Title: Determinants of genetic diversity and species richness of North American amphibians

Running title:

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Abstract: We know little about the general links between broad-scale biodiversity patterns at the nuclear genetic and species levels. Recent work in mammals suggests environmental carrying capacity and ecological opportunity link these two base levels of biodiversity. Energy- and resource-rich environments are thought to support larger populations with higher genetic diversity and species richness. Niche availability is expected to limit population size causing drift while increasing genetic differentiation due to environmental specialization. Several of the processes underlying these links are temperature-dependent, so we might expect different patterns for endotherms and ectotherms. We use a database comprised of raw microsatellite genotypes for 13616 individuals of 18 species sampled at 548 locations in the United States and Canada. We analyzed salamander and frog species separately and simultaneously fit our hypotheses with structural equation models. Similar to mammals, niche availability was the primary contributor to diversity at both the genetic and species levels in frogs, and energy availability was an important predictor of species richness for both taxa. Different than mammals, environmental energy availability was not linked to genetic diversity. There are shared underlying mechanisms linking genetic and species-level diversity but the processes are not entirely general across these species groups.

Keywords: latitudinal diversity gradient, biogeography, heterozygosity, frogs, salamanders, more individuals hypothesis

46   **Introduction**

47   Although species richness is higher in the tropics for most taxa, diversity patterns differ between

48   species groups. In North America for instance, vertebrate richness generally increases with

49   energy availability, but mammals and birds tend to have higher species richness in dry,

50   mountainous areas, and reptiles and amphibians are more diverse in wet, low-elevation regions

51   (Currie 1991). This finding suggests that while richness increases with greater energy

52   availability, taxon-specific traits may cause richness patterns to diverge from a strictly latitudinal

53   gradient. Because several hypotheses link species richness gradients to temperature-dependent

54   processes (Currie et al. 2004), we should expect different patterns for endotherms and

55   ectotherms. Similar broad-scale patterns for genetic diversity have only recently been identified

56   due to the accumulation of open data in public repositories (mtDNA: Miraldo et al. 2016; Manel

57   et al. 2020; Theodoridis et al. 2020; nDNA: Schmidt et al. 2020). Genetic diversity is typically

58   thought of as the most fundamental level of biodiversity because it influences the potential for

59   adaptive evolution in response to environmental change (Frankham 1995). Recent analyses of

60   mammals suggest that environments simultaneously shape species richness and genetic diversity

61   on continental scales, suggesting that genetic diversity and species richness together form the

62   base level of the biodiversity hierarchy (Schmidt et al. 2020). Whether this is also true in

63   ectothermic taxa is unknown. Understanding common processes underlying variation in

64   biogeographic patterns across taxa with different environmental requirements can help us move

65   toward a general understanding of the drivers of biodiversity.

66   Recent empirical tests incorporating estimates of genome-wide diversity from mammals

67   (Schmidt et al. 2020) demonstrate the importance of carrying capacity and ecological

68   opportunity in shaping broad-scale patterns of genetic diversity and species richness. Energy-

69   and resource-rich environments supported larger populations with higher genetic diversity and

70   species richness, while niche availability in heterogeneous habitats reduced population sizes,

71   increased genetic differentiation, and promoted species coexistence because specialization

72   reduces available resources. These processes are related to two prominent hypotheses for the

73   latitudinal species richness gradient: the more-individuals hypothesis (Wright 1983) and the

74   heterogeneity hypothesis (Allouche et al. 2012). The more individuals hypothesis posits that

75   resource-rich regions near the equator are capable of supporting larger populations and

76   communities, and thus more species than more temperate regions. The heterogeneity hypothesis

77   suggests that greater niche availability in more complex heterogeneous environments allow more

78   species to coexist, but with smaller population sizes because resources are partitioned.

79   The effects of environmental heterogeneity are generally applicable across taxa (Stein et al.

80   2014); however, the relevance of the more-individuals hypothesis for ectotherms is disputed

81   (Buckley and Jetz 2010). Compared to endotherms, ectotherms have lower energy requirements

82   and can behaviorally thermoregulate—meaning their abundances are less likely to be limited by

83   energy-related carrying capacities (Buckley and Jetz 2010). Instead, ectotherm distributions, and

84   therefore species richness, appear to be more directly constrained by environmental temperature

85   because fewer species have evolved thermal adaptations required for expanding into cooler

86   regions (Buckley and Jetz 2010). Further, the evolution of traits associated with better survival in

87   temperate regions may have additional effects on speciation dynamics. For example, species

88   turnover tends to be higher among viviparous squamate reptiles (Pyron and Burbrink 2014).

89   Species richness gradients in ectotherms may thus be less strongly governed by energy limits on

90   population size than mammals, whose temperature independence allows them to colonize a

91   wider range of habitats. If true, then we might expect the effects of habitat heterogeneity to be

92   more pronounced in ectotherms. In mammals, the effects of energy availability on species

93   richness appear to dominate in low-energy regions, but once a minimum energy threshold is

94   reached (1000 mm/yr potential evapotranspiration), habitat heterogeneity becomes the main

95   cause of richness (Kerr and Packer 1997). The effects of heterogeneity may thus be more

96   pronounced in ectotherms because fewer species inhabit low-energy regions.

