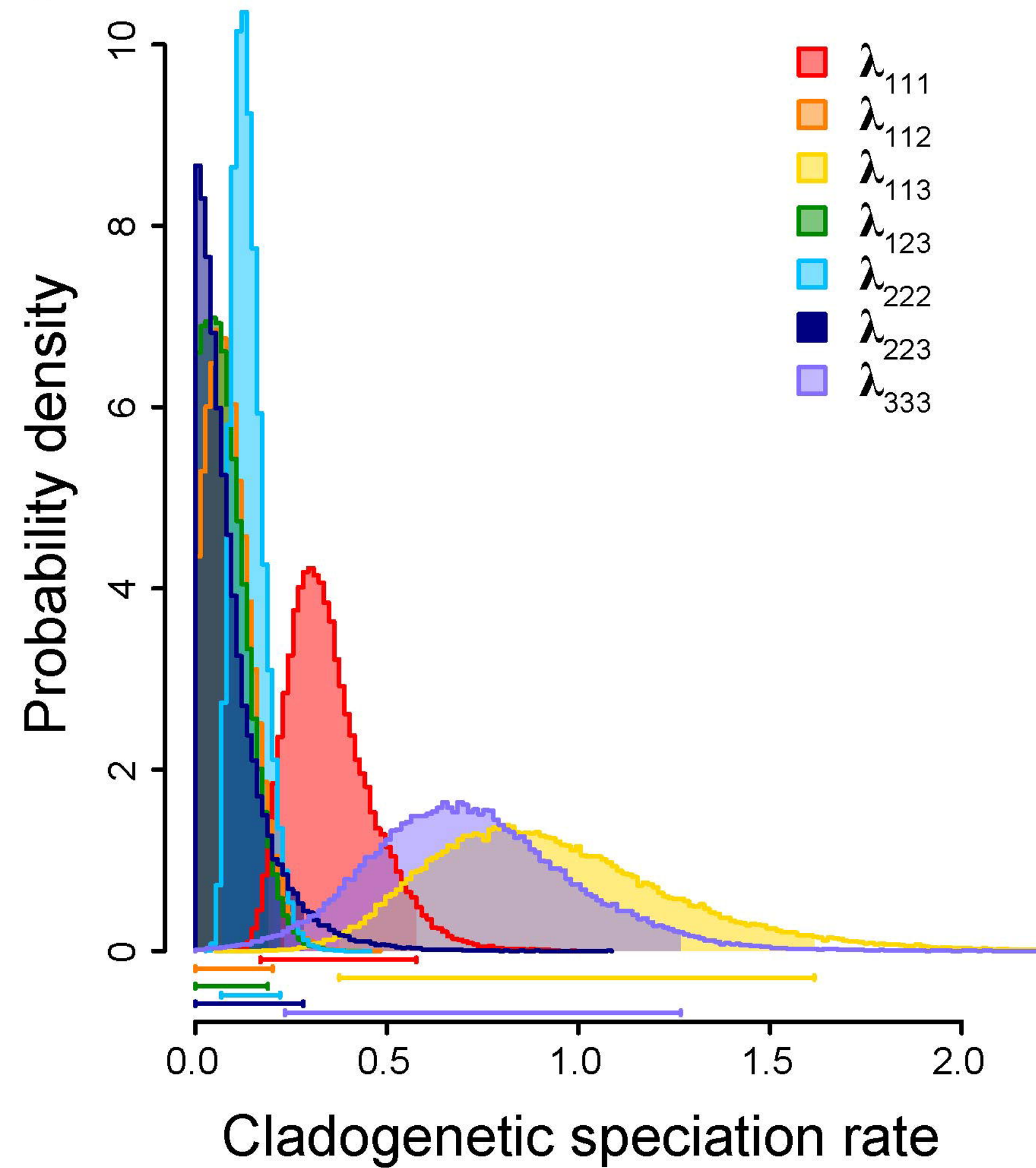
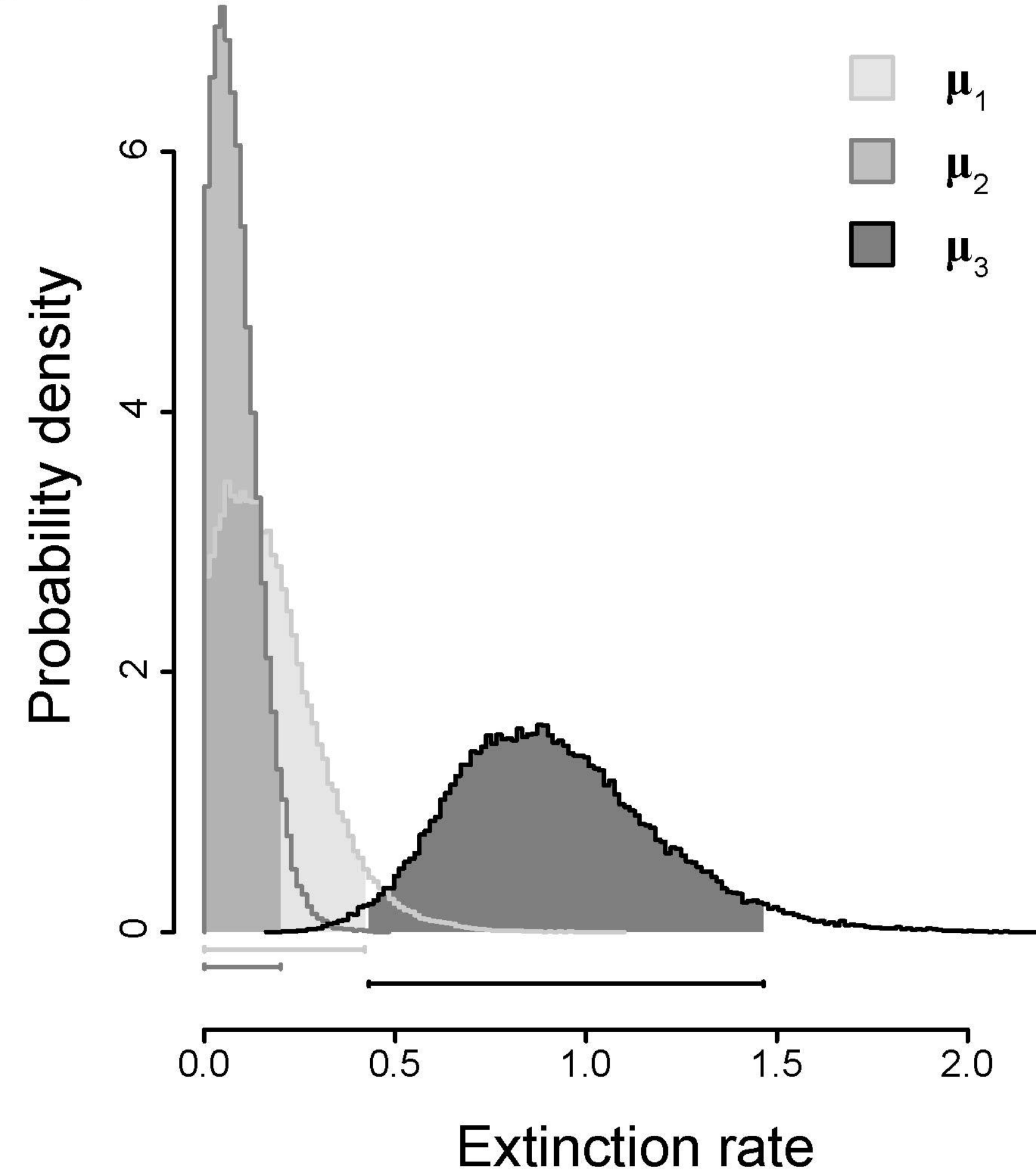
