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RESEARCH ARTICLE

Partitioning genetic structure of a subterranean rodent at multiple spatial scales: accounting for isolation by barriers, distance, and environment

Nathan Alexander · Bradley J. Cosentino · Robert L. Schooley

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Abstract

Context Understanding genetic structure at multiple spatial scales and identifying drivers of genetic isolation are important for developing comprehensive conservation plans including for grassland conservation efforts. However, few studies account for multiple genetic isolation processes nor partition genetic variance among these processes.

Objectives We assess key processes that can create spatial genetic patterns including isolation by barrier (IBB), isolation by distance (IBD), and isolation by environment (IBE) for a widespread pocket gopher species (*Geomys bursarius*) and a spatially restricted subspecies (*Geomys bursarius illinoensis*). We further partition genetic variation to each isolating effect and identify genetic variation that was shared between processes.

Methods We used seven microsatellites to determine spatial genetic clustering and identify environmental factors impacting genetic similarities. Then, we used redundancy analysis to partition variance explained by IBB, IBD, and IBE.

Results Major rivers including the Mississippi River acted as barriers and explained the most genetic variation across the species. In contrast, IBD explained the most genetic variation for *G. b. illinoensis*. Gophers had genetic associations to soil sand percent and soil color, but IBE uniquely explained a small amount of genetic structure for *G. bursarius,* with additional variation shared with other isolating processes.

Conclusions Gopher genetic structure resulted from barriers, distance, and environmental factors at the species range as well as for a subspecies' region, but the relative amount of genetic variance assigned to unique isolating processes differed between scales. Delineation of conservation units should consider major rivers as natural boundaries, and finer-scale management should identify and protect areas close to source populations with similar soil friability. Our study exemplifies how analyzing gene flow at rangewide and regional scales can aid managers in developing localized strategies that fit within broader conservation units.

Keywords Genetic variation partitioning · *Geomys bursarius* · Isolation by barrier · Isolation by distance · Isolation by environment · Spatial scale

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N. Alexander (✉) · R. L. Schooley
Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA
e-mail: nba2@illinois.edu

B. J. Cosentino
Department of Biology, Hobart and William Smith Colleges, Geneva, NY, USA

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Introduction

Genetic diversity is a central component of biodiversity (DeWoody et al. 2021). To protect biodiversity, therefore, we must understand how different processes structure genetic variation across temporal and spatial scales (Hewitt 2004; Cushman and Landguth 2010; Lucati et al. 2020). Key processes include isolation by barrier (IBB) in which impermeable landscape elements cause genetic isolation by restricting gene flow (Manel et al. 2003; Holderegger and Wagner 2008; Manel and Holderegger 2013), isolation by distance (IBD) that produces continuous genetic differentiation due to spatially limited dispersal (Wright 1943; Jenkins et al. 2010; van Strien et al. 2015), and isolation by environment (IBE) in which heterogeneous environments may further impact gene flow beyond IBD due to local adaptation or natal-site selection (Shafer and Wolf 2013; Sexton et al. 2014; Wang and Bradburd 2014).

These three processes may all contribute to spatial genetic variation, however, few studies have quantified their relative effects. For instance, Muñoz-Valencia et al. (2023) found that IBD and IBE effects from precipitation and temperature explained the most genetic variance of leaf-cutter ants (*Atta cephalotes*), but additional genetic variance was explained by the Andes Mountains acting as a barrier. Likewise, genetic variance of American badgers (*Taxidea taxus*) was explained by geographic distance, the Wisconsin River as a barrier, and agricultural land cover (Kierepka and Latch 2016a). Through partitioning genetic variation to these distinct processes, we can gain a holistic understanding of spatial genetic structure (Weber et al. 2016; Priadka et al. 2019; Muñoz-Valencia et al. 2023).

IBB patterns commonly arise from mountain ranges (Muñoz-Valencia et al. 2023), rivers (Pfau et al. 2001; Musher et al. 2022), or other impassible landscape structures. However, semi-permeable barriers may exist, including from anthropogenic causes (e.g., roads), where gene flow may be reduced but not completely disrupted (Esperandio et al. 2019; Lecis et al. 2022). IBD results from short dispersal distances across geographic space. Genetic similarities may be greater than expected under IBD, however, because of rare long-distance dispersal that maintains infrequent gene flow (Centeno-Cuadros et al. 2011; Alexander et al. 2019). Whereas IBB and IBD arise from dispersal processes, gene flow can also be driven by characteristics within a species' home range, leading to genetic patterns in neutral markers. IBE arises when local adaptation or natal habitat selection creates genetic structure due to associations with specific environmental conditions rather than solely spatial processes (Shafer and Wolf 2013; Orsini et al. 2013; Wang and Bradburd 2014). A combination of these processes affecting gene flow may be intertwined in species that span large riverways or other barriers, persist across a large geographic range, or exhibit strong environmental associations.

Furthermore, these genetic isolating effects may be spatially dependent with different processes dominating rangewide versus within a specific region (Anderson et al. 2010; Keller et al. 2013). For instance, IBB is expected to be most consequential at larger scales that may include multiple substantial barriers to movement. IBD and IBE may be strongest at smaller scales reflecting dispersal and habitat selection occurring within regions defined by barriers. IBD is more pronounced for species with low dispersal (Orsini et al. 2013), but may exhibit a threshold pattern, where eventually geographic distance does not contribute to added genetic distance (van Strien et al. 2015). Similarly, IBE may vary across spatial scales either due to environmental spatial autocorrelation (Shafer and Wolf 2013), or dispersal swamping out local adaptation (Richardson et al. 2014). Determining the relative strengths and scales of the isolating processes can guide efforts to maintain or restore suitable habitat and landscape connectivity.

Multi-scale landscape genetic studies can clarify management units and identify the spatial extent that matches conservation goals (Keller et al. 2015). By retaining large-scale management considerations, restoration or habitat management at small scales can be leveraged for broad-scale planning (Augustine et al. 2021, Gilby et al. 2021). This perspective is important for managing prairie ecosystems as large-scale, connected grasslands are needed for both migratory species and sedentary species with metapopulation dynamics (Augustine et al. 2021). However, grasslands often are small and fragmented patches with highly altered vegetation communities, thus identifying how local management practices fits within a rangewide context is necessary to promote connectivity (Augustine et al. 2021; Warner 1994).

The plains pocket gopher (*Geomys bursarius*) is a grassland species that provides an excellent opportunity to examine multiple drivers of gene flow across spatial scales because it is a subterranean species that may have strong, hierarchical genetic structuring (e.g., Mapelli et al. 2020). *Geomys bursarius* ranges across the Great Plains from Texas to southern Canada, with only *G. b. illinoensis* and *G. b. wisconsinensis* occurring east of the Mississippi River (Connior 2011), likely following post-glacial range expansion similar to many other taxa (Smith 1957). Rivers and other water bodies are likely effective barriers that prevent gene flow for many subterranean rodents (Visser et al. 2018; Mapelli et al. 2020; Austrich et al. 2020). *G. bursarius* cannot swim well (Komarek and Spencer 1931; Kennerly 1963), and the Mississippi River aligns with a subspecies boundary for *G. b. illinoensis* (Connior 2011). Furthermore, most subterranean rodents have limited gene flow, even over short distances, and develop IBD over time (Mapelli et al. 2012; Fasanella et al. 2013; Gómez Fernández et al. 2016; Visser et al. 2018). *Geomys bursarius* also exhibits strong soil associations (Reichman and Seabloom 2002), and anthropogenic land use prevents gophers from persisting in historically occupied soils (Alexander et al. 2022). Understanding these isolating effects may help to designate management units and ensure conservation efforts are enacted close enough to an already established population.