97   The major determinants of richness across all terrestrial vertebrates are generally shown to be

98   related to energy (potential evapotranspiration, primary productivity), water-energy balance

99   (actual evapotranspiration, precipitation), and environmental heterogeneity (elevation variability,

100   land cover) (Currie 1991; Kerr and Packer 1997; Hawkins et al. 2003; Rodríguez et al. 2005;

101   Buckley and Jetz 2007; Stein et al. 2014; Jiménez-Alfaro et al. 2016). Amphibians are an

102   interesting case because they are doubly constrained by water availability and temperature.

103   Water availability is consistently identified as an important driver of diversity in amphibians

104   (Rodríguez et al. 2005; Buckley and Jetz 2007). Indeed in Europe, the best predictors of species

105   richness in mammals and birds shifted from energy to water availability at decreasing latitudes,

106   but amphibian richness was strongly related to water-energy balance regardless of latitude

107   (Whittaker et al. 2007).

108   The causes of population genetic diversity are rarely studied at the same time or scale as patterns

109   of species richness (but see Marshall and Camp 2006; Schmidt et al. 2020), yet the presumed

110   mechanisms related to more-individuals and environmental heterogeneity hypotheses are

111   intricately related to carrying capacity and population-level processes. The more-individuals

112   mechanism predicts a positive relationship between species richness and population genetic

113   diversity because bigger populations and communities tend to have higher levels of genetic and

114   species diversity (Kimura 1983; Hubbell 2001). With higher population-level carrying capacities,

115   more species persist because they can reach minimal viable population sizes. On the other hand,

116   heterogeneity causes negative correlations between genetic diversity and species richness by

117   increasing the number of species a given area can support which in turn reduces population size

118   and limits gene flow due to increased niche specialization. Heterogeneous environments also

119   facilitate population divergence due to spatially varying selection. In mammals, evolutionary

120   processes acting on the population level scaled up and interacted with environmental factors to

121   produce previously identified species richness patterns (Schmidt et al. 2020).

122   Whether carrying capacity mechanisms related to energy and niche availability predict patterns

123   of species richness in ectotherms is unclear. To test this idea, we repurposed raw microsatellite

124   data from 18 North American amphibian species (8 frogs, 10 salamanders), with >13000

125   individuals sampled at 548 sites. Our first objective was to identify spatial patterns in genetic

126   diversity and quantify the extent to which genetic diversity and species richness covary spatially.

127   We then tested whether limits on energy and niche availability jointly determined genetic

128   diversity and species richness using structural equation models, which allowed us to evaluate and

129   assess the relative importance of both hypotheses at both levels of biodiversity simultaneously.

130   Finally, we compare our results to previous results in mammals (Schmidt et al. 2020) to infer

131   whether similar environmental features contribute to diversity gradients across endothermic and

132   ectothermic taxa in North America.

133

134   **Methods**

135   **Data**

136   *Genetic diversity.* We used a database of genetic metrics in North American amphibians

137   compiled by Schmidt and Garroway (2020). This database was assembled by calculating metrics

138   of genetic diversity and differentiation from raw microsatellite datasets publicly archived in the

139   Dryad repository. To build the database we conducted a systematic search of the Dryad data

140   repository with the following keywords: species name (e.g., *Plethodon cinereus*), "microsat*",

141   "short tandem*", and "single tandem*". We used the IUCN Red List database to obtain a list of

142   amphibian species native to North America for the search. We excluded datasets that lacked

143   spatial reference, were not located in North America, did not sample neutral microsatellite loci,

144   or had study designs that may have affected genetic diversity (including sampling island

145   populations, or captive or managed populations). The database includes data from 13616

146   individuals of 18 species sampled at 548 locations in the contiguous United States and Canada.

147   Here, we used gene diversity (Nei 1973) as a measure of genetic diversity because it is

148   minimally affected by sample size (Charlesworth and Charlesworth 2010). Gene diversity is a

149   measure of heterozygosity which measures the evenness of alleles in a population (Nei 1973).

150   *Population size.* Because species censuses are not widely available, we used body size as a proxy

151   for species population size. Abundance typically scales negatively with body size (Peters and

152   Wassenberg 1983; Damuth 1987). Body size is also correlated with several life history traits

153   which partly determine the effective population size, and is generally negatively related to

154   neutral genetic variation across diverse taxa (Romiguier et al. 2014; Brüniche-Olsen et al. 2018;

155   Mackintosh et al. 2019). We used body length (mm) as our metric of body size, obtained from

156   the AmphiBIO v1 database (Oliveira et al. 2017).

157   *Species richness.* We estimated species richness using amphibian range extent data from the

158   IUCN RedList (IUCN 2019), applying filters for native, extant species ranges. For each species

159   included in our dataset, we counted the number of overlapping species ranges as a measure of

160   species richness at the species level. We took this approach because we were interested in

161   environmental factors operating at broad spatial scales, and to avoid over- or under-sampling

162   areas due to irregular site placement in the aggregated dataset. To account for biases due to range

163   size, species with larger ranges having more overlaps, we divided the number of overlapping

164   ranges by range area (km^2). To generate the maps in Figure 1, we used a site-level measure of

165   species richness calculated by summing the number of species ranges overlapping each genetic

166   sample site.

