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# Determinants of genetic diversity and species richness of North American amphibians

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

3 **Running title:**

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26 **Abstract:** Ecological limits on the population sizes and number of species a region is capable of  
27 supporting are thought to simultaneously produce spatial patterns in genetic diversity and species  
28 richness. However, we do not know the extent to which resource-based environmental limits  
29 jointly determine these patterns of biodiversity in ectotherms because of their low energy  
30 requirements compared to endotherms. Here, we adapt a framework for the ways ecological  
31 limits may shape genetic diversity and species richness previously tested in mammals for  
32 amphibians to determine whether similar processes produce continental patterns of biodiversity  
33 across both taxa. Repurposing open, raw microsatellite data from 19 species sampled at 554 sites  
34 in North America we found that spatial patterns of genetic diversity run opposite to patterns of  
35 species richness and genetic differentiation. However, while measures of resource availability  
36 and niche heterogeneity predict 89% of the variation in species richness, these landscape metrics  
37 are poor predictors of genetic diversity. Although heterogeneity appears to be an important driver  
38 of genetic and species biodiversity patterns in both amphibians and mammals, our results suggest  
39 that variation in genetic diversity both within and across species makes it difficult to infer  
40 general processes producing spatial patterns of amphibian genetic diversity.

41

42 **Keywords:** latitudinal diversity gradient, biogeography, heterozygosity, frogs, salamanders,  
43 more individuals hypothesis, macrogenetics

44

45 **Introduction**

46 Although species richness is higher in the tropics for most taxa, the details of diversity patterns  
47 differ among species groups. In North America for instance, vertebrate richness generally  
48 increases with resource availability, but mammals and birds tend to have higher species richness  
49 in dry, mountainous areas, and reptiles and amphibians are more diverse in wet, lower elevation  
50 regions (Currie 1991). This pattern suggests that while richness increases with resource  
51 availability, taxon-specific traits may cause richness patterns to diverge from a strictly latitudinal  
52 gradient. Broad-scale patterns of biodiversity at the genetic level have only recently begun to be  
53 mapped thanks to the accumulation of open data in public repositories (Miraldo et al. 2016;  
54 Manel et al. 2020; Schmidt et al. 2020; Theodoridis et al. 2020). Genetic diversity is typically  
55 thought of as the most fundamental level of biodiversity because it bears on populations'  
56 capacities to evolve adaptively in response to environmental change (Frankham 1995). Recent  
57 analyses of mammals suggest that environments can simultaneously shape species richness and  
58 genetic diversity on continental scales (Schmidt et al. 2020). Whether this is also true in  
59 ectothermic taxa is unknown. Understanding whether common processes underlie variation in  
60 biogeographic patterns of diversity across taxa with different environmental requirements can  
61 help us move toward a general understanding of the drivers of biodiversity at multiple levels.  
62 In mammals, continental scale multi-species patterns of nuclear genetic diversity and species  
63 richness could be inferred with estimates of resource and niche availability, or ecological  
64 opportunity (Schmidt et al. 2020). This suggested that ecological limits placed on the number of  
65 individuals and species an environment can support are important drivers of broad-scale  
66 biodiversity patterns. Resource-rich environments supported larger populations with higher  
67 genetic diversity and species richness, while niche availability in heterogeneous habitats

68 promoted species coexistence but reduced population sizes and genetic diversity because  
69 specialization restricts available resources. These processes are related to two well-supported  
70 hypotheses for the production of biogeographic patterns of species richness: the more-individuals  
71 hypothesis (Wright 1983), and the effects of environmental and resource heterogeneity (Allouche  
72 et al. 2012; Stein et al. 2014). The more individuals hypothesis posits that resource-rich regions  
73 near the equator are capable of supporting larger populations and communities, and thus more  
74 species than temperate regions. Heterogeneity is thought to increase species richness due to  
75 greater niche availability in more complex heterogeneous environments which allows more  
76 species to coexist, but with smaller population sizes because resources are partitioned.

77 The effects of resource heterogeneity on species richness and genetic diversity seem likely to be  
78 generally applicable across taxa (Stein et al. 2014; Schmidt et al. 2020), however the general  
79 relevance of the more-individuals hypothesis for ectotherms is uncertain (Buckley and Jetz  
80 2010). This is because compared to endotherms, ectotherms have lower energy requirements and  
81 can behaviorally thermoregulate, meaning their abundances are less likely to be limited by  
82 resource-related ecological limits (Pough 1980; Buckley and Jetz 2010). Instead, ectotherm  
83 distributions, and therefore species richness, seem more directly constrained by environmental  
84 temperature because fewer species have evolved thermal adaptations required for expanding into  
85 cooler regions (Buckley and Jetz 2007, 2010). Further, the evolution of traits associated with  
86 better survival in temperate regions may have additional effects on speciation dynamics. For  
87 example, species turnover tends to be higher among viviparous squamate reptiles, which  
88 typically occupy cooler regions (Pyron and Burbrink 2014). Reaching a more comprehensive  
89 understanding of how biodiversity patterns emerge requires knowledge about whether

90 relationships between genetic diversity, species richness, and environments are consistent across  
91 endothermic and ectothermic taxa.

92 The determinants of species richness across all terrestrial vertebrates are generally related to  
93 resource availability as estimated by energy (e.g., potential evapotranspiration, primary  
94 productivity), water-energy balance (e.g., actual evapotranspiration, precipitation), and  
95 heterogeneity (e.g., elevation variability, land cover diversity) (Currie 1991; Kerr and Packer  
96 1997; Hawkins et al. 2003; Rodríguez et al. 2005; Buckley and Jetz 2007; Stein et al. 2014;  
97 Jiménez-Alfaro et al. 2016). Amphibians are interesting because they are constrained both by  
98 water availability and temperature and water availability is consistently identified as an  
99 important driver of diversity in amphibians (Rodríguez et al. 2005; Buckley and Jetz 2007).

100 Indeed in Europe, the best predictors of species richness in mammals and birds shift from energy  
101 to water availability at decreasing latitudes, but amphibian species richness remains strongly  
102 related to water-energy balance regardless of latitude (Whittaker et al. 2007).

103 The causes of population genetic diversity are rarely studied at the same time or scale as patterns  
104 of species richness (but see Marshall and Camp 2006; Schmidt et al. 2020), yet the presumed  
105 mechanisms related to the more-individuals hypothesis and heterogeneity are closely related to  
106 carrying capacity and population-level processes (Schmidt et al. 2020). The more-individuals  
107 mechanism predicts a positive relationship between species richness and population genetic  
108 diversity because bigger populations and communities tend to have higher levels of genetic and  
109 species diversity (Kimura 1983; Hubbell 2001; Schmidt et al. 2020). With higher carrying  
110 capacities, more species persist because they can reach minimal viable population sizes. On the  
111 other hand, heterogeneity is predicted to cause negative correlations between genetic diversity  
112 and species richness by increasing the number of species a given area can support which in turn

113 reduces population size and limits gene flow due to increased niche specialization.

114 Heterogeneous environments also facilitate population differentiation due to spatially varying  
115 selection. In mammals, evolutionary processes acting on the population level scaled up and  
116 interacted with resource availability and heterogeneity to produce genetic diversity and species  
117 richness patterns (Schmidt et al. 2020).