Burrowing rodents often display local adaptation to soil structure, potentially generating IBE patterns (Barbosa et al. 2021). In fact, soil properties act as distribution boundaries that delineate gopher species (Hoffman et al. 2007; Hoffman and Choate 2008). Gophers may exhibit IBE patterns based on energetic costs related to soil friability (Vleck 1979, 1981) and selection of familiar soil types. Also, gophers exhibit pelage matching to soil color (Hendricksen 1972; Krupa and Geluso 2000; Rios and Álvarez-Castañeda 2012), which decreases predation risk even for subterranean rodents (Krupa and Geluso 2000; Rios and Álvarez-Castañeda 2012; Singaravelan et al. 2013). Although gopher pelage color has not been linked to a single locus (Wlasiuk and Nachman 2007), neutral loci may identify how soil color influences gene flow due to presumed fitness costs.

There are two possible reasons for pelage color matching soil color. First, gophers occasionally disperse above ground (Warren et al. 2017; Pynne et al. 2019), and matching surface soil color would increase crypsis during dispersal events. Second, crypsis during burrow construction may impact fitness. How mound creation correlates to time above ground is unclear, but gophers may create three mounds per day with ~ 60% of excavated soil deposited on the surface (Andersen 1987). Gopher foraging tunnels are close to the surface, whereas nest chambers may be deeper (~ 50 to 100 cm below the surface; Wilkins and Roberts 2007). Crypsis during soil removal from burrows may drive color-matching adaptation. However, genetic patterns resulting from fitness benefits of crypsis in gophers has not been assessed. Identifying genetic associations with soil properties is also important for conservation as gophers may be excluded from historically occupied soils due to land-use intensification (Alexander et al. 2022).

Because IBB, IBD, and IBE may all contribute to genetic structure, we examine the relative effects of these isolating processes on plains pocket gophers. Also, because genetic differentiation is scale dependent, we explore isolating effects both for *G. bursarius* across the geographic range and for a subspecies, *G. b. illinoensis*, within a region including Illinois and Indiana. *Geomys bursarius illinoensis* should be considered an Evolutionary Significant Unit (Alexander 2023), and it occurs in a region historically dominated by tallgrass prairie and oak savannah but now dominated by agriculture (Augustine et al. 2021; Alexander et al. 2022). For *G. bursarius*, we predict that IBB effects will occur due to the Mississippi River and other major waterways, and that there will be strong IBD due to limited dispersal. We also hypothesize there will be IBE from soil sand percentage affecting friability and from soil surface color affecting crypsis during above ground dispersal and creating mounds. For *G. b. illinoensis,* we predict no IBB effects due to no large riverways within the subspecies range, but we expect IBD from dispersal limitations and colonization history, and IBE due to similar processes for the species across the range.

Methods

Tissue samples and DNA extraction

We collected toe samples from 241 museum specimens of *G. bursarius* from the Illinois Natural

History Survey including 8 subspecies from across the range, only omitting *G. b. ozarkensis* (Table S1). All museum specimens were collected between 1921 and 1985 and included subspecies identification except for samples from Wisconsin (n = 30), which we classified as *G. b. wisconsinensis* based on the range map (Connior 2011). We also collected tissue samples from 27 live-trapped *G. bursarius illinoensis* (UIUC IACUC #17190) in 2018–2019, following appropriate guidelines (Sikes and The Animal Use and Care Committee of the American Society of Mammalogists 2016). To improve amplification, we used a 1× Sodium Choride-Tris–EDTA buffer to increase solubility of DNA and lysed the sample in an incubator with a rotisserie at 56 ˚C. We used QIAquick spin columns to capture DNA. We successfully sampled and amplified a total of 267 *G. bursarius*, 170 of which were *G. b. illinoensis*.

Microsatellite amplification and verification

To identify genetic structure, neutral genetic markers (e.g., microsatellites) are commonly used as they are not under selection and have a high mutation rate (Epps and Keyghobadi 2015). Although genomic or other markers (e.g., SNPs) are gaining use, microsatellites can still identify similar environmental-genetic associations and underlying processes (Hauser et al. 2021; Skey et al. 2023). We attempted amplification of 12 microsatellites (*GBR06*, *GBR09*, *GBR10*, *GBR15*, *GBR25*, *GBR26*, *GBR27*, *GBR33*, *GBR36*, *TM1*, *TM6*, and *TM7*; Steinberg 1999; Welborn et al. 2012). We optimized polymerase chain reactions by trying different starting conditions, and fragment analysis was done on an ABI Prism 370xl Analyzer. Alleles were scored manually using Geneious v.11.1.5 (https://www.geneious.com).

To assess population heterozygosity statistics and determine that genetic markers were not closely associated with one another, we initially assigned individuals to populations a priori based on spatial proximity, using major rivers in the United States as population boundaries (Supplemental 1; Esri et al. 2010). We assessed Hardy–Weinberg Equilibrium (HWE), linkage disequilibrium (LD), and null alleles using R code adapted from Wagner (2022). To test for HWE, we used a conservative $\alpha = 0.05$ with a chi-square test using the function "hw.test" in the R package *pegas* (Paradis 2010). If a locus was consistently out of HWE across populations, the locus was dropped. We tested LD via an index association analysis, using the "ia" function (Brown et al. 1980) and loci association using "pair.ia" (Agapow and Burt 2001) in *poppr* (Kamvar et al. 2014) with a permutation of 999. If the measure of correlation ($\bar{r}_d$) was ≤ 0.3 from pairwise comparisons between loci, the loci were retained as this would approximate a 10% linear correlation threshold (Wagner 2022). We estimated null alleles in *PopGenReport* (Adamack and Gruber 2014) using the "null.all" function, implementing the Brookfield (1996) method that effectively estimates null alleles at loci that could falsely appear homozygous. If there is spatial structuring and fixed alleles from Wahlund or other isolating effects, there may be an overestimation of null alleles, as null allele estimates assume HWE (Dabrowski et al. 2014). Thus, we assessed null alleles rangewide and for each region independently. We also estimated observed and expected heterozygosity and calculated rarefied allelic richness in *PopGenReport* (Adamack and Gruber 2014) and fixation index (F_{ST}) and inbreeding coefficient (F_{IS}) in *hierfstat* (Goudet 2005). Two regions had low sample sizes ($n \leq 8$). Given that this could impact our region-specific estimates of heterozygosity, rarefied allelic richness, and Fst, we estimated gene flow and population structure relying on individual-based analyses.

