INTRODUCTION

Although species richness is higher in the tropics for most taxa, the details of diversity patterns differ among species groups. In North America for instance, vertebrate richness generally increases with resource availability, but mammals and birds tend to have higher species richness in dry, mountainous areas, and reptiles and amphibians are more diverse in wet, lower elevation regions (Buckley & Jetz, 2007; Currie, 1991; Hawkins et al., 2012; Jenkins et al., 2013; Marin & Hedges, 2016; Roll et al., 2017). These different patterns suggest that while species richness generally increases with resource availability, taxon-specific traits that determine the types of resources species can exploit cause richness patterns to diverge from a strictly latitudinal gradient. Such macroecological patterns...
ultimately arise from processes operating at the population level (e.g. demographic factors; Charlesworth et al., 1982; Schmidt, Dray, & Garroway, 2022; Schmidt, Muñoz, et al., 2022); thus, we might achieve a more cohesive understanding of the general factors that underlie species richness gradients across taxonomic groups by linking them to microevolutionary processes. Biogeographic patterns of population genetic diversity can suggest links between population processes and macroecological patterns (Lawrence & Fraser, 2020; Leigh et al., 2021; Schmidt, Dray, & Garroway, 2022; Schmidt, Muñoz, et al., 2022). Broad-scale patterns of population genetic biodiversity across species have only recently begun to be mapped thanks to the accumulation of open data in public repositories. We are still in the early stages of understanding the links between microevolutionary processes and macroecological patterns (Manel et al., 2020; Mirlaldo et al., 2016; Schmidt, Dray, & Garroway, 2022; Theodoridis et al., 2020).

Genetic diversity is typically thought of as the most fundamental level of biodiversity because it bears on populations’ capacities to evolve adaptively in response to environmental change (Frankham, 1995). Patterns of genetic diversity and species richness are both products of population demography and micro-scale eco-evolutionary processes (Vellend & Geber, 2005), thus we might expect that environments can simultaneously shape the spatial distribution of both these levels of biodiversity on biogeographic scales (Lawrence & Fraser, 2020; Schmidt, Dray, & Garroway, 2022). Common environmental causes should therefore generate spatial covariation between patterns of genetic diversity and species richness, as has been recently demonstrated in North American mammals (Schmidt, Dray, & Garroway, 2022). Whether this is also true in other taxa is currently unknown.

The most well-supported hypotheses explaining the processes underlying species richness gradients are those related to ecological limits (Etienne et al., 2019; Hagen et al., 2021; Rabosky, 2009). Hypotheses based on ecological limits suggest that broad-scale diversity patterns can arise from spatial variation in resources and how those resources are divided across local environments—simply put, resource availability sets limits on the number of individuals and species a region can support (Kerr & Packer, 1997). The local availability and distribution of resources are the basis for two specific ecological limits hypotheses: the more individuals hypothesis (Wright, 1983), and the environmental and resource heterogeneity hypothesis (Allouche et al., 2012; Stein et al., 2014). The more individuals hypothesis posits that resource-rich regions can support larger populations and communities, and thus should have more species than resource poor regions. The heterogeneity hypothesis suggests that environmental and resource heterogeneity can increase species richness due to greater niche availability which allows more species to coexist, but with smaller population sizes because resources are partitioned among niches. These hypotheses are not mutually exclusive: total energy availability and heterogeneity are thought to interactively produce biodiversity patterns (Kerr & Packer, 1997; Schmidt, Dray, & Garroway, 2022). Although the more individuals and heterogeneity hypotheses both invoke population processes, they are rarely extended to or tested on the genetic level (but see Lawrence & Fraser, 2020; Schmidt, Dray, & Garroway, 2022; Schmidt, Muñoz, et al., 2022).