118 Whether mechanisms related to ecological limits and niche availability predict patterns of  
119 species richness and genetic diversity in ectotherms is unclear. To test this prediction, we  
120 analyzed previously-published microsatellite genotype data from 19 North American amphibian  
121 species (8 frogs, 11 salamanders), with >13000 individuals sampled at 554 sites. Our first  
122 objective was to identify existing spatial patterns in genetic diversity and differentiation and  
123 quantify the extent to which genetic diversity and species richness covary. We then tested  
124 whether limits on resources and niche availability jointly determined genetic diversity and  
125 species richness using structural equation models, which allowed us to evaluate multiple  
126 hypotheses at genetic and species level biodiversity simultaneously. We based our conceptual  
127 framework on previous findings in mammals (Schmidt et al. 2020) and adapted it for  
128 amphibians. First, we excluded human presence because previous investigation shows it did not  
129 have a clear effect on amphibian genetic diversity (Schmidt and Garroway 2021a). Second, we  
130 included a measure of water availability as an additional indicator of resource availability. Third,  
131 body size was used as a proxy for whole species census size in mammals, however we do not  
132 know the extent to which body size is correlated with effective population size or genetic  
133 diversity within amphibians. We therefore first tested for a relationship between body size and  
134 genetic diversity for species in our sample to determine whether it should be incorporated into  
135 the model. Finally, we compare our results to previous results in mammals (Schmidt et al. 2020)

136 to infer whether similar environmental features contribute to diversity gradients across  
137 endothermic and ectothermic taxa in North America.

138

## 139 **Methods**

### 140 **Biodiversity data**

141 *Genetic diversity and differentiation.* We used raw genotypes (i.e. called allele sizes) of North  
142 American amphibians compiled by (Schmidt and Garroway 2021a). This data set was assembled  
143 from raw microsatellite datasets publicly archived in Dryad (DataDryad.org). To identify data  
144 sets we conducted a systematic search of the Dryad data repository with the following keywords:  
145 species name (e.g. *Plethodon cinereus*), “microsat\*”, “short tandem\*”, and “single tandem\*”.  
146 We used the IUCN Red List database to obtain a list of amphibian species native to North  
147 America for the search. We excluded datasets that lacked spatial reference, were not located in  
148 North America, did not sample neutral microsatellite loci, or had study designs that may have  
149 affected genetic diversity (including sampling island populations, or captive or managed  
150 populations). In total the data sets include genotypes for 13680 individuals spanning 19 species  
151 sampled at 554 locations in the contiguous United States and Canada. We used gene diversity as  
152 a measure of genetic diversity because it is minimally affected by sample size (Nei 1973;  
153 Charlesworth and Charlesworth 2010). Our measure of differentiation was population-specific  
154  $F_{ST}$  (Weir and Goudet 2017). Population-specific  $F_{ST}$  differs from pairwise  $F_{ST}$  in that it  
155 measures how far single populations in a sample have diverged from a common ancestor—this  
156 means it is comparable across studies and species. Population-specific  $F_{ST}$  was not estimable at 2  
157 sites because it requires a minimum of 2 sample sites in the original dataset ( $n = 552$ ).



158 *Species richness.* We estimated species richness at each of our genetic diversity sample sites  
159 using amphibian range extent data from the IUCN RedList (IUCN 2019), applying filters for  
160 native, extant species ranges. We measured species richness as the number of species' ranges  
161 overlapping each genetic sample site.

162

### 163 **Diversity maps and spatial variation partitioning**

164 We used distance-based Moran's eigenvector maps (MEMs) to detect spatial patterns in genetic  
165 diversity and differentiation and compare these to patterns of species richness. MEMs are  
166 orthogonal spatial eigenvectors with eigenvalues that are directly proportional to Moran's  $I$ .  
167 They measure spatial autocorrelation at all scales present in the data. We computed MEMs in the  
168 R package *adespatial* (Dray et al. 2017). We used the forward selection procedure described in  
169 Blanchet et al. (2008) to select two sets of MEMs describing important patterns in genetic  
170 diversity, differentiation, and species richness. We then selected MEMs which explained the  
171 broadest spatial patterns in these data (Moran's  $I > 0.25$ ). To create maps of genetic diversity,  
172 differentiation, and species richness (Fig. 1), we used the fitted values for gene diversity,  
173 population-specific  $F_{ST}$ , and species richness regressed on broad-scale MEMs. This approach  
174 allowed us to visualize broad purely spatial patterns in genetic diversity, differentiation, and  
175 species richness without variation due to other sources, such as local environments, species  
176 identity, or population history. Maps of raw values are presented in Fig. S1.

177 Next, we determined the extent to which spatial patterns in genetic diversity and species richness  
178 were shared using variation partitioning. Because our MEM analysis for both levels of  
179 biodiversity had the same input distance matrix, the resulting spatial MEMs were directly

180 comparable. This was not the case for genetic differentiation, which had fewer sample sites. We  
181 therefore did not partition variation in genetic differentiation because these MEMs are not the  
182 same as those computed for genetic diversity and species richness.

183 We determined the fraction of total variation explained by spatial structure, shared spatial  
184 structure, and non-spatial variation using variation partitioning as follows. We ran a series of  
185 linear regressions with either species richness ( $y_{SR}$ ) or gene diversity ( $y_{GD}$ ) as the response  
186 variable using all MEMs selected for that variable (Equations 1 and 2), or only MEMs shared by  
187 both variables as predictors (Equations 3 and 4):

$$188 \quad y_{SR} \sim \alpha + \beta_{1S}(\text{MEM}_{1S}) + \beta_{2S}(\text{MEM}_{2S}) + \dots + \beta_{8S}(\text{MEM}_{8S}) + \epsilon \quad \text{Eq. 1}$$

$$189 \quad y_{GD} \sim \alpha + \beta_{1G}(\text{MEM}_{1G}) + \beta_{2G}(\text{MEM}_{2G}) + \dots + \beta_{5G}(\text{MEM}_{5G}) + \epsilon \quad \text{Eq. 2}$$

$$190 \quad y_{SR} \sim \alpha + \beta_{1SG}(\text{MEM}_{1SG}) + \beta_{2SG}(\text{MEM}_{2SG}) + \dots + \beta_{5SG}(\text{MEM}_{5SG}) + \epsilon \quad \text{Eq. 3}$$

$$191 \quad y_{GD} \sim \alpha + \beta_{1SG}(\text{MEM}_{1SG}) + \beta_{2SG}(\text{MEM}_{2SG}) + \dots + \beta_{5SG}(\text{MEM}_{5SG}) + \epsilon \quad \text{Eq. 4}$$

192

193 where  $\alpha$  is the grand mean, and  $\text{MEM}_{iS}$  and  $\text{MEM}_{iG}$  are the set of MEMs selected for species  
194 richness (8 MEMs) and genetic diversity (5 MEMs), respectively. The coefficients of variation  
195 ( $R^2$ ) from Eqs. 1 and 2 give the total amount of variation explained by spatial patterns for species  
196 richness and genetic diversity. Subtracting these values from 1 gives the amount of non-spatial  
197 variation.  $\text{MEM}_{iSG}$  represents the set of MEMs shared by both species richness and genetic  
198 diversity (5 MEMs).  $R^2$  values from Eqs. 3 and 4 tell us the amount of variation in each response  
199 variable which can be explained by spatial variation shared at both levels of diversity. When  
200 subtracted from the total spatial variation in genetic diversity or species richness (Eqs. 1 and 2),  
201 we get the proportion of non-shared spatial variation.

202

203 **Structural equation modeling**

204 *Environmental data.* We measured resource and niche heterogeneity by computing Simpson's  
205 Diversity Index for landcover categories within buffers at each site. We obtained a 30 m  
206 resolution map of North American landcover data from the Commission for Environmental  
207 Cooperation (CEC et al. 2015). This map is based on 2015 satellite imagery and has 19 standard  
208 land cover classifications which includes forests, shrubland, grassland, wetlands, cropland,  
209 barren land, and built-up land. Because this variable is scale-dependent, we recorded  
210 heterogeneity within 4 buffer sizes (10, 25, 40, and 80 km) around each site.