167   *Environmental variables.* Amphibians are habitat-limited by both temperature and water

168   availability. Water availability can be measured by evapotranspiration, or the amount of water

169   removed from the Earth's surface through soil or open water evaporation and plant transpiration

170   processes. Potential evapotranspiration (PET) measures the atmospheric demand for water,

171   depending on factors such as temperature and wind (Peng et al. 2019). It is strongly correlated

172   with temperature. PET is the maximum amount of water that would be removed in the absence

173   of biophysical limitations (Peng et al. 2019). The amount of water actually removed, actual

174   evapotranspiration (AET), reflects water availability and soil moisture levels. Actual

175   evapotranspiration has also been shown to be one of the strongest predictors of amphibian

176   species richness (Buckley and Jetz 2007). PET can be viewed as a measure of energy

177   availability, and AET one of water-energy balance (see Currie 1991; Buckley and Jetz 2007;

178   Kreft and Jetz 2007). We measured mean PET and AET (mm/yr) values across each species'

179   range using data from the CGIAR Consortium for Spatial Information (Trabucco and Zomer

180   2019).

181   Finally, we measured habitat heterogeneity by calculating the range (m) in elevation across each

182   species range using a topography map obtained from NOAA (National Oceanic and Atmospheric

183   Administration (NOAA) and U.S. National Geophysical Data Center). Larger ranges generally

184 have greater topographical variation, thus we corrected for species range area to avoid potential

185 biases.

186 **Analysis**

187 *Detecting shared spatial structure in genetic diversity and species richness.* We used distance-

188 based Moran's eigenvector maps (MEMs) to detect spatial patterns in genetic diversity and

189 compare these to patterns of species richness. MEMs are orthogonal spatial eigenvectors with

190 eigenvalues that are directly proportional to Moran's *I*. They measure spatial autocorrelation at

191 all scales present in the data. We computed dbMEMs in the R package adespatial (Dray et al.

192 2017). We used the forward selection procedure described in (Blanchet et al. 2008) to select two

193 sets of MEMs describing important patterns in genetic diversity and species richness. To create

194 maps of genetic diversity and species richness (Fig. 1), we used the predicted values for gene

195 diversity and species richness regressed on selected MEMs. Next, we determined the extent to

196 which spatial patterns in genetic diversity and species richness were shared using variation

197 partitioning. Because our dbMEM analysis for both levels of biodiversity had the same input

198 distance matrix, the resulting spatial MEMs were directly comparable. We determined the

199 fraction of total variation explained by spatial structure, shared spatial structure, and non-spatial

200 variation using variation partitioning as follows. We ran a series of linear regressions with either

201 species richness (y_{SR}) or gene diversity (y_{GD}) as the response variable using all MEMs selected

202 for that variable (Equations 1 and 2), or only MEMs shared by both variables as predictors

203 (Equations 3 and 4):

204 $$y_{SR} \sim \alpha + \beta_{1S}(\text{MEM}_{1S}) + \beta_{2S}(\text{MEM}_{2S}) + \cdots + \beta_{iS}(\text{MEM}_{iS}) + \epsilon \qquad \text{Eq. 1}$$

205 $$y_{GD} \sim \alpha + \beta_{1G}(\text{MEM}_{1G}) + \beta_{2G}(\text{MEM}_{2G}) + \cdots + \beta_{iG}(\text{MEM}_{iG}) + \epsilon \qquad \text{Eq. 2}$$

206 $$y_{SR} \sim \alpha + \beta_{1SG}(\text{MEM}_{1SG}) + \beta_{2SG}(\text{MEM}_{2SG}) + \cdots + \beta_{iSG}(\text{MEM}_{iSG}) + \epsilon \qquad \text{Eq. 3}$$

207 $$y_{GD} \sim \alpha + \beta_{1SG}(\text{MEM}_{1SG}) + \beta_{2SG}(\text{MEM}_{2SG}) + \cdots + \beta_{iSG}(\text{MEM}_{iSG}) + \epsilon \qquad \text{Eq. 4}$$

208

209 where α is the grand mean, and MEM_{iS} and MEM_{iG} are the set of MEMs selected for species

210 richness and genetic diversity, respectively. The coefficients of variation (R^2) from Eqs. 1 and 2

211 give the total amount of variation explained by spatial patterns for species richness and genetic

212 diversity. Subtracting these values from 1 gives the amount of non-spatial variation. MEM_{iSG}

213 represents the set of MEMs shared by both species richness and genetic diversity. R^2 values from

214 Eqs. 3 and 4 tell us the amount of variation in each response variable which can be explained by

215 spatial variation shared at both levels of diversity. When subtracted from the total spatial

216 variation in genetic diversity or species richness (Eqs. 1 and 2), we get the proportion of non-

217 shared spatial variation.