The relationship between resource heterogeneity and species richness seems likely to hold across taxa (Brown, 1981; Schmidt, Dray, & Garroway, 2022; Stein et al., 2014); however, the general relevance of the more individuals hypothesis for ectotherms is uncertain (Buckley & Jetz, 2010). Compared to endotherms, ectotherms have lower energy requirements and can behaviorally thermoregulate, meaning their abundances are less likely to be limited by resource-related ecological limits (Buckley & Jetz, 2010; Pough, 1980). Instead, ectotherm distributions, and therefore species richness, seem more directly constrained by environmental temperature because relatively few species have evolved thermal adaptations required for expanding into cooler regions (Buckley & Jetz, 2007, 2010). Further, the evolution of traits associated with better survival in temperate regions may have additional effects on speciation dynamics in ectotherms. For example, species turnover tends to be higher among viviparous squamate reptiles, which typically occupy cooler regions (Pyron & Burbrink, 2014).

The determinants of species richness across terrestrial vertebrates are widely reported to be related to resource availability as estimated by ecosystem-wide energy availability (e.g. potential evapotranspiration, PET; primary productivity), water-energy balance (e.g. actual evapotranspiration, AET; precipitation), and heterogeneity (e.g. elevation variability, land cover diversity) (Buckley & Jetz, 2007; Currie, 1991; Hawkins et al., 2003; Jiménez-Alfaro et al., 2016; Kerr & Packer, 1997; Rodríguez et al., 2005; Stein et al., 2014). Amphibians are interesting because they are constrained both by water availability and temperature. Water availability is consistently identified as an important driver of diversity in amphibians (Buckley & Jetz, 2007; Rodríguez et al., 2005). Indeed in Europe, the best predictors of species richness in mammals and birds shift from energy to water availability at decreasing latitudes, but amphibian species richness remains strongly related to water-energy balance regardless of latitude (Whittaker et al., 2007). Water availability is thus an important point of distinction in the major determinants of resource availability between amphibians and other terrestrial species that allows us to explore how the causes of species richness and genetic diversity might diverge across taxa with very different ecological and physiological constraints.

The causes of population genetic diversity are rarely studied at the same time or scale as patterns of species richness (but see Marshall & Camp, 2006; Schmidt, Dray, & Garroway, 2022; Schmidt, Muñoz, et al., 2022), yet the presumed mechanisms related to the more individuals hypothesis and heterogeneity hypotheses are closely related to population-level processes (Schmidt, Dray, & Garroway, 2022). The more individuals hypothesis predicts a positive relationship between species richness and population genetic diversity because bigger populations and communities tend to have higher levels of genetic and species diversity, respectively (Hubbell, 2001; Kimura, 1983; Schmidt, Dray, & Garroway, 2022). On the other hand, heterogeneity is predicted to cause negative
correlations between genetic diversity and species richness by increasing the number of species a given area can support, which in turn reduces population size and limits gene flow due to increased niche specialization. Heterogeneous environments also facilitate population differentiation due to spatially varying selection. In mammals, evolutionary processes acting on the population level scaled up and interacted with resource availability and heterogeneity to produce biogeographic patterns of genetic diversity and species richness (Schmidt, Dray, & Garroway, 2022).

Whether the above-described mechanisms related to energy and niche availability predict patterns of species richness and genetic diversity in ectotherms is unclear. To test the extent to which ecological limits predict amphibian biodiversity patterns on genetic and species levels, we analysed publically archived, previously published microsatellite genotype data from 19 North American amphibian species (8 frogs, 11 salamanders), with 13,680 individuals sampled at 554 sites (Figure 1, Table 1). Our first objective was to identify existing spatial patterns in genetic diversity and population differentiation, and quantify the extent to which genetic diversity and species richness spatially covary. We then tested whether limits on resources and niche availability jointly determined genetic diversity and species richness using structural equation models, which allowed us to evaluate the more individuals hypothesis and the heterogeneity hypothesis simultaneously across genetic and species level biodiversity. We based our conceptual structural equation model framework (Figure 2a) on previous findings in mammals (Schmidt, Dray, & Garroway, 2022) and adapted it for amphibians, allowing us to explore whether similar environmental features contribute to diversity gradients across two major endothermic and ectothermic taxa in North America.