Isolation by barriers

To determine whether genetic barriers exist across the geographic range of *G. bursarius*, we used STRUCTURE v. 2.3.4, which identifies genetic clusters through maximizing HWE (Pritchard et al. 2000; Falush et al. 2003). STRUCTURE performs well at identifying the highest level of discrete genetic structures (Evanno et al. 2005; Chen et al. 2007), and it can also identify hierarchical genetic structures (Warnock et al. 2010).

We ran STRUCTURE with an admixture model without location priors (Hubisz et al. 2009). We tested 1–10 populations (K) with 5 trials at each value of K with 300,000 iterations and a 100,000-iteration burn-in. To determine the number of genetic clusters, we used the Evanno method that relies on the change in likelihood (Evanno et al. 2005) using STRUCTURE HARVESTER (Earl and vonHoldt 2012). Because STRUCTURE's log probability often overestimates K, the Evanno method

identifies the highest hierarchical genetic clustering based on the log probability's rate of change between successive K values (Evanno et al. 2005). We then visually inspected assignment of individuals to each cluster using CLUMPAK (Kopelman et al. 2015) using a 0.5 population assignment threshold (Kierepka and Latch 2016a). Because STRUCTURE resolves the highest level of genetic structure, we re-ran STRUCTURE on each identified cluster to determine if there was hierarchical genetic structure until no spatial pattern was evident (Vähä et al. 2007; Warnock et al. 2010). Then, we re-ran STRUCTURE just on *G. b. illinoensis* samples to determine genetic clusters within a single subspecies across a smaller spatial scale.

Uneven sampling can impact STRUCTURE clustering (Puechmaille 2016), so we thinned the *G. b. illinoensis* samples by 10 km (n = 40), keeping only one sample if multiple individuals were sampled within that distance, and re-ran STRU CTURE. Given the smaller sample size of the other seven subspecies (n = 97 total), we maintained all samples that were not *G. b. illinoensis* regardless of nearest-neighbor distance. *G. b. illinoensis* still had the highest sample size but was comparable to other subspecies (e.g., n = 32 for *G. b. wisconsinensis*). *GBR06* amplification failed mostly in northern samples, so we ran STRUCTURE using the thinned sampling of *G. b. illinoensis* and all other *Geomys bursarius* samples excluding *GBR06* to ensure amplification failure did not bias clustering.

To further understand how rivers may act as barriers for gophers, we calculated F_{ST} (Weir and Cokerham 1984) between a priori regions (see Section "Microsatellite amplification and verification"). Whereas STRUCTURE can create clusters based on genetics with landscape features being identified post hoc, estimating F_{ST} between regions allows for testing of our IBB hypothesis directly. For *G. bursarius* rangewide, we assigned individuals to populations with major rivers acting as population boundaries (Supplemental 1, Esri et al. 2010). For *G. b. illinoensis*, we assigned individuals to populations with rivers that form major watersheds (Illinois State Water Survey 2011) serving as population boundaries. To calculate F_{ST}, we used "pairwise.WCfst" and estimated bootstrap values with "boot.ppfst" and 1000 bootstraps in the R package *hierfstat* (Goudet 2005).

Isolation by distance

To detect genetic spatial autocorrelation for *G. bursarius* (rangewide) and for *G. b. illinoensis* (within Illinois and Indiana), we used Moran Eigenvector Maps (MEMs), which rely on regression of genetic distances in a multivariate framework (Galpern et al. 2014). MEMs identify genetic clines across geographic distances, making them a complementary approach to STRUCTURE (Galpern et al. 2014; Priadka et al. 2019). We calculated proportion of shared alleles (D_{PS}; Bowcock et al. 1994) to estimate genetic dissimilarity between individuals (Shirk et al. 2017) using the "codomToPropShared," function and then calculated and visualized Moran eigenvectors using the "mgQuick" and "mgMap" functions in the R package *memgene* (Galpern et al. 2014), mapping the first two axes. We also ran a leave-one-out sensitivity analysis of MEMs, iteratively omitting one locus to determine potential effects of null alleles.

Isolation by environment

We tested if gene flow was associated with soil conditions using redundancy analysis (RDA) with a Principal Components Analysis (PCA) of allele frequencies as a dependent matrix (Muñoz-Valencia et al. 2023). We conducted the analysis for *G. bursarius* and at the regional scale for *G. b. illinoensis*. In our global model, we included sand percent (Soil Survey Staff 2018) as a metric of soil friability resulting from glacial processes (Dobos et al. 2023), soil color at 5 cm and 75 cm depths (Soil Survey Staff 2022) to assess genetic signatures of pelage-soil color matching, and geographic distance to account for IBD genetic patterns. Although multiple soil properties may impact friability (e.g., percent clay, particle size), these parameters were all correlated with soil sand percent. Thus, soil sand percent should be considered as a proxy for multiple contributors to soil friability. Land cover is an increasingly important driver for *G. bursarius* (Alexander et al. 2022), however, given the dynamic nature of land cover and the temporal breadth of our genetic samples, we retained only predictors that should be largely stable over the past 100 years. To determine if there were temporal effects on genetic similarity, we included the year of sample collection in the model.

We included soil color at two depths as color matching may occur for above ground dispersal (5 cm) or for excavation of deeper soils to create nest or other chambers (75 cm) and depositing those soils on the surface. We converted soil color maps to RGB color maps in ArcMap 10.8.1 using the "copy raster" function, in which a unique raster was generated for red, green, and blue pixel values. We then calculated Euclidean distances in three-dimensional space between the raster values at gopher locations for soil color at 5 cm and 75 cm depths to quantify soil color similarity. To convert distance matrices to linear vectors, we used principal coordinates of neighborhood matrices (PCNM; Borcard and Legendre 2002) for geographic distance and soil color distances using the "pcnm" function in the R package *vegan* (Oksanen et al. 2022). All continuous variables were scaled by centering means to 0 and dividing by the standard deviation. We used "dudi.pca" from the *ade4* R package (Dray and Dufour 2007) and retained two principal components (PCs) of the allele frequencies PCA. We then performed RDAs using the "rda" function to create a global and a null model and used "ordistep" in *vegan* to select the top models for *G. bursarius* and then for *G. b. illinoensis*. We assessed model fit using an Analysis of Variance using the "anova.cca" function and estimated the adjusted R^2 using the "RsquareAdj" function in *vegan*. We assessed variation inflation factors (VIFs) using the "vif.cca" function in *vegan*. We also estimated IBE following the above methods omitting each locus iteratively in a leave-one-out sensitivity analysis to determine potential impacts of null alleles.

Variation partitioning

To determine the relative effects of IBB, IBD, and IBE on gopher genetic structure, we conducted variation partitioning of the two PCs for *G. bursarius* and for *G. b. illinoensis* (see 'Isolation by Environment'). We ran "rda," "vif.cca," and "varpart" in *vegan* (see Section "Isolation by environment"), separating out a priori regions for IBB (see Section "Microsatellite amplification and verification"), PCNM of distance for IBD, and then PCNM of soil colors (5 and 75 cm) and sand percent for IBE. We also included the year of sample collection to account for genetic similarity based on time. We estimated variation partitioning omitting each locus iteratively in a leave-one-out sensitivity analysis to determine potential impacts of null alleles.