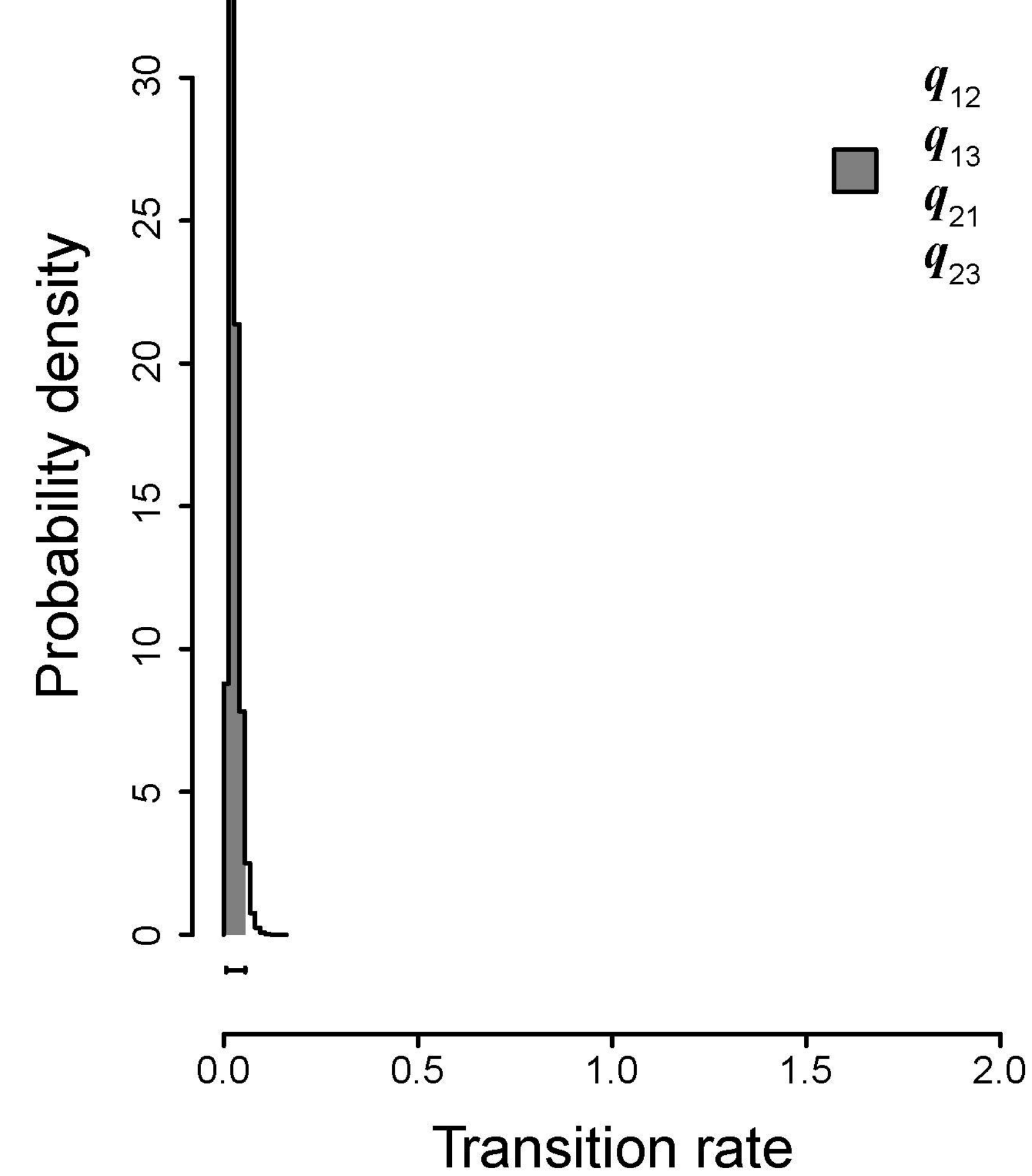