2 | MATERIALS AND METHODS

2.1 | Biodiversity data

2.1.1 | Genetic diversity and differentiation

We used genotypes (i.e. called allele sizes) of North American amphibians compiled by (Schmidt & Garroway, 2021b). This dataset was assembled from raw microsatellite datasets publicly archived in Dryad (DataDryad.org). To identify datasets, we conducted a systematic search of the Dryad data repository with the following keywords: species name (e.g. *Plethodon cinereus*), "microsat*", "short tandem*" and "single tandem*". We used the IUCN Red List database to obtain a list of amphibian species native to North America for the search. Our inclusion criteria required that datasets had spatial references for sample localities, were located in

![FIGURE 1](Images/figure1.png) (Top row) Maps of predicted genetic diversity, species richness, and genetic differentiation at genetic sample sites (points) of North American amphibians (frogs and salamanders) based on spatial MEMs. MEMs were able to recover known patterns of species richness, which are negatively correlated with spatial patterns of genetic diversity. Patterns of genetic differentiation mirror those of species richness. (Bottom row) Maps depicting the environmental variables predicted to have simultaneous effects on genetic diversity and species richness, and variation partitioning results. Note land cover heterogeneity is a categorical variable and this map represents different land cover classes. Maps are a North America Albers equal area conic projection.
North America, and sampled neutral microsatellite loci. In total the datasets included genotypes for 13,680 individuals spanning 19 species sampled at 554 locations in the contiguous United States and Canada (Table 1). We estimated genetic diversity for each species at each sample locality. We used gene diversity as a measure of genetic diversity because it is minimally affected by sample

### Table 1: Data Summary

| Species (Number of Sites) | Gene Diversity | Loci | Individuals | Species richness | PET | AET | Heterogeneity |
|--------------------------|----------------|------|-------------|-----------------|-----|-----|--------------|
| **Anura**                |                |      |             |                 |     |     |              |
| Ascaphus montanus (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) |
| Lithobates pipiens (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) |
| Lithobates sylvaticus (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) |
| Pseudacris crucifer (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) |
| Pseudacris streckeri (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) |
| Rana draytonii (17)      | 0.51 (0.27–0.66) | 15   | 13 (7–83)   | 9.41 (7–12)     | 1776.46 (1586.25–1925.78) | 405.28 (331.77–493.63) | 0.71 (0.55–0.8) |
| Rana luteiventris (25)   | 0.55 (0.37–0.69) | 8    | 15 (6–205)  | 9.16 (5–11)     | 1200.7 (1077.99–1041.02) | 494.77 (254–579.38) | 0.66 (0.41–0.74) |
| Rana pretiosa (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) |
| **Caudata**              |                |      |             |                 |     |     |              |
| Ambystoma barbouri (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) |
| Ambystoma maculatum (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) |
| Desmognathus fuscus (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) |
| Dicamptodon aterrimus (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) |
| Dicamptodon copei (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) |
| Ensatina eschscholtzii (4) | 0.8 (0.75–0.84) | 10   | 11.5 (5–19) | 10 (9–11)      | 2140.32 (2039.14–2308.24) | 366.69 (341.99–380.48) | 0.63 (0.6–0.72) |
| Hydromantes brunus (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) |
| Hydromantes platycephalus (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) |
| Plethodon albagula (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) |
| Plethodon cinereus (1)   | 0.6             | 7    | 122         | 22             | 1348.41                     | 843.96                | 0.77              |
| Taricha granulosa (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) |