211 We used two measures of resource availability because amphibians are habitat-limited by both  
212 temperature and water availability. Water availability can be measured by evapotranspiration, or  
213 the amount of water removed from the Earth's surface through soil or open water evaporation  
214 and plant transpiration processes. Potential evapotranspiration (PET) measures the atmospheric  
215 demand for water, depending on factors such as temperature and wind (Peng et al. 2019). It is  
216 strongly correlated with temperature. PET is the maximum amount of water that would be  
217 removed in the absence of biophysical limitations (Peng et al. 2019). The amount of water  
218 actually removed, actual evapotranspiration (AET), reflects water availability and soil moisture  
219 levels. Actual evapotranspiration has also been shown to be one of the strongest predictors of  
220 amphibian species richness (Buckley and Jetz 2007). PET can be viewed as a measure of energy  
221 availability, and AET one of water-energy balance (see Currie 1991; Buckley and Jetz 2007;  
222 Kreft and Jetz 2007). Together these variables represent total ecosystem resource availability at  
223 sites. We measured mean PET and AET (mm/yr) at sites within 10, 25, 40, and 80 km buffers  
224 using data from the CGIAR Consortium for Spatial Information (Trabucco and Zomer 2019).

225 *Population size.* Body size is a readily measured trait that is typically negatively correlated with  
226 long-term effective population size across a diversity of species (Frankham 1996; Romiguier et  
227 al. 2014; Mackintosh et al. 2019). To explore whether body size was a useful substitute for long-  
228 term effective population size in amphibians, we obtained body length data from the AmphiBIO  
229 v1 database (Oliveira et al. 2017). We then regressed it on gene diversity in a hierarchical model  
230 with species as a random effect allowing intercepts to vary and predicted a negative relationship.  
231 We found no relationship between body size and genetic diversity in our data (Fig. S2), thus we  
232 excluded population size from our proposed causal framework. Without population size as an  
233 intermediate variable, we predicted that resource availability would have direct positive effects  
234 on both genetic diversity and species richness, and that heterogeneity would have direct negative  
235 and positive effects on genetic diversity and species richness, respectively. We hypothesize that  
236 these direct effects would act through population size were such data available.

237 *Analysis.* We used structural equation modeling (SEM) to determine whether genetic diversity  
238 and species richness are shaped by differential ecological limits due to limits on resources and  
239 niche availability. Structural equation modeling begins with a causal diagram, or conceptual  
240 model, where paths between variables represent hypothesized causal relationships (Fig. 2a).  
241 Hypotheses are envisioned as a network where variables are nodes, and paths connecting them  
242 represent causal relationships. In SEM, the effects of multiple predictors are simultaneously  
243 assessed for multiple response variables (Shipley 2016). We implemented structural equation  
244 models using the piecewiseSEM package (version 2.0.2), which uses a local estimation approach  
245 for models in the hypothesis network allowing for the incorporation of more complex model  
246 types (Lefcheck et al. 2019). Model fit is evaluated using tests of directed separation (Shipley  
247 2016), which determine whether an association exists between two variables in the network

248 conditional on each of their causes. If two variables are not conditionally independent, the model  
249 is updated by adding a path between them to make the model more consistent with the data. *P*-  
250 values from tests of directed separation are used to calculate Fisher's *C*, which is then used to  
251 calculate an overall *p*-value for the model network. Models are a good fit to the data when *p* >  
252 0.05, indicating the null hypothesis—the proposed hypothesis network—is not rejected.

253 Our model network consisted of two models with genetic diversity and species richness as  
254 response variables. Our genetic data is hierarchical with multiple sites nested within species. To  
255 account for variation in mean genetic diversity across species we modeled random intercepts for  
256 species. Additionally, we allowed the responses of genetic diversity to resource availability and  
257 heterogeneity to vary across species by including a random slope term for each environmental  
258 predictor (PET, AET, and land cover diversity). With this random effect structure, we thus allow  
259 relationships to vary among species and do not assume effects will be of the same magnitude or  
260 direction for all species. We fit the hierarchical model for genetic diversity using the lme4  
261 package (Bates et al. 2015) within piecewiseSEM. We scaled and centered all variables before  
262 analysis so path coefficients were comparable. Finally, we checked model residuals for spatial  
263 autocorrelation using Moran tests. We conducted SEM analyses in parallel for each of our 4  
264 buffer sizes.

265

### 266 **Effect of heterogeneity on population differentiation**

267 Finally, we tested whether landscape heterogeneity was related to increased population  
268 differentiation. We regressed heterogeneity on population-specific  $F_{ST}$  using a hierarchical model  
269 with a random effect for species accounting for differences in mean  $F_{ST}$  (intercepts) while

270 allowing the strength and direction of the effect of heterogeneity (slopes) to vary across species.  
271 To account for spatial variation we included MEMs describing spatial patterns in  $F_{ST}$  as  
272 covariates. We performed these analyses in parallel across all four heterogeneity buffers.

273

## 274 **Results**

275 *Spatial patterns in genetic variation.* We detected spatial patterns across genetic diversity,  
276 genetic differentiation, and species richness (Fig. 1). The major axis of broad-scale variation is  
277 longitudinal, and across all three biodiversity metrics the western sample sites show little  
278 variation whereas spatial patterns were more complex in the east and appear to vary latitudinally.  
279 This broad longitudinal pattern is consistent with environmental variation in North America (Fig.  
280 1) as well as genetic diversity and species richness patterns in mammals (Schmidt et al. 2020).  
281 We recovered known patterns of amphibian species richness with MEMs, where richness was  
282 highest in the southeastern United States (Fig. 1) (Currie 1991). Western USA, which is hotter  
283 and drier, had a comparatively low number of species. In eastern sample sites, genetic diversity  
284 increased with latitude, while differentiation and species richness increase towards the tropics.  
285 Genetic diversity and differentiation in the western samples were in the mid-range of values  
286 across sample sites (Fig. 1).

287 In general, species richness was more spatially structured than genetic diversity, with 85% and  
288 23% of variation explained by spatial patterns, respectively (Fig. 1). We detected shared spatial  
289 patterns between both levels of biodiversity, however, while shared patterns accounted for the  
290 entirety of the spatial variation in genetic diversity, they explained less of the variation in species  
291 richness (18%).

292 *Common causes of genetic diversity and species richness.* Our conceptual model (Fig 2a) fit the  
293 data well (Fisher's  $C = 0.49$ ,  $p = 0.78$ , 2 degrees of freedom) with no additional links suggested  
294 at any scale. Note that for SEM,  $p > 0.05$  means that our conceptual model is not rejected. We  
295 present results from the 40 km buffer in the main text; results from all models can be found in  
296 tables S1 – 4. Species richness was well explained ( $R^2 = 0.89$ ) and increased with water  
297 availability, environmental heterogeneity, and species body size (Fig 2, Table S3). Water  
298 availability had the strongest effect on species richness. Genetic diversity was not well predicted  
299 by any variables in our model ( $R^2 = 0.04$ ; Fig. 2). Residuals from genetic diversity models did  
300 not exhibit spatial autocorrelation. Species richness residuals were spatially autocorrelated at  
301 local scales (Moran's  $I = 0.06$ ). In general, the environmental covariates in our models captured  
302 broad spatial patterns well, and we did not incorporate fine-scale spatial structure into our  
303 models as this was likely due to clustered sampling of some species. Lastly, genetic  
304 differentiation within species decreased with heterogeneity ( $\beta = -0.32 \pm 0.10$  SE) at the most  
305 local spatial scale we tested (10 km buffer), but this relationship disappeared at larger buffer  
306 sizes.