218 *Identifying environmental determinants of spatial patterns in genetic diversity and species*

219 *richness.* Our next aim was to determine whether genetic diversity and species richness are

220 shaped by differential environmental carrying capacities due to limits on energy and niche

221 availability. To do this we used structural equation modeling (SEM). Hypotheses in structural

222 equation models are envisioned as a hypothesis network representing a conceptual model, where

223 paths between variables represent predicted causal relationships (Fig 2a). In SEM, the effects of

224 multiple predictors are simultaneously assessed for multiple response variables (Shipley 2016).

225 We implemented structural equation models using the piecewiseSEM package (version 2.0.2),

226 which uses a local estimation approach for models in the hypothesis network allowing for the

227 incorporation of more complex model types (Lefcheck et al. 2019). Model fit is evaluated using

228 tests of directed separation (Shipley 2016), which determine whether an association exists

11

229   between two variables in the network conditional on each of their causes. If two variables are not

230   conditionally independent, the model is updated by adding a path between them to make the

231   model more consistent with the data. In general, using causal diagrams and examining

232   independence relationships between variables considerably helps to reduce confounder and

233   collider bias in statistical models (McElreath 2015). *P*-values from tests of directed separation

234   are used to calculate Fisher's *C* which follows a chi-squared distribution. Models are a good fit

235   to the data when $p > 0.05$, indicating the null hypothesis—the proposed hypothesis network—is

236   not rejected.

237   We based our conceptual model on previous findings in mammals (Schmidt et al. 2020). This

238   model supposes that limitations on energy (mean potential evapotranspiration) and niche

239   availability (elevation range) set limits on supportable population sizes (body size), and therefore

240   also the number of species that can coexist in a given habitat (Fig 2a). We amended this

241   conceptual model for amphibians. First, we excluded human presence because previous

242   investigation shows it did not have a clear effect on amphibian genetic diversity (Schmidt and

243   Garroway 2020). Second, we included mean actual evapotranspiration as an additional measure

244   of energy, because water availability is an important environmental constraint on amphibian

245   ranges and site occupancy. All variables except genetic diversity were measured at the species

246   level because we are primarily interested in the processes underlying diversity gradients at broad

247   spatial scales. We log-transformed elevation range, and scaled and centered all variables before

248   analysis so path coefficients could be compared across models. We tested our conceptual model

249   using the entire dataset with frogs and salamanders combined, and also analyzed frogs and

250   salamanders separately. There is some evidence that body size, our measure of population size,

251   in these orders may have different relationships to temperature (Olalla-Tárraga and Rodríguez

252   2007), and there appears to be little overlap in body size between orders (Fig. S1). We tested

253   residuals from frog and salamander SEMs for spatial autocorrelation using Moran tests.

254   Following results from SEM analysis, we tested for effects of heterogeneity on population

255   differentiation in frogs. We measured differentiation using a population-specific F_{ST} metric

256   (Weir and Goudet 2017) included in the genetic database. Population-specific F_{ST} differs from

257   pairwise F_{ST} (Weir and Cockerham 1984) in that it measures how far single populations in a

258   sample have diverged from a common ancestor. We tested for the effects of heterogeneity on

259   population differentiation with a mixed effects model controlling for spatial structure using

260   MEMs and including species as a random effect allowing intercepts to vary.

261

262   **Results**

263   *Spatial patterns in genetic diversity.* We recovered previously identified patterns of amphibian

264   species richness with MEMs, where richness was highest in the high-energy, moist, low

265   elevation region in the southeastern United States (Fig. 1; Currie 1991). Western USA, which is

266   hotter and drier, had a comparatively low number of species. As in mammals, the major species

267   richness gradient in North American amphibians is longitudinal rather than latitudinal. We found

268   no broad spatial trends in genetic diversity. Spatial patterns of genetic diversity were more

269   complex in the east, while western populations all had similarly high levels of genetic diversity

270   (Fig. 1). In general, species richness was more spatially structured than genetic diversity, with

271   85% and 56% of variation explained by spatial patterns, respectively (Fig. S2). We detected

272   shared spatial patterns between both levels of biodiversity, however, while shared patterns

273   explained 98% of the spatial variation in genetic diversity, they explained very little of the

274   variation in species richness (Fig. S2).

275   *Common causes of genetic diversity and species richness.* Our conceptual model (Fig 2a) fit the

276   data well with no additional links suggested (combined: Fisher's C = 3.56, *p* = 0.47, *n* = 548,

277   Table S1; frogs: Fisher's C = 2.46, *p* = 0.65, *n* = 288; salamanders: Fisher's C = 2.05, *p* = 0.73,

278   *n* = 260, Table S2). Note that for SEM, *p* > 0.05 means our null hypothesis is not rejected. Here

279   we discuss results from the separate frog and salamander analyses; results from the combined

280   model are given in Table S1. In both frogs and salamanders, species richness was well explained

281   (frog R^2 = 0.86; salamander R^2 = 0.95), and increased with water availability, environmental

282   heterogeneity, and species body size (Fig 2, Table S2). Heterogeneity was the most important

283   determinant of amphibian species richness (frog β = 0.59 ± 0.04 SE; salamander β = 1.16 ± 0.03

284   SE; Fig 2, Table S2). Frog species richness was unrelated to energy availability and inversely

285   related to genetic diversity (Fig 2b). In salamanders, species richness increased with energy

286   availability and genetic diversity (Fig 2c). Across both groups body size was inversely related to

287   water availability and environmental heterogeneity, and in salamanders energy availability

288   additionally had a negative effect on body size. We note that in the combined model, water

289   availability had a positive effect on body size (Table S1) which may be due to general size

290   differences between frogs and salamanders, the latter tending to be larger (Fig. S1). In frogs,

291   heterogeneity had a strong negative effect on genetic diversity (β = -0.77 ± 0.28 SE), however

292   genetic diversity in salamanders was not well predicted by any variables in our model (Fig. 2).