**Note:** Summary of aggregated raw genetic data: mean gene diversity, mean number of loci, median number of individuals at sites per species. Species richness is the mean species richness at sites. Environmental variables (40 km buffer): potential evapotranspiration (PET); actual evapotranspiration (AET); heterogeneity is the mean land cover diversity measured with Simpson's Index. Ranges of values are given in parentheses where applicable.
size (Charlesworth & Charlesworth, 2010; Nei, 1973). Gene diversity is equivalent to expected heterozygosity at Hardy–Weinberg equilibrium, but the term gene diversity is used when estimating the average probability that two sampled alleles are different in non-random mating populations (Nei, 1973). Our measure of differentiation was population-specific $F_{ST}$, which estimates the probability of identity by descent between alleles in a population compared to pairs of alleles from all populations in a sample (Weir & Goudet, 2017). Population-specific $F_{ST}$ differs from pairwise $F_{ST}$ in that it measures how far single populations in a sample have diverged from a common ancestral population—this means it is comparable across studies and species. We estimated population-specific $F_{ST}$ using the ‘betas’ function in the ‘hierfstat’ package in R (Goudet & Jombart, 2015). Because it is a measure of divergence, population-specific $F_{ST}$ requires a minimum of two sample sites to be estimated. Due to this restriction, we were unable to estimate population-specific $F_{ST}$ for two studies where only a single site was sampled ($n = 552$).

### 2.1.2 Species richness

We estimated species richness at each of our genetic diversity sample sites using amphibian range extent data (shapefiles) for all native, extant species in North America available from the IUCN RedList database (274 species; IUCN, 2019). We measured species richness as the number of species’ ranges overlapping each genetic sample site using a spatial join in ArcMap 10.3.1 (ESRI).

### 2.2 Diversity maps and spatial variation partitioning

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

Next, we determined the extent to which spatial patterns in genetic diversity and species richness were shared using variation partitioning. Because our MEM analysis for both levels of biodiversity had the same spatial coordinates of sites as inputs, the resulting spatial MEMs were directly comparable. This was not the case for genetic differentiation, which had fewer sample sites. We therefore did not partition variation in genetic differentiation because these
MEMs are not the same as those computed for genetic diversity and species richness.

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

\[ y_{SR} - a_1 + \beta_{S,1}(MEM_{S,1}) + \beta_{S,2}(MEM_{S,2}) + ... + \beta_{S,8}(MEM_{S,8}) + \epsilon \]  
\[ (1) \]

\[ y_{GD} - a_2 + \beta_{G,1}(MEM_{G,1}) + \beta_{G,2}(MEM_{G,2}) + ... + \beta_{G,5}(MEM_{G,5}) + \epsilon \]  
\[ (2) \]

\[ y_{SR} - a_1 + \beta_{SG,1}(MEM_{SG,1}) + \beta_{SG,2}(MEM_{SG,2}) + ... + \beta_{SG,8}(MEM_{SG,8}) + \epsilon \]  
\[ (3) \]

\[ y_{GD} - a_2 + \beta_{SG,1}(MEM_{SG,1}) + \beta_{SG,2}(MEM_{SG,2}) + ... + \beta_{SG,5}(MEM_{SG,5}) + \epsilon \]  
\[ (4) \]

where $a_1$ is the grand mean, and $MEM_{S,j}$ and $MEM_{SG,j}$ are the set of MEMs selected for species richness (8 MEMs) and genetic diversity (5 MEMs), respectively. The coefficients of variation ($R^2$) from Equations 1 and 2 give the total amount of variation explained by spatial patterns for species richness and genetic diversity. Subtracting these values from 1 gives the amount of non-spatial variation. $MEM_{SG,j}$ represents the set of MEMs shared by both species richness and genetic diversity (5 MEMs). $R^2$ values from Equations 3 and 4 tell us the amount of variation in each response variable which can be explained by spatial variation shared at both levels of diversity. When subtracted from the total spatial variation in genetic diversity or species richness (Equations 1 and 2), we get the proportion of non-shared spatial variation.

2.3 | Structural equation modelling

2.3.1 | Environmental data

We measured resource and niche heterogeneity by computing Simpson’s Diversity Index for landcover categories within buffers at each site. We obtained a 30 m resolution map of North American landcover data from the Commission for Environmental Cooperation (CEC, NRCan/CCMEO, USGS, INEGI, CONABIO, and CONAFOR, 2015). This map is based on 2015 satellite imagery and has 19 standard land cover classifications including forests, shrubland, grassland, wetlands, cropland, barren land and built-up land. Because this variable is scale-dependent, we recorded heterogeneity within 4 buffer sizes (10, 25, 40 and 80 km) around each site.