307

## 308 **Discussion**

309 We found spatial variation shared across genetic diversity, differentiation, and species richness at  
310 broad spatial scales (Fig. 1). In general, areas with high species richness tended to have  
311 genetically differentiated populations with relatively low genetic diversity. There was a  
312 latitudinal gradient in genetic diversity across eastern sites which varied in the opposite direction  
313 of the gradient in species richness and genetic differentiation. These patterns are consistent with  
314 our predicted effects of heterogeneity, however, these relationships were not well-reflected by

315 our structural equation model (Fig. 2). Our variation partitioning suggests that all spatial  
316 variation in genetic diversity was shared with species richness, but the environments that  
317 predicted species richness did not predict genetic diversity well. This finding suggests that other  
318 processes drive the genetic diversity patterns we detect. These processes are likely species-  
319 specific, and not generalizable at continental scales as they were in mammals (Schmidt et al.  
320 2020).

321 We suspect the general lack of relationship between genetic diversity and species richness and  
322 climate we find in our structural equation model may come down to amphibian population  
323 dynamics. Ecological limits hypotheses assume that communities are in equilibrium with respect  
324 to speciation, colonization, and extinction dynamics (Storch et al. 2018)— extending this to the  
325 genetic level, we also assume populations are in an equilibrium state with regards to gene flow,  
326 mutation, and genetic drift. However, amphibians have variable local population sizes which can  
327 sometimes fluctuate by orders of magnitude from year to year (Collins et al. 2009), high rates of  
328 species turnover at local and regional scales (Werner et al. 2007; Buckley and Jetz 2008), and  
329 relatively low occupancy within potential distributions (Munguía et al. 2012). Species turnover at  
330 sites is associated with environmental heterogeneity in freshwater habitats, especially  
331 hydroperiod variation in temporary ponds (Urban 2004). Varying population dynamics could  
332 obscure general relationships between population genetic diversity and population size, species  
333 richness, and the climatic factors we explore here.

334 Interestingly, although genetic diversity was not affected by environmental heterogeneity at any  
335 scale, genetic differentiation decreased with heterogeneity at the most local scale we tested;  
336 genetic differentiation was relatively low in the northeast where landscape heterogeneity was  
337 higher (Fig. 1). If heterogeneity increases niche availability and creates opportunities for



338 specialization and divergence, we predicted that it would increase genetic differentiation.  
339 However, the pattern we detect here may be expected if species that were capable of recolonizing  
340 northern regions following glaciation tend to be widely distributed generalists that maintain  
341 population connectivity over relatively large geographic distances (Smith et al. 2005; Zeisset and  
342 Beebee 2008). Niche partitioning in amphibians may also occur at finer scales within suitable  
343 habitats (Karlin et al. 1984; Cloyd and Eason 2017)—for example by modifying microhabitat  
344 usage, diets, foraging strategies, or behaviors across species. Thus, the landscape level metric we  
345 use here may not capture the varying ways that environmental heterogeneity affects genetic  
346 diversity and population differentiation if species respond to environments in different ways.  
347 These patterns point to the importance of species-specific responses to environmental conditions  
348 and environmental instability in generating biogeographic patterns of genetic diversity and  
349 species richness in amphibians (Urban 2004).

350 Previous exploration of the relationships between nuclear genetic diversity, species richness, and  
351 environments in plethodontid salamanders produced similarly mixed results (Marshall and Camp  
352 2006). Genetic diversity was positively associated with temperature and rainfall across all eight  
353 species studied, but topographic heterogeneity had both positive and negative effects on genetic  
354 diversity depending on species. Allelic richness was only correlated with species richness for  
355 *Desmognathus* species and *Plethodon jordani*, but relationships were positive and negative  
356 (Marshall and Camp 2006). In another example, Karlin et al. (1984) report a negative  
357 relationship between species richness and genetic diversity across populations of *D. fuscus*.  
358 Similar to our findings, it appears resource availability and heterogeneity simultaneously affect  
359 biodiversity on genetic and species levels, but genetic diversity in general is less well predicted

360 by environments alone. Variation between species may reduce our ability to detect general  
361 relationships across species.

362 Although we did not detect clear latitudinal or longitudinal gradients in genetic diversity across  
363 North America, previous findings suggest mitochondrial genetic diversity in amphibians and  
364 other ectotherms varies latitudinally and mirrors species richness patterns (Miraldo et al. 2016;  
365 Manel et al. 2020). Miraldo et al. (2016) found that amphibian mitochondrial genetic diversity in  
366 North America was highest in the species-rich southeastern United States. They suggested this  
367 pattern may be related to the evolutionary speed hypothesis, where presumably high  
368 environmental temperature increases rates of population divergence and speciation through its  
369 effects on mutation rate and generation time (Miraldo et al. 2016). In addition to a negative  
370 correlation between patterns of nuclear genetic diversity and species richness, we detected no  
371 effect of temperature on nuclear genetic diversity in our SEM, casting doubt on this hypothesis  
372 for amphibians. Furthermore, a lack of latitudinal gradient indicates that nuclear genetic diversity  
373 is not related to temperature in a straightforward way. Environmental temperature has varied and  
374 complex effects on metabolism and mitochondrial processes (Munro and Treberg 2017; Zhang  
375 and Wong 2021), making relationships with nuclear and mitochondrial mutation rates unlikely to  
376 be generalizable (Lanfear et al. 2007; Schmidt and Garroway 2021b). More generally  
377 mitochondrial DNA alone is not a reliable marker for detecting intraspecific demographic  
378 patterns (Bazin et al. 2006; Galtier et al. 2009; Schmidt and Garroway 2021b). Thus, marker  
379 choice is very likely responsible for the diverging patterns we find here. This also appears to be  
380 true in mammals, where patterns of genetic diversity measured using mitochondrial DNA and  
381 nuclear DNA also trended in opposite directions (Schmidt et al. 2020).

382 Despite limitations modeling population size, it appears that spatial patterns in genetic diversity  
383 and species richness in amphibians are driven by processes similar to mammals. Interestingly,  
384 similar proportions of variation in genetic diversity (~ 25%) could be attributed to spatial  
385 processes across both taxa. Additionally, it was the case in both groups that spatial variation in  
386 genetic diversity was primarily due to factors that also shaped species richness, but other factors  
387 contribute to unique spatial variation in species richness. The overall negative correlation  
388 between spatial patterns of species richness and genetic diversity in amphibians and mammals  
389 (Schmidt et al. 2020) suggests heterogeneity and niche partitioning are major contributors to  
390 diversity across genetic and species levels in endo- and ectothermic vertebrates. Heterogeneity  
391 has previously been put forth as a universal driver of species richness (Stein et al. 2014). In  
392 general, similar environmental factors seem capable of generating an overall species richness  
393 gradient across taxa, but slight deviations from this general pattern are mediated by differential  
394 interactions between environments, species traits, and population processes.