293   There was no residual spatial autocorrelation in the salamander model. In frogs, body size and

294   species richness residuals were spatially autocorrelated at very local scales (body size Moran's I

295   = 0.01, species richness Moran's I = 0.03). In general, the environmental covariates in our

296   models captured broad spatial patterns well, and we did not incorporate fine-scale spatial

297   structure into our model for frogs as this was likely due to the hierarchical structure of the data.

298   Environmental heterogeneity had a negative effect on genetic diversity in frogs; however,

299   genetic diversity is not an indicator of population divergence. We tested the idea that

300   heterogeneity also increased divergence using population-specific F_{ST}. Frog populations indeed

301   tended to be more genetically differentiated in heterogeneous environments ($\beta = 0.99 \pm 0.40$ SE).

302

Discussion

Patterns of amphibian biodiversity

305   The clearest effects in our models suggest that heterogeneity is a prominent determinant of

306   biodiversity in both frogs and salamanders. We did not detect an obvious gradient in genetic

307   diversity in North American amphibians, and it appears the relationships between intraspecific

308   genetic diversity and species richness in these taxa are different than they are in mammals

309   (Schmidt et al. 2020). Notably, our variation partitioning analysis suggests that the spatial

310   patterns shared between genetic diversity and species richness explain most of the variation in

311   genetic diversity, but almost none of the variation in species richness (Fig. S2). This finding

312   suggests that the processes affecting intraspecific genetic diversity and population structure

313   contribute little to broader gradients in species richness.

314   We suspect the general disconnect between genetic diversity and species richness and climate we

315   find may come down to amphibian population dynamics. Carrying capacity hypotheses assume

316   that communities are in equilibrium between speciation, colonization, and extinction (Storch et

317   al. 2018)—by extending this to the genetic level, we also assume populations are in an

318   equilibrium state with regard to gene flow, mutation and genetic drift, as is often assumed by

319   neutral population genetic models. However, amphibians have highly variable local population

320   sizes which can sometimes fluctuate between orders of magnitude from year to year (Collins et

321   al. 2009). Frequent bottlenecks and founder effects due to recolonization from nearby areas in

322   sampled populations could obscure a general relationship of population genetic diversity with

323   population size, species richness, and the climatic factors we explore here.

324   Interestingly, genetic diversity had opposite effects on species richness in frogs and salamanders.

325   In frogs, populations in more heterogeneous environments tended to be less genetically diverse

326   and more differentiated. This pattern, in turn, was associated with greater species richness. These

327   are the predictions of the heterogeneity hypothesis, where increased niche availability reduces

328   population sizes and facilitates population divergence and species coexistence. However,

329   heterogeneity had no detectable effect on salamander genetic diversity, which was positively

330   related to species richness. These relationships suggest that more diverse populations, which are

331   presumably larger, lead to an increase in species richness in line with predictions for the more

332   individuals hypothesis. It thus appears that broad-scale heterogeneity seems to have different

333   effects on salamander and frog population genetic diversity. In general, frogs have better

334   dispersal capabilities than salamanders (Smith and Green 2005). Our dataset included several

335   wide-ranging, northern-adapted generalist species, including the wood frog (*Lithobates*

336   *sylvaticus*), northern leopard frog (*L. pipiens*), and spring peeper (*Pseudacris crucifer*). Such

337   species have greater potential for population isolation and divergence. The spotted salamander

338   (*Ambystoma maculatum*) is likewise a northern-adapted species with a broad distribution,

339   however its range is comparatively smaller (Fig. S3). In salamanders the importance of niche

340   conservatism—the tendency for closely related species to occupy similar niches—for speciation

341   declines with latitude (Kozak and Wiens 2006). Thus, speciation in temperate North American

342   salamanders may primarily have been a product of geographic isolation and niche conservatism

343   rather than ecological divergence (Kozak and Wiens 2006). However, the same pattern is not

344   true for frogs, where niche conservatism does not appear to be generally important for allopatric

345   speciation (Hua and Wiens 2010).