Results

Microsatellite amplification and verification

Four microsatellites *(GBR15*, *GBR26*, *GBR33*, and *TM7*) either had amplification failure or ambiguous genotypes, and one microsatellite (*GBR36*) was monomorphic. These five microsatellites were excluded from analyses, leaving a total of seven microsatellites. Other studies have effectively identified genetic structure with a similar number of markers (e.g., Cosentino et al. 2015; McCluskey et al. 2022). We also identified substantial genetic structuring (see below), but we acknowledge that additional fine-scale structure may have gone undetected.

All retained loci were out of HWE globally; however, no locus was consistently out of HWE across all a priori populations determined by major rivers indicating Hardy–Weinberg disequilibrium was a result of spatial isolation and drift rather than other mechanisms. The population of *G. b. illinoensis* deviated from HWE at all loci, but this is likely due to increased sample size compared to the other regions. The Wisconsin samples only had one individual that amplified at *GBR06*, so *GBR06* was omitted from the Wisconsin null allele analysis. One locus, *TM6*, had null alleles in regions with higher sample sizes, so we estimated observed heterozygosity, expected heterozygosity, and rarefied allelic richness excluding *TM6* as well (Supplemental 2). Null allele frequencies ranged from 0.1 to 0.34 (Supplemental 2). Deficiencies of heterozygotes can increase the signal null alleles (Dabrowski et al. 2014; Meeûs 2018), and identification of null alleles at the rangewide scale is likely due in part to population structure (i.e., Wahlund Effects). Within regions, null alleles were generally lower, with estimates often not distinguishable from 0 (Supplemental 2). Given the low dispersal distances of *G. bursarius* and likely Wahlund effects, we kept the remaining 7 microsatellites in the analyses. The overall loci index association was 0.22 and an $\bar{r}_d = 0.038$. However, because all pairwise $\bar{r}_d$ were ≤ 0.3, we assumed no linkage between loci. Sample sizes from *apriori* populations ranged from 4 to 170 (mean = 38, SE = 22; Supplemental 2).

Observed heterozygosity was lower than expected heterozygosity across all loci globally (Ho = 0.477, He = 0.702, F_{ST} = 0.167, F_{IS} = 0.320; Supplemental 2). Rarefied allelic richness ranged from 2.14 to 2.88 (Supplemental 2).

Isolation by barrier

For *G. bursarius*, we identified 2 populations across the range with the STRUCTURE analysis. At this highest level, *G. b. illinoensis* clustered separately from the rest of *G. bursarius* with the separation mostly aligning with the Mississippi River (Fig. 1a). We then observed genetic clustering west of the Mississippi River (n = 103) with two populations identified mostly along subspecies and latitudinal gradients (n_{K1} = 57, n_{K2} = 47), generally separated by the Missouri River (Fig. 1b). For the *G. b. illinoensis* population east of the Mississippi River (n = 164), two subpopulations were identified (n_{K1} = 101, n_{K2} = 63) but admixture in the individual assignment plots revealed a clinal structure rather than discrete populations (Fig. 1c). These two subpopulations within *G. b. illinoensis* are likely spurious clusters due to STRUCTURE's algorithm and more likely represent an IBD pattern.

Using a thinned sample of *G. b. illinoensis* but with all other samples included, we still identified two populations, but the clusters resolved around a south-north barrier. The structure included populations north (n = 52) and south (n = 85) of the Missouri and Illinois Rivers, with *G. b. illinoensis* clustering with the southern samples (Fig. 2a). Despite two populations having the strongest support, three populations were also well supported. We visually inspected individual assignments with K = 3, which identified *G. b. illinoensis* as a third cluster (Supplemental 3).

For the two clusters identified with the thinned sampling method, no further spatial structure resolved in the northern population, indicating that the seven microsatellites used here could not separate the subspecies *G. b. majusculus, G. b. wisconsinensis*, and *G. b. bursarius*. For the southern cluster, there was added structure with *G. b. illinoensis* clustering separately, and the Mississippi River acting as a boundary (Fig. 2b). When *G. b. illinoensis* was analyzed alone, we identified 2 populations (Fig. 1c). However, individuals showed a south-north gradient of admixture indicating that IBD is more likely than IBB for *G. b. illinoensis*.

For *G. bursarius*, F_{ST} values ranged from 0.048 to 0.320 based on a priori defined regions divided by major rivers (Supplemental 2). The populations divided by the Canadian River in the southern region had relatively low F_{ST} (0.049), whereas populations divided by the Arkansas, Missouri, Mississippi, and Illinois Rivers all had pairwise $F_{ST} > 0.1$. F_{ST} for only *G. b. illinoensis* ranged from 0.035 to 0.145 based on region, with only the populations divided by the Canadian River having an Fst of 0.049 (Supplemental 2). The Canadian River divides the Kiowa and Red Hills regions, which both had a low sample size, and further sampling may better identify genetic structure created by the river.

Isolation by distance

For *G. bursarius*, geographic distance was correlated with genetic distance (r^2 = 0.34) based on the MEM. The MEM identified structuring along the first axis similar to STRUCTURE, with two populations divided by the Mississippi River (proportion of variation = 0.41). The second axis revealed a south-north clinal genetic structure (proportion of variation = 0.27; Fig. 3a). The leave-one-out sensitivity analysis displayed the same pattern (Supplemental 4), so only the analysis retaining all loci is included here.

For *G. b. illinoensis*, geographic distance also was correlated with genetic distance (r^2 = 0.17). Gophers in southwest Illinois were genetically similar to gophers in the northeast along the first axis (proportion of variation = 0.46). However, gophers in southwest Illinois were genetically similar to gophers in western Illinois along the second axis (proportion of variation = 0.26; Fig. 3b).

Isolation by environment

For the IBE models, all VIFs were < 2 indicating no multicollinearity and the first two principal components explained genetic variance well (Supplemental 5). For *G. bursarius*, the top model included soil color at 5 cm ($p < 0.001$), sand percent ($p < 0.001$), and geographic distance ($p < 0.001$) with an adjusted R^2 of 0.36 (Table 1). The rangewide leave-one-out analysis mostly included the same parameters, with the model omitting *TM6* only including distance and sand