395 Genetic diversity and species richness are two important metrics for biodiversity conservation  
396 because they contribute to the resilience of populations and communities in rapidly changing  
397 environments (Oliver et al. 2015). Neutral genetic diversity is indicative of population mean  
398 fitness and the efficiency of selection in response to environmental change—although it is only  
399 weakly correlated with additive genetic diversity, it is nevertheless informative for conservation  
400 purposes because it reflects levels of inbreeding and the efficiency of selection due to its  
401 relationship to the effective population size (Frankham 1995; Mittell et al. 2015). Amphibians  
402 are among the most imperiled vertebrates (Stuart et al. 2004) and are especially susceptible to  
403 environmental change. Macrogenetics approaches mapping multispecies patterns of genetic  
404 diversity at broad scales have great potential for incorporation into conservation policies

405 targeting regional conservation of genetic diversity. However, complex ecophysiological  
406 requirements, life histories, and population dynamics may render this approach impractical for  
407 amphibians because the environmental factors affecting genetic diversity may differ depending  
408 on species (Schmidt and Garroway 2021a). Species-specific measures of environmental  
409 heterogeneity, resource availability, and habitat suitability may prove to be more reliable  
410 predictors of genetic diversity, but may be less relevant for species richness. We are only  
411 beginning to explore broad scale patterns of intraspecific genetic diversity across several species,  
412 but it is already apparent that they are not as consistently clear as gradients in species richness  
413 (Miraldo et al. 2016; Manel et al. 2020; Schmidt et al. 2020; Theodoridis et al. 2020). We look  
414 forward to the continued exploration of these patterns in other taxonomic groups to help build a  
415 comprehensive picture of the distribution of genetic biodiversity across the globe.

416

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418 designed the study and C.S. conducted the analyses with input from S.D. and C.J.G. All authors  
419 contributed to data interpretation. C.S. wrote the first draft of the manuscript and all authors  
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421

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427 **Data availability:** Synthesized genetic data is available from the Dryad Data Repository (DOI:  
428 10.5061/dryad.qv9s4mwf0). Species range boundary files and environmental data are available  
429 from open online sources (see Methods).

430

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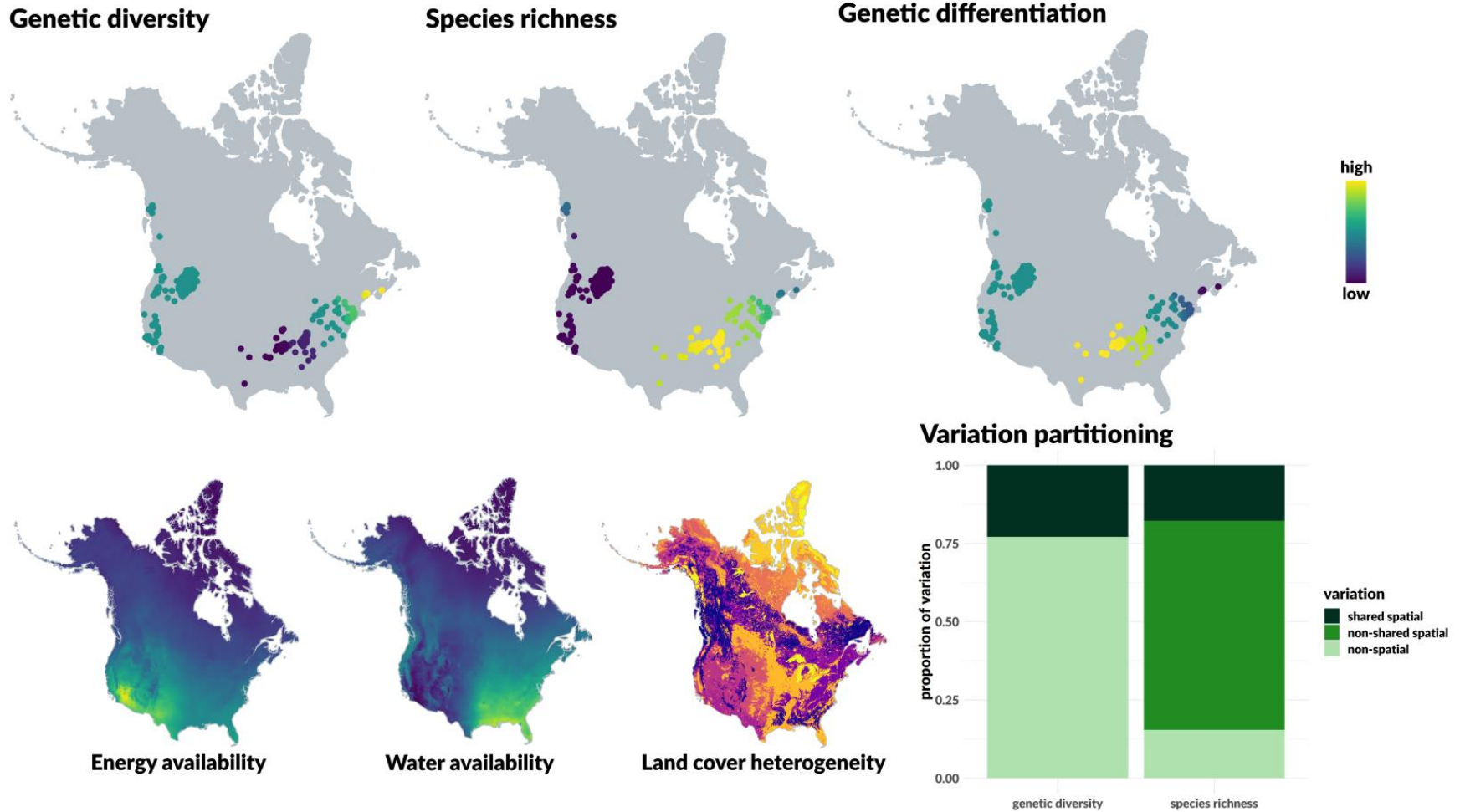
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581 **Table 1.** Data summary. Summary of aggregated raw genetic data: mean gene diversity, mean number of loci, median number of  
 582 individuals at sites per species. Species richness is the mean species richness at sites. Environmental variables (40 km  
 583 buffer): potential evapotranspiration (PET); actual evapotranspiration (AET); heterogeneity is the mean land cover diversity measured  
 584 with Simpson's Index. Ranges of values are given in parentheses where applicable.