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

We lacked an appropriate general measure of population size for the sites where genetic diversity was sampled, thus we did not incorporate it into our proposed causal framework. Without population size mediating the effects of environments on genetic diversity and species richness in our structural equation models, we predicted that resource availability would have direct positive effects on both genetic diversity and species richness, and that heterogeneity would have direct negative and positive effects on genetic diversity and species richness, respectively.

2.3.2 | Analysis

We used structural equation modelling (SEM) to determine whether genetic diversity and species richness are shaped by differential ecological limits due to limits on resources and niche availability. Structural equation modelling begins with a causal diagram, or conceptual model, where paths between variables represent hypothesized causal relationships (Figure 2a). Hypotheses are envisioned as a network where variables are nodes, and paths connecting them represent causal relationships. In SEM, the effects of multiple predictors are simultaneously assessed for multiple response variables (Shipley, 2016). We implemented structural equation models using the piecewiseSEM package (2.0.2), which uses a local estimation approach for models in the hypothesis network allowing for the incorporation of more complex model types (Lefcheck et al., 2019). Model fit is evaluated using tests of directed separation (Shipley, 2016), which determine whether an association exists between two variables in the network conditional on each of their causes. If two variables are not conditionally independent, the model is updated by adding a path between them to make the model more consistent with the data. p-values from tests of directed separation are used to calculate Fisher’s C, which is then used to calculate an overall p-value for the model network. Models are a good fit to the data when $p > 0.05$, indicating the null hypothesis—the proposed hypothesis network—is not rejected.

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

2.4 | Effect of heterogeneity on population differentiation

Finally, we tested whether landscape heterogeneity was related to increased population differentiation. We regressed heterogeneity on population-specific FST using a hierarchical model with a random effect for species accounting for differences in mean FST (intercepts) while allowing the strength and direction of the effect of heterogeneity (slopes) to vary across species. To account for spatial variation we included MEMs describing spatial patterns in FST as covariates. We performed these analyses in parallel across all four heterogeneity buffers.

3 | RESULTS

3.1 | Spatial patterns in genetic variation

We detected spatial patterns across genetic diversity, genetic differentiation, and species richness (Figure 1). The major axis of broad-scale variation was longitudinal, and across all three biodiversity metrics the western sample sites showed little variation whereas spatial patterns were more complex in the east and appeared to vary latitudinally. This broad longitudinal pattern is consistent with environmental variation in North America (Figure 1) as well as genetic diversity and species richness patterns in mammals (Schmidt, Dray, & Garroway, 2022). We recovered known patterns of amphibian species richness (Currie, 1991) with MEMs, where richness was highest in the southeastern United States (Figure 1). The western United States, which is hotter and drier, had a comparatively low number of species. In eastern sample sites, genetic diversity increased with latitude, while differentiation and species richness increase towards the tropics. Genetic diversity and differentiation in the western samples were in the mid-range of values across sample sites (Figure 1). We note that due to the spatial arrangements of sample sites, we were unable to make inferences about genetic variation across the centre of the continent.

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

3.2 | Common causes of genetic diversity and species richness

Our conceptual model (Figure 2a) fit the data well (Fisher's C = 0.49, p = 0.78, 2 degrees of freedom) with no additional links suggested at any scale. Note that for SEM, p > 0.05 means that our conceptual model is not rejected. We present results from the 40 km buffer in the main text; results from all models can be found in Tables S1–S4. Species richness was well explained (R² = 0.89) and increased with water availability and environmental heterogeneity (Figure 2, Table S3). Water availability had the strongest effect on species richness. Genetic diversity was not well predicted by any variables in our model (R² = 0.04; Figure 2). Residuals from genetic diversity models did not exhibit spatial autocorrelation. Species richness residuals were spatially autoregressive at local scales (Moran’s I = 0.06). In general, the environmental covariates in our models captured broad spatial patterns well, and we did not incorporate fine-scale spatial structure into our models as this was likely due to clustered sampling of some species. Lastly, genetic differentiation within species decreased with heterogeneity (μ = −0.32 ± 0.10 SE) at the most local spatial scale we tested (10 km buffer), but this relationship disappeared at larger buffer sizes.