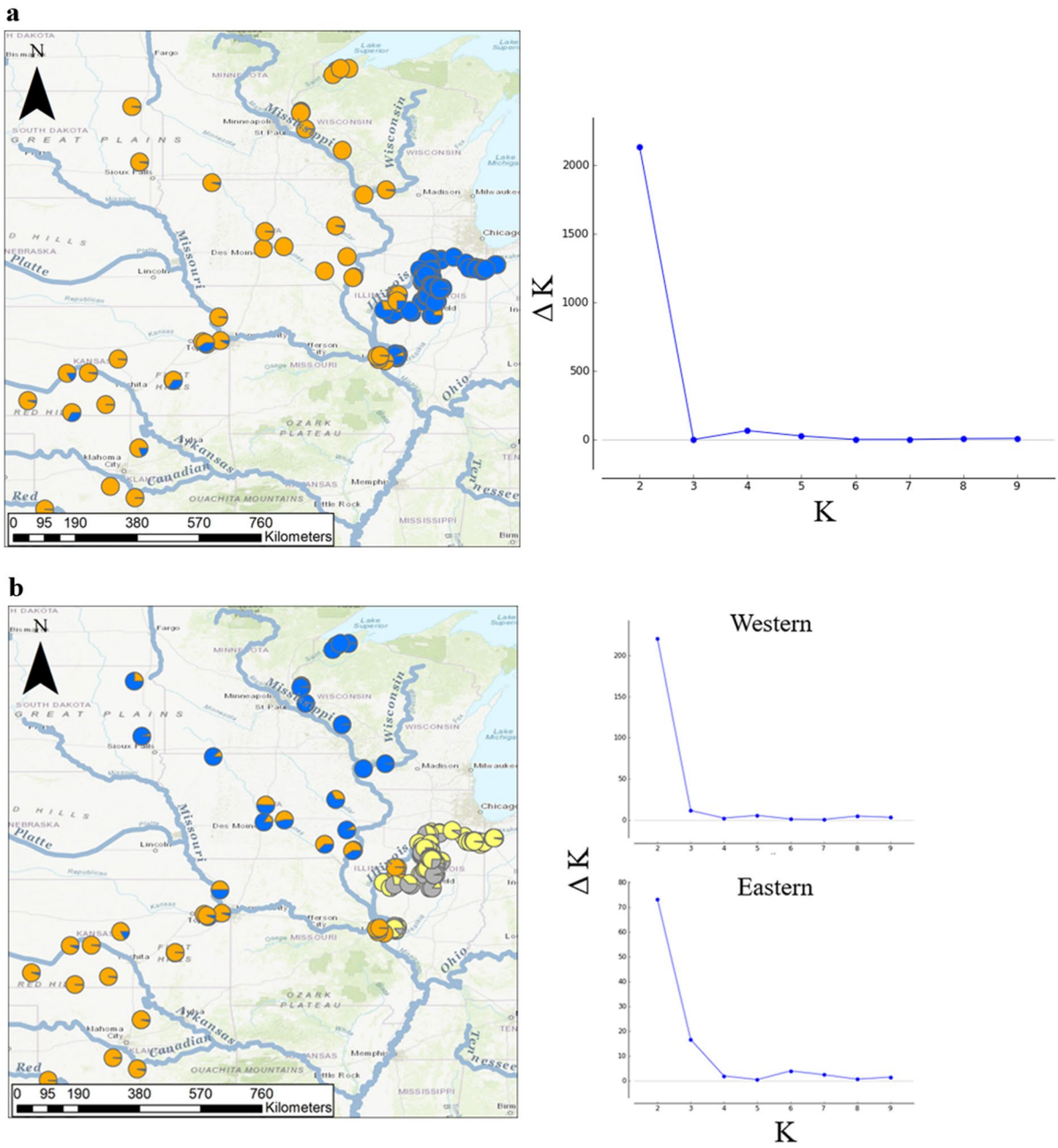

Fig. 1 Individual population assignments with admixture from STRUCTURE (left), and number of populations estimated using the Evanno method (right) without spatial thinning for **a** all samples of *Geomys bursarius* (n = 267), **b** with further structuring for the western (n = 103) and eastern (n = 164) populations, and **c** only *G. b. illinoensis* (n = 170). Individual assignments viewed on the base World Topography Map (ESRI et al. 2017)

percent, and the model omitting *GBR25* including distance, soil color at 5 cm, and soil color at 75 cm (Supplemental 6). For *G. b. illinoensis*, the top model included soil color at 75 cm ($p = 0.029$), sand percent ($p = 0.005$), and geographic distance ($p < 0.001$) with an adjusted R^2 of 0.31 (Table 1). For *G. b. illinoensis*, all of the leave-one-out models included distance, five out of the seven top models included sand color at

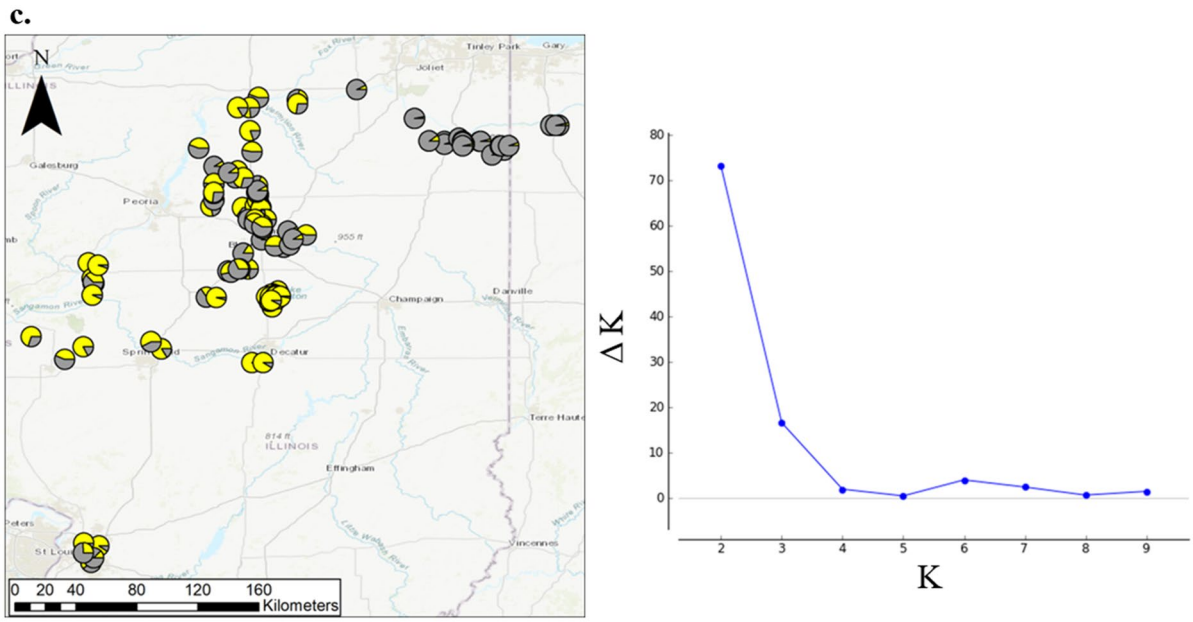

Fig. 1 (continued)

75 cm, and four out of the seven top models included sand percent (Supplemental 6).

Variation partitioning

For *G. bursarius*, IBB explained the most variance independently (adjusted $R^2 = 0.26$), but there was high variance shared by IBB and IBD (adjusted $R^2 = 0.27$; Fig. 4a). Variance partitioned to IBE minimally explained genetic patterns independently (adjusted $R^2 = 0.01$). However, additional variance was shared by IBB and IBE (adjusted $R^2 = 0.04$) and by IBB, IBD, and IBE (adjusted $R^2 = 0.03$). IBD did not explain any genetic variance independently rangewide, but shared variance with IBB and IBE. Year did not have any variation partitioned independently, but had variance shared by IBB and IBD (adjusted $R^2 = 0.02$). Similar patterns emerged from the leave-one-out sensitivity analysis (Supplemental 6).