585

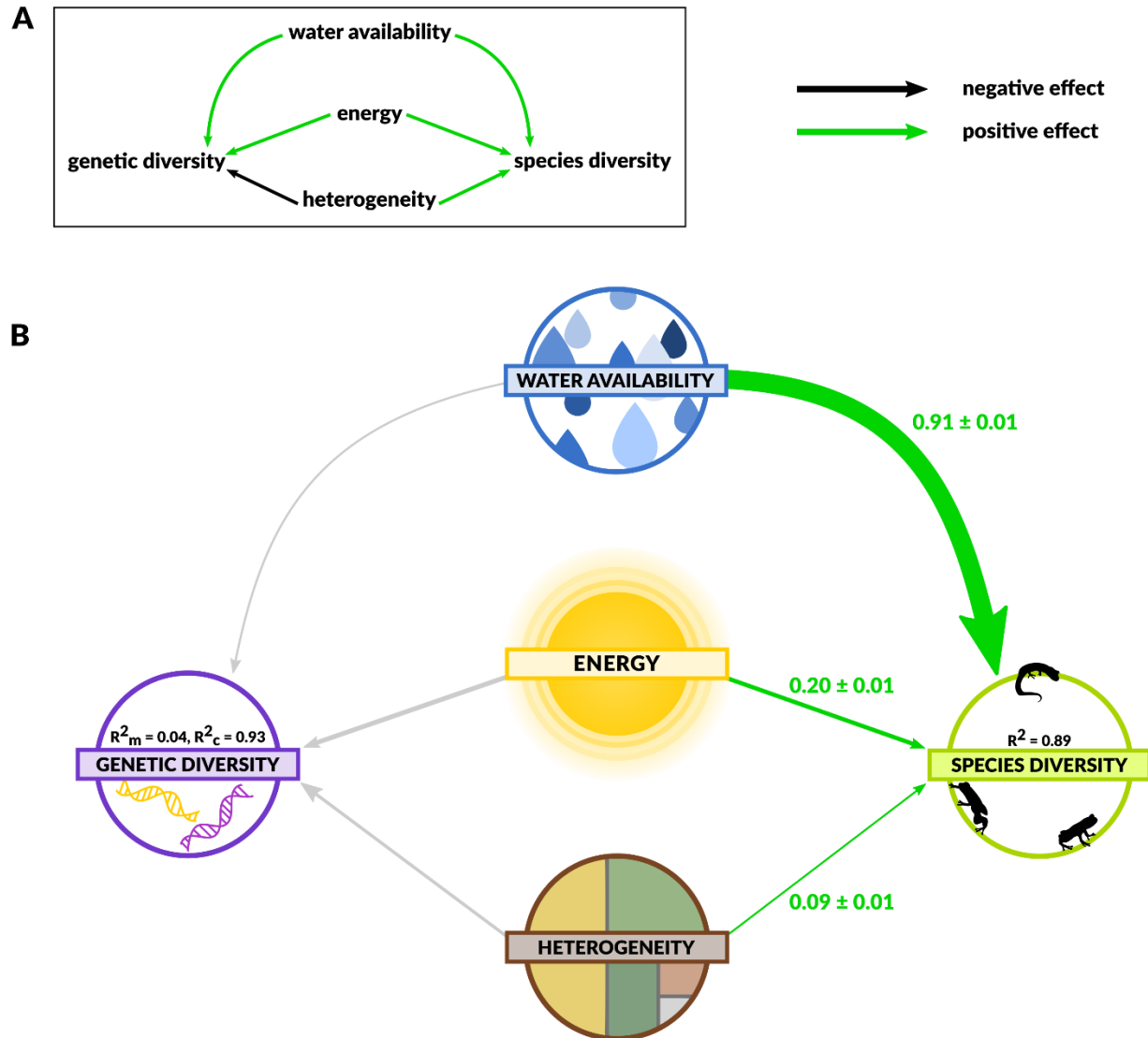
586

Species (# sites)	Gene diversity	Loci	Individuals	Species richness	PET	AET	Heterogeneity
<b>Anura</b>							
<i>Ascaphus montanus</i> (100)	0.74 (0.41–0.87)	13	20 (10–41)	7.35 (3–11)	1097.84 (1006.52–1327.35)	506.86 (341.66–601.4)	0.54 (0.31–0.72)
<i>Lithobates pipiens</i> (5)	0.86 (0.83–0.9)	9	40 (20–46)	18.4 (16–21)	961.02 (924.69–1041.02)	656.24 (614.39–711.78)	0.74 (0.69–0.78)
<i>Lithobates sylvaticus</i> (90)	0.8 (0.7–0.9)	10.57 (7–15)	24 (5–36)	21.4 (11–38)	1062.37 (843.19–1437.5)	759.82 (669.33–1050.57)	0.76 (0.63–0.84)
<i>Pseudacris crucifer</i> (11)	0.75 (0.65–0.81)	11	28 (5–114)	16.18 (15–17)	984.96 (913.86–1122.62)	656.36 (569.9–689.03)	0.53 (0.27–0.8)
<i>Pseudacris streckeri</i> (17)	0.59 (0.38–0.82)	14	8 (5–22)	24.71 (15–34)	1479.33 (1257.25–1925.78)	878.45 (521.88–1001.36)	0.63 (0.3–0.86)
<i>Rana draytonii</i> (17)	0.51 (0.27–0.66)	15	13 (7–83)	9.41 (7–12)	1776.46 (1586.25–2179.73)	405.28 (331.77–493.63)	0.71 (0.55–0.8)
<i>Rana luteiventris</i> (25)	0.55 (0.37–0.69)	8	15 (6–205)	9.16 (5–11)	1200.7 (1077.99–1563.67)	494.77 (254–579.38)	0.66 (0.41–0.74)
<i>Rana pretiosa</i> (23)	0.32 (0.16–0.55)	10.39 (7–13)	34 (26–299)	9.35 (5–18)	1195.73 (924.34–1341.85)	515.32 (424.91–631.07)	0.55 (0.37–0.81)
<b>Caudata</b>							
<i>Ambystoma barbouri</i> (76)	0.78 (0.54–0.88)	11	22 (8–43)	28.28 (21–34)	1274.78 (1189.33–1337.51)	889.87 (811.77–967.52)	0.68 (0.55–0.75)
<i>Ambystoma maculatum</i> (97)	0.69 (0.45–0.75)	9.16 (7–14)	24 (8–36)	20.74 (16–35)	1074.81 (961.91–1406.61)	752.9 (682.96–931.33)	0.74 (0.37–0.8)
<i>Desmognathus fuscus</i> (5)	0.39 (0.15–0.65)	5	26 (22–35)	26.4 (26–27)	1258.69 (1224.99–1271.15)	827 (813.24–849.54)	0.62 (0.58–0.67)
<i>Dicamptodon aterrimus</i> (3)	0.48 (0.43–0.53)	9	91 (90–180)	6.33 (5–7)	1039.28 (1029.16–1046.55)	527.8 (469.46–564.46)	0.36 (0.32–0.41)
<i>Dicamptodon copei</i> (29)	0.69 (0.48–0.87)	11	18 (6–81)	13.86 (13–15)	905.66 (826.03–1074.59)	652.43 (628.31–678.36)	0.57 (0.48–0.74)
<i>Ensatina eschscholtzii</i> (4)	0.8 (0.75–0.84)	10	11.5 (5–19)	10 (9–11)	2140.32 (2039.14–2308.24)	368.69 (341.99–380.48)	0.63 (0.6–0.72)
<i>Hydromantes brunus</i> (6)	0.52 (0.38–0.61)	10	10 (7–18)	9.5 (8–11)	2021.39 (2008.05–2037.4)	347.91 (291.31–424.01)	0.7 (0.63–0.75)
<i>Hydromantes platycephalus</i> (15)	0.44 (0.25–0.64)	10	13 (6–21)	6.93 (4–9)	1786.55 (1523.97–2159.97)	466.68 (326.23–576.76)	0.65 (0.55–0.73)
<i>Plethodon albagula</i> (21)	0.48 (0.44–0.55)	20	16 (10–24)	31 (31–31)	1349.69 (1348.91–1350.48)	833.13 (832.48–833.85)	0.66 (0.66–0.66)
<i>Plethodon cinereus</i> (1)	0.6	7	122	22	1348.41 (1348.41–1348.41)	843.96	0.77
<i>Taricha granulosa</i> (9)	0.4 (0.26–0.77)	6	16 (6–32)	4 (2–13)	746.76 (614.95–1612.18)	522.65 (480.12–609.21)	0.73 (0.69–0.82)



587

588 **Figure 1.** (Top row) Maps of predicted genetic diversity, species richness, and genetic differentiation at genetic sample sites (points)  
 589 based on spatial MEMs. MEMs were able to recover known patterns of species richness, which are negatively correlated with spatial  
 590 patterns of genetic diversity. Patterns of genetic differentiation mirror those of species richness. (Bottom row) Maps depicting the  
 591 environmental variables predicted to have simultaneous effects on genetic diversity and species richness, and variation partitioning  
 592 results. Note land cover heterogeneity is a categorical variable and this map represents different land cover classes.