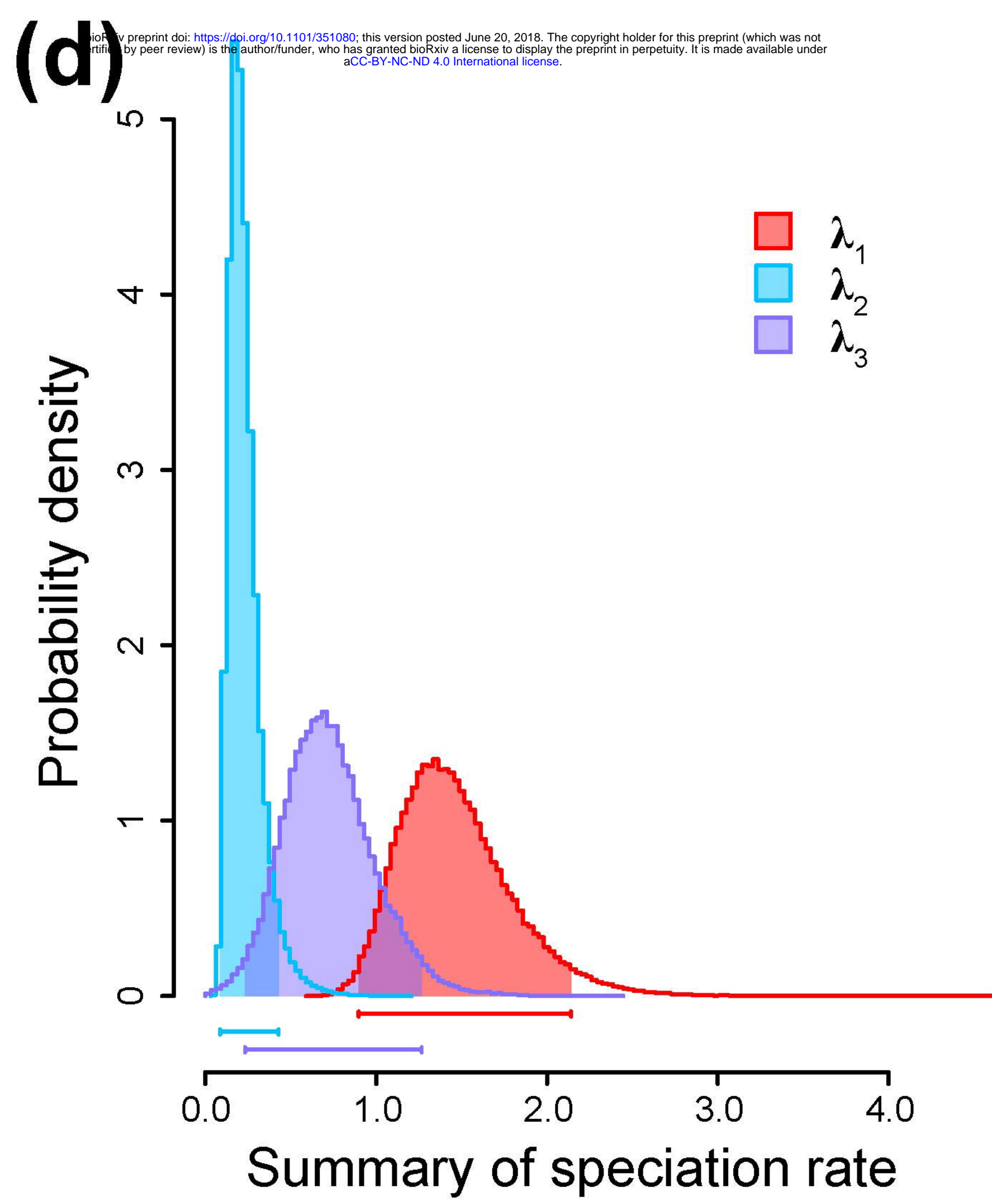
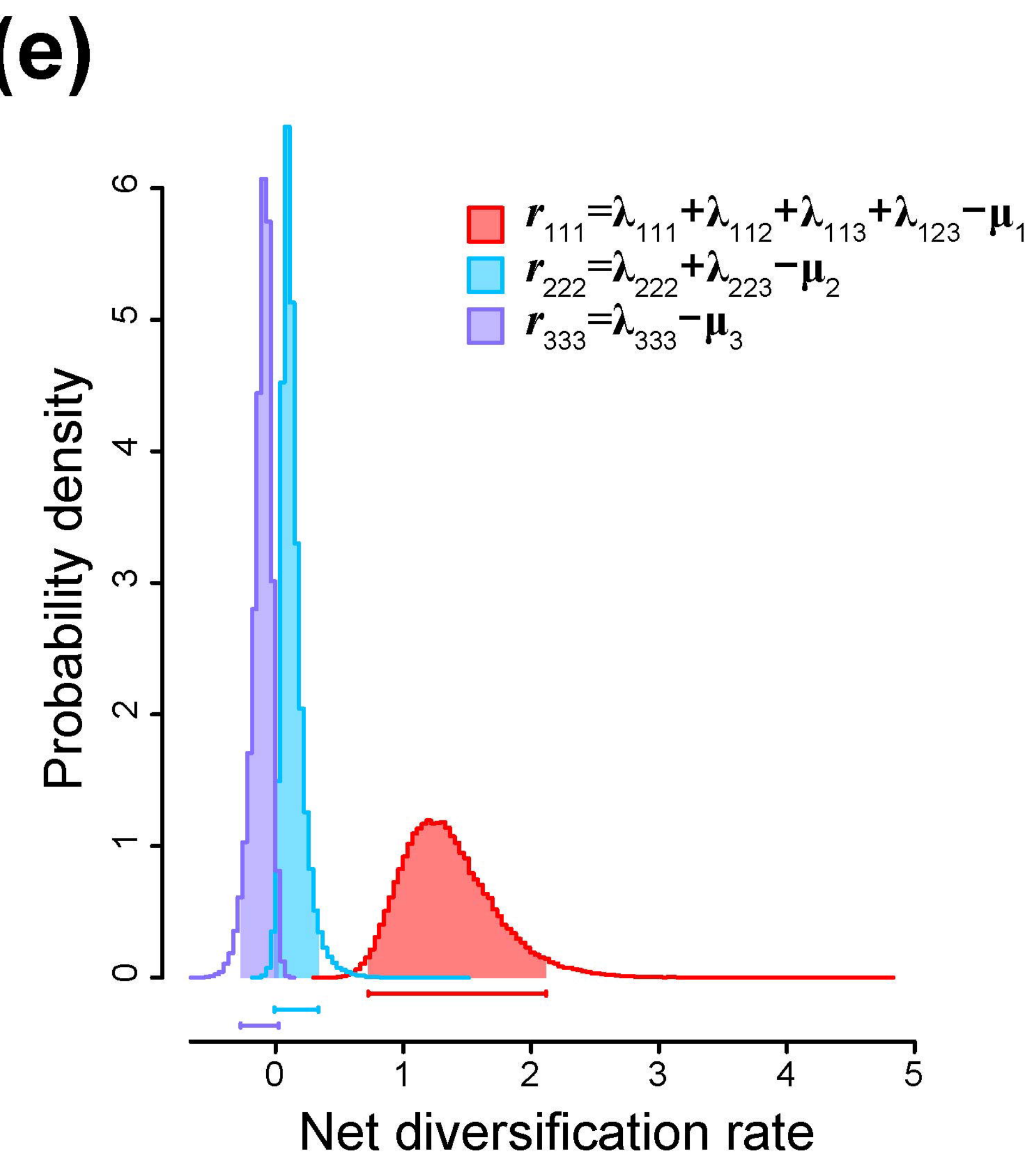
Mackinnonia
cladeCore *Athyrium**Athyrium*Andean *Diplazium*
cladeSubgenus *Callipteris**Diplazium*

(a)



(b)



(a)**(b)****(c)****(d)****(e)****(f)**