For *G. b. illinoensis*, variance shared by IBB and IBD was relatively strong (adjusted $R^2 = 0.18$), but IBD had the most variation partitioned as a single factor (adjusted $R^2 = 0.06$; Fig. 4b). IBE did not explain any genetic variation independently, but IBE shared variance with IBB and IBD (adjusted $R^2 = 0.04$). Year again had minimal variation partitioned to it. Overall, IBB and IBD processes explained the most genetic variation for the gopher subspecies. Similar patterns emerged from the leave-one-out sensitivity analysis (Supplemental 6).

Discussion

By considering the relative effects of isolation by barriers, distance, and environment, we showed that large-scale and regional gene flow for a fossorial rodent were driven by different processes. For the range-wide analysis of *G. bursarius*, more genetic variation was partitioned uniquely to IBB than to IBD or IBE. In contrast, when analyses were restricted to *G. b. illinoensis* in Illinois and Indiana, IBD uniquely explained the most genetic variation. However, substantial genetic variation could not be partitioned between IBB and IBD at either scale likely due to both processes creating north–south isolating effects. Isolation by environment minimally explained genetic structure at both scales, although IBE shared variance with IBB and IBD.

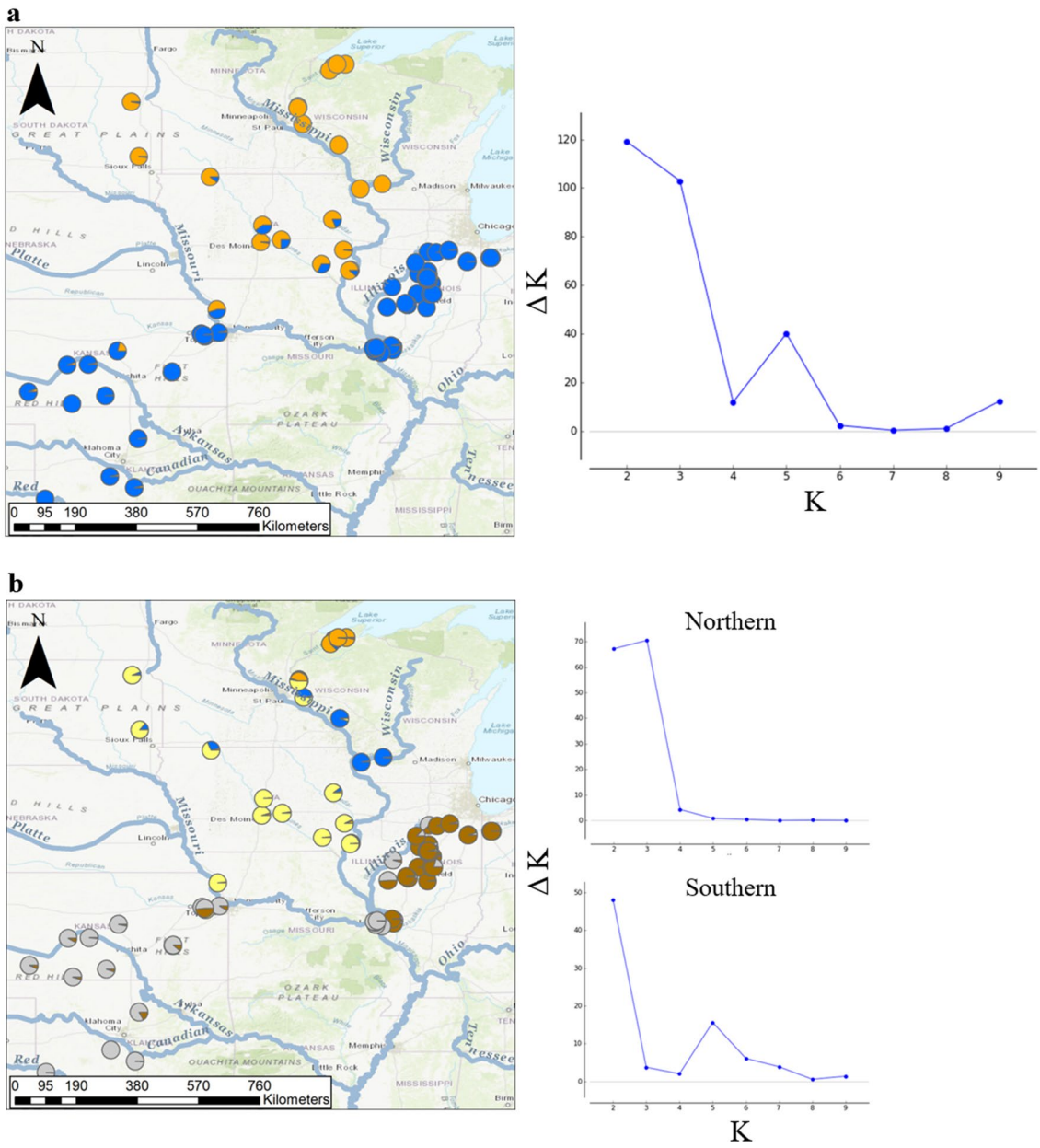

Fig. 2 Population assignments from STRUCTURE (left) and number of populations estimated using the Evanno method (right) with (**a**) spatial thinning of *Geomys bursarius illinoensis* (n = 137), and **b** samples from the resulting substructures in the northern population (n = 52) and the southern population (n = 85) identified from the initial clustering with K = 3 in the north and K = 2 in the south. There is close support for K = 3 for the initial clustering (**a**), and close support for K = 2 for the northern population (**b**). Individual assignments viewed on the base World Topography Map (ESRI et al. 2017) with major rivers in the US (Esri 2010)