**Figure 2.** Structural equation model results. (A) Our hypothesized conceptual model. Line color denotes predicted directions of effect. (B) Results for model using environments within 40 km of sites (results for all spatial scales are in Tables S1-S4). Regression coefficients with standard errors are shown along each path. Paths between variables where no effect was detected are colored in gray (see Table S3 for a complete summary of all paths).  $R^2$ , the proportion of variation explained by the model, is given for genetic diversity and species richness. 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.

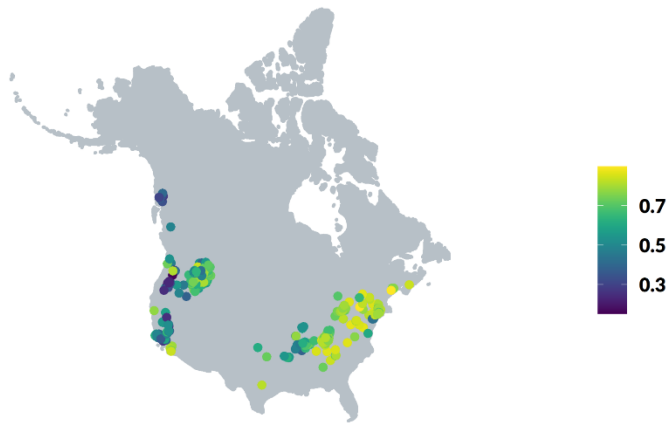
## **Determinants of genetic diversity and species richness of North American amphibians**