4 | DISCUSSION

We found spatial variation shared across genetic diversity, differentiation and species richness at broad spatial scales (Figure 1). In general, species richness and genetic diversity were negatively correlated: areas with high species richness tended to have genetically differentiated populations with relatively low genetic diversity, and vice versa. Sample sites for which we obtained genetic data were spatially distributed into western and eastern clusters with a gap in the centre of the continent. There was a latitudinal gradient in genetic diversity across eastern sites which varied in the opposite direction of the gradient in species richness and genetic differentiation. These patterns are consistent with our predicted effects of ecological limits related to heterogeneity, however, these relationships were not well-reflected by our structural equation model (Figure 2). Our variation partitioning suggests that all spatial variation in genetic diversity was shared with species richness (Figure 1). However, the environments that predicted species richness did not predict genetic diversity well. This finding suggests that environmental factors have species-specific effects on genetic diversity that do not generalize well at multispecies, continental scales, or that the environmental variables we selected are too coarsely resolved to predict genetic diversity. Nevertheless, variation partitioning results
and the broad-scale patterns of genetic diversity and differentiation we detected indicate that ecological limits on the sizes of populations and communities likely play a role in determining biodiversity across population genetic and species levels in amphibians.

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

Interestingly, although genetic diversity was not affected by environmental heterogeneity at any scale, genetic differentiation decreased with heterogeneity at the most local scale we tested (10 km), and genetic differentiation was relatively low in the northeast where landscape heterogeneity was higher (Figure 1). If heterogeneity increases niche availability and creates opportunities for specialization and divergence, we predicted that it would increase genetic differentiation. However, the pattern we detect here may be expected if species that were capable of recolonizing northern regions following glaciation tend to be widely distributed generalists that maintain population connectivity over relatively large geographic distances (Smith et al., 2005; Zeisset & Beebee, 2008). Niche partitioning in amphibians may also occur at finer scales within suitable habitats (Cloyd & Eason, 2017; Karlin et al., 1984)—for example by modifying microhabitat usage, diets, foraging strategies, or behaviours across species. Thus, the landscape level metric we use here may not capture the varying ways that environmental heterogeneity affects genetic diversity and population differentiation if species respond to environments in different ways. These patterns point to the importance of species-specific responses to environmental conditions and environmental instability (Urban, 2004) in generating biogeographic patterns of genetic diversity and species richness in amphibians.

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

Although we did not detect clear latitudinal or longitudinal gradients in nuclear genetic diversity across North America, previous findings suggest mitochondrial genetic diversity in amphibians and other ectotherms varies latitudinally and mirrors species richness patterns (Mandel et al., 2020; Miraldo et al., 2016). Miraldo et al. (2016) found that amphibian mitochondrial genetic diversity in North America was highest in the species-rich southeastern United States. They suggested this pattern may be related to the evolutionary speed hypothesis for the species richness gradient, where presumably high environmental temperature increases rates of population divergence and speciation through its effects on mutation rate and generation time (Mraldo et al., 2016). In addition to a negative correlation between patterns of nuclear genetic diversity and species richness, we detected no effect of temperature on nuclear genetic diversity in our SEM, casting doubt on this hypothesis for amphibians. Furthermore, a lack of latitudinal gradient indicates that nuclear genetic diversity is not related to temperature in a straightforward way. Environmental temperature has varied and complex effects on metabolism and mitochondrial processes (Munro & Treberg, 2017; Zhang & Wong, 2021), making relationships with nuclear and mitochondrial mutation rates unlikely to be generalizable (Lanfear et al., 2007; Schmidt & Garroway, 2021a). More generally, mitochondrial DNA alone is not a reliable marker for detecting intra-specific demographic patterns (Bazin et al., 2006; Galtier et al., 2009; Schmidt & Garroway, 2021a). Thus, marker choice is very likely responsible for the diverging patterns we find here. This also appears to be true in mammals, where patterns of genetic diversity measured using mitochondrial DNA (Miraldo et al., 2016; Theodoridis et al., 2020) and nuclear DNA (Schmidt, Dray, & Garroway, 2022) also trended in opposite directions.