346   Previous evidence in support of the more individuals hypothesis was reported in Plethodontid

347   salamanders (Marshall and Camp 2006), where the authors found a positive correlation between

348   genetic diversity, species richness, and water and energy availability. Topographic heterogeneity

349   made a secondary contribution to diversity (Marshall and Camp 2006). Salamanders' limited

350   dispersal capability relative to frogs may mean population genetic diversity is affected by

351   environmental heterogeneity at finer scales not captured by our model, but that broad-scale

352   heterogeneity is sufficient to resolve diversity patterns at the species level. Bringing this idea

353   back to spatial variation partitioning, it could be that while broad- and fine-scale processes both

354   contribute to species richness, fine-scale processes contribute less. The same fine-scale

355   processes, however, are the primary drivers of spatial genetic diversity patterns. The prominence

356   of heterogeneity in our model contrasts with previous studies reporting species richness most

357   strongly varies with water availability, with heterogeneity being a secondary cause of diversity

358   (Rodríguez et al. 2005; Marshall and Camp 2006; Buckley and Jetz 2007).

359   The relevance of carrying capacity limits for amphibians and ectotherms more generally is

360   debatable due to their low energy usage relative to endotherms. Given the caveats noted above,

361   body size may not be a good proxy for population size in amphibians. It has also been suggested

362   elsewhere that habitat availability does not have an effect on body size in frogs (Olalla-Tárraga

363   et al. 2009). Opposite to our expectations under the more individuals hypothesis, our models

364   suggest that species richness in amphibians is positively correlated with body size, but greater

365   resource availability favors smaller body sizes. In contrast to mammals, body size in amphibians

366 may be more directly related to environmental conditions because it affects thermoregulation and

367 moisture balance. Amphibians can behaviorally thermoregulate and avoid desiccation to an

368 extent by selecting microhabitats with suitable temperature and humidity levels, and by changing

369 their posture (Pough et al. 1983; Olalla-Tárraga et al. 2009; Rozen-Rechels et al. 2019).

370 However, smaller species desiccate faster than large species due to evaporative water loss and

371 higher surface-to-volume ratios (Olalla-Tárraga et al. 2009; Levy and Heald 2016), meaning

372 larger body sizes are favored in drier climates.

373 Although we did not detect any consistent latitudinal or longitudinal gradients in genetic

374 diversity, previous findings suggest mitochondrial genetic diversity in amphibians and other

375 ectotherms varies latitudinally and mirrors species richness patterns (Miraldo et al. 2016; Manel

376 et al. 2020). Amphibian mitochondrial genetic diversity in North America was highest in the

377 species-rich southeastern United States, supporting the evolutionary speed hypothesis, where

378 high environmental temperature increases rates of population divergence and speciation through

379 its effects on mutation rate and generation time (Miraldo et al. 2016). However, we detected no

380 effect of temperature on nuclear genetic diversity in our SEM, casting doubt on this hypothesis

381 for amphibian nuclear genetic diversity. Furthermore, a lack of latitudinal gradient indicates that

382 nuclear genetic diversity is not related to temperature in a straightforward way, likely due to

383 strong temporal instability in amphibian population sizes. Our nuclear genetic data suggests

384 heterogeneity is a major determinant of genetic diversity at broad scales in frogs, but

385 mitochondrial DNA alone is not a reliable marker for detecting intraspecific patterns of

386 population structure (Galtier et al. 2009). Disagreement between biogeographic patterns of

387 mitochondrial and nuclear DNA diversity is relatively common, and often arises from

388 demographic disparities and sex-biased dispersal (Toews and Brelsford 2012). Thus, marker

389   choice is very likely responsible for the divergent patterns we find here. This also appears to be

390   true in mammals, where patterns of genetic diversity measured using mitochondrial DNA and

391   nuclear DNA trended in opposite directions (Schmidt et al. 2020).

392   **Generality of causal mechanisms**

393   Despite limitations modeling population size in amphibians, it appears that genetic diversity and

394   species richness in frogs are driven by processes similar to mammals. Although Schmidt et al.

395   (2020) found support for both the more individuals hypothesis and the role of heterogeneity in

396   mammals, heterogeneity was the main contributor to diversity across both genetic and species

397   levels. Heterogeneity has previously been put forth as a universal driver of species richness

398   (Stein et al. 2014). Our findings combining genetic diversity and species richness in amphibians

399   and previous findings in mammals (Schmidt et al. 2020) indeed suggest that heterogeneity is a

400   major determinant of biodiversity on species and genetic levels in both endothermic and

401   ectothermic vertebrates. However, the underlying mechanisms are not universal, and appear to

402   vary depending on species groups. While in mammals shared spatial patterns between genetic

403   diversity and species richness pointed to common causes, patterns of species richness in

404   amphibians were not well predicted by spatial patterns shared with genetic diversity. Thus, the

405   role of population-level processes in determining amphibian species richness is unclear. Energy

406   and water limitations likely act directly on species richness, and not genetic diversity, by

407   imposing physiological limits on amphibian distributions (Buckley and Jetz 2007). The

408   demographic effects of carrying capacity and niche availability may then only be borne out

409   within regions with suitable environmental conditions. It appears that similar environmental

410   factors are capable of generating an overall latitudinal species richness gradient across taxa, but

411   slight deviations from this general pattern are mediated by differential interactions between

412   environments, species traits, and population processes.