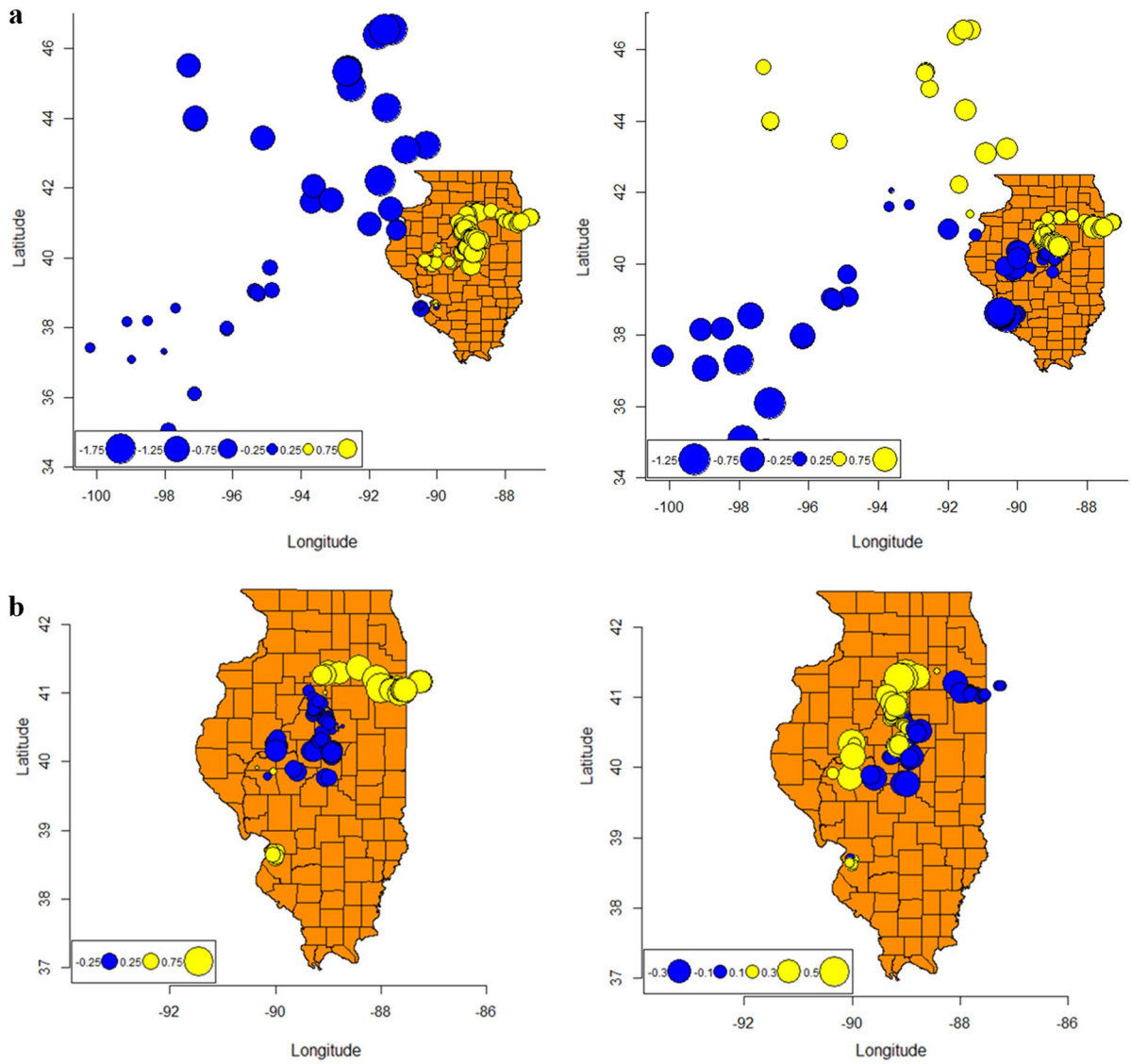

Fig. 3 First axis (left) and second axis (right) for Moran Eigenvector Maps for (a) *Geomys bursarius* (n = 267, $R^2 = 0.34$), and (b) the *G. b. illinoensis* subspecies (n = 170, $R.^2 = 0.17$)

Isolation by barriers

Subterranean species, including gophers, are often isolated by major rivers (Connior 2011; Cutrera et al. 2013; Visser et al. 2018). Fossorial rodents have unique proximal limb morphology compared to rodents with other locomotor ecologies (Hedrick et al. 2020), possibly impacting swimming ability. For instance, *G. bursarius* can swim for ≤ 6.5 min (Hickman 1977). Major rivers acted as barriers for *G. bursarius* rangewide, with the Mississippi River, Illinois River, and Missouri River aligning closely with genetic separation identified by STRUCTURE. Both the Missouri and Mississippi Rivers have been identified as gene flow barriers for other taxa including badgers (Kierepka and Latch 2016b), northern leopard frogs (*Rana pipiens*; Waraniak et al. 2019), and Nearctic milksnakes (*Lampropeltis triangulum*; Burbrink et al. 2022), and the Illinois River is considered the northern range barrier for *G. b. illinoensis* (Hoffmeister 1989; Alexander et al. 2022). *Geomys bursarius illinoensis* likely colonized Illinois from

Table 1 Top model parameters from redundancy analysis for gene flow due to isolation by environment

Data set	n	PCs	Model parameters	Adjusted R^2
G. bursarius	267	2	Color (5 cm)*** + sand percent*** + distance***	0.36
G. b. illinoensis	170	2	Color (75 cm)* + sand percent** + distance***	0.31

We assessed genetic associations to environmental parameters using redundancy analysis across the full data set (*G. bursarius*) and a subset (*G. b. illinoensis*) including sample size (n) number of genetic principal components retained (PCs), the included model parameters (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), and adjusted R^2. We assessed genetic correlations to soil color at depths of 5 cm and 75 cm, sand percent, geographic distance, and year. Only the top models are shown, with model selection done through a stepwise function

individuals coming from southern populations of *G. bursarius*. After a dispersal event across the Mississippi during the Pleistocene (Smith 1957; Alexander 2023), rivers have remained important barriers. Moreover, the low observed heterozygosity supports a Wahlund effect of gopher populations isolated by barriers (Penney and Zimmerman 1976), and F_{IS} indicated that there is likely further population structure within each region. With higher resolution markers, further genetic structuring may be detected.

Even major riverways, however, may not have consistent barrier effects. *Geomys bursarius* on the eastern side of the Mississippi River in Wisconsin clustered with gophers to the west of the Mississippi rather than with *G. b. illinoensis*. However, this pattern is probably due to colonization history. There were likely two instances of *G. bursarius* crossing the Mississippi River, once from Missouri into Illinois, and a second within Wisconsin (Elrod et al. 2000). The Illinois River then acted as a barrier between *G. b. illinoensis* and *G. b. wisconsinensis*. The Mississippi River is also narrower in the north compared to the Missouri-Illinois divide, so colonization events may have been easier there. With smaller rivers there is likely reduced gene flow, but not complete isolation (Roratto et al. 2015; Painter et al. 2022). This pattern is similar to genetic structure of badgers in Wisconsin, with the Mississippi River acting as a barrier broadly (Kierepka and Latch 2016a), yet the smaller Wisconsin River does not (Kierepka and Latch 2016b).

When restricting the analyses to *G. b. illinoensis* in Illinois and Indiana, STRUCTURE and MEMs did not identify genetic discontinuity based on rivers. Moreover, most genetic variance was not uniquely associated with IBB and instead could not be partitioned between IBB and IBD. Hence, our prediction of IBB effects being scale-dependent was supported.

Isolation by distance

Although river barriers explained broad genetic variation, IBD patterns also emerged from the MEMs and variation partitioning analyses. For *G. bursarius*, however, genetic variation could not be partitioned between IBD and IBB due to the barriers isolating regions in an almost linear manner from the southwest to the northeast. The inability to partition between geographic distance and barrier effects may be due to northward colonization post glaciation, where colonization occurred in a largely linear manner. On a finer scale for *G. b. illinoensis*, IBD emerged as a contributor to genetic structure that could be parsed, but there was still considerable variation shared between IBD and IBB.