### **Supplementary Information**

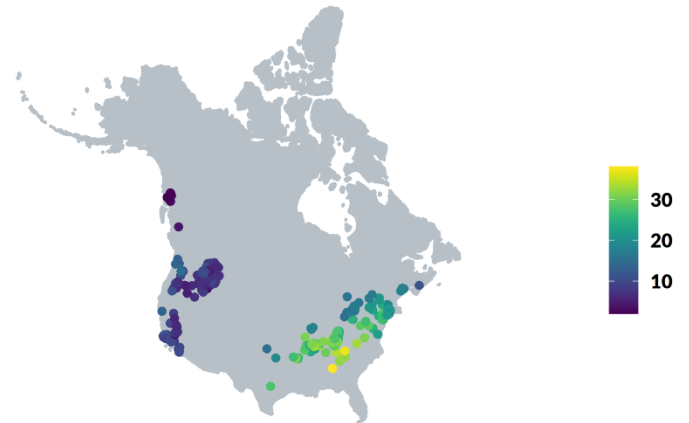
Figures S1-3

Tables S1-4

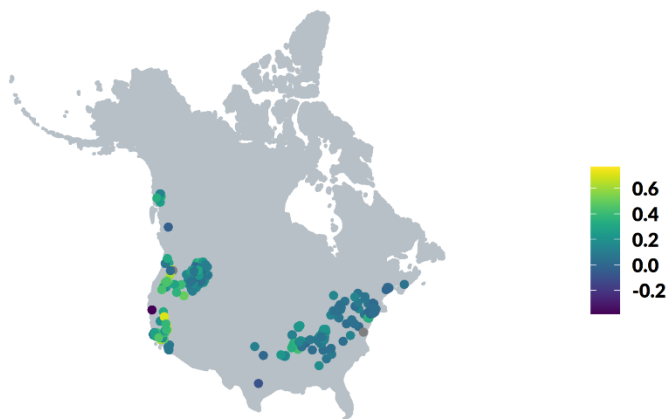
### Genetic diversity



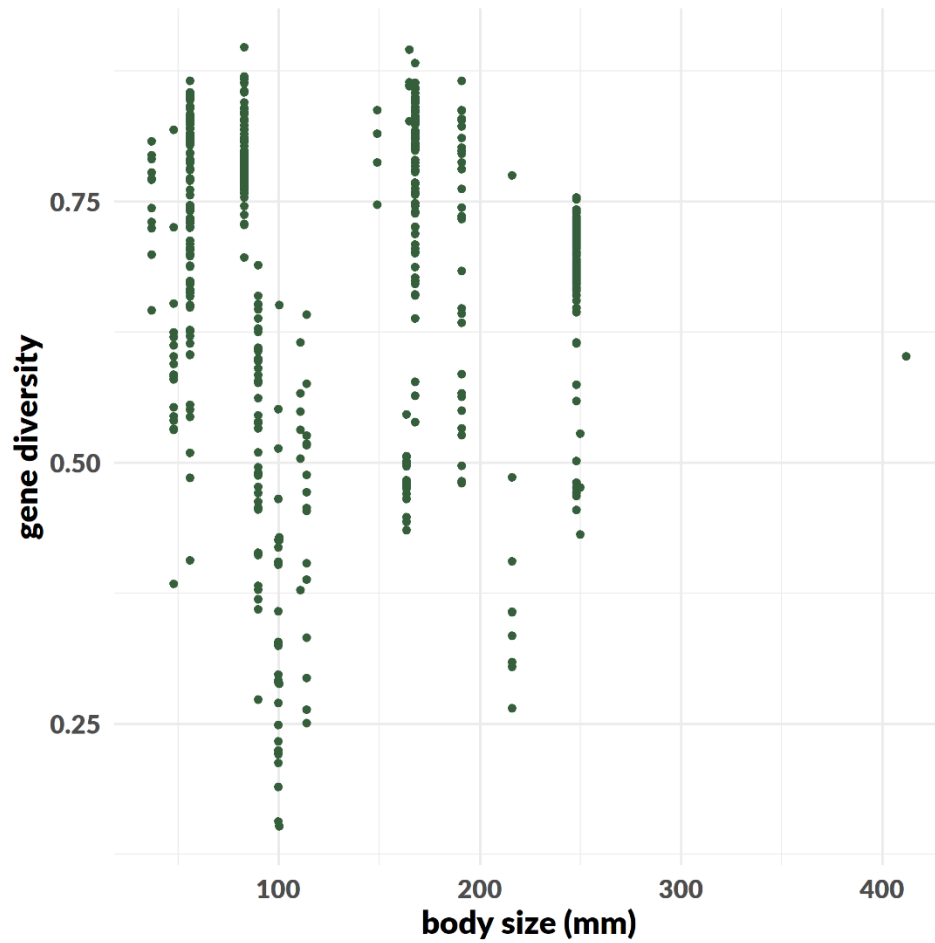
### Species richness



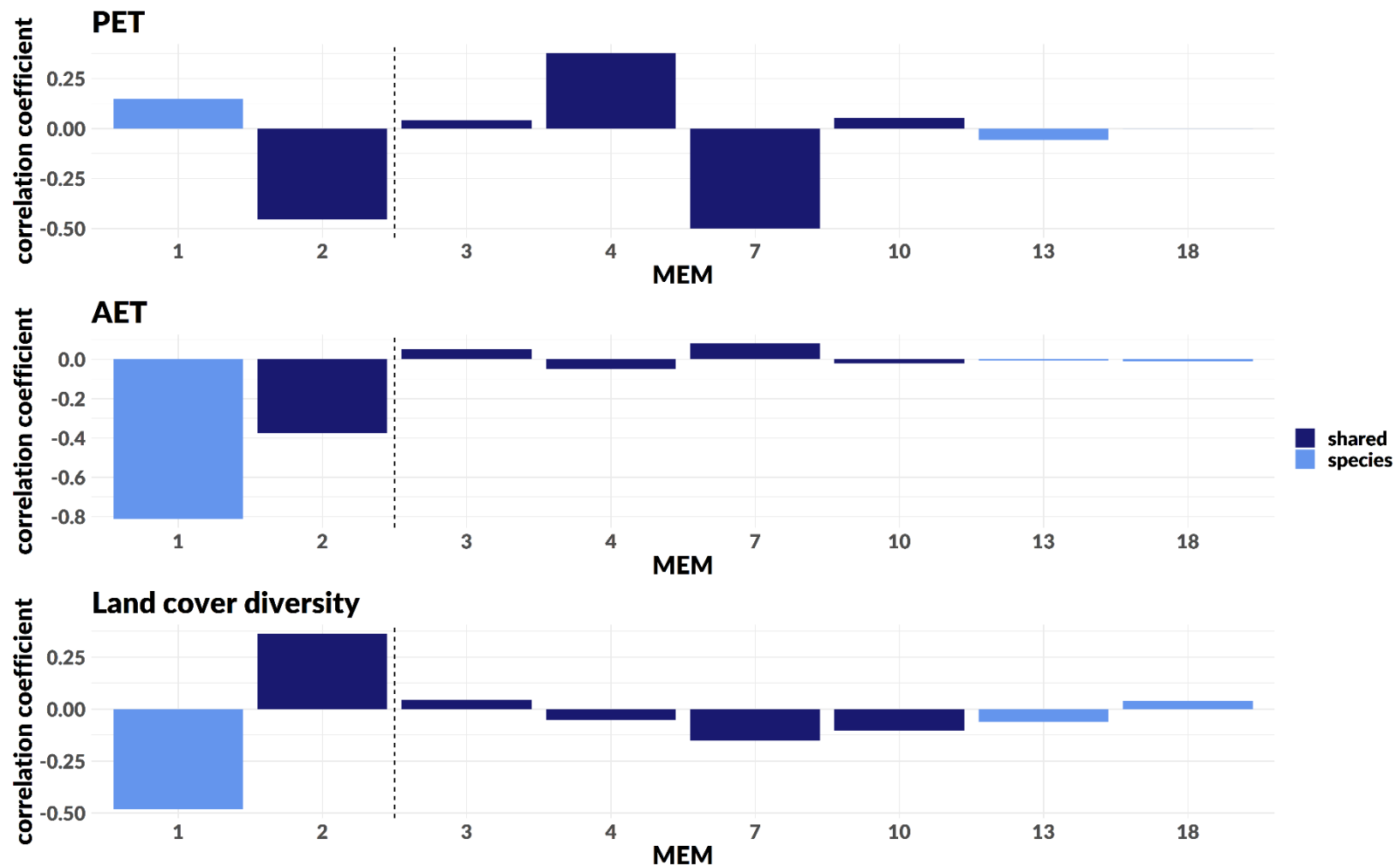
### Genetic differentiation



**Figure S1.** Maps of raw data for genetic diversity, species richness, and genetic differentiation. Genetic diversity is Nei's gene diversity; species richness is the number of amphibian species' ranges overlapping each site; genetic differentiation is site-specific  $F_{ST}$ .



**Figure S2.** Relationship between genetic diversity and body size (snout-vent length). We detected no relationship using a mixed-effects model including a random intercept for species ( $\beta = -0.03 \pm 0.2$  SE).



**Figure S3.** Spatial patterns (MEMs) for genetic diversity and species richness are correlated with environments: energy availability (potential evapotranspiration; PET), water availability (actual evapotranspiration; AET), and heterogeneity (land cover diversity). MEMs are ordered along the x-axis according to spatial scale explained, from broadest (MEM1) to finest (MEM18). MEMs to the left of the dashed lines indicate the broadest-scale patterns with Moran's  $I > 0.25$  used to produce maps of genetic diversity and species richness. Light blue bars are MEMs explaining spatial patterns of species richness, and dark blue bars are MEMs explaining spatial patterns shared by genetic diversity and species richness. No MEMs explained spatial patterns in genetic diversity only.



1 **Table S1.** Path coefficients and standard errors for SEM model with heterogeneity measured  
2 within a 10 km buffer around sites (Fisher's  $C = 0.28$ ,  $p = 0.87$ , 2 degrees of freedom). Genetic  
3 diversity  $R^2_m = 0.04$  (variance explained by fixed effects);  $R^2_c = 0.92$  (variance explained by  
4 fixed and random effects); species richness  $R^2 = 0.87$ .

5

<b>Response</b>	<b>Predictor</b>	<b>Estimate <math>\pm</math> SE</b>
gene diversity	potential evapotranspiration	0.16 $\pm$ 0.16
gene diversity	actual evapotranspiration	0.13 $\pm$ 0.14
gene diversity	heterogeneity	0.16 $\pm$ 0.09
species richness	potential evapotranspiration	0.18 $\pm$ 0.02
species richness	actual evapotranspiration	0.90 $\pm$ 0.02
species richness	heterogeneity	0.09 $\pm$ 0.02

6

7 **Table S2.** Path coefficients and standard errors for SEM model with heterogeneity measured  
8 within a 25 km buffer around sites (Fisher's  $C = 0.59$ ,  $p = 0.74$ , 2 degrees of freedom). Genetic  
9 diversity  $R^2_m = 0.04$  (variance explained by fixed effects);  $R^2_c = 0.92$  (variance explained by  
10 fixed and random effects); species richness  $R^2 = 0.88$ .

11

<b>Response</b>	<b>Predictor</b>	<b>Estimate <math>\pm</math> SE</b>
gene diversity	potential evapotranspiration	0.20 $\pm$ 0.16
gene diversity	actual evapotranspiration	0.07 $\pm$ 0.16
gene diversity	heterogeneity	0.19 $\pm$ 0.12
species richness	potential evapotranspiration	0.19 $\pm$ 0.01
species richness	actual evapotranspiration	0.90 $\pm$ 0.02
species richness	heterogeneity	0.10 $\pm$ 0.02

12

13 **Table S3.** Path coefficients and standard errors for SEM model with heterogeneity measured  
14 within a 40 km buffer around sites (Fisher's  $C = 0.49$ ,  $p = 0.78$ , 2 degrees of freedom). Genetic  
15 diversity  $R^2_m = 0.04$  (variance explained by fixed effects);  $R^2_c = 0.93$  (variance explained by  
16 fixed and random effects); species richness  $R^2 = 0.89$ .

17

<b>Response</b>	<b>Predictor</b>	<b>Estimate <math>\pm</math> SE</b>
gene diversity	potential evapotranspiration	0.22 $\pm$ 0.17
gene diversity	actual evapotranspiration	0.10 $\pm$ 0.17
gene diversity	heterogeneity	0.15 $\pm$ 0.11
species richness	potential evapotranspiration	0.20 $\pm$ 0.01
species richness	actual evapotranspiration	0.91 $\pm$ 0.01
species richness	heterogeneity	0.09 $\pm$ 0.01

18

19

20 **Table S4.** Path coefficients and standard errors for SEM model with heterogeneity measured  
21 within an 80 km buffer around sites (Fisher's  $C = 0.40$ ,  $p = 0.82$ , 2 degrees of freedom). Genetic  
22 diversity  $R^2_m = 0.03$  (variance explained by fixed effects);  $R^2_c = 0.92$  (variance explained by  
23 fixed and random effects); species richness  $R^2 = 0.90$ .

24

<b>Response</b>	<b>Predictor</b>	<b>Estimate <math>\pm</math> SE</b>
gene diversity	potential evapotranspiration	0.16 $\pm$ 0.19
gene diversity	actual evapotranspiration	0.24 $\pm$ 0.20
gene diversity	heterogeneity	0.09 $\pm$ 0.09
species richness	potential evapotranspiration	0.22 $\pm$ 0.01
species richness	actual evapotranspiration	0.95 $\pm$ 0.01
species richness	heterogeneity	0.07 $\pm$ 0.01

25

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27