413   Genetic diversity and species richness are two important metrics for biodiversity conservation

414   because they contribute to the resilience of populations and communities in rapidly changing

415   environments (Oliver et al. 2015). Genetic diversity in particular helps ensure population

416   viability, fitness, and capacity to respond to environmental change through adaptation (Frankham

417   1995). Amphibians are among the most imperiled vertebrates (Stuart et al. 2004) and are

418   especially susceptible to environmental change. Macrogenetics approaches to mapping

419   multispecies patterns of genetic diversity at broad scales have great potential for incorporation

420   into conservation policies targeting regional conservation of genetic diversity. However,

421   complex ecophysiological requirements, life histories, and population dynamics may render this

422   approach impractical for amphibians. Finer-scale measures of environmental heterogeneity,

423   energy availability, and habitat suitability may prove to be more reliable predictors of genetic

424   diversity but may do less well at the species level. Population size fluctuation in amphibians

425   could pose a significant roadblock for macrogenetics studies that do not take into account

426   temporal sampling, whether due to study design or lack of available data for this purpose. We are

427   only beginning to explore broad-scale patterns of intraspecific nuclear genetic diversity across

428   several species, but it is already apparent that they are not as consistently clear as gradients in

429   species richness (Miraldo et al. 2016; Manel et al. 2020; Schmidt et al. 2020; Theodoridis et al.

430   2020). We look forward to the continued exploration of these patterns in other taxonomic groups

431   to build a comprehensive picture of the distribution of genetic biodiversity across the globe.

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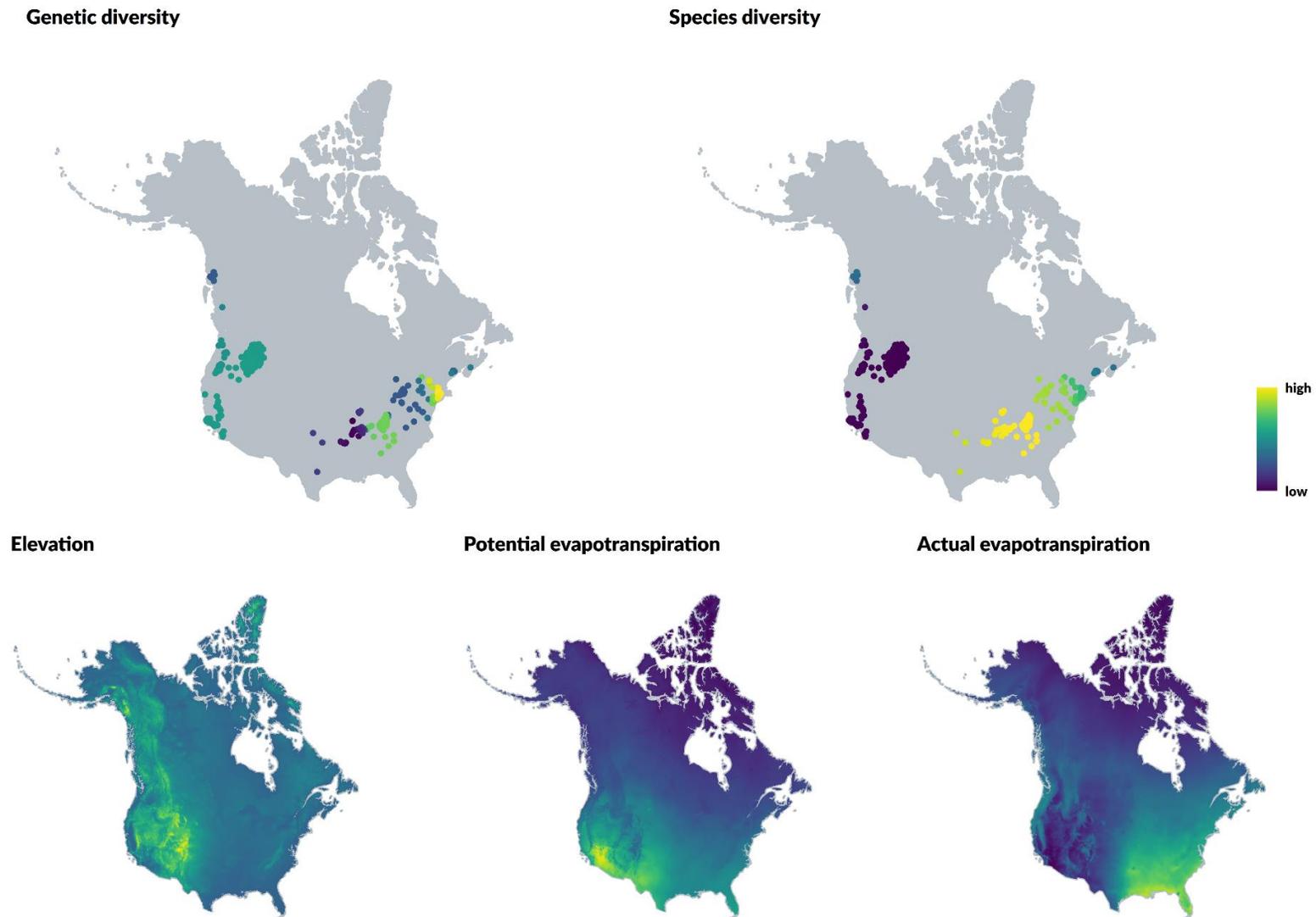594   496–506.