An interesting pattern emerged from the MEM of *G. b. illinoensis* in which the southwestern gophers were genetically similar to the northeastern gophers along the 1st axis but displayed similarities with other western individuals along the 2nd axis. This pattern further indicates that genetic structure remains from colonization history in which the *G. b. illinoensis* population in the southwest crossed the Mississippi River and expanded northeastward along the southern boundary of the Illinois River (Elrod et al. 2000). Although inferring colonization history from seven microsatellites should be done cautiously, this result is supported from phylogenetics indicating post-glaciation range expansion of *G. bursarius* across the Mississippi River (Smith 1957; Alexander 2023). An IBD pattern for subterranean species is expected as they likely exhibit short-distance dispersal (Welborn and Light 2014; Warren et al. 2017). Gophers disperse short distances above ground (< 800 m), generally with smaller individuals or juveniles dispersing and recruiting within < 50 m or until they encounter suitable habitat (Vaughan 1963; Williams and Cameron 1984; Daly and Patton 1990; Warren et al. 2017).

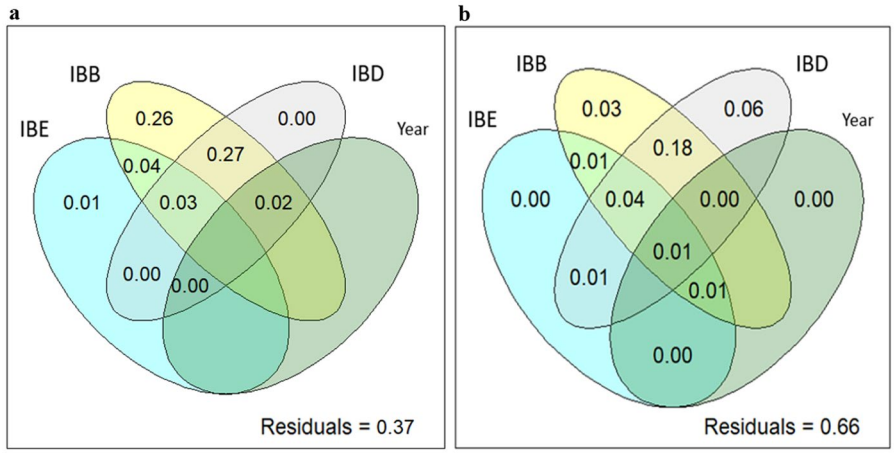

Fig. 4 Partitioning of genetic variation between isolation by environment (IBE), isolation by barrier (IBB), isolation by distance (IBD), and year. Results are for **a** *Geomys bursarius* using 2 principal components (PCs) of genetic variation, and **b** *G. b. illinoensis* using 2 PCs of genetic variation. Values < 0 are not shown and values of 0.00 indicate low partitioning values rounded to 0

Isolation by environment

Isolation by environment also contributed to genetic structure of *G. bursarius*, although minimally compared to other isolating processes. A key concept of IBE is that neutral loci can detect outcomes of processes driven by habitat selection or local adaptation without necessarily identifying the specific process (Sexton et al. 2014; Wang and Bradburd 2014). Although local adaptation may impact neutral processes, genetic drift and isolation drive mostly swamped any adaptive processes that might be identified. Soil dependency varies among *Geomys* species (Davis et al. 1938; Wilkins and Swearingen 1990; Alexander et al. 2022) and can maintain genetic and morphometric structure (Hendricksen 1972; Sudman et al. 1987; Mauk et al. 1999; Genoways et al. 2008). An interesting phenomenon that may underly the MEM for *G. b. illinoensis* in Illinois and Indiana is that soil properties vary across the distribution, with western regions having sandier soils and the northeastern and southwestern regions having a higher clay content. *G. b. illinoensis* has a bimodal selection for sand percent (Alexander et al. 2022), and with sand percent contributing to genetic variation across spatial scales based on IBE models, soil friability may affect genetic structure of gophers, fitting roughly with the MEM analysis.

Although texture and friability affect genetic structure of gophers, soil color also contributed to the IBE models. Pelage color matching soil color occurs across gopher species, likely due to predation risk (Hendricksen 1972; Krupa and Geluso 2000; Rios and Álvarez-Castañeda 2012). For *G. bursarius*, soil color at 5 cm depth was included in the top model, indicating soil matching affects genetic structure and there is predation pressure during above-ground dispersal for a predominantly subterranean species (Williams and Cameron 1984; Warren et al. 2017; Pynne et al. 2019). On a smaller scale for *G. b. illinoensis*, soil color at 75 cm depth contributed to genetic structure, but not at 5 cm. However, this outcome is likely due to limited variation of soil color at 5 cm at genetic sample locations for *G. b. illinoensis*. Soil color is impacted by soil texture, organic matter, minerals, and hydrology (Wascher et al. 1960; Schulze et al. 1993). Further, oxidation may convert blue-gray soil colors to more of an olive-brown (Donald McKay et al. 1986), thus limiting variation in soil color at 5 cm. *G. b. illinoensis* has experienced a niche reduction and shift in relation to soil sand percent and texture due to agricultural intensification, with a contemporary bimodal response to soil sand percent and a general shift towards sandier soils (Alexander et al. 2022). The impact of surface soil color on gene flow might be more pronounced with more contemporary samples.

Conclusions

Our complementary analyses demonstrated commonalities as well as differences in genetic structure and environmental associations across spatial scales of a species complex. We illustrated hierarchical genetic structures for a fossorial species in which IBB explained most of the genetic variation. However,

IBD and IBE also were consequential processes. Major rivers act as barriers; geographic distance creates clinal structure, at least within our focal subspecies, and soil traits promote genetic structure across spatial scales. As gopher relationships with soils have changed over time due to land use (Alexander et al. 2022), understanding potential gene flow reduction associated with loss of habitable soils can inform management decisions.

Whereas management of *G. bursarius* rangewide should focus on discrete management units bounded by rivers acting as natural barriers, management of *G. b. illinoensis* should consider ensuring gophers can reach suitable sites and that have similar soil properties as the source population. This allows managers to focus local efforts to bolster connectivity for populations within management units. Specifically, the Conservation Reserve Program is a strong tool to help manage grassland patch connectivity (Augustine et al. 2021), but managers applying these and similar conservation programs also should consider soil friability and distance to source populations when considering site prioritization. As gophers are one of many species that expanded northward post-glaciation, colonizing east of the Mississippi River during the Pleistocene (Smith 1957), partitioning genetic isolating effects can identify conservation priorities for a multitude of species.

More generally, recognizing genetic-environmental associations is increasingly important for conservation efforts and can help maintain adaptive potential (Capblancq et al. 2018; Capblancq and Forester 2021; Muñoz-Valencia et al. 2023). It is critical to consider multiple processes because genetic structure can be a result of colonization history, landscape connectivity, local adaptation, demography, or an interaction between processes (Orsini et al. 2013). Although genetic variance produced by each isolating process may not parse to unique drivers (Nadeau et al. 2016), RDA is a promising tool to identify what variance can or cannot be partitioned (Capblancq and Forester 2021). As the field of landscape genetics continues to develop, integrated approaches can guide conservation practices (Ruiz-Gonzalez et al. 2015; Priadka et al. 2019) and may prevent inflated correlations that can emerge if only a single process is considered.

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Declarations

Competing interests The authors have no competing interests, financial or otherwise, to disclose.

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