Although the environmental variables we selected did not predict genetic diversity well in our SEM, our maps of genetic diversity and species richness (Figure 1) and variation partitioning results suggest that genetic diversity and species richness are likely related to ecological limits, as previously reported in mammals (Schmidt, Dray, & Garroway, 2022). Interestingly, similar proportions of variation in genetic diversity (~25%) could be attributed to spatial processes across both taxa. Additionally, variation partitioning indicated it was the case in both groups that spatial variation in genetic diversity was primarily due to factors that also shaped species richness, but other factors contribute to unique spatial variation in species richness. The overall negative correlation between spatial patterns of species richness and genetic diversity in amphibians and mammals (Schmidt, Dray, & Garroway, 2022) suggests heterogeneity and niche partitioning may be major contributors to diversity across genetic and species
levels in endothermic and ectothermic vertebrates, despite difference in total energy use (Buckley & Jetz, 2010). Heterogeneity has previously been suggested as a universal driver of species richness across taxa (Stein et al., 2014) however the extent to which it simultaneously shapes population genetic diversity, differentiation and species richness remains to be tested across more species groups. In general, environmental factors and population-level processes related to ecological limits seem capable of generating an overall species richness gradient across taxa, but deviations from this general gradient are mediated by species’ differential interactions between environments, species traits, and population processes.

Genetic diversity and species richness are two important metrics for biodiversity conservation because they contribute to the resilience of populations and communities in rapidly changing environments (Oliver et al., 2015). Neutral genetic diversity is indicative of population mean fitness and the efficiency of selection in response to environmental change—although it is only weakly correlated with additive genetic diversity, it is nevertheless informative for conservation purposes because it reflects levels of inbreeding and the efficiency of selection due to its relationship to the effective population size (Frankham, 1995; Mittell et al., 2015). Amphibians are among the most imperilled vertebrates (Stuart et al., 2004) and are especially susceptible to environmental change. Macrogenetics approaches mapping multispecies patterns of genetic diversity at broad scales have great potential for incorporation into conservation policies targeting regional conservation of genetic diversity. However, complex ecological, physiological requirements, life histories and population dynamics may render this approach impractical for amphibians because the environmental factors affecting genetic diversity may differ depending on species (Schmidt & Garroway, 2021b). Species-specific measures of environmental heterogeneity, resource availability, and habitat suitability may prove to be more reliable predictors of genetic diversity, but may be less relevant for species richness. We are only beginning to explore broad scale patterns of intraspecific genetic diversity across several species, but it is already apparent that they are not as consistently clear as gradients in species richness (Manel et al., 2020; Miraldo et al., 2016; Schmidt, Dray, & Garroway, 2022; Theodoridis et al., 2020). The continued exploration of these patterns in other taxonomic groups will help build a more comprehensive picture of the distribution of genetic biodiversity across the globe.

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DATA AVAILABILITY STATEMENT

Data used in analyses are available from the Dryad Data Repository (DOI: 10.5061/dryad.1g1jwsv0m). Species range boundary files and environmental data are available from open online sources (see Methods). Code to reproduce analyses is available on GitHub: https://github.com/chloewsch/amphibians_genetics_species/

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BIOSKETCH
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Author contributions: C.J.G., and C.S. conceived of the study. C.S., J.M.S, S.D. and C.J.G. designed the study and C.S. conducted the analyses with input from S.D. and C.J.G. All authors contributed to data interpretation. C.S. wrote the first draft of the manuscript and all authors participated in editing subsequent manuscript drafts.

SUPPORTING INFORMATION
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