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Figure 1. *(Top row)* Maps of predicted genetic diversity and species richness at genetic sample sites (points) based on spatial MEMs for frogs and salamanders combined. No obvious pattern was detected in genetic diversity, but MEMs were able to recover known patterns of species richness. *(Bottom row)* Maps depicting the environmental variables predicted to have simultaneous effects on genetic diversity and species richness.
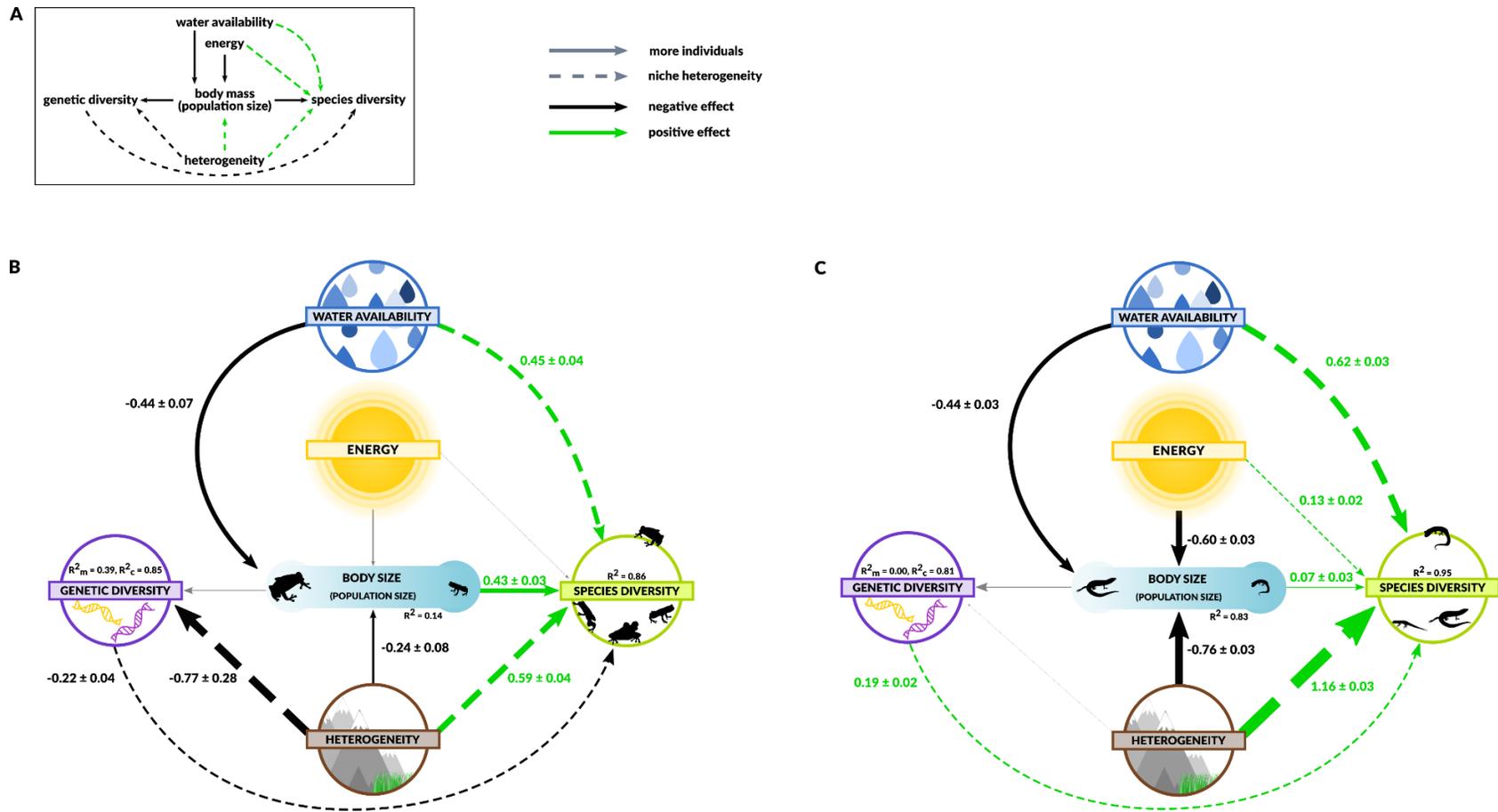
Figure 2. Structural equation models. (A) Our hypothesized conceptual model based on findings in mammals; solid and dashed lines correspond to processes related to the more individuals and heterogeneity hypotheses, respectively. Line width is proportional to path coefficients. Model results are shown for (B) frogs, and (C) salamanders. Results from the overall model with both orders are in Table S1. Regression coefficients with standard errors are shown along each path. Paths between variables where no effect was detected are colored in gray (see Table S2 for a complete summary of all paths). The proportion of variation explained (R^2) is given for all dependent variables. For genetic diversity, R^2_m is the variation explained by fixed effects and R^2_c is the variation explained by both fixed effects and the random